Protein Isolation from Tomato Seed Meal, Extraction Optimization

GEORGE N. LIADAKIS, CONSTANTINA TZIA, VASSILIKI OREOPOULOU, and CHRISTOS D. THOMOPOULOS

ABSTRACT

Water extraction of tomato seed meal proteins was studied to find optimal conditions for protein extraction and isolation. A central composite design including temperature, pH, time and water/solids was used and second order models were employed. Optimum conditions were: 50°C, pH 11.5, 20 min and water/solids = 30/1 (v/w). Experimental values were: extraction yield (extracted protein to that in raw material) 66.1%, protein content of product 72.0%, and total protein yield (protein in isolated product to that in raw material) 43.6%. Estimated values were in good agreement with experimental values. Optimum conditions were confirmed by a larger scale experiment.

Key Words: tomato, protein extraction, seed meal, optimization

INTRODUCTION

ENVIRONMENTAL POLLUTION caused by food processing wastes could be reduced by appropriate recovery of edible nutrients (Birch et al., 1976; Green and Kramer, 1979; Knorr, 1983). Tomato processing wastes, primarily skins and seeds, comprise 10 to 30% of raw fruit weight (Ben-Gera and Kramer, 1969; Geisman, 1981). Tomato seeds represent 50–55% of the pomace. Tomato pomace is mainly disposed of as animal feed or fertilizer (Tsatsaronis and Boskou, 1975; Canella et al., 1979; Cantarelli et al., 1989). A small fraction of the seeds is used by the oil industry (Canella et al., 1979; Geisman, 1981). About 1×10^6 metric tons of tomatoes are processed into products annually in Greece (NSSG, 1990), generating ≈100,000 tons of tomato seeds.

The potential of tomato seeds as a food source has been reported (Ammerman et al., 1963; Drouliskos, 1976; Kramer and Kwee, 1977a,b; Abdel-Rahman, 1982; Al-Wandawi et al., 1985; Lasztity et al., 1986; Rahma et al., 1986). The approximate composition of tomato seeds (dry basis) is: fat 11–20%, protein 15–22% and ash 3–7%. The high unsaturated fatty acid content of tomato seed oil ($C_{18:1}$ 20%, $C_{18:2}$ 55–60%, $C_{18:3}$ 2%) and the nutritive value of the protein compare favorably with soybeans (Rymal, 1973; Rymal et al., 1974; Brodowski and Geisman, 1980; Lazos and Kalathenos, 1988). The high lysine content (8–10 g/16 g N) of tomato seed protein (Rymal et al., 1974; Cantarelli et al., 1989) makes it suitable for supplementing proteins in cereal products (Brodowski and Geisman, 1980; Carlson et al., 1981; Yaseen et al., 1991). In addition the functionality of tomato seed proteins may have many uses in food systems (Kramer and Kwee, 1977a; Moharram et al., 1984; Doxastakis et al., 1988a,b; Doxastakis et al., 1988; Kiosseoglu et al., 1989). Tomato seeds lack antinutritional factors or toxic substances often found in other non-conventional protein sources (Rahma et al., 1986). Thus, the recovery and utilization of tomato seed protein for human consumption has been studied (Kwee, 1970; Canella et al., 1979; Doxastakis et al., 1988b; Cantarelli et al., 1989; Kiosseoglu et al., 1989).

Protein has been isolated from tomato seeds using a 3-step process: extraction, precipitation and drying of protein precipitate (Kramer and Kwee, 1977b; Fazio et al., 1983). Canella and Castriota (1980) examined the effects of several individual factors on protein extraction from tomato seed meal. Latlief and Knorr (1983a,b) studied the protein precipitation step using commercial tomato seeds.

Our objective was to determine the optimal conditions for protein extraction from defatted tomato seed meal, examining simultaneously effects of temperature, pH, time and water-to-solids ratio. The effect of extraction conditions on protein yield and on protein content of isolated product was also determined.

The authors are affiliated with the Laboratory of Food Chemistry & Technology, Dept. of Chemical Engineering, National Technical University of Athens, 5 Heroon Polytechniou St., Zografou, 15780 Athens, Greece.

MATERIALS & METHODS

Materials

Tomato pomace was obtained from a tomato processing plant (KOPAIS S.A. Aliartos, Greece). It was sundried (25–30°C, 3–4 days) and ground in a blender (Waring Commercial Blendor, Dynamics Co., New Hartford, CO). The major part of the skins was removed using a 1 mm sieve. The skins remaining on the sieve were separated from seeds with a fan blowing an upward airstream. The seed fraction was ground (Ultra-Centrifugal Mill, Type ZM1, F.K. Retsch GmbH & Co, Haan, Germany) to pass a 1 mm sieve. Tomato seed meal was prepared by defatting ground seeds with n-hexane in a Soxhlet apparatus and grinding (Ultra-Centrifugal Mill, Type ZM1, F.K. Retsch GmbH & Co, Haan, Germany) to pass a 0.5 mm sieve.

Protein isolation from tomato seed

Tomato seed meal (10 g) was extracted with deionized water (10:1–30:1 ratio) in a stirred glass vessel. The pH of the suspension (7.5–11.5) was kept constant during the extraction by adjusting with 0.5N NaOH. Temperature (30–50°C) was regulated within ±0.2°C by a water bath. The slurry was centrifuged at $2600 \times g$ for 20 min, the supernatant was collected and the pH was adjusted to the isoelectric point (3.9) using 0.5N HCl. The protein precipitate was separated by centrifugation at $2600 \times g$ for 25 min and freeze dried. The solid residue after protein extraction was dried at 60°C under vacuum and was used for protein determination.

Isoelectric point (pI)

The pI of tomato seed proteins was determined as the pH value of maximal precipitation. 20g of tomato seed meal was extracted as described, under the conditions: water-to-solids ratio 20:1 (v/w), pH 10, 40°C, 30 min. The pI was found by titrating aliquots of the collected extract to specific pH values and determining the protein content of the supernatant after centrifugation. The protein content was determined according to the method of Lowry et al. (1951).

Analytical methods

Moisture, crude fat, ash, total dietary fiber and crude protein (Nx6.25) were determined according to standard methods (AOAC, 1990). Minerals were determined by atomic absorption/emission spectroscopy (Perkin-Elmer Model 2380, Perkin Elmer Co., Norwalk, CT). Phosphorus was determined photometrically by the ascorbic acid method (Hach Company, 1989), after digestion with concentrated sulfuric acid and hydrogen peroxide (50%) in a Digesdahl apparatus (Hach Company, Loveland, CO). Total sugars were measured according to the phenol-sulfuric acid method (Dubois et al., 1956) using glucose as standard.

Table 1—Variables and levels for central composite design

Variable	Symbol	Coded variables levels[a] −2	−1	0	1	2
Temperature (°C)	x_1	30	35	40	45	50
pH	x_2	7.5	8.5	9.5	10.5	11.5
Time (min)	x_3	20	30	40	50	60
Water-to-solids ratio (v/w)	x_4	10:1	15:1	20:1	25:1	30:1

[a] Passage from coded variable (X_i) level to natural variable (x_i) level is given by the following equations: $x_1=5X_1 + 40$; $x_2=X_2 + 9.5$; $x_3=10X_3 + 40$; $x_4=(5X_4 + 20):1$.

Table 2—Proximate composition and mineral content of tomato seed meal (dry basis)

	Tomato seed	Tomato seed meal
Moisture (%)	8.3	8.1
Protein (%)	25.5	31.3
Crude fat (%)	18.2	1.3
Ash (%)	3.7	4.6
Total sugars (%)	2.9	3.2
Total dietary fiber (%)		54.1
K (mg/100 g)		1046
Na (mg/100 g)		70
Ca (mg/100 g)		294
Mg (mg/100 g)		491
P (mg/100 g)		903
Fe (mg/100 g)		10
Mn (mg/100 g)		6
Cu (mg/100 g)		2
Zn (mg/100 g)		4

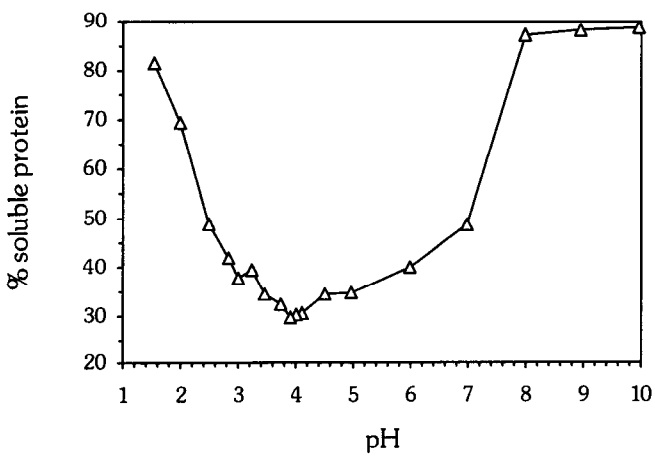

Fig. 1—Precipitation of extracted proteins from tomato seed meal as related to pH.

Experimental design and statistical analysis

The process variables (factors) and the responses (dependent variables) were defined from preliminary studies and published data (Latlief and Knorr, 1983a; Rustom et al., 1991). The process variables (x_i) were: temperature (x_1), pH (x_2), and time of extraction (x_3) and water-to-solids ratio (x_4). Each variable was coded at five levels: −2, −1, 0, 1, 2 (Table 1).

Selected responses which evaluate the extraction process included protein extraction yield (EY) defined as the ratio of total extracted protein to total protein in the raw material, expressed as percentage. Also included were protein content of the product (PR) and total protein yield (TY) defined as the ratio of total protein in the isolated product to total protein in the raw material, expressed as percentage.

A central composite design (CCD) was arranged to allow for fitting of a second-order model (Cohran and Cox, 1957; Adler et al., 1975). The CCD combined the vertices of a hypercube whose coordinates were given by the 2^n factorial design (runs 1–16) with the "star" points (runs 17–24). The star points were added to the factorial design to provide for estimation of curvature of the model (Joklegar and May, 1987). Seven replicates at the center point of the design (runs 25–31) were used to allow for estimation of the "pure error" sum of squares. All experiments were carried out in a randomized order to minimize any effects of extraneous factors on the observed responses. The regression coefficients and the ANOVA tables were computed using the Data analysis-Regression option of EXCEL 5.0 (Microsoft Corporation) program.

Table 3—Central composite design arrangement and responses (1–31) and experimental runs at the optimum conditions (32–39)

Run	Variable levels[a] X_1	X_2	X_3	X_4	Responses EY	PR	TY
1	1	1	1	1	56.60	73.38	37.64
2	1	−1	1	1	49.91	67.66	26.16
3	−1	1	1	1	57.31	74.90	38.87
4	−1	−1	1	1	48.53	71.34	34.62
5	1	1	−1	1	57.16	72.58	40.98
6	1	−1	−1	1	49.98	70.60	30.84
7	−1	1	−1	1	53.06	72.39	34.84
8	−1	−1	−1	1	50.02	69.77	29.57
9	1	1	1	−1	48.74	74.64	30.55
10	1	−1	1	−1	43.91	73.50	30.82
11	−1	1	1	−1	50.60	76.22	37.84
12	−1	−1	1	−1	48.11	72.04	30.43
13	1	1	−1	−1	54.02	74.95	28.77
14	1	−1	−1	−1	50.14	74.48	26.17
15	−1	1	−1	−1	48.30	70.35	29.03
16	−1	−1	−1	−1	46.49	71.75	32.12
17	2	0	0	0	46.63	73.94	24.48
18	−2	0	0	0	51.45	69.37	30.75
19	0	2	0	0	55.41	69.83	35.96
20	0	−2	0	0	48.37	69.32	26.95
21	0	0	2	0	49.02	75.23	36.24
22	0	0	−2	0	51.71	74.36	29.37
23	0	0	0	2	53.32	71.56	35.83
24	0	0	0	−2	42.83	72.64	24.90
25	0	0	0	0	54.49	71.97	35.38
26	0	0	0	0	51.35	75.00	33.35
27	0	0	0	0	50.20	74.05	31.90
28	0	0	0	0	47.85	74.34	29.80
29	0	0	0	0	51.38	73.24	34.41
30	0	0	0	0	46.07	74.48	29.35
31	0	0	0	0	48.13	72.18	30.44
32	−2	2	2	2	63.67	71.79	41.05
33	−2	2	2	2	65.62	71.81	39.93
34	−2	2	2	2	62.51	72.45	42.46
35	−2	2	2	2	64.29	72.07	40.28
36	2	2	−2	2	67.23	69.16	40.07
37	2	2	−2	2	67.73	71.92	42.82
38	2	2	−2	2	65.19	73.37	46.13
39	2	2	−2	2	64.19	73.42	45.31

[a] Coded variables

Optimization

Optimum extraction conditions were estimated by the steepest ascent method (Adler et al., 1975) using a computer program written in BASIC. The program using the fitted model for each response searched the experimental space for optimum responses that were generated by feasible combinations of all factors simultaneously. Several experimental runs were conducted at the predicted optimum conditions; these runs in combination with the earlier ones were used for estimation of new coefficients for fitted models.

Contour plots

Variables with significant linear terms were chosen for axes of contour plots for each response. Contour plots were generated by assigning constant (zero) values to two of the four variables and solving the fitted equations as a quadratic equation in the remaining two variables.

RESULTS & DISCUSSION

Dried tomato pomace consisted of about 53% seeds and 47% skins (weight basis). The seeds were separated from skins, not only because they contained most of the proteins but also because the essential amino acid content and the biological value of seed proteins are higher than those of skin proteins (Lasztity et al., 1986).

The seeds had a protein content of 25.5% (dry basis) considered adequate for protein recovery. Seeds were defatted, since tomato seed oil is recognized as an edible oil (Canella et al., 1979). The proximate composition of tomato seeds and tomato

Table 4—Regression coefficients for the fitted second-order models

Coefficients	Initial models			New models		
	EY	PR	TY	EY	PR	TY
b_0	49.924	73.609	32.090	50.295	73.860	32.792
b_1	−0.067	0.507*	−1.164*	−0.021	0.590**	−1.154**
b_2	2.199****	0.804***	2.325****	2.127****	0.755**	2.189****
b_3	−0.452	0.356	1.181**	−0.497	0.274	1.171**
b_4	2.218****	−0.728**	2.069***	2.146****	−0.777***	1.932****
b_{11}	−0.061	−0.405	−0.803	−0.189	−0.492*	−1.047**
b_{22}	0.652	−0.925***	0.157	0.523	−1.012****	−0.087
b_{33}	0.271	0.380	0.494	0.142	0.293	0.250
b_{44}	−0.302	−0.293	−0.116	−0.431	−0.380	−0.360
b_{12}	0.404	0.022	0.632	0.541	0.270	0.662
b_{13}	−1.176*	−0.854**	−1.112	−0.960**	−0.708**	−0.702
b_{14}	0.089	−0.712**	0.677	0.226	−0.464	0.707
b_{23}	0.430	0.683*	0.497	0.293	0.435	0.467
b_{24}	0.793	0.593*	1.531**	0.576	0.447	1.121**
b_{34}	0.608	−0.163	−0.781	0.471	−0.431	−0.711

* P<0.1
** P<0.05
*** P<0.01
**** P<0.001

Table 5—F values, coefficient of determination and coefficient of variation for the fit of experimental data to models

	Initial models			New models		
	EY	PR	TY	EY	PR	TY
F values	3.91***	4.41***	3.97***	27.15****	3.90***	14.75****
R^2	0.774	0.795	0.778	0.941	0.695	0.896
CV(%)	4.48	1.80	8.55	3.94	1.90	7.23

*** P<0.01
**** P<0.001

Table 6—Observed and predicted responses at the optimum conditions

	EY		PR		TY	
Point	Predicted	Observed[a]	Predicted	Observed[a]	Predicted	Observed[a]
−2,2,2,2	64.2	64.0	72.2	72.0	41.2	40.9
2,2,−2,2	66.1	66.1	71.9	72.0	45.6	43.6

[a] Mean values of four replicates

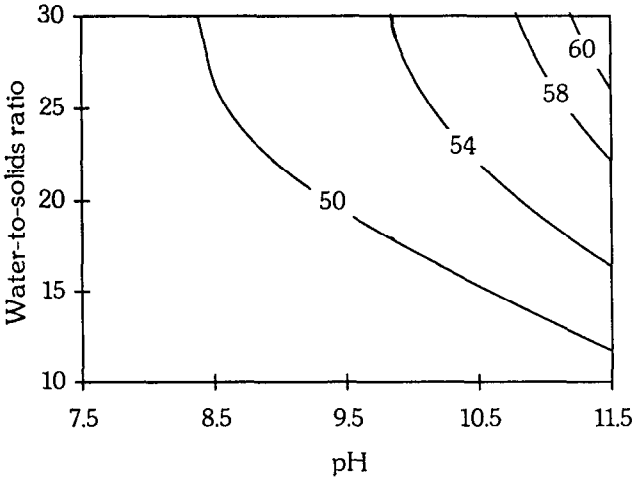

Fig. 2—Contour plots for EY as related to pH and water-to-solids ratio; the other two variables were fixed at zero coded levels.

seed meal were compared (Table 2). Protein content of tomato seeds was higher but ash and crude fat content were slightly lower than reported values (Brodowski and Geisman, 1980; Latlief and Knorr, 1983a; Moharram et al., 1984). This may be due to differences in tomato cultivars and processes.

Tomato seed meal was rich in protein (31.3% d.b.) and compared favorably with other oilseed meals as a potential nonconventional source of protein (Yazicioglu et al., 1981; Liadakis et al., 1993). According to Cantarelli et al., (1989), tomato seed meal is more suitable for protein isolates production because of high fiber content.

Protein precipitation was done at the pI which was 3.9 (Fig. 1). Other values of tomato seed proteins pI have been reported to be between 3.8 and 4.6 (Kramer and Kwee, 1977b; Canella and Castriota, 1980; Latlief and Knorr, 1983a,b; Fazio et al., 1983).

Protein extraction yield (EY), protein content of the product (PR) and total protein yield (TY) obtained by different combinations of the extraction conditions were compared (Table 3). Widely dispersed values of EY were obtained with different combinations of extraction conditions, varying from 42.8 to 57.3%. TY also showed widely dispersed values, from 24.5 to 41%. As protein extraction increased, more proteins were in solution, and more proteins could be precipitated, contributing to total yield increase, thus explaining the same pattern of EY and TY values. However, PR of isolated products did not vary much, ranging from 67.7 to 76.2%, relatively high for such products (Kramer and Kwee, 1977b; Tchorbanov et al., 1986). Nevertheless, these products were characterized as concentrates.

Model fitting

Regression coefficients for the fitted models were compared (Table 4). pH and water-to-solids ratio were the most significant factors for all models. Especially, for the PR model, pH showed highly significant ($P < 0.01$) linear and quadratic terms. This implies that pH and water-to-solids ratio were the predominant factors for modeling of protein isolation from tomato seed meal, as reported by Rustom et al., (1991) for peanuts. Note that temperature was not a significant factor in any models; Canella and Castriota, (1980) reported a similar effect, however, others have reported that temperature influenced protein extraction from proteinaceous sources (Drawert et al., 1979; Rustom et al., 1991).

The adequacy of each model was tested by the lack of fit test and the coefficient of determination R^2 (Table 5). All three models were significant by the F-test at the 1% confidence level and none of the models exhibited lack of fit. Our three models for EY, PR and TY showed R^2 values of 77.4, 79.5 and 77.6% respectively, adequate for models of this type. Reproducibilities of our models were very good (CV 4.5, 1.8 and 8.6% respectively).

Optimization

Fitted models were introduced as objective functions in an optimization program based on the steepest ascent method. Predicted optimum EY values were 70.3 and 67.5 at (−2,2,2,2) and (2,2,−2,2) respectively (variables in coded levels). For PR optimum values of 80.4 and 75.0 were predicted at (2,−0.9,−2,−2) and (−2,1.7,2,2) respectively. For TY the predicted optimum values were 52.0 and 50.5 at (2,2,−2,2) and (−0.5,2,2,2) respectively. As seen from these values, both EY

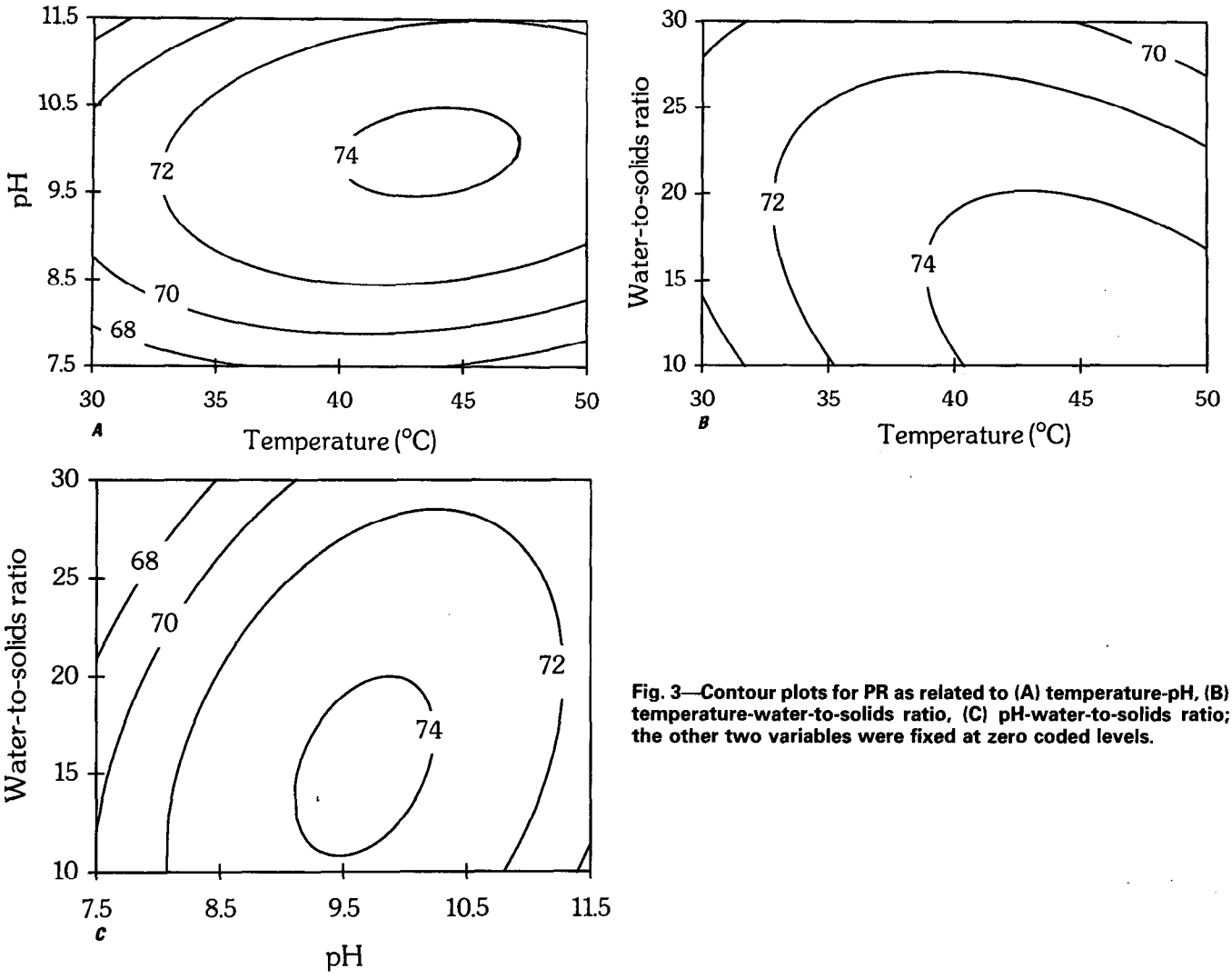

Fig. 3—Contour plots for PR as related to (A) temperature-pH, (B) temperature-water-to-solids ratio, (C) pH-water-to-solids ratio; the other two variables were fixed at zero coded levels.

and TY showed their optimum values at the same factor region. Because protein extraction is the predominant step for protein isolation, we decided to examine further the optimum conditions for EY. Observing optimum EY conditions, note that maximum EY values were obtained at the high level of pH and water-to-solids ratio, low temperature-high time level as well as high temperature-low time level. As is well known, protein solubility increases as pH increases above 7.5 (Kwee, 1970; Kramer and Kwee, 1977b; Canella and Castriota, 1980; Latlief and Knorr, 1983a; Fazio et al., 1983). In addition increased water-to-solids ratio facilitates protein extraction. The combinations of high temperature-short time as well as low temperature-long time give better results of protein extraction, avoiding protein denaturation.

In order to verify the predicted values a series of experimental runs at the optimum conditions was conducted, at the points (−2,2,2,2) and (2,2,−2,2) (Table 3). The observed value for EY compared favorably with the predicted one only at (2,2,−2,2). Because of the discrepancies found between predicted and observed values, we decided to reevaluate the coefficients of fitted equations using all 39 experimental runs (1-31 CCD runs + 32-39 optimum points runs) (Table 4). For all models no essential change was observed for significant factors and interactions; coefficients had slightly modified values, keeping the same sign. The refitted equations were also adequate. All adequacy tests were improved for EY and TY models, especially R^2 being 94.1 and 89.6% for EY and TY respectively. Only PR model showed a slight decrease in R^2 (Table 5).

Predicted values from the refitted equations (Table 6) and observed values were in very good agreement. The (2,2,−2,2) point was selected as conditions giving the maximum EY and TY values.

Contour plots

Variables with significant effect were chosen as axes for contour plots for each response; the other variables were fixed at the central (zero) level. (Fig. 2–4).

EY increased with increasing water-to-solids ratio and pH level (Fig. 2). The highest EY resulted at high pH and water-to-solids ratio. PR showed maximum values at temperature >40°C (Fig. 3A,B), pH between 9.5–10.5 (Fig. 3A,C) and low water-to-solids ratio (Fig. 3B,C). TY increased as pH (Fig. 4A,C) and water-to-solids ratio (Fig. 4B,C) increased. A significant interaction between pH and water-to-solids ratio was observed for TY (Fig. 4C).

A larger scale experiment was conducted at optimum conditions (2,2,−2,2). Tomato seed meal (130 g) was treated following the same procedure. The experiment gave results in close agreement with those of the small scale, with an EY of 64.1%, and a TY of 41.2%. The product proximate composition and the solid residue remaining after protein extraction were analyzed (Table 7). The economic feasibility of protein isolation process also depends on utilization of by-products. The whey remaining after protein precipitation could be concentrated or

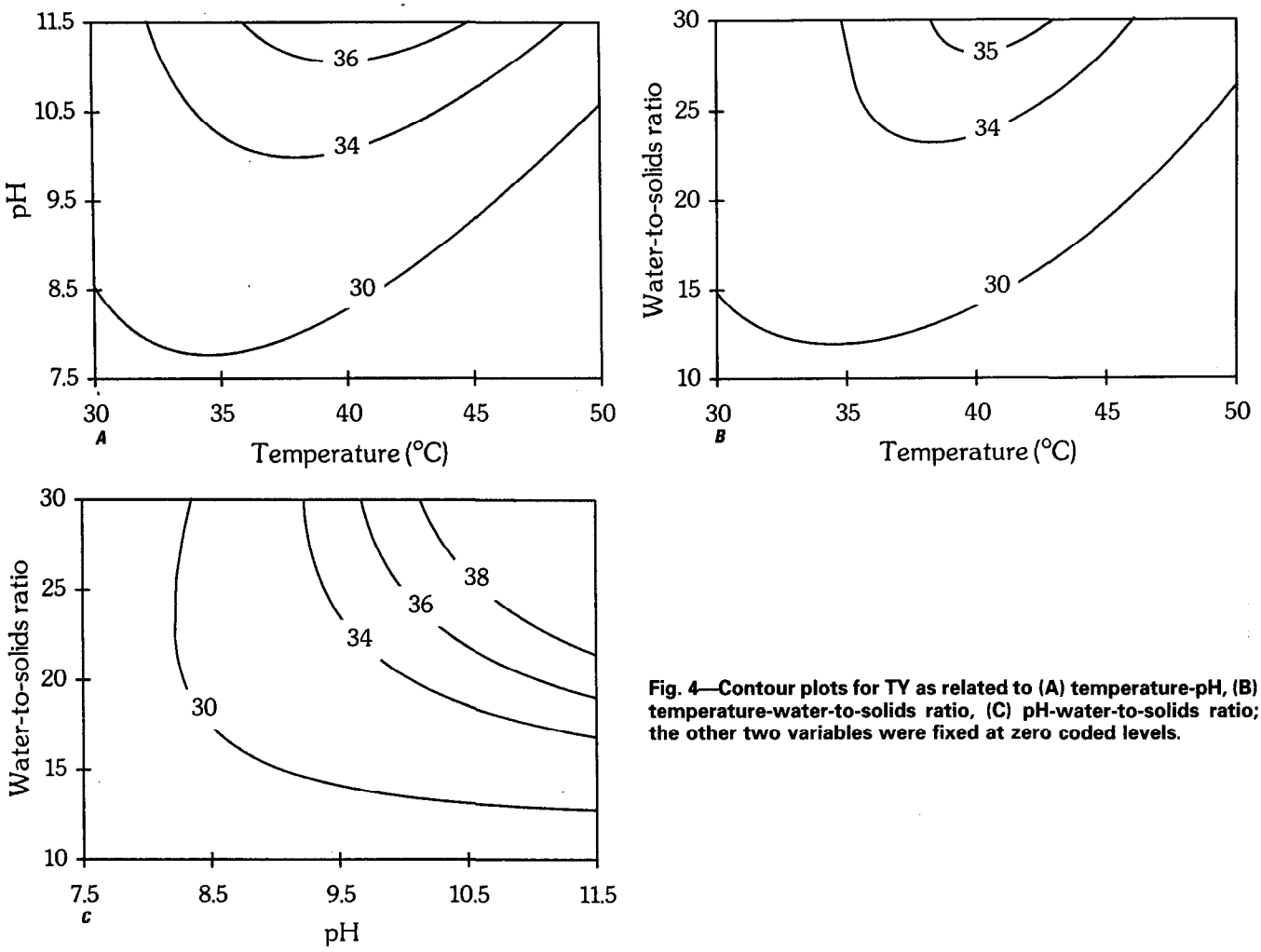

Fig. 4—Contour plots for TY as related to (A) temperature-pH, (B) temperature-water-to-solids ratio, (C) pH-water-to-solids ratio; the other two variables were fixed at zero coded levels.

Table 7—*Proximate composition and mineral content of tomato seed protein concentrate and solid residue after protein extraction (dry basis)*

	Tomato seed protein conc	Solid residue
Crude protein (%)	71.3	18.5
Ash (%)	3.4	5.5
Total sugars (%)	0.8	0.7
Total dietary fiber (%)	16.1	71.0
K (mg/100 g)	193	400
Na (mg/100 g)	596	912
Ca (mg/100g)	525	214
Mg (mg/100 g)	102	282
P (mg/100 g)	570	473
Fe (mg/100 g)	15	13
Mn (mg/100 g)	2	6
Cu (mg/100 g)	2	1
Zn (mg/100 g)	3	5

spray-dried to recover proteins and the solid residue of the extraction could be used as animal feed.

CONCLUSION

OPTIMUM EXTRACTION of tomato seed proteins with water for protein isolation, could be achieved by extracting one part of tomato seed meal with 30 parts of water (w/v ratio) at pH 11.5 at 50°C for 20 min. These conditions resulted in extracting 66.1% of the proteins contained in tomato seed meal, at a total protein yield of 43.6%. Isolated product had a protein content of 72%.

REFERENCES

Abdel-Rahman, A.Y. 1982. The chemical constituents of tomato seeds. Food Chem. 9: 315–318.

Adler, Y.P., Markova, E.V., and Granovsky, Y.V. 1975. *The Design of Experiments to Find Optimal Conditions.* Mir Publishers, Moscow, Russia.

Al-Wandawi, H., Abdul-Rahman, M., and Al-Shaikhly, K. 1985. Tomato processing wastes as essential raw materials source. J. Agric. Food Chem. 33: 804–807.

Ammerman, C.B., Arrington, L.R., Loggins, P.E., McCall, J.T., and Davis, G.K. 1963. Nutritive value of dried tomato pulp for ruminants. J. Agric. Food Chem. 11: 347–349.

AOAC. 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Washington, DC.

Ben-Gera, I. and Kramer, A. 1969. The utilization of food industries wastes. Adv. Food Res. 17: 77–85.

Birch, G.G., Parker, K.J., and Worogan, J.T. 1976. *Food from Waste.* Applied Science Publishers Ltd., London, England.

Brodowski, D., and Geisman, J.R. 1980. Protein content and amino acid composition of protein of seeds from tomatoes at various stages of ripeness. J. Food Sci. 45: 228–229, 235.

Canella, M., Cardinali, F., Castriotta, G., and Nappuci, R. 1979. Caratteri chimici dei semi di pomodoro di differenti varieta. Riv. Ital. Sost. Grasse. 56: 8–11.

Canella, M. and Castriota, G. 1980. Protein composition and solubility of tomato seed meal. Lebensm. Wiss. u.-Technol. 13: 18–21.

Cantarelli, P.R., Parma, E.R., and Caruso, J.G.B. 1989. Composition and amino acid profiles of tomato seeds from canning wastes. Acta Alimentaria. 18: 13–18.

Carlson, B.L., Knorr, D., and Watkins, T.R. 1981. Influence of tomato seed addition on the quality of wheat flour breads. J. Food Sci. 46: 1029–1031, 1042.

Cohran, W.G. and Cox, G.M. 1957. *Experimental Designs.* John Wiley & Sons Inc., New York.

Doxastakis, G., Kiosseoglou, V., and Boskou, D. 1988a. Emulsifying properties of tomato seed proteins obtained from processing waste. Sciences des Aliments. 8: 259–267.

Doxastakis, G., Kiosseoglou, V., and Boskou, D. 1988b. Functional properties of tomato seed protein obtained from processing waste. In *Frontiers of Flavor*, Proceedings of the 5th International Flavor Conference, Porto Karras, Chalkidiki, Greece, 1–3 July 1987, G. Charalambous (Ed.), p. 373–378. Elsevier Science Publishers B.V., Amsterdam, Netherlands.

Doxastakis, G., Kiosseoglou, V., Boskou, D., and Stalidis, G. 1988. Adsorption behaviour of tomato seed proteins at the corn oil-water interface. Lebensm. Wiss. u.-Technol. 21: 96–99.
Drawert, F., El-Refai, A.A., and Radola, B.J. 1979. Extraction of cottonseed proteins with urea. Chem. Mikrobiol. Technol. Lebensm. 6: 87–89.
Drouliskos, N.J. 1976. Nutritional evaluation of the protein of dried tomato pomace in the rat. Br. J. Nutr. 36: 449–456.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.
Eggers, L.K. 1974. Some biochemical and electron microscopic studies of the protein present in seeds recovered from tomato cannery waste. Ph.D. dissertation. The Ohio State Univ., Columbus, OH.
Fazio, G., Arcoleo, G., and Pirrone, L. 1983. Sul contenuto proteico del seme di pomodoro. Riv. Soc. Ital. Sci. Aliment. 12: 195–200.
Geisman, J.R. 1981. Protein from tomato seeds. Ohio Report on Research and Development. 66(6): 92–94.
Green, J.H. and Kramer, A. 1979. *Food Processing Waste Management.* AVI Publishing Co. Inc., Westport, CT.
Hach Company. 1989. *Water Analysis Handbook.* Hach Company, Loveland, CO.
Joglekar, A.M., and May, A.T. 1987. Product excellence through design of experiments. Cereal Foods World 32: 857–868.
Kiosseoglou, V., Theodorakis, K., and Doxastakis, G. 1989. The rheology of tomato seed protein isolate films at the corn oil-water interface. Colloid Polym. Sci. 267: 834–838.
Knorr, D. 1983. Recovery of functional proteins from food processing wastes. Food Technol. 37(2): 71–76.
Kramer, A. and Kwee, W.H. 1977a. Functional and nutritional properties of tomato protein concentrates. J. Food Sci. 42: 207–211.
Kramer, A. and Kwee, W.H. 1977b. Utilization of tomato processing wastes. J. Food Sci. 42: 212–215.
Kwee, W.H. 1970. Recovery of nutrients from tomato cannery wastes and vines- their functional and nutritional properties. Ph.D. dissertation, Univ. of Maryland, College Park, MD.
Lasztity, R., Abd El Samei, M., and El Shafei, M. 1986. Biochemical studies on some non conventional sources of protein. Part 1. Tomato seeds and peels. Nahrung. 6: 615–620.
Latlief, S.J. and Knorr, D. 1983a. Tomato seed protein concentrates: Effects of methods of recovery upon yield and compositional characteristics. J. Food Sci. 48: 1583–1586.
Latlief, S.J. and Knorr, D. 1983b. Effect of chitin as coagulating aid on protein yield, composition and functionality of tomato seed protein concentrates. J. Food Sci. 48: 1587–1590.
Lazos, E.S. and Kalathenos, P. 1988. Technical note: Composition of tomato processing wastes. Int. J. Food Sci. Technol. 23: 649–652.
Liadakis, G.N., Floridis, A., Tzia, C., and Oreopoulou, V. 1993. Protein isolates with reduced gossypol content from screw-pressed cottonseed meal. J. Agric. Food Chem. 41: 918–922.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randal, R.J. 1951. Protein analysis with Folin phenol reagent. J. Biol. Chem. 193: 265–275.
Moharram, V.G., Rahma, E.H., Mostafa, M.M., and Messalam, S.F. 1984. Utilization of tomato cannery wastes (seeds) in food purposes. Minufiya J. Agric. 8: 291–306.
NSSG. 1990. *Agricultural Statistics.* National Statistical Service of Greece. Athens, Greece.
Rahma, E.H., Moharram, Y.G., and Mostafa, M.M. 1986. Chemical characterization of tomato seed proteins (var. Pritchard). Egypt. J. Food Sci. 14: 221–230.
Rustom, I.Y.S., Lopez-Leiva, M.H., and Nair, B.M. 1991. Optimization of extraction of peanut proteins with water by response surface methodology. J. Food Sci. 56: 1660–1663.
Rymal, K.S. 1973. The seeds of *Lycopersicon esculentum* cultivars; their flavor, carotenoids, fatty acids and amino acids. Ph.D. dissertation. Univ. of Georgia, Athens, GA.
Rymal, K.S., Smit, C.J.B., and Nakayama, T.O.M. 1974. Fatty acid and amino acid composition of the seeds of three cultivars of the tomato, *Lycopersicon esculentum* L.J. Amer. Soc. Hort. Sci. 99: 12–15.
Tchorbanov, B., Ushanova, G., and Litchev, V. 1986. Lipid-protein concentrations from tomato and paprika seeds. Nahrung. 30: 408–410.
Tsatsaronis, G.C., and Boskou, D.G. 1975. Amino acid and mineral salt content of tomato seed and skin waste. J. Sci. Food Agric. 26: 421–423.
Yaseen, A.A.E., Shams El-Din, M.H.A., and Abd El-Latif, A.R. 1991. Fortification of balady bread with tomato seed meal. Cereal Chem. 68: 159–161.
Yazicioglu, T., Gokcen, J., and Karaali, A. 1981. Studies on extraction of protein isolates from Turkish sunflowerseed and cottonseed extraction cakes and on their functional properties. Fette Seifen Anstrichm. 83: 474–477.

Ms received 7/18/94; revised 1/23/95; accepted 1/29/95.

Author G.N. Liadakis acknowledges the receipt of a scholarship by the National Foundation of Scholarships of Greece. Mr. E. Kakoulides is gratefully acknowledged for assistance in mineral analyses. We thank Dr. G. Aravantinos-Zafiris for critical reading of the manuscript.

MANOTHERMOSONICATION OF PEROXIDASE. . .

From page 445

Ganthavorn, C., Nagel, C.W., and Powers, J.R. 1991. Thermal inactivation of asparagus lipoxygenase and peroxidase. J. Food Sci. 56: 47–49.
Gould, G.W. (Ed.). 1995. *New Methods of Food Preservation.* Blackie, Glasgow.
Graber, M., and Combes, D. 1989. Effects of polyols on fungal-amilase thermostability. Enz. Microb. Technol. 11: 673–681.
Kella, N.K., and Poola. 1985. Sugars decrease the thermal denaturation and aggregation of arachin. Int. J. Pep. Prot. Res. 26: 390–397.
Kon, S. and Whitaker, J.R. 1965. Separation and partial characterization of the peroxidases of *Ficus glabrata* latex. J. Food Sci. 30: 977–985.
Lee, J.C., and Timasheff, S.N. 1981. The stabilization of proteins by sucrose. J. Biol. Chem. 256: 7193–7201.
Lopez, P. and Burgos, J. 1995. J. Agric. Food Chem. (In press).
Lopez, P., Sala, F.J., de la Fuente, J.L., Condon, S., Raso, J., and Burgos, J. 1994. Inactivation of peroxidase, lipoxygenase, and polyphenoloxidase by manothermosonication. J. Agric. Food Chem. 42: 252–256.
Lu, A.L. and Whitaker, J.R. 1974. Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. J. Food Sci. 39: 1173–1178.
Macris, B.J. and Markakis, P. 1971. Postirradiation inactivation of horseradish peroxidase. J. Food Sci. 36: 812–815.
Maehly, A.C. 1952. Splitting of horseradish peroxidase into prosthetic group and protein as a mean of studying the linkage between hemin and protein. Biochim. Biophys. Acta 8: 1–17.
Maehly, A.C. 1953. Experiments with peroxidase. 2. Further observations on splitting the enzyme at extreme pH values. Arch. Biochem. Biophys. 44: 430–442.
Mertens, B. and Knorr, D. 1992. Developments of nonthermal processes for food preservation. Food Technol. 46: 124–133.
Metodiewa, D., Gebicka, L., and Bachman, S. 1987. The radiation induced inactivation and post irradiation effects of horseradish peroxidase solutions. J. Radioanal. Nucl. Chem. Lett. 119: 87–93.
Naveh, D., Mizhari, S., and Kopelman, I.J. 1988. Kinetics of peroxidase deactivation in blanching of corn in the cob. J. Agric. Food Chem. 30: 967–970.
Putnam, F.W. 1953. Protein denaturation. Ch. 9, in *The Proteins*, Vol. II part B, H. Neurath and K. Bailey (Ed.), p. 807–892. Academic Press, New York.
Robinson, D.S. 1991. Peroxidases and their significance in fruits and vegetables. Ch. 10, in *Food Enzymology*, Vol I, P.F. Fox (Ed.), p. 399–426. Elsevier Applied Science. New York.
Sala, F.J., Burgos, J., Condon, S., Lopez, P., and Raso, J. 1994. Effect of heat and ultrasonic waves on microorganisms and enzymes. Ch. 9 in *New Methods of Food Preservation*, G.W. Gould (Ed), p. 176–204. Blackie. Glasgow.
Stauffer, C.E. (Ed.). 1989. *Enzyme Assay for the Food Scientists.* Van Nostrand Reinhold, New York.
Timasheff, S. 1993. The control of protein stability and association by weak interactions with water: How do solvents affect the processes? Annu. Rev. Biophys. Biomol. Struct. 22: 67–96.
Timasheff, S., and Arakawa, T. 1989. Stabilization of protein structure by solvents. Ch. 14, in *Protein Structure, A Practical Approach*, T.E. Creighton (Ed.), p. 331–345. IRL Press, Oxford, England.
Webb, F.C. 1964. *Biochemical Engineering.* Van Nostrand Reinhold, New York.
Weissler, A. 1960. Effects of ultrasonic irradiation on hemoglobin. J. Acoust. Soc. Am. 32: 1208–1212.
Williams, D.C., Lim, M.H., Chen, A.O., Pangborn, R.M., and Whitaker J.R. 1986. Blanching of vegetables for freezing. Which indicator enzyme to choose. Food Technol. 40: 130–140.
Winter, E. 1971. Hitzebeständigkeit der Peroxidase. Z. Lebensm. Unters. Forsch. 145: 3–6.
Yoovidhya, T., Combes, D., and Monsan, P. 1986. Kinetics and thermal stability studies of rulactine, a proteolytic enzyme from *Micrococcus caseolyticus*. Biochem. Lett. 8: 333–338.

Ms received 7/30/94; revised 11/21/94; accepted 12/14/94.