OPTIMIZING A LACTIC FERMENTATION OF SLICED CARROTS

REGINA M. NABAIS[1] and F. XAVIER MALCATA[2]

Escola Superior de Biotecnologia
Universidade Catolica Portuguesa
Rua Dr. Antonio Bernardino de Almeida
4200 Porto, Portugal

ABSTRACT

Response surface methods were utilized in the statistical optimization of several quality factors pertaining to the preservation of carrot slices using a brine containing lactic acid produced by in situ fermentation. These factors were the concentrations of salt, acid, and reducing sugars, and the texture of the carrot material, and the pH, concentrations of acid, reducing sugars, and biomass (at two different times) of the brine. The processing variables considered were the temperature, the salt concentration of the brine, and the ratio of volumes of brine to the carrot material. The starting (corner and center) factorial design has indicated that, within the experimental range of practical interest, a linear model failed to provide a good fit to the experimental data; hence, this design was complemented with an axial design. Checks of the residuals and estimates of third-order parameters have indicated that no apparent reason existed to question the statistical adequacy of the quadratic empiric model. The loci of the optima (and the characterization of such optima) were then obtained for this model, and the general directions for the variation of the values of the processing parameters were presented. This study has indicated that temperature may be manipulated to give rise to any desired increase or decrease of the quality factor chosen, a conclusion that may be relevant in attempts to industrially improve carrot preservation processes based on lactic acid fermentation.

[1]Current address: Escola Superior Agraria de Coimbra, S. Martinho do Bispo, 3000 Coimbra, Portugal.
[2]To whom all correspondence should be addressed.

INTRODUCTION

The preservation of such vegetables as carrots in low pH brines has been employed extensively using mainly one of two alternative routes: addition of food grade organic acids to the brines or in situ acidification by fermentation. The latter route possesses the particular advantage that the major factor responsible for the extension of the shelf life of the carrots, i.e., the low pH brought about by the presence of organic acids, is obtained at the expense of the metabolic use by the microorganisms of the sugars contributed to the solution by the carrots themselves, thus reducing undesirable secondary fermentations (Nicketic-Aleksic 1973; Fleming *et al.* 1983).

Lactic acid is an important tool in food grade fermentations because *Lactobacillus plantarum* (the most common strain employed), a facultative heterofermentative strain (Bergey 1989), possesses a very high acid tolerance (Fleming 1982), lactic acid is not toxic for human consumption (Adam and Hall 1988), and the low pH in the brines brought about by lactic acid prevents infections (Bell *et al.* 1972). Since the initial common pH of brines is ca. 5, most of the lactic acid remains in dissociated form ($pK_a=3.86$), which is known to have a low inhibitory effect in desired microorganisms due to its slow rates of transport across their membrane (Adams and Hall 1988). The above reasoning, coupled with the observation that high acid concentrations in the medium strongly correlate with low pH values in the vegetable edible tissue (Moreira *et al.* 1988), make it of crucial importance to tailor a pickling fermentation process in order to achieve high rates of acid production immediately upon start up of the brining process (Adams and Hall 1988). Since lactic acid is a growthassociated product, such high rates of acid production are obtained at the expense of high growth rates of the lactic acid bacteria during, say, the first 24 to 48 h of brining.

Salt deliberately added to the brines plays an important role in pickling (Binsted *et al.* 1962). In fact, this solute helps leaching of water and reducing sugars from the vegetable tissues, and together with the organic acids helps inhibit the growth of undesirable microorganisms (Fleming 1982; Steinkraus 1983). However, excess of this solute has been implicated with such major changes in the vegetable structure as membrane shrinkage (Schwartzberg and Chao 1982), as well as the development of unwanted osmophilic microorganisms (Diez *et al.* 1985) and the inhibition of lactic acid production (Diez 1985). The current trend is towards the reduction of the overall inventory of salt in brining processes due to its pollution potential and to adverse health effects upon ingestion by consumers (Diez *et al.* 1985).

In addition to the detrimental effect of salt upon the vegetable cell membranes, softening enzymes initially present in the plant tissue or attack by microbial enzymes also cause loss of texture of fermented pickles (Fleming *et* *al.* 1983). Hence, it is a good practice in the manufacture of fermented pickles to generate as high a concentration of acid as possible not only because of its inhibitory effect on undesirable microorganisms during the primary fermentation but also because of the increase in the extent of sugar leaching with concomitant effects in the prevention of growth of spore-forming, spoilage bacteria and secondary fermentation yeasts on the fermentable sugars remaining in the carrot material (Fleming *et al.* 1983; Diez *et al.* 1985; Adam *et al.* 1988). However, low pH values also cause calcium shifts in the pectic substances (Diez *et al.* 1985), which may directly lead to extensive losses of texture, and so a compromise must be reached in order to obtain final pickles with good quality.

The transport of sugars from the carrot cells to the brine has been studied previously for relatively low temperatures by a number of authors (Steinkraus 1983; Potts *et al.* 1986; Nabais *et al.* 1995; Nabais and Malcata 1995), as well as the transport of lactic acid (Potts *et al.* 1986; Nabais *et al.* 1995; Nabais and Malcata 1995), and sodium chloride from the brine to the carrots (Nabais *et al.* 1995; Nabais and Malcata 1995). The fermentation of the sugars upon leaching from the vegetables to the brines by deliberately added microbial inocula has received the attention of several researchers (Nicketic-Aleksic 1973; Etchells 1973; Fleming 1982; Diez *et al.* 1985; Andersson *et al.* 1990). However, the conditions which lead to optimal values of predefined quality factors of the carrots has not been established to date in a simultaneously comprehensive and statistically sound manner.

It is the aim of this communication to report optimization efforts using response surface methodology for nine different quality factors using as predictive variables three processing factors which are easily manipulated.

EXPERIMENTAL METHODS

Previous experience has indicated that the temperature, the concentration of salt in the brine, and the ratio of the volumes of the brine and that of the carrots were three operating variables which should deserve careful study not only because their values can be easily manipulated, but also because they affect the properties of the final pickled carrots in a significant fashion. However, it should be borne in mind that practical limits exist for the ranges of variation of such processing variables because of organoleptic constraints posed by the final consummer.

In terms of quality factors, the choice is obviously much wider, and can be either associated with the properties of the brine or the properties of the carrots after a reasonable batch time has elapsed. Four quality factors associated with the carrots were considered in this analysis: (1) the concentration of chloride

TABLE 1.

STARTING (FIRST ORDER) EXPERIMENTAL DESIGN FOR TEMPERATURE (T), SALT CONCENTRATION IN THE BRINE (C), AND VOLUME VOLUME OF THE BRINE (V); EXPERIMENTAL DATA OBTAINED FOR THE CONCENTRATION OF CHLORIDE SALTS IN THE CARROT (S, %W/W), CONCENTRATION OF ACIDS IN THE CARROT (A, %W/W), CONCENTRATION OF REDUCING SUGARS IN THE CARROT (R, %W/W), TEXTURE OF THE CARROT (N, Gp/CM^2), PH OF THE BRINE (p), CONCENTRATION OF ACIDS IN THE BRINE (a, %W/V), CONCENTRATON OF REDUCING SUGARS IN THE BRINE (r, %W/V), CONCENTRATION OF BIOMASS AFTER 24 H IN THE BRINE (f, %W/V), AND FINAL CONCENTRATION OF BIOMASS IN THE BRINE (x, %W/V); VALUES OF MAIN EFFECTS; CHECS OF SECOND ORDER INTERACTIONS (12, 13, AND 23); AND CHECKS OF QUADRATIC CURVATURE (11+22+33). (RUNS WERE MADE IN RANDOM ORDER.)

		E				LoF				MV CM				MVB				
	U	(I)	(1)	(2)	(3)	(12)	(13)	(23)	(11)+(22)+(33)	S	A	R	N	p	a	r	f	x
C	1	+1	-1	-1	-1					0.42	0.87	0.51	0.95	3.20	1.04	1.56	0.31	1.36
	2	+1	-1	-1	+1					0.82	0.50	0.19	0.75	3.50	0.67	0.39	0.27	0.65
	3	+1	-1	+1	-1					2.87	0.45	0.65	0.94	3.50	0.52	3.04	0.26	0.98
	4	+1	-1	+1	+1					3.80	0.18	0.42	0.87	4.40	0.35	2.43	0.24	0.60
	5	+1	+1	-1	-1					0.51	0.69	0.74	0.90	3.30	0.70	4.46	0.53	0.62
	6	+1	+1	-1	+1					0.99	0.30	0.31	0.61	3.90	0.40	1.92	0.36	0.48
	7	+1	+1	+1	-1					3.50	0.22	0.73	0.98	3.50	0.39	5.89	0.36	0.67
	8	+1	+1	+1	+1					4.37	0.09	0.49	0.65	4.60	0.12	6.50	0.25	0.39
c	9	+1	0	0	0					2.33	0.41	0.33	0.86	3.60	0.35	1.05	0.41	0.56
	10	+1	0	0	0					2.31	0.43	0.34	1.08	3.65	0.39	1.00	0.41	0.54
	11	+1	0	0	0					2.33	0.43	0.31	1.10	3.60	0.39	0.95	0.40	0.57
EE ±t.σ	S	2.2045 ±0.0077	-0.1825 ±0.0094	1.4750 ±0.0094	0.3350 ±0.0094	0.1175 ±0.0094	0.0025 ±0.0094	0.1150 ±0.0094	-0.1633 ±0.0177									
	A	0.4155 ±0.0077	-0.0875 ±0.0094	-0.1775 ±0.0094	-0.1450 ±0.0094	0.0075 ±0.0094	0.0150 ±0.0094	0.04500 ±0.0094	-0.0108 ±0.0177									
	R	0.4564 ±0.0101	0.0625 ±0.0125	0.0675 ±0.0125	-0.1525 ±0.0125	-0.0250 ±0.0125	-0.0150 ±0.0125	0.0350 ±0.0125	0.1783 ±0.0234									
	N	0.8809 ±0.0884	-0.0463 ±0.1086	0.0288 ±0.1086	-0.1113 ±0.1086	0.0013 ±0.1086	-0.0438 ±0.1086	0.0113 ±0.1086	-0.1821 ±0.1639									
	P	3.7045 ±0.0192	0.0875 ±0.0235	0.2625 ±0.0235	0.3625 ±0.0235	-0.0375 ±0.0235	0.0625 ±0.0235	0.1375 ±0.0235	0.1208 ±0.0442									
	a	0.4836 ±0.0153	-0.1213 ±0.0188	-0.1788 ±0.0188	-0.1388 ±0.0188	0.0313 ±0.0188	-0.0038 ±0.0188	0.0288 ±0.0188	0.1471 ±0.0354									
	r	2.6536 ±0.0332	1.4188 ±0.0408	1.1913 ±0.0408	-0.4638 ±0.0408	0.3113 ±0.0408	-0.0188 ±0.0408	0.4638 ±0.0408	2.2738 ±0.0766									
	f	0.3455 ±0.0038	0.0525 ±0.0047	-0.0450 ±0.0047	-0.0425 ±0.0047	-0.0250 ±0.0047	-0.0275 ±0.0047	0.0100 ±0.0047	-0.0842 ±0.0088									
	x	0.6745 ±0.0101	-0.1788 ±0.0125	-0.0588 ±0.0125	-0.1888 ±0.0125	0.0488 ±0.0125	0.0838 ±0.0125	0.0238 ±0.0125	0.1621 ±0.0234									

Note: c - Center points; C - Corner points; EE - Estimated Effects; E - Effects (linear); I - grand average; LoF - Lack of Fit analysis; MV CM - Measured Values for the Carrot Material; MV B - Measured Values for the Brine; *t* - probability point of Student's *t*-distribution; U - run; 1 - Normalized value of temperature, defined as (T-30)/10, where T is expressed in °C; 2 - Normalized concentration of salt in the brine, defined as (C-4)/3, where C is expressed in g/L; 3 - Normalized volume of brine, defined as (V-375)/275, where V is expressed in mL; and σ - standard deviation.

salt(s), (2) the concentration of acid(s), (3) the concentration of reducing sugars, and (4) the texture. Five quality factors associated with the brine were also considered: (1) the pH, (2) the concentration of acid(s), (3) the concentration of reducing sugars, (4) the concentration of biomass after 24 h, and (5) the concentration of biomass at the end of the batch. The experimental results for all eight properties obtained according to the starting design are tabulated in Table 1, whereas the results obtained following the composite design are listed in Table 2.

Materials

Fresh carrots were bought at random at local markets (dominating cultivar: Nantes). Sodium chloride, sodium hydroxide, potassium dichromate, copper sulphate, potassium permanganate, and silver nitrate were purchased from Merck (Frankfurt, Germany). Dinitrosalicilic acid was obtained from BDH Laboratory Supplies (Dorset, England). The inocula of *Lactobacillus plantarum* (LP no. 91249 G-2) were a gift from Textel/Lactolabo from Marshall (Madrid, Spain). MRS broth and YNB were purchased from Oxoid (Hampshire, UK). Yeast Extract was obtained from Difco (Detroit, USA) and anhydrous D(+)-glucose from Merck (Frankfurt, Germany).

Equipment

Centrifugations were performed in a Universal Hettich Centrifuge (Tuttlingen, Germany). Titrations were done with a titroprocessor model 682 connected to a Dosimat Model 665 equipped with the combined glass electrode 60202100, all from Metrohm (Herisau, Switzerland). Measurements of pH were done with a titrator from Crisson (Barcelona, Spain) equipped with the combined electrode 104023311 from Ingold (Steinbach, Switzerland). Isothermal conditions and stirring were achieved using thermostatted shaker baths Kotterman 3047 from Labortechnik (Amtsmtsgerich Burgdorf, Germany). Spectrophotometric measurements were made with a Spectrophotometer Model 350 from Pye Unicam (Cambridge, UK). Sterilization was achieved in a laboratory retort Austester Model 437G from Selecta (Barcelona, Spain). A scale Model PC 2000 from Mettler (Greifensee, Switzerland) was used for rough weight determinations, whereas accurate weight determinations were done with an analytical balance Model S 2000 from Bosch (Jungingen, Germany). A sensor thermometer Model G90200 from Cole Parmer (Niles, Illinois, USA) was employed to measure the temperature of the solutions before calibration of the titroprocessor. Isothermal chambers Model B80 from Memmert (Schwabach, Germany) and

Model 750E from Fitoclima (Lisboa, Portugal) were used for drying and incubating, respectively. The texture of the carrots (hardness) was evaluated with a texture analyser from Stevens LFRA (Essex, UK) using the probe TA9.

Methods

Preparation of the Inocula. Following opening of the package, the inoculum microorganisms were mainted at 7C and weekly reseeded in Petri dishes on MRS agar. From these dishes, a portion was removed and incubated for 12 h in MRS medium (50 ml) at 30C, and then for an extra 12 hr at room temperature. Following this period, the suspension of cells was sequentially centrifugated and washed with 0.85%(w/v) of sodium chloride. The cell precipitate was resuspended in sterilized deionized water in a volume sufficient to obtain the desired concentration of inoculum, which was then used for actual fermentation batches in the ratio of 1 ml of inoculum per 100 ml of solution; the concentration corresponded to an optical density at 640 nm (OD_{640}) of ca. 0.100.

Performance of Experimental Fermentations. Brines were prepared at 25C using the appropriate amounts of sodium chloride (previously dried overnight to constant weight) in deionized water. The brines were sterilized for 15 min in a laboratory retort.

Before starting each experiment, the carrots had both tops cut off, and were thoroughly washed with tap water, submerged for 1.5 min in a 0.2% (w/v) copper sulphate solution, submerged for 2 min in a 0.2% (w/v) potassium permanganate solution, and finally rinsed with an aqueous solution of the same salt concentration to be used in that experiment. Carrots were then sliced.into 1-cm high pieces, and 12 pieces of the disinfected carrots were submerged in the aforementioned brines. Soon after thermal equilibrium was reached, the brines were inoculated with 1 ml of inoculum, and gently stirred in an uniform fashion using a shaker bath (orbital setting speed: 5).

Analysis of Sodium Chloride. After removal from the brines, the carrot slices were quickly rinsed with deionized water, slightly dried with tissue paper, cut into very small pieces, submerged in 50 ml of deionized water in a stoppered flask, and heated for 30 min in a laboratory retort according to the method by McKnee (1985). Upon cooking, the carrot pieces together with the aqueous extract were homogenized and centrifuged for 10 min at 5,000 rpm. Finally, 2 ml of the clarified supernatant liquid were titrated with a standard solution of 0.05 N silver nitrate using potassium dichromate as indicator.

Analysis of Lactic Acid. The lactic acid was measured via determination of the total acidity of the solution expressed as lactic acid equivalent. An aliquot of 2 ml of the clarified extract (obtained as described in the previous subsection) was diluted in 50 ml of deionized water, titrated with 0.01 N solution of sodium hydroxide until a final pH of 8.2 is reached and maintained for 99 s using an automatic titrator; the titre of the blank was deducted accordingly.

Analysis of pH. The pH of the brine was determined using an automatic pH meter with a combined glass electrode.

Analysis of Reducing Sugars. Aliquots of the brines were periodically taken, diluted with deionized water, and analyzed following the dinitrosalicilic acid method of Miller (1959). The results were expressed as glucose equivalent. The concentration of reducing sugars remaining in the carrot material was determined by analyzing, using the same method, the clarified extract (obtained as described in the subsection *Analysis of sodium chloride*).

Analysis of Texture. The texture of the carrots, defined for our purposes as the hardness of the carrots, was evaluated with the texture analyser.

Analysis of Biomass. The concentration of cells (in g/L) was estimated from a calibration curve. The resuspended inoculum at the adequate dilution was used in the proportion of 1 ml per 100 ml of sterilized broth of YNB added with 10 g of glucose per L and 2.5 g of yeast extract per L so that the initial optical density of the culture medium at 640 nm (OD_{640}) was ca. 0.100 (deionized water used as blank). The growth of the microorganisms was followed by reading the optical density at the same wavelength in the range 0.1–0.5. The samples obtained at each reading were subject to multiple centrifugation and washing steps, and the biomass dried at 60C until a constant final weight (ca. 48 h). A fit of a linear model yielded the equation $BM(g/L)=0.289\ OD_{640}+0.0605$, where BM is the biomass concentration expressed as dry cell weight (correlation coefficient of 0.95).

STATISTICAL METHODS

The experiments were designed in a sequential fashion starting with an eight-point, full, two-way factorial design coupled with three center points (i.e., three replicates used as estimators of variance) (Box *et al.* 1978) aiming at fitting the following four-parameter linear model:

$$\hat{y}_{i,j} = b_{0;j} + b_{1;j}\, x_{1,j} + b_{2;j}\, x_{2,j} + b_{3;j}\, x_{3,j} \tag{1}$$

Here $\hat{y}_{i,j}$ is the estimated value for the *j*-th quality factor (i=1,2,...,8) in the *i*-th experiment (j=1,2,...,11), the $b_{i;j}$'s (i=0,1,2,3) are parameters associated with the *j*-th quality factor to be fitted by linear regression analysis, and the x_{ij}'s (i=1,2,3) are the predictors associated with the *j*-th quality factor and the *i*-th experiment. The results obtained for the parameters are tabulated in Table 1 together with their 95% marginal confidence intervals (the formulas utilized are listed as Appendix). Diagnostic checks were performed on the values of each of the three two-way interaction effects and on the magnitude of the sum of the three quadratic effects. The values of these diagnostics are also tabulated in Table 1.

The results of the measurements for all quality factors tested obtained from the data set generated according to the starting design have indicated that the linear model was inappropriate; hence, a further set of eight experiments was performed in order to form a composite design with nineteen experimental points. The eight new experiments were performed as three sets of two experiments laid out as star points (as opposed to the corner points used in the previous design) coupled with two extra center points (which were combined with the previous three replicates in the calculation of a new improved estimate of variance with fewer degrees of freedom) (Box *et al.* 1978). In this case, the following ten-parameter quadratic model was entertained:

$$\hat{y}_{i,k} = b_{0;k} + b_{1;k}\, x_{1,k} + b_{2;k}\, x_{2,k} + b_{3;k}\, x_{3,k} + b_{11;k}\, x_{1,k}^2 + b_{22;k}\, x_{2,k}^2 + \ldots \quad (2)$$
$$\ldots\; b_{33;k}\, x_{3,k}^2 + b_{12;k}\, x_{1,k}\, x_{2,k} + b_{13;k}\, x_{1,k}\, x_{3,k} + b_{23;k}\, x_{2,k}\, x_{3,k}$$

where the $b_{j;k}$'s (j=0,1,2,3; k=1,2,...,8) are linear parameters and the $b_{jl;k}$'s (j=1,2,3; l=1,2,3; k=1,2,...,8) are quadratic (or second order) parameters to be fitted by linear regression analysis. The results obtained for the parameters using the full composite design are tabulated in Table 2 together with their 95% marginal confidence intervals (the formulas utilized are listed as Appendix). Diagnostic checks were performed in order to assess the validity of the quadratic model in view of the possibility of employing a cubic model. The value of the three-way interaction effect and the magnitude of the cubic effects along each of the three spatial directions (corresponding to the three predictors x_1, x_2, and x_3) are tabulated in Table 2. The magnitude of the block effect between the first and the second part of the design is also included in this table.

Since statistical considerations have indicated that no need exists for a cubic model, the best estimates of the parameters in Eq.(2) were further utilized to find the analytical expressions for the loci of the critical points and to decide on the nature of such points. The results of this analysis are depicted in Table 3 (the formulas utilized are listed as Appendix).

DISCUSSION AND CONCLUSIONS

In the designing of the experiments, a sequential methodology was followed. This means that a block of eleven experiments was performed initially. Since the data generated by the initial design revealed general inadequacy of the linear model, the design was complemented with another block of eight experiments at a later stage. The possible existence of block to block differences was checked via comparison of the average of the three center points of the starting design with the average of the two center points of the secondary design (see column block in Table 2), which, in all cases, has shown to be statistically not significant. The general adequacy of the assumptions underlying the validity of the regression analyses pertaining to the quadratic model (i.e., normal distribution of residuals and constant variance) was double checked via diagnostics of the residuals (see Fig.1) which did not reveal significant bias.

The major goal of the above analysis was to determine the equations of the lines which contain all maxima (or all minima, depending on the type of optima as depicted in Table 3) for every predictor given the values for the other two predictors. Such equations give at once the best value for the predictor in question if the other two are set by any other processing or heuristic constraint. Inspection of Table 3 indicates that a true maximum for all three predictors considered together exists only for the salt concentration in the carrot, although the location of such overall maximum is beyond physical realizability; remember that, given the definition of variables x_1, x_2, and x_3 as linear functions of **T**, **C**, and **V**, respectively, practical constraints read $-3<x_1<+7$, $-4/3<x_2<+10$, and $-15/11<x_3$. For all quality factors but **S**, no overall local optimum exists, which mathematically is equivalent to say that the coordinates in the **MM** column define saddle points (Box *et al.* 1978); in these cases, the maximum (or the minimum) values of the optima lie on physical constraints.

The objectives underlying the improvement of the processing conditions of the manufacture of carrot pickles are to increase the values of **A**, **N**, **a**, **x**, and **f**, and to decrease the values of **S**, **R**, **p**, and **r**. From inspection of Table 3, it is clear that only x_1 should be manipulated to obtain the desired optimum in the case of **A** and **a**, either x_1 or x_2 in the cases of **R**, **p**, **r**, **f**, and **x**, and only x_2 in the case of **N**. The directions of manipulation are given by the analytical expressions of the optima tabulated in the **LO** column of Table 3.

It is interesting to note that temperature is ubiquitous in the optimization procedures except for the texture, which makes it a particularly suitable variable for manipulation in view of the preset objective functions. Therefore, within the limits of practical interest for the processing variable in question (and assuming that both x_2 and x_3 do not violate the constraints set forth before), factors **S**, **A**, **a**, **f**, and **x** pass through true local maxima and factors **R**, **p**, and **r** pass through

TABLE 2.

COMPOSITE (SECOND ORDER) EXPERIMENTAL DESIGN FOR TEMPERATURE (T), SALT CONCENTRATION IN THE BRINE (C), AND VOLUME OF THE BRINE (V); EXPERIMENTAL DATA OBTAINED FOR THE CONCENTRATION OF CHLORIDE SALTS IN THE CARROT (S, %W/W), CONCENTRATION OF ACIDS IN THE CARROT (A, %W/W), CONCENTRATION OF REDUCING SUGARS IN THE CARROT (R, %w/w), TEXTURE OF THE CARROT (N, G_F/CM^2), PH OF THE BRINE (p), CONCENTRATION OF ACIDS IN THE BRINE (a, %W/V), CONCENTRATON OF REDUCING SUGARS IN THE BRINE (r, %W/V),CONCENTRATION OF BIOMASS AFTER 24 H IN THE BRINE (f, %W/V), AND FINAL CONCENTRATION OF BIOMASS IN THE BRINE (x, %W/V); VALUES OF MAIN LINEAR EFFECTS (1, 2, AND 3); VALUES OF MAIN QUADRATIC EFFECTS (11, 22, AND 33); VALUES OF SECOND-ORDER INTERACTIONS (12, 13, AND 23); CHECKS OF THIRD-ORDER INTERACTIONS (123); CHECKS OF CUBIC CURVATURE (111-122/3-133/3, 222-112/3-233/3, AND 333-113/3-223/3); AND CHECKS OF BLOCK EFFECTS. (RUNS WERE MADE IN RANDOM ORDER.)

		E&I									
	U	(I)	(1)	(2)	(3)	(11)	(22)	(33)	(12)	(13)	(23)
C	1	+1	-1	-1	-1	+1	+1	+1	+1	+1	+1
	2	+1	-1	-1	+1	+1	+1	+1	+1	-1	-1
	3	+1	-1	+1	-1	+1	+1	+1	-1	+1	-1
	4	+1	-1	+1	+1	+1	+1	+1	-1	-1	+1
	5	+1	+1	-1	-1	+1	+1	+1	-1	-1	+1
	6	+1	+1	-1	+1	+1	+1	+1	-1	+1	-1
	7	+1	+1	+1	-1	+1	+1	+1	+1	-1	-1
	8	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
c	9	+1	0	0	0	0	0	0	0	0	0
	10	+1	0	0	0	0	0	0	0	0	0
	11	+1	0	0	0	0	0	0	0	0	0
c	12	+1	0	0	0	0	0	0	0	0	0
	13	+1	0	0	0	0	0	0	0	0	0
ax	14	+1	-1	0	0	+1	0	0	0	0	0
	15	+1	+1	0	0	+1	0	0	0	0	0
	16	+1	0	-1	0	0	+1	0	0	0	0
	17	+1	0	+1	0	0	+1	0	0	0	0
	18	+1	0	0	-1	0	0	+1	0	0	0
	19	+1	0	0	+1	0	0	+1	0	0	0
EE ±t.σ	S	2.4449±0.0478	0.2200±0.0702	1.504±0.0702	0.349±0.0702	-0.0411±0.0702	-0.0511±0.0702	-0.1761±0.0702	0.1175±0.0812	0.0025±0.0812	0.115±0.0812
	A	0.3482±0.0292	-0.0900±0.0429	-0.1980±0.0429	-0.1540±0.0429	-0.1286±0.0429	0.0714±0.0429	0.1114±0.0429	0.0075±0.0496	0.0150±0.0496	0.0450±0.0496
	R	0.2702±0.0395	0.0650±0.0580	0.0830±0.0580	-0.1390±0.0580	0.2395±0.0580	0.0295±0.0580	-0.0505±0.0580	-0.0250±0.0671	-0.0150±0.0671	0.0350±0.0671
	T	0.9438±0.0564	-0.0310±0.0828	0.0610±0.0828	-0.0940±0.0828	0.0489±0.0828	-0.1711±0.0828	0.0039±0.0828	0.0013±0.0958	-0.0438±0.0958	0.0113±0.0958
	p	3.3077±0.1476	0.1600±0.2166	0.3400±0.2166	0.2800±0.2166	0.3201±0.2166	0.3201±0.2166	-0.2799±0.2166	-0.0375±0.2506	0.0625±0.2506	0.1375±0.2506
	a	0.3696±0.0299	-0.1130±0.0439	-0.2060±0.0439	-0.1570±0.0439	-0.1441±0.0439	0.1509±0.0439	0.1359±0.0439	0.0313±0.0508	-0.0038±0.0508	0.0288±0.0508
	r	1.5911±0.4041	1.7200±0.5929	1.3750±0.5929	-0.3500±0.5929	1.3049±0.5929	0.4899±0.5929	-0.0450±0.5929	0.3113±0.6861	-0.0188±0.6861	0.4638±0.6861
	f	0.3508±0.0232	0.0350±0.0340	-0.0550±0.0340	-0.0600±0.0340	-0.0969±0.0340	-0.0069±0.0340	0.0681±0.0340	-0.0250±0.0394	-0.0275±0.0394	0.0100±0.0394
	x	0.6342±0.0527	-0.1580±0.0773	-0.0630±0.0773	-0.2140±0.0773	-0.0870±0.0773	-0.0020±0.0773	0.1730±0.0773	0.0488±0.0894	0.0838±0.0894	0.0238±0.0894

TABLE 2. (cont.)

	U	LoF (123)	LoF (111)	LoF (222)	LoF (333)	LoF block	MV CM S	MV CM A	MV CM R	MV CM T	MV B p	MV B a	MV B r	MV B f	MV B x
C	1						0.42	0.87	0.51	0.95	3.20	1.04	1.56	0.31	1.36
	2						0.82	0.50	0.19	0.75	3.50	0.67	0.39	0.27	0.65
	3						2.87	0.45	0.65	0.94	3.50	0.52	3.04	0.26	0.98
	4						3.80	0.18	0.42	0.87	4.40	0.35	2.43	0.24	0.60
	5						0.51	0.69	0.74	0.90	3.30	0.70	4.46	0.53	0.62
	6						0.99	0.30	0.31	0.61	3.90	0.40	1.92	0.36	0.48
	7						3.50	0.22	0.73	0.98	3.50	0.39	5.89	0.36	0.67
	8						4.37	0.09	0.49	0.65	4.60	0.12	6.50	0.25	0.39
e	9						2.33	0.41	0.33	0.86	3.60	0.35	1.05	0.41	0.56
	10						2.31	0.43	0.34	1.08	3.65	0.39	1.00	0.41	0.54
	11						2.33	0.43	0.31	1.01	3.60	0.49	0.95	0.40	0.57
e	12						2.45	0.33	0.20	0.88	3.80	0.35	1.00	0.30	0.77
	13						2.54	0.30	0.43	0.89	3.00	0.45	2.88	0.35	0.74
ax	14						2.10	0.28	0.37	0.94	2.90	0.26	0.24	0.26	0.62
	15						2.84	0.08	0.52	1.00	3.80	0.10	6.09	0.19	0.47
	16						0.84	0.66	0.09	0.56	2.70	0.79	0.24	0.41	0.71
	17						4.08	0.10	0.38	0.94	4.00	0.16	4.46	0.22	0.55
	18						1.93	0.61	0.24	0.95	2.80	0.69	1.71	0.52	1.12
	19						2.74	0.23	0.07	0.90	2.70	0.23	1.92	0.26	0.49
EE $\pm t.\sigma$	S	-0.0175±0.0812	0.1875±0.1816	0.1450±0.1816	0.0700±0.1816	0.1717±0.2893									
	A	0.0200±0.0496	-0.0125±0.1109	-0.1025±0.1109	-0.0450±0.1109	-0.1083±0.1767									
	R	0.0125±0.0671	0.0125±0.1500	0.0775±0.1500	0.0675±0.1500	-0.0117±0.2390									
	T	-0.0213±0.0958	0.0763±0.2143	0.1613±0.2143	0.0863±0.2143	-0.1283±0.3415									
	p	0.0125±0.2506	0.3625±0.5604	0.3875±0.5604	-0.4125±0.5604	-0.2167±0.8929									
	a	-0.0213±0.0508	0.0413±0.1136	-0.1363±0.1136	-0.0913±0.1136	-0.0100±0.1809									
	r	0.3238±0.6861	1.5063±1.5341	0.9188±1.5341	0.5688±1.5341	0.9400±2.4444									
	f	0.0050±0.0394	-0.0875±0.0880	-0.0500±0.0880	-0.0875±0.0880	-0.0817±0.1402									
	x	-0.0588±0.0894	0.1038±0.1999	-0.0213±0.1999	-0.1263±0.1999	0.1983±0.3186									

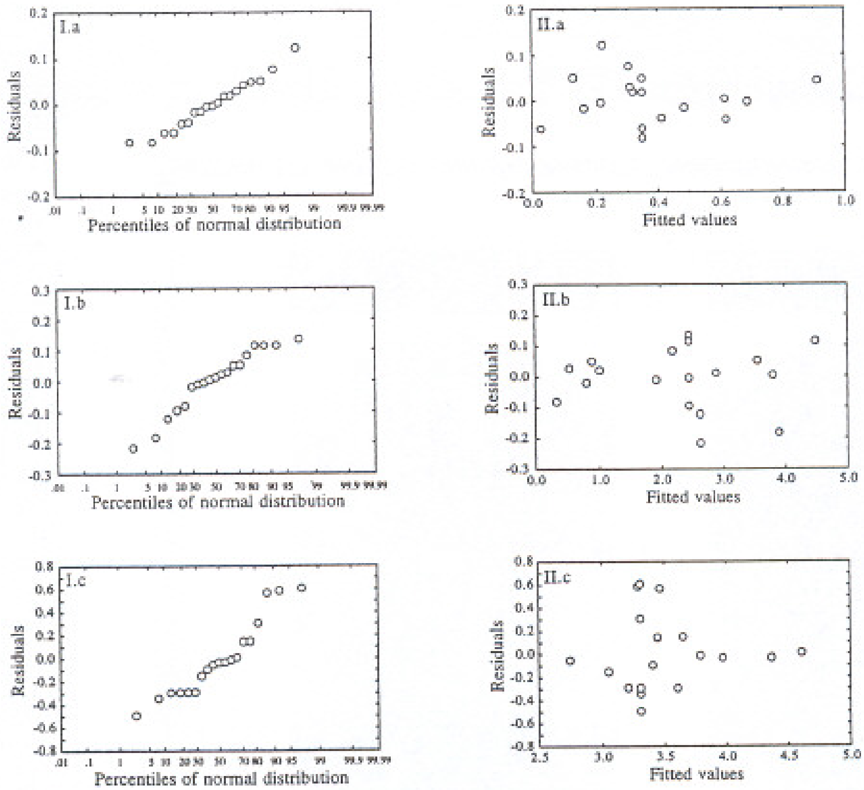
Note: ax - axial points; e - Center points; C - Corner points; **EE** - Estimated Effects; **E&I** - Effects (linear and quadratic) and Interactions (second order); I - grand average; **LoF** - Lack of Fit analysis; **MV CM** - Measured Values for the Carrot Material; **MV B** - Measured Values for the Brine;t - probability point of Student's t-distribution; U - rUn; 1 - Normalized value of temperature, defined as (T-30)/10, where **T** is expressed in C; 2 - Normalized concentration of salt in the brine, defined as (C-4)/3, where C is expressed in g/L; 3 - Normalized volume of brine, defined as (V-375)/275, where V is expressed in mL; and σ - standard deviation.

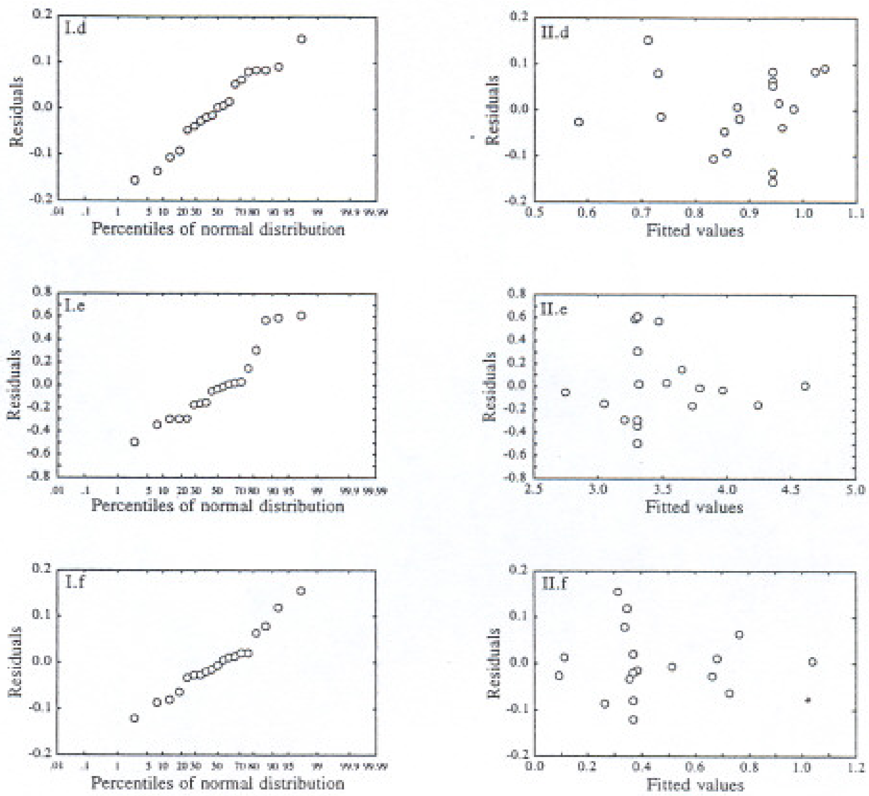
TABLE 3.

LOCI AND TYPE OF OPTIMA IN EACH OF THE OPERATING VARIABLES TEMPERATURE (x_1), SALT CONCENTRATION IN THE BRINE (x_2), AND VOLUME OF THE BRINE (x_3) ASSOCIATED WITH THE QUADRATIC MODELS FITTED TO THE DATA OBTAINED FOR THE CONCENTRATION OF CHLORIDE SALTS IN THE CARROT (S), CONCENTRATION OF ACIDS IN THE CARROT (A), CONCENTRATION OF REDUCING SUGARS IN THE CARROT (R), TEXTURE OF THE CARROT (N), PH OF THE BRINE (p), CONCENTRATION OF ACIDS IN THE BRINE (a), CONCENTRATON OF REDUCING SUGARS IN THE BRINE (r), AND CONCENTRATION OF BIOMASS IN THE BRINE (x).

		LO			TO			MM		
		x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3
EE	S	$2.6739 + 1.4281 x_2 + 0.0304 x_3$	$14.7065 + 1.1489 x_1 + 1.1245 x_3$	$0.9907 + 0.0071 x_1 + 0.3265 x_2$	max	max	max	-23.7278	-18.3771	-5.1771
	A	$- 0.3500 + 0.0292 x_2 + 0.0583 x_3$	$1.3857 - 0.0525 x_1 - 0.3149 x_3$	$0.6909 - 0.0673 x_1 - 0.2019 x_2$	max	min	min	-0.2867	1.2570	0.4564
	R	$- 0.1357 + 0.0522 x_2 + 0.0313 x_3$	$- 1.4051 + 0.4232 x_1 - 0.5925 x_3$	$- 1.3772 - 0.1486 x_1 + 0.3468 x_2$	min	min	max	-0.2143	-0.5796	-1.5464
	N	$0.3169 - 0.0128 x_2 + 0.4472 x_3$	$0.1783 + 0.0037 x_1 + 0.0329 x_3$	$11.9926 + 5.5817 x_1 - 1.4353 x_2$	min	max	min	-3.8590	-0.1430	-9.3421
	p	$- 0.2499 + 0.0586 x_2 - 0.0976 x_3$	$- 0.5311 + 0.0586 x_1 - 0.2148 x_3$	$0.5002 + 0.1117 x_1 + 0.2456 x_2$	min	min	max	-0.3166	-0.6169	0.3133
	a	$- 0.3921 + 0.1085 x_2 - 0.0130 x_3$	$0.6824 - 0.1035 x_1 - 0.0952 x_3$	$0.5775 + 0.0138 x_1 - 0.1058 x_2$	max	min	min	-0.3262	0.6684	0.5023
	r	$- 0.6590 - 0.1193 x_2 + 0.0072 x_3$	$- 1.4032 - 0.3176 x_1 - 0.4733 x_3$	$- 3.8849 - 0.2081 x_1 + 5.1475 x_2$	min	min	max	-0.7000	0.1713	-2.8572
	f	$0.1807 - 0.1291 x_2 - 0.1420 x_3$	$4.0113 - 1.8233 x_1 + 0.7293 x_3$	$0.4402 + 0.2018 x_1 - 0.0734 x_2$	max	max	min	0.6355	-4.5139	0.8997
	x	$- 0.9079 + 0.2801 x_2 + 0.4813 x_3$	$- 15.6687 + 12.1246 x_1 + 5.9068 x_3$	$0.6185 - 0.2421 x_1 - 0.0686 x_2$	max	max	min	3.5648	18.5768	-1.5196

Note: **EE** - Estimated Effects; **LO** - Loci of Optima; **MM** - Minimum of Maxima, Minimum of Minima, Maximum of Minima, or Maximum of Maxima; **TO** - Type of Optima.


I.a
Residuals
Percentiles of normal distribution
II.a
Residuals
Fitted values
I.b
Residuals
Percentiles of normal distribution
II.b
Residuals
Fitted values
I.c
Residuals
Percentiles of normal distribution
II.c
Residuals
Fitted values


I.d
II.d
I.e
II.e
I.f
II.f
Residuals
Percentiles of normal distribution
Fitted values

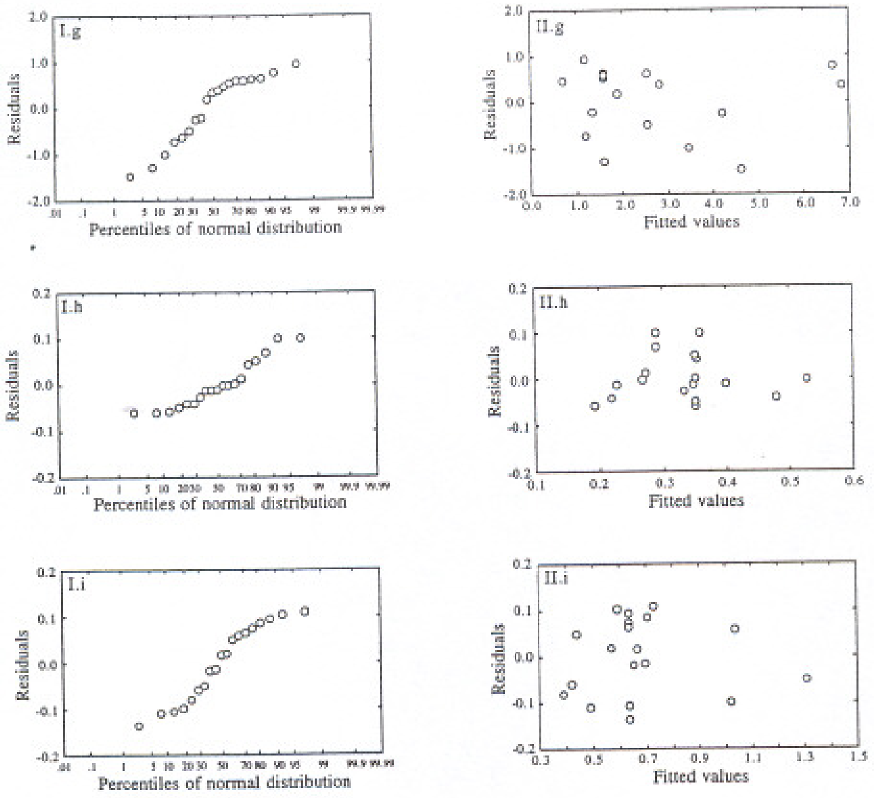


FIG. 1. PLOT OF RESIDUALS VERSUS (I) THE PERCENTILES OF A NORMAL DISTRIBUTION AND (II) THE FITTED VALUES, FOR DATA PERTAINING TO THE ACID CONTENT OF THE CARROTS (a), THE SALT CONTENT OF THE CARROTS (b), THE SUGAR CONTENT OF THE CARROTS (c), THE TEXTURE OF THE CARROTS (d), THE pH OF THE BRINE (e), THE CONCENTRATION OF ACID IN THE BRINE (f), THE CONCENTRATION OF REDUCING SUGARS IN THE BRINE (g), THE CONCENTRATION OF BIOMASS IN THE BRINE AFTER 24 H (h), AND THE CONCENTRATION OF BIOMASS IN THE BRINE AFTER COMPLETION OF THE BATCH (i)

local minima. It should be realized that for every combination of x_2 and x_3, a different value of the aforementioned optimum is obtained as given by the equations listed in the first row of Table 3; for the sake of information, it can be said that for factors **A** and **a** the worst of the optima, i.e. the minimum value for the maxima (as can be concluded from inspection of the last two rows of Table 3) is obtained when temperature takes the values 27.1 and 26.7C, respectively. By the same token, if the salt concentration is the processing variable elected for manipulation, texture passes through a true local maximum, and the worst of the optima thus obtained corresponds to having a brine 3.6 g/L in sodium chloride. The information generated in this research effort is useful in predesigning carrot preservation processes based on lactic fermentation.

NOMENCLATURE

a = concentration of acids in the brine (%w/v)
A = concentration of acids in the carrot (%w/w)
$b_{i,j}$ = parameter associated with the linear effect of x_i in the *j*-th quality factor
$b_{ii;j}$ = parameter associated with the quadratic effect of x_i in the *j*-th quality factor
$b_{ij;k}$ = parameter associated with the effect of the interaction of x_i and x_j in the *k*-th quality factor
C = salt concentration in the brine (g/L)
f = concentration of biomass after 24 h in the brine (%w/v)
N = texture of the carrot (g_f/cm^2)
p = pH of the brine
r = concentration of reducing sugars in the brine (%w/v)
R = concentration of reducing sugars in the carrot (%w/w)
T = temperature (C)
V = volume of the brine (ml)
S = concentration of chloride salts in the carrot (%w/w)
x = final concentration of biomass in the brine (%w/v)
x_1 = normalized value of temperature, (**T**-30)/10
x_2 = normalized concentration of salt in the brine, (**C**-4)/3
x_3 = normalized volume of brine, (**V**-375)/275
$y_{i,j}$ = estimated value for the *j*-th quality factor obtained in the *i*-th experiment
$\hat{y}_{i,j}$ = experimental value for the *j*-th quality factor obtained in the *i*-th experiment

FORMULAE UTILIZED TO GENERATE THE CANONICAL FORM OF TABLE 1

$$b_{i;0} = \frac{\sum_{j=1}^{11} y_{i,j}}{11}, \quad i=1,2,\ldots,9$$

$$b_{i;j} = \frac{\sum_{k=1}^{8} x_{j,k} \cdot y_{i,k}}{8}, \quad i=1,2,\ldots,9, \; j=1,2,3$$

$$b_{i;jk} = \frac{\sum_{l=1}^{8} x_{jk,l} \cdot y_{i,l}}{8}, \quad i=1,2,\ldots,9, \; j=1,2, \; k=j+1,\ldots,3$$

$$b_{i;11,22,33} = \frac{\sum_{j=1}^{8} y_{i,j}}{8} - \frac{\sum_{j=9}^{11} y_{i,j}}{3}, \quad i=1,2,\ldots,9$$

$$t.\sigma_{i;0} = t(\alpha=5\%,\nu=11) \cdot \sigma \sqrt{\frac{1}{11}}, \quad i=1,2,\ldots,9 \; ; \; t(\alpha=5\%,\nu=11) = 2.201$$

$$t.\sigma_{i;j} = t(\alpha=5\%,\nu=8) \cdot \sigma \sqrt{\frac{1}{8}}, \quad i=1,2,\ldots,9, \; j=1,2,3 \; ; \; t(\alpha=5\%,\nu=8) = 2.306$$

$$t.\sigma_{i;jk} = t(\alpha=5\%,\nu=8) \cdot \sigma \sqrt{\frac{1}{8}}, \quad i=1,2,\ldots,9, \; j=1,2, \; k=i+1,\ldots,3 \; ; \; t(\alpha=5\%,\nu=8) = 2.306$$

$$t.\sigma_{i;11,22,33} = t(\alpha=5\%,\nu=9) \cdot \sigma \sqrt{\frac{1}{8}+\frac{1}{3}}, \quad i=1,2,\ldots,9 \; ; \; t(\alpha=5\%,\nu=9) = 2.262$$

$$x_{ij,k} = x_{i,k} \cdot x_{j,k}, \quad i=1,2, \; j=i+1,\ldots,3, \; k=1,2,\ldots,11$$

$$\sigma = \sqrt{\frac{\sum_{j=9}^{11} \left(y_{i,j} - \frac{\sum_{k=9}^{11} y_{i,k}}{3} \right)}{2}}$$

FORMULAE UTILIZED TO GENERATE THE CANONICAL FORM OF TABLE 3

$$\left(\frac{\partial \hat{y}_i}{\partial x_{1,i}}\right)_{x_{2,i},x_{3,i}} (x_{1,i}=x_{1,i,opt}) = 2b_{11}\, x_{1,i,opt} + b_{12}\, x_{2,i} + b_{13}\, x_{3,i} + b_1 = 0, \quad i=1,2,\ldots,9$$

$$\left(\frac{\partial \hat{y}_i}{\partial x_{2,i}}\right)_{x_{1,i},x_{3,i}} (x_{2,i}=x_{2,i,opt}) = b_{12}\, x_{1,i} + 2b_{22}\, x_{2,i,opt} + b_{23}\, x_{3,i} + b_2 = 0, \quad i=1,2,\ldots,9$$

$$\left(\frac{\partial \hat{y}_i}{\partial x_{3,i}}\right)_{x_{1,i},x_{2,i}} (x_{3,i}=x_{3,i,opt}) = b_{13}\, x_{1,i} + b_{23}\, x_{2,i} + 2b_{33}\, x_{3,i,opt} + b_3 = 0, \quad i=1,2,\ldots,9$$

$$\text{minimum: } \left(\frac{\partial^2 \hat{y}_i}{\partial x_{j,i}^2}\right)_{x_{k \neq j}} (x_{j,i}=x_{j,i,opt}) = 2b_{jj} > 0, \quad i=1,2,\ldots,9, \; j=1,2,3$$

$$\text{maximum: } \left(\frac{\partial^2 \hat{y}_i}{\partial x_{j,i}^2}\right)_{x_{k \neq j}} (x_{j,i}=x_{j,i,opt}) = 2b_{jj} < 0, \quad i=1,2,\ldots,9, \; j=1,2,3$$

$$\left(\frac{\partial \hat{y}_i}{\partial x_{1,i}}\right)_{x_{2,i},x_{3,i}} (x_{1,i}=x_{1,i,Mopt}, x_{2,i}=x_{2,i,Mopt}, x_{3,i}=x_{3,i,Mopt}) = 2b_{11}\, x_{1,i,Mopt} + b_{12}\, x_{2,i,Mopt} + b_{13}\, x_{3,i,Mopt} + b_1 = 0, \quad i=1,2,\ldots,9$$

$$\left(\frac{\partial \hat{y}_i}{\partial x_{2,i}}\right)_{x_{1,i},x_{3,i}} (x_{1,i}=x_{1,i,Mopt}, x_{2,i}=x_{2,i,Mopt}, x_{3,i}=x_{3,i,Mopt}) = b_{12}\, x_{1,i,Mopt} + 2b_{22}\, x_{2,i,Mopt} + b_{23}\, x_{3,i,Mopt} + b_2 = 0, \quad i=1,2,\ldots,9$$

$$\left(\frac{\partial \hat{y}_i}{\partial x_{3,i}}\right)_{x_{1,i},x_{2,i}} (x_{1,i}=x_{1,i,Mopt}, x_{2,i}=x_{2,i,Mopt}, x_{3,i}=x_{3,i,Mopt}) = b_{13}\, x_{1,i,Mopt} + b_{23}\, x_{2,i,Mopt} + 2b_{33}\, x_{3,i,Mopt} + b_3 = 0, \quad i=1,2,\ldots,9$$

FORMULAE UTILIZED TO GENERATE THE CANONICAL FORM OF TABLE 2.

$$b_{i;0} = \frac{\sum_{j=1}^{19} y_{i,j}}{19} \text{ , } i=1,2,\ldots,9$$

$$b_{i;j} = \frac{\sum_{k=1}^{19} x_{j,k} \cdot y_{i,k}}{10} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2,3$$

$$b_{i;11} = \frac{19\left(\sum_{k=1}^{19} x_{1,k}^{2} \cdot y_{i,k}\right) - 10\left(\sum_{k=1}^{19} y_{i,k}\right) - 52\, b_{i;22} - 52\, b_{i;33}}{90} \text{ , } i=1,2,\ldots,9$$

$$b_{i;22} = \frac{1{,}710\left(\sum_{k=1}^{19} x_{2,k}^{2} \cdot y_{i,k}\right) - 988\left(\sum_{k=1}^{19} x_{1,k}^{2} \cdot y_{i,k}\right) - 380\left(\sum_{k=1}^{19} y_{i,k}\right) - 1{,}976\, b_{i;33}}{1{,}976} \text{ , } i=1,2,\ldots,9$$

$$b_{i;33} = \frac{9{,}227{,}160\left(\sum_{k=1}^{19} x_{3,k}^{2} \cdot y_{i,k}\right) - 3{,}378{,}960\left(\sum_{k=1}^{19} x_{2,k}^{2} \cdot y_{i,k}\right) - 3{,}378{,}960\left(\sum_{k=1}^{19} x_{1,k}^{2} \cdot y_{i,k}\right) - 1{,}299{,}600\left(\sum_{k=1}^{19} y_{i,k}\right)}{25{,}212{,}240} \text{ , } i=1,2,\ldots,9$$

$$b_{i;jk} = \frac{\sum_{l=1}^{8} x_{jk,l} \cdot y_{i,l}}{8} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2 \text{ , } k=j+1,\ldots,3$$

$$b_{i;111} = \frac{y_{i,15}-y_{i,14}}{2} - \frac{(y_{i,5}-y_{i,1}) + (y_{i,6}-y_{i,2}) + (y_{i,7}-y_{i,3}) + (y_{i,8}-y_{i,4})}{8} \text{ , } i=1,2,\ldots,9$$

$$b_{i;123} = \frac{\sum_{j=1}^{8} x_{123,j} \cdot y_{i,j}}{8} \text{ , } i=1,2,\ldots,9$$

$$b_{i;222} = \frac{y_{i,17}-y_{i,16}}{2} - \frac{(y_{i,3}-y_{i,1}) + (y_{i,4}-y_{i,2}) + (y_{i,7}-y_{i,5}) + (y_{i,8}-y_{i,6})}{8} \text{ , } i=1,2,\ldots,9$$

$$b_{i;333} = \frac{y_{i,19}-y_{i,18}}{2} - \frac{(y_{i,2}-y_{i,1}) + (y_{i,4}-y_{i,3}) + (y_{i,6}-y_{i,5}) + (y_{i,8}-y_{i,7})}{8} \text{ , } i=1,2,\ldots,9$$

$$B_{i,I \to II} = \frac{\sum_{j=12}^{13} y_j}{2} - \frac{\sum_{j=9}^{11} y_j}{3} \text{ , } i=1,2,\ldots,9$$

$$t.\sigma_{i;0} = t_{(\alpha=5\%, v=19)} \cdot \sigma \sqrt{\frac{1}{19}} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2,3 \text{ ; } t_{(\alpha=5\%, v=19)} = 2.093$$

$$t.\sigma_{i;j} = t_{(\alpha=5\%, v=10)} \cdot \sigma \sqrt{\frac{1}{10}} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2,3 \text{ ; } t_{(\alpha=5\%, v=10)} = 2.228$$

$$t.\sigma_{i;jj} = t_{(\alpha=5\%, v=10)} \cdot \sigma \sqrt{\frac{1}{10}} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2,3 \text{ ; } t_{(\alpha=5\%, v=10)} = 2.228$$

$$t.\sigma_{i;jk} = t_{(\alpha=5\%, v=8)} \cdot \sigma \sqrt{\frac{1}{8}} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2 \text{ , } k=i+1,\ldots,3 \text{ ; } t_{(\alpha=5\%, v=8)} = 2.306$$

$$t.\sigma_{i;jjj} = t_{(\alpha=5\%, v=8)} \cdot \sigma \sqrt{\frac{1}{2} + \frac{1}{8}} \text{ , } i=1,2,\ldots,9 \text{ , } j=1,2,3 \text{ ; } t_{(\alpha=5\%, v=8)} = 2.306$$

$$t.\sigma_{i;123} = t_{(\alpha=5\%, v=8)} \cdot \sigma \sqrt{\frac{1}{8}} \text{, } i=1,2,\ldots,9 \text{ ; } t_{(\alpha=5\%, v=8)} = 2.306$$

$$x_{ij,k} = x_{i,k} \cdot x_{j,k} \text{ , } i=1,2 \text{ , } j=i+1,\ldots,3 \text{ , } k=1,2,\ldots,19$$

$$x_{123,i} = x_{1,i} \cdot x_{2,i} \cdot x_{3,i} \text{ , } i=1,2,\ldots,19$$

$$t.\sigma_{i,I \to II} = t_{(\alpha=5\%, v=3)} \cdot \sigma \sqrt{\frac{1}{3} + \frac{1}{2}} \text{ , } i=1,2,\ldots,9 \text{ ; } t_{(\alpha=5\%, v=3)} = 3.182$$

$$\sigma = \sqrt{\frac{\sum_{j=9}^{13}\left(y_{i,j} - \frac{\sum_{k=9}^{13} y_{i,k}}{5}\right)}{4}}$$

CANONICAL FORM OF TABLE 1.

		E&I								MV CM				MVB				
	U	I	(1)	(2)	(3)	(12)	(13)	(23)	(11)+(22)+(33)	S	A	R	T	p	a	r	f	x
C	1	+1	$x_{1,1}$	$x_{2,1}$	$x_{3,1}$					$y_{1,1}$	$y_{2,1}$	$y_{3,1}$	$y_{4,1}$	$y_{5,1}$	$y_{6,1}$	$y_{7,1}$	$y_{8,1}$	$y_{9,1}$
	2	+1	$x_{1,2}$	$x_{2,2}$	$x_{3,2}$					$y_{1,2}$	$y_{2,2}$	$y_{3,2}$	$y_{4,2}$	$y_{5,2}$	$y_{6,2}$	$y_{7,2}$	$y_{8,2}$	$y_{9,2}$
	3	+1	$x_{1,3}$	$x_{2,3}$	$x_{3,3}$					$y_{1,3}$	$y_{2,3}$	$y_{3,3}$	$y_{4,3}$	$y_{5,3}$	$y_{6,3}$	$y_{7,3}$	$y_{8,3}$	$y_{9,3}$
	4	+1	$x_{1,4}$	$x_{2,4}$	$x_{3,4}$					$y_{1,4}$	$y_{2,4}$	$y_{3,4}$	$y_{4,4}$	$y_{5,4}$	$y_{6,4}$	$y_{7,4}$	$y_{8,4}$	$y_{9,4}$
	5	+1	$x_{1,5}$	$x_{2,5}$	$x_{3,5}$					$y_{1,5}$	$y_{2,5}$	$y_{3,5}$	$y_{4,5}$	$y_{5,5}$	$y_{6,5}$	$y_{7,5}$	$y_{8,5}$	$y_{9,5}$
	6	+1	$x_{1,6}$	$x_{2,6}$	$x_{3,6}$					$y_{1,6}$	$y_{2,6}$	$y_{3,6}$	$y_{4,6}$	$y_{5,6}$	$y_{6,6}$	$y_{7,6}$	$y_{8,6}$	$y_{9,6}$
	7	+1	$x_{1,7}$	$x_{2,7}$	$x_{3,7}$					$y_{1,7}$	$y_{2,7}$	$y_{3,7}$	$y_{4,7}$	$y_{5,7}$	$y_{6,7}$	$y_{7,7}$	$y_{8,7}$	$y_{9,7}$
	8	+1	$x_{1,8}$	$x_{2,8}$	$x_{3,8}$					$y_{1,8}$	$y_{2,8}$	$y_{3,8}$	$y_{4,8}$	$y_{5,8}$	$y_{6,8}$	$y_{7,8}$	$y_{8,8}$	$y_{9,8}$
c	9	+1	$x_{1,9}$	$x_{2,9}$	$x_{3,9}$					$y_{1,9}$	$y_{2,9}$	$y_{3,9}$	$y_{4,9}$	$y_{5,9}$	$y_{6,9}$	$y_{7,9}$	$y_{8,9}$	$y_{9,9}$
	10	+1	$x_{1,10}$	$x_{2,10}$	$x_{3,10}$					$y_{1,10}$	$y_{2,10}$	$y_{3,10}$	$y_{4,10}$	$y_{5,10}$	$y_{6,10}$	$y_{7,10}$	$y_{8,10}$	$y_{9,10}$
	11	+1	$x_{1,11}$	$x_{2,11}$	$x_{3,11}$					$y_{1,11}$	$y_{2,11}$	$y_{3,11}$	$y_{4,11}$	$y_{5,11}$	$y_{6,11}$	$y_{7,11}$	$y_{8,11}$	$y_{9,11}$
EE $\pm t.\sigma$	S	$b_{1;0}\pm t.\sigma_{1;0}$	$b_{1;1}\pm t.\sigma_{1;1}$	$b_{1,2}\pm t.\sigma_{1,2}$	$b_{1,3}\pm t.\sigma_{1,3}$	$b_{1,12}\pm t.\sigma_{1,12}$	$b_{1,13}\pm t.\sigma_{1,13}$	$b_{1,23}\pm t.\sigma_{1,23}$	$b_{1;11,22,33}\pm t.\sigma_{1;11,22,33}$									
	A	$b_{2;0}\pm t.\sigma_{2;0}$	$b_{2;1}\pm t.\sigma_{2;1}$	$b_{2,2}\pm t.\sigma_{2,2}$	$b_{2,3}\pm t.\sigma_{2,3}$	$b_{2,12}\pm t.\sigma_{2,12}$	$b_{2,13}\pm t.\sigma_{2,13}$	$b_{2,23}\pm t.\sigma_{2,23}$	$b_{2;11,22,33}\pm t.\sigma_{2;11,22,33}$									
	R	$b_{3;0}\pm t.\sigma_{3;0}$	$b_{3;1}\pm t.\sigma_{3;1}$	$b_{3,2}\pm t.\sigma_{3,2}$	$b_{3,3}\pm t.\sigma_{3,3}$	$b_{3,12}\pm t.\sigma_{3,12}$	$b_{3,13}\pm t.\sigma_{3,13}$	$b_{3,23}\pm t.\sigma_{3,23}$	$b_{3;11,22,33}\pm t.\sigma_{3;11,22,33}$									
	T	$b_{4;0}\pm t.\sigma_{4;0}$	$b_{4;1}\pm t.\sigma_{4;1}$	$b_{4,2}\pm t.\sigma_{4,2}$	$b_{4,3}\pm t.\sigma_{4,3}$	$b_{4,12}\pm t.\sigma_{4,12}$	$b_{4,13}\pm t.\sigma_{4,13}$	$b_{4,23}\pm t.\sigma_{4,23}$	$b_{4;11,22,33}\pm t.\sigma_{4;11,22,33}$									
	p	$b_{5;0}\pm t.\sigma_{5;0}$	$b_{5;1}\pm t.\sigma_{5;1}$	$b_{5,2}\pm t.\sigma_{5,2}$	$b_{5,3}\pm t.\sigma_{5,3}$	$b_{5,12}\pm t.\sigma_{5,12}$	$b_{5,13}\pm t.\sigma_{5,13}$	$b_{5,23}\pm t.\sigma_{5,23}$	$b_{5;11,22,33}\pm t.\sigma_{5;11,22,33}$									
	a	$b_{6;0}\pm t.\sigma_{6;0}$	$b_{6;1}\pm t.\sigma_{6;1}$	$b_{6,2}\pm t.\sigma_{6,2}$	$b_{6,3}\pm t.\sigma_{6,3}$	$b_{6,12}\pm t.\sigma_{6,12}$	$b_{6,13}\pm t.\sigma_{6,13}$	$b_{6,23}\pm t.\sigma_{6,23}$	$b_{6;11,22,33}\pm t.\sigma_{6;11,22,33}$									
	r	$b_{7;0}\pm t.\sigma_{7;0}$	$b_{7;1}\pm t.\sigma_{7;1}$	$b_{7,2}\pm t.\sigma_{7,2}$	$b_{7,3}\pm t.\sigma_{7,3}$	$b_{7,12}\pm t.\sigma_{7,12}$	$b_{7,13}\pm t.\sigma_{7,13}$	$b_{7,23}\pm t.\sigma_{7,23}$	$b_{7;11,22,33}\pm t.\sigma_{7;11,22,33}$									
	f	$b_{8;0}\pm t.\sigma_{8;0}$	$b_{8;1}\pm t.\sigma_{8;1}$	$b_{8,2}\pm t.\sigma_{8,2}$	$b_{8,3}\pm t.\sigma_{8,3}$	$b_{8,12}\pm t.\sigma_{8,12}$	$b_{8,13}\pm t.\sigma_{8,13}$	$b_{8,23}\pm t.\sigma_{8,23}$	$b_{8;11,22,33}\pm t.\sigma_{8;11,22,33}$									
	x	$b_{9;0}\pm t.\sigma_{9;0}$	$b_{9;1}\pm t.\sigma_{9;1}$	$b_{9,2}\pm t.\sigma_{9,2}$	$b_{9,3}\pm t.\sigma_{9,3}$	$b_{9,12}\pm t.\sigma_{9,12}$	$b_{9,13}\pm t.\sigma_{9,13}$	$b_{9,23}\pm t.\sigma_{9,23}$	$b_{9;11,22,33}\pm t.\sigma_{9;11,22,33}$									

CANONICAL FORM OF TABLE 2.

		E&I									
	run	I	(1)	(2)	(3)	(11)	(22)	(33)	(12)	(13)	(23)
C	1	+1	$x_{1,1}$	$x_{2,1}$	$x_{3,1}$	$x_{11,1}$	$x_{22,1}$	$x_{33,1}$	$x_{12,1}$	$x_{13,1}$	$x_{23,1}$
	2	+1	$x_{1,2}$	$x_{2,2}$	$x_{3,2}$	$x_{11,2}$	$x_{22,2}$	$x_{33,2}$	$x_{12,2}$	$x_{13,2}$	$x_{23,2}$
	3	+1	$x_{1,3}$	$x_{2,3}$	$x_{3,3}$	$x_{11,3}$	$x_{22,3}$	$x_{33,3}$	$x_{12,3}$	$x_{13,3}$	$x_{23,3}$
	4	+1	$x_{1,4}$	$x_{2,4}$	$x_{3,4}$	$x_{11,4}$	$x_{22,4}$	$x_{33,4}$	$x_{12,4}$	$x_{13,4}$	$x_{23,4}$
	5	+1	$x_{1,5}$	$x_{2,5}$	$x_{3,5}$	$x_{11,5}$	$x_{22,5}$	$x_{33,5}$	$x_{12,5}$	$x_{13,5}$	$x_{23,5}$
	6	+1	$x_{1,6}$	$x_{2,6}$	$x_{3,6}$	$x_{11,6}$	$x_{22,6}$	$x_{33,6}$	$x_{12,6}$	$x_{13,6}$	$x_{23,6}$
	7	+1	$x_{1,7}$	$x_{2,7}$	$x_{3,7}$	$x_{11,7}$	$x_{22,7}$	$x_{33,7}$	$x_{12,7}$	$x_{13,7}$	$x_{23,7}$
	8	+1	$x_{1,8}$	$x_{2,8}$	$x_{3,8}$	$x_{11,8}$	$x_{22,8}$	$x_{33,8}$	$x_{12,8}$	$x_{13,8}$	$x_{23,8}$
c	9	+1	$x_{1,9}$	$x_{2,9}$	$x_{3,9}$	$x_{11,9}$	$x_{22,9}$	$x_{33,9}$	$x_{12,9}$	$x_{13,9}$	$x_{23,9}$
	10	+1	$x_{1,10}$	$x_{2,10}$	$x_{3,10}$	$x_{11,10}$	$x_{22,10}$	$x_{33,10}$	$x_{12,10}$	$x_{13,10}$	$x_{23,10}$
	11	+1	$x_{1,11}$	$x_{2,11}$	$x_{3,11}$	$x_{11,11}$	$x_{22,11}$	$x_{33,11}$	$x_{12,11}$	$x_{13,11}$	$x_{23,11}$
c	12	+1	$x_{1,12}$	$x_{2,12}$	$x_{3,12}$	$x_{11,12}$	$x_{22,12}$	$x_{33,12}$	$x_{12,12}$	$x_{13,12}$	$x_{23,12}$
	13	+1	$x_{1,13}$	$x_{2,13}$	$x_{3,13}$	$x_{11,13}$	$x_{22,13}$	$x_{33,13}$	$x_{12,13}$	$x_{13,13}$	$x_{23,13}$
ax	14	+1	$x_{1,14}$	$x_{2,14}$	$x_{3,14}$	$x_{11,14}$	$x_{22,14}$	$x_{33,14}$	$x_{12,14}$	$x_{13,14}$	$x_{23,14}$
	15	+1	$x_{1,15}$	$x_{2,15}$	$x_{3,15}$	$x_{11,15}$	$x_{22,15}$	$x_{33,15}$	$x_{12,15}$	$x_{13,15}$	$x_{23,15}$
	16	+1	$x_{1,16}$	$x_{2,16}$	$x_{3,16}$	$x_{11,16}$	$x_{22,16}$	$x_{33,16}$	$x_{12,16}$	$x_{13,16}$	$x_{23,16}$
	17	+1	$x_{1,17}$	$x_{2,17}$	$x_{3,17}$	$x_{11,17}$	$x_{22,17}$	$x_{33,17}$	$x_{12,17}$	$x_{13,17}$	$x_{23,17}$
	18	+1	$x_{1,18}$	$x_{2,18}$	$x_{3,18}$	$x_{11,18}$	$x_{22,18}$	$x_{33,18}$	$x_{12,18}$	$x_{13,18}$	$x_{23,18}$
	19	+1	$x_{1,19}$	$x_{2,19}$	$x_{3,19}$	$x_{11,19}$	$x_{22,19}$	$x_{33,19}$	$x_{12,19}$	$x_{13,19}$	$x_{23,19}$
EE $\pm t.\sigma$	S	$b_{1;0}\pm t.\sigma_{1;0}$	$b_{1;1}\pm t.\sigma_{1;1}$	$b_{1;2}\pm t.\sigma_{1;2}$	$b_{1;3}\pm t.\sigma_{1;3}$	$b_{1;11} t.\sigma_{1;11}$	$b_{1;22}\pm t.\sigma_{1;22}$	$b_{1;33}\pm t.\sigma_{1;33}$	$b_{1;12}\pm t.\sigma_{1;12}$	$b_{1;13}\pm t.\sigma_{1;13}$	$b_{1;23}\pm t.\sigma_{1;23}$
	A	$b_{2;0}\pm t.\sigma_{2;0}$	$b_{2;1}\pm t.\sigma_{2;1}$	$b_{2;2}\pm t.\sigma_{2;2}$	$b_{2;3}\pm t.\sigma_{2;3}$	$b_{2;11}\pm t.\sigma_{2;11}$	$b_{2;22}\pm t.\sigma_{2;22}$	$b_{2;33}\pm t.\sigma_{2;33}$	$b_{2;12}\pm t.\sigma_{2;12}$	$b_{2;13}\pm t.\sigma_{2;13}$	$b_{2;23}\pm t.\sigma_{2;23}$
	R	$b_{3;0}\pm t.\sigma_{3;0}$	$b_{3;1}\pm t.\sigma_{3;1}$	$b_{3;2}\pm t.\sigma_{3;2}$	$b_{3;3}\pm t.\sigma_{3;3}$	$b_{3;11}\pm t.\sigma_{3;11}$	$b_{3;22}\pm t.\sigma_{3;22}$	$b_{3;33}\pm t.\sigma_{3;33}$	$b_{3;12}\pm t.\sigma_{3;12}$	$b_{3;13}\pm t.\sigma_{3;13}$	$b_{3;23}\pm t.\sigma_{3;23}$
	T	$b_{4;0}\pm t.\sigma_{4;0}$	$b_{4;1}\pm t.\sigma_{4;1}$	$b_{4;2}\pm t.\sigma_{4;2}$	$b_{4;3}\pm t.\sigma_{4;3}$	$b_{4;11}\pm t.\sigma_{4;11}$	$b_{4;22}\pm t.\sigma_{4;22}$	$b_{4;33}\pm t.\sigma_{4;33}$	$b_{4;12}\pm t.\sigma_{4;12}$	$b_{4;13}\pm t.\sigma_{4;13}$	$b_{4;23}\pm t.\sigma_{4;23}$
	p	$b_{5;0}\pm t.\sigma_{5;0}$	$b_{5;1}\pm t.\sigma_{5;1}$	$b_{5;2}\pm t.\sigma_{5;2}$	$b_{5;3}\pm t.\sigma_{5;3}$	$b_{5;11}\pm t.\sigma_{5;11}$	$b_{5;22}\pm t.\sigma_{5;22}$	$b_{5;33}\pm t.\sigma_{5;33}$	$b_{5;12}\pm t.\sigma_{5;12}$	$b_{5;13}\pm t.\sigma_{5;13}$	$b_{5;23}\pm t.\sigma_{5;23}$
	a	$b_{6;0}\pm t.\sigma_{6;0}$	$b_{6;1}\pm t.\sigma_{6;1}$	$b_{6;2}\pm t.\sigma_{6;2}$	$b_{6;3}\pm t.\sigma_{6;3}$	$b_{6;11}\pm t.\sigma_{6;11}$	$b_{6;22}\pm t.\sigma_{6;22}$	$b_{6;33}\pm t.\sigma_{6;33}$	$b_{6;12}\pm t.\sigma_{6;12}$	$b_{6;13}\pm t.\sigma_{6;13}$	$b_{6;23}\pm t.\sigma_{6;23}$
	r	$b_{7;0}\pm t.\sigma_{7;0}$	$b_{7;1}\pm t.\sigma_{7;1}$	$b_{7;2}\pm t.\sigma_{7;2}$	$b_{7;3}\pm t.\sigma_{7;3}$	$b_{7;11}\pm t.\sigma_{7;11}$	$b_{7;22}\pm t.\sigma_{7;22}$	$b_{7;33}\pm t.\sigma_{7;33}$	$b_{7;12}\pm t.\sigma_{7;12}$	$b_{7;13}\pm t.\sigma_{7;13}$	$b_{7;23}\pm t.\sigma_{7;23}$
	f	$b_{8;0}\pm t.\sigma_{8;0}$	$b_{8;1}\pm t.\sigma_{8;1}$	$b_{8;2}\pm t.\sigma_{8;2}$	$b_{8;3}\pm t.\sigma_{8;3}$	$b_{8;11}\pm t.\sigma_{8;11}$	$b_{8;22}\pm t.\sigma_{8;22}$	$b_{8;33}\pm t.\sigma_{8;33}$	$b_{8;12}\pm t.\sigma_{8;12}$	$b_{8;13}\pm t.\sigma_{8;13}$	$b_{8;23}\pm t.\sigma_{8;23}$
	x	$b_{9;0}\pm t.\sigma_{9;0}$	$b_{9;1}\pm t.\sigma_{9;1}$	$b_{9;2}\pm t.\sigma_{9;2}$	$b_{9;3}\pm t.\sigma_{9;3}$	$b_{9;11}\pm t.\sigma_{9;11}$	$b_{9;22}\pm t.\sigma_{9;22}$	$b_{9;33}\pm t.\sigma_{9;33}$	$b_{9;12}\pm t.\sigma_{9;12}$	$b_{9;13}\pm t.\sigma_{9;13}$	$b_{9;23}\pm t.\sigma_{9;23}$

CANONICAL FORM OF TABLE 2 (cont.).

		LoF					MV CM				MVB				
	run	(123)	(111)	(222)	(333)	block	S	A	R	T	p	a	r	f	x
C	1						$y_{1,1}$	$y_{2,1}$	$y_{3,1}$	$y_{4,1}$	$y_{5,1}$	$y_{6,1}$	$y_{7,1}$	$y_{8,1}$	$y_{9,1}$
	2						$y_{1,2}$	$y_{2,2}$	$y_{3,2}$	$y_{4,2}$	$y_{5,2}$	$y_{6,2}$	$y_{7,2}$	$y_{8,2}$	$y_{9,2}$
	3						$y_{1,3}$	$y_{2,3}$	$y_{3,3}$	$y_{4,3}$	$y_{5,3}$	$y_{6,3}$	$y_{7,3}$	$y_{8,3}$	$y_{9,3}$
	4						$y_{1,4}$	$y_{2,4}$	$y_{3,4}$	$y_{4,4}$	$y_{5,4}$	$y_{6,4}$	$y_{7,4}$	$y_{8,4}$	$y_{9,4}$
	5						$y_{1,5}$	$y_{2,5}$	$y_{3,5}$	$y_{4,5}$	$y_{5,5}$	$y_{6,5}$	$y_{7,5}$	$y_{8,5}$	$y_{9,5}$
	6						$y_{1,6}$	$y_{2,6}$	$y_{3,6}$	$y_{4,6}$	$y_{5,6}$	$y_{6,6}$	$y_{7,6}$	$y_{8,6}$	$y_{9,6}$
	7						$y_{1,7}$	$y_{2,7}$	$y_{3,7}$	$y_{4,7}$	$y_{5,7}$	$y_{6,7}$	$y_{7,7}$	$y_{8,7}$	$y_{9,7}$
	8						$y_{1,8}$	$y_{2,8}$	$y_{3,8}$	$y_{4,8}$	$y_{5,8}$	$y_{6,8}$	$y_{7,8}$	$y_{8,8}$	$y_{9,8}$
c	9						$y_{1,9}$	$y_{2,9}$	$y_{3,9}$	$y_{4,9}$	$y_{5,9}$	$y_{6,9}$	$y_{7,9}$	$y_{8,9}$	$y_{9,9}$
	10						$y_{1,10}$	$y_{2,10}$	$y_{3,10}$	$y_{4,10}$	$y_{5,10}$	$y_{6,10}$	$y_{7,10}$	$y_{8,10}$	$y_{9,10}$
	11						$y_{1,11}$	$y_{2,11}$	$y_{3,11}$	$y_{4,11}$	$y_{5,11}$	$y_{6,11}$	$y_{7,11}$	$y_{8,11}$	$y_{9,11}$
`c	12						$y_{1,12}$	$y_{2,12}$	$y_{3,12}$	$y_{4,12}$	$y_{5,12}$	$y_{6,12}$	$y_{7,12}$	$y_{8,12}$	$y_{9,12}$
	13						$y_{1,13}$	$y_{2,13}$	$y_{3,13}$	$y_{4,13}$	$y_{5,13}$	$y_{6,13}$	$y_{7,13}$	$y_{8,13}$	$y_{9,13}$
ax	14						$y_{1,14}$	$y_{2,14}$	$y_{3,14}$	$y_{4,14}$	$y_{5,14}$	$y_{6,14}$	$y_{7,14}$	$y_{8,14}$	$y_{9,14}$
	15						$y_{1,15}$	$y_{2,15}$	$y_{3,15}$	$y_{4,15}$	$y_{5,15}$	$y_{6,15}$	$y_{7,15}$	$y_{8,15}$	$y_{9,15}$
	16						$y_{1,16}$	$y_{2,16}$	$y_{3,16}$	$y_{4,16}$	$y_{5,16}$	$y_{6,16}$	$y_{7,16}$	$y_{8,16}$	$y_{9,16}$
	17						$y_{1,17}$	$y_{2,17}$	$y_{3,17}$	$y_{4,17}$	$y_{5,17}$	$y_{6,17}$	$y_{7,17}$	$y_{8,17}$	$y_{9,17}$
	18						$y_{1,18}$	$y_{2,18}$	$y_{3,18}$	$y_{4,18}$	$y_{5,18}$	$y_{6,18}$	$y_{7,18}$	$y_{8,18}$	$y_{9,18}$
	19						$y_{1,19}$	$y_{2,19}$	$y_{3,19}$	$y_{4,19}$	$y_{5,19}$	$y_{6,19}$	$y_{7,19}$	$y_{8,19}$	$y_{9,19}$
EE $\pm t.\sigma$	S	$b_{1;123} \pm t.\sigma_{1;123}$	$b_{1;111} \pm t.\sigma_{1;111}$	$b_{1;222} \pm t.\sigma_{1;222}$	$b_{1;333} \pm t.\sigma_{1;333}$	$B_{1,I \to II} \pm t.\sigma_{1,I \to II}$									
	A	$b_{2;123} \pm t.\sigma_{2;123}$	$b_{2;111} \pm t.\sigma_{2;111}$	$b_{2;222} \pm t.\sigma_{2;222}$	$b_{2;333} \pm t.\sigma_{2;333}$	$B_{2,I \to II} \pm t.\sigma_{2,I \to II}$									
	R	$b_{3;123} \pm t.\sigma_{3;123}$	$b_{3;111} \pm t.\sigma_{3;111}$	$b_{3;222} \pm t.\sigma_{3;222}$	$b_{3;333} \pm t.\sigma_{3;333}$	$B_{3,I \to II} \pm t.\sigma_{3,I \to II}$									
	T	$b_{4;123} \pm t.\sigma_{4;123}$	$b_{4;111} \pm t.\sigma_{4;111}$	$b_{4;222} \pm t.\sigma_{4;222}$	$b_{4;333} \pm t.\sigma_{4;333}$	$B_{4,I \to II} \pm t.\sigma_{4,I \to II}$									
	p	$b_{5;123} \pm t.\sigma_{5;123}$	$b_{5;111} \pm t.\sigma_{5;111}$	$b_{5;222} \pm t.\sigma_{5;222}$	$b_{5;333} \pm t.\sigma_{5;333}$	$B_{5,I \to II} \pm t.\sigma_{5,I \to II}$									
	a	$b_{6;123} \pm t.\sigma_{6;123}$	$b_{6;111} \pm t.\sigma_{6;111}$	$b_{6;222} \pm t.\sigma_{6;222}$	$b_{6;333} \pm t.\sigma_{6;333}$	$B_{6,I \to II} \pm t.\sigma_{6,I \to II}$									
	r	$b_{7;123} \pm t.\sigma_{7;123}$	$b_{7;111} \pm t.\sigma_{7;111}$	$b_{7;222} \pm t.\sigma_{7;222}$	$b_{7;333} \pm t.\sigma_{7;333}$	$B_{7,I \to II} \pm t.\sigma_{7,I \to II}$									
	f	$b_{8;123} \pm t.\sigma_{8;123}$	$b_{8;111} \pm t.\sigma_{8;111}$	$b_{8;222} \pm t.\sigma_{8;222}$	$b_{8;333} \pm t.\sigma_{8;333}$	$B_{8,I \to II} \pm t.\sigma_{8,I \to II}$									
	x	$b_{9;123} \pm t.\sigma_{9;123}$	$b_{9;111} \pm t.\sigma_{9;111}$	$b_{9;222} \pm t.\sigma_{9;222}$	$b_{9;333} \pm t.\sigma_{9;333}$	$B_{9,I \to II} \pm t.\sigma_{9,I \to II}$									

CANONICAL FORM OF TABLE 3.

		LO			TO			MM		
		x_1	x_2	x_3	x_1	x_2	x_3	x_1	x_2	x_3
EE	S	$x_{1,1,opt}$	$x_{2,1,opt}$	$x_{3,1,opt}$				$x_{1,1,Mopt}$	$x_{2,1,Mopt}$	$x_{3,1,Mopt}$
	A	$x_{1,2,opt}$	$x_{2,2,opt}$	$x_{3,2,opt}$				$x_{1,2,Mopt}$	$x_{2,2,Mopt}$	$x_{3,2,Mopt}$
	R	$x_{1,3,opt}$	$x_{2,3,opt}$	$x_{3,3,opt}$				$x_{1,3,Mopt}$	$x_{2,3,Mopt}$	$x_{3,3,Mopt}$
	N	$x_{1,4,opt}$	$x_{2,4,opt}$	$x_{3,4,opt}$				$x_{1,4,Mopt}$	$x_{2,4,Mopt}$	$x_{3,4,Mopt}$
	p	$x_{1,5,opt}$	$x_{2,5,opt}$	$x_{3,5,opt}$				$x_{1,5,Mopt}$	$x_{2,5,Mopt}$	$x_{3,5,Mopt}$
	a	$x_{1,6,opt}$	$x_{2,6,opt}$	$x_{3,6,opt}$				$x_{1,6,Mopt}$	$x_{2,6,Mopt}$	$x_{3,6,Mopt}$
	r	$x_{1,7,opt}$	$x_{2,7,opt}$	$x_{3,7,opt}$				$x_{1,7,Mopt}$	$x_{2,7,Mopt}$	$x_{3,7,Mopt}$
	f	$x_{1,8,opt}$	$x_{2,8,opt}$	$x_{3,8,opt}$				$x_{1,8,Mopt}$	$x_{2,8,Mopt}$	$x_{3,8,Mopt}$
	x	$x_{1,9,opt}$	$x_{2,9,opt}$	$x_{3,9,opt}$				$x_{1,9,Mopt}$	$x_{2,9,Mopt}$	$x_{3,9,Mopt}$

REFERENCES

ADAMS, M.R. and HALL, C.J. 1988. Growth inhibition of food-borne pathogens by lactic and acetic acid and their mixtures. Int. J. Food Sci. Technol. *23*, 287-292.

ANDERSSON, R.E., ERIKSSON, C.E., SALOMONSSON, A.C. and THEANDE, O. 1990. Lactic acid fermentation of fresh and stored carrot: chemical, microbial and sensory evaluation of products. Lebensmitt. Wiss. u-Technol. *23*, 34-40.

BELL, T., TURNEY, L. and ETCHELLS, J. 1972. Influence of different organic acids on firmness of fresh-packed pickles. J. Food Sci. *37*, 446-449.

BERGEY, D. H. 1989. *Bergey's Manual of Systematic Bacteriology*. p. 1229. Williams and Wilkins, Baltimore.

BINSTED, R., DEVEY, J. D. and DAKIN, J. C. 1962. *Pickle and Sauce Making*. p. 106. Food Trade, London.

BOX, G.E.P., HUNTER, W.G. and HUNTER, J.S. 1978. *Statistics for Experimenters*. John Wiley & Sons, New York.

DIEZ, M.J., *et al.* 1985. *Biotecnologia de la Aceituna de Mesa*. Instituto de la Grasa. Sevilla.

ETCHELLS, M., BELL, T.A., FLEMING, H. and POTTS, E. 1973. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers - use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pak. Sci. *3*, 4-14.

FLEMING, H.P. 1982. Fermented vegetables. In *Economic Microbiology and Fermented Food*. p. 277, (A.H. Rose, ed.) Academic Press, New York.

FLEMING, H.P., McFEETERS, R., THOMPSON, R. and SANDERS, D. 1983. Storage stability of vegetables fermented with pH control. J. Food Sci. *49*, 975-981.

McKNEE, J.M.T. 1985. A single method for the extraction of reducing sugars and nonreducing sugars from carrots and other storage vegetables. J. Sci. Food Agric. *36*, 55-58.

MOREIRA, L.A., OLIVEIRA, F.A.R. and SILVA, T.R. 1992. Prediction of pH change in processed acidified turnips. Int. J. Food Sci. Technol. *57*, 928-931.

NABAIS, R.M., and MALCATA, F.X. 1995. Modelling the transport of lactic acid, sodium chloride and reducing sugars in carrot slices submerged in carrot slices submerged in brines. Part 2: Multivariate approach. J. Food Eng. In Press.

NABAIS, R.M., VIEIRA, M.C. and MALCATA, F.X. 1995. J. Food Eng. Modelling the transport of lactic acid, sodium chloride and reducing sugars in carrot slices submerged in brines. Part I: Multivariate approach. In Press.

NIKETIC-ALEKSIC, G.K., BOURNE, M.C. and STAMER, J.R. 1973. Preservation of carrots by lactic acid fermentation. J. Food Sci. *38*, 84-86.

SCHWARTZBERG, H.B. and CHAO, R.Y. 1982. Solute diffisivities in leaching processes. Food Technol. *36*, 73-86.

STEINKRAUS, K.H. 1983. Acid fermented vegetables. In *Handbook of Indigenous Fermented Food Involving an Acid Fermentation*, p. 127, Marcel Dekker, New York.