NP

JOURNAL OF FOOD PROCESSING AND PRESERVATION

D.B. LUNI
EDITOR

FOOD & NUTRITION PRESS, INC.

LUME 9, NUMBER 4 198

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Dr. D. B. Lund, University of Wisconsin, Department of Food Science, 1605 Linden Drive, Madison, Wisconsin 53706 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc. P.O. Box 71, Westport, Connecticut 06881 USA.

One volume of four issues will be published annually. The price for Volume 8 is $70.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $82.00 per year via surface mail, and $90.00 per year via airmail.

Subscriptions for individuals for their own personal use are $50.00 for Volume 8 which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $62.00 per year via surface mail, and $70.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

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

The *Journal of Food Processing and Preservation* (ISSN: 0145-8892) is published quarterly by Food & Nutrition Press, Inc.—Office of Publication is 155 Post Road East, Westport, Connecticut 06881 USA. Current issue is December 1985.

Second class postage paid at Westport, CT 06881.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., P.O. Box 71, Westport, CT 06881.

JOURNAL OF FOOD PROCESSING AND PRESERVATION

JOURNAL OF FOOD PROCESSING AND PRESERVATION

Journal of
FOOD PROCESSING
and
PRESERVATION

VOLUME 9
NUMBER 4

Editor: D. B. LUND

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

ISSN 0145-8892

Printed in the United States of America

CONTENTS

SORPTION OF VINYLCHLORIDE ONTO POLYVINYLCHLORIDE BY CLASSICAL PARTITION AND INVERSE GAS CHROMATOGRAPHY: COMPARISON OF TWO METHODS

M. G. KONTOMINAS[1] and P. G. DEMERTZIS

Lab of Food Chemistry
Dept. of Chemistry
Univ. of Ioannina
Ioannina-Greece

and
S. G. GILBERT

Dept. of Food Science
Cook College, Rutgers Univ.
New Brunswick N.J. 08903

Received for Publication July 22, 1985

ABSTRACT

Sorption of vinylchloride onto Polyvinylchloride was studied using both classical partition and inverse gas chromatography. The effect of temperature, polymer particle size and monomer concentration were particularly examined. Data from both methods showed that at very low concentrations, the monomer was strongly retained by the polymer with equilibrium distribution in favor of the polymer increasing as concentration decreased. Increase in temperature resulted in increase of the kinetic energy of VCM molecules hence desorption was thermodynamically more favored than at lower temperatures. Decrease in polymer particle size resulted in a greater binding of the monomer by the polymer, due to uncovering of additional active sites in the polymer. Values for the thermodynamic parameters calculated using the two methods were in fair agreement. Results were in accordance with the active site hypothesis.

[1]*M. G. Kontominas: author whom inquiries should be addressed to.*

Journal of Food Processing and Preservation 9 (1985) 223-234. All Rights Reserved.

INTRODUCTION

Polyvinylchloride (PVC) has been the issue of great controversy, especially during the past decade, due to the carcinogenic nature of its monomer, vinylchloride (VCM), (Hefner *et al.* 1975), (Maltoni and Lefemine 1975a), (Maltoni 1980). It is for this reason that considerable work has been done on the possible migration of VCM from PVC into various food contact phases, (Figge 1972), (Daniels and Proctor 1975), (Gilbert 1976), (Bellebono *et al.* 1981), (Gilbert *et al.* 1982), (Kontominas *et al.* 1982), (Kontominas *et al.* 1985). Despite the fact that modern production techniques for PVC have succeeded in significantly lowering the monomer residue in the polymer to the low ppb range, vinylchloride migration work still continues in an effort to elucidate the nature of the PVC/VCM interaction.

Sorption/desorption phenomena are generally related to equilibrium distribution of the migrant in the food and polymer phases respectively, which inturn depends on the relative solubilities of the migrant in each phase. Attaining equilibrium will therefore be a function of the respective thermodynamic parameters involved, namely: free energy, enthalpy, entropy, and activity coefficient of interaction, while rate of attaining equilibrium will be related to diffusion.

Previous work, (Morano *et al.* 1977), (Kashtock *et al.* 1980), (Kontominas 1980), (Demertzis 1984), (Demertzis *et al.* 1985), on the migration of vinylchloride from polyvinylchloride in the presence of distilled water, hexane, corn oil and olive oil showed a concentration dependent distribution of the monomer in favor of the polymer especially at very low monomer concentration levels. Similar results, (Kinigakis 1979), (Kontominas *et al.* 1982), were obtained, using inverse gas chromatography, a dynamic equilibrium technique which has been successfully used recently to study polymer/monomer interaction (Gilbert 1984), (Strobel 1973), (Berezkin *et al.* 1977).

It is the objective of this paper to study the effect of temperature, polymer particle size and monomer concentration on sorption of VCM by PVC using both classical partition and inverse gas chromatography and attempt to correlate the data between the two methods.

Thermodynamic parameters for sorption of VCM onto PVC by inverse gas chromatography and classical partition respectively were calculated as follows.

Inverse Gas Chromatography

The partial molar enthalpy of solution of vapor in the column stationary phase (ΔH_s) can be calculated from the specific retention volume of the monomer in the column (V_g^o), using the Clausius-Clapeyron equation:

$$\frac{d\,(\ln V_g^o)}{dT} = \frac{\Delta H_s}{RT^2} \tag{1}$$

The specific retention volume is given by Eq. (2):

$$V_g^o = \frac{J\dot{V}\,(t_r - t_f)}{W_s} \times \frac{273}{T} \tag{2}$$

Where:

J = compression factor taking into account the pressure drop along the column length, given by Eq. (3).

$$J = \frac{3}{2}\,\frac{(P_i/P_o)^2 - 1}{(P_i/P_o)^3 - 1} \tag{3}$$

V = flow rate of carrier gas (ml/s)
t_r = retention time of compound under study (monomer)
t_f = retention time of unadsorbed species (air)
W_s = weight of stationary phase (9)
P_i = column inlet pressure (atm)
P_o = column outlet pressure equal to 1 atm

The partial molar enthalpy and entropy are related to the column capacity coefficient (k) through Eq. (4):

$$\ln k = \ln f + \frac{\Delta H}{RT} - \left(\frac{\Delta S}{R}\right) \quad (4)$$ (Knox and Vasvari 1973)

where:

ΔH = partial molar enthalpy of solute when transferring from the stationary to the mobile phase (apparently $\Delta H_s = -\Delta H$) (5)

ΔS = partial molar entropy of solute when transferring from the stationary to the mobile phase, $\Delta S_s = -\Delta S$) (6)

f = ratio of volume of stationary phase to mobile phase

k = column capacity coefficient, defined as the ratio $\frac{V_g^o}{V_o}$ where V_o is the dead volume of the column, associated with lg of stationary phase.

Combination of equations (4), (5) and (6) gives:

$\Delta S_s = R\,(\ln k - \ln f) + \frac{\Delta H_s}{T}$ (7). Equations (1) and (7) provide the ΔH_s and ΔS_s values necessary to calculate Gibb's free energy (ΔG_s) of the solute-solvent interaction using Eq. (8).

$$\Delta G_s = \Delta H_s - T\Delta S_s \tag{8}$$

If we define as "excess" free energy of mixing (ΔG_{xs}) for a real solution (ΔG_s) in comparison to an ideal solution (ΔG_i) then:

$$\Delta G_{xs} = \Delta G_s - \Delta G_i \tag{9}$$

$$\Delta G_s = RT\ln(\gamma P_i^o) \quad (10) \quad \text{and} \quad \Delta G_i = RT\ln P_i^o \tag{11}$$

where γ = activity coefficient
P_i^o = vapor pressure of pure solute
Combination of equations (9), (10) and (11) gives:

$$\Delta G_{xs} = RT\ln\gamma \tag{12}$$

Equation (1), (7), (8), (9) and (12) were used to calculate the thermodynamic parameters of the PVC/VCM interaction.

Classical Partition

ΔG_i was calculated from Eq. (11) using vapor pressure tables.
ΔG_s was calculated from Eq. (13)

$$\Delta G_s = RT\ln k_p \tag{13}$$

where $$k_p = \frac{\text{(conc. of VCM in PVC) equil.}}{\text{(conc. of VCM in food phase) equil.}}$$

ΔG_{xs} and γ were calculated using Eq. (9) and (12), respectively.

ΔH_s was calculated graphically by plotting $\frac{\Delta G_s}{T}$ vs. $\frac{1}{T}$. The slope of these curves is equal to ΔH_s. Finally ΔS_s was calculated using Eq. (14)

$$\Delta S_s = \frac{\Delta H_s - \Delta G_s}{T} \qquad (14)$$

EXPERIMENTAL

Materials

A commercial powdered unplasticized PVC resin (VC 47, BE-1) of particle sizes: 60-80, 100-120 and 150-200 Mesh (supplied by Borden Chemical Co., North Andover, MA) was used in all experiments. The VCM was 99.9% pure gas from Matheson gas products (East Rutherford N.J.).

(1) Food grade olive oil packaged in glass was purchased locally.
(2) Distilled water.

Methods

Procedures for classical sorption experiments were those described previously in the literature (Kontominas *et al.* 1982).

GC operational conditions were as follows:
Instrument: Varian GC, model 3700, equipped with a dual F.I.D.
Column: 10% SE-30 on Anakrom ABS 90-100 Mesh, 6ft. × ¼ in. O.D.
Tcol: 80°C
Tdet: 240°C
Tinj: 80°C
Carrier gas: high purity N_2, flow rate = 30 ml/min.
Sorption was carried out at 8, 15, 22 and 30°C.

Procedures for inverse gas chromatography experiments were as follows: Three different columns, containing unplasticized PVC resin of

three different mesh sizes mentioned previously, were prepared. The resin was stripped of its residual monomer using the following method: (Morano *et al.* 1977): A limited amount of resin was placed in a wide-mouth container and the container was held under ventilation in a hood. Resin samples were treated in this manner for a two week period. The concentration of residual monomer following this treatment was below 1 ppb w/w. The columns were weighed before and after filling with the PVC resin to determine the weight of the polymer in each one. A series of standard VCM samples were prepared by direct injection of a known volume of VCM gas in glass serum vials of 70 and 90 ml capacity. The columns were connected to the GC and known amounts of (gas VCM) were injected into the instrument. All injections were made using a gas-tight syringe, equipped with a two way luer lock valve.

GC operational conditions were as follows:

Instrument: same

Columns: 6ft. × ¼in. O.D. stainless steel, containing the polymer.

Tdet: 200°C

Tinj: 40°C

Carrier gas: same

Temperatures used were 15, 22, 30 and 40°C.

Measurement of retention times were accurate to ±0.05 s. The retention time of air was taken as the retention time of the unadsorbed species.

Equilibrium concentrations of $[VCM]_{PVC}$ ranged from 0.28 to 23.8 ppm w/w for the PVC/VCM/water system, from 0.05 to 4.12 ppm w/w for the PVC/VCM/olive oil system and from 0.05 to 25.5 ppm w/w for the PVC/VCM/air system.

RESULTS AND DISCUSSION

Results for systems PVC/VCM/water and PVC/VCM/olive oil at 8, 15, 22 and 30°C and two resin particle sizes are shown in Tables 1 and 2.

Results for system PVC/VCM/air at 15, 22, 30 and 40°C and three resin particle sizes are shown in Table 3.

The main thermodynamic parameters of PVC/VCM interaction considered, were the partial molar free energy (ΔG_s), partial molar excess free energy (ΔG_{xs}) and activity coefficient (γ) of sorption. Both ΔG_s and ΔG_{xs} are generally negative, an indication of the spontaneous nature of the PVC/VCM interaction. Activity coefficient values ($\gamma < < 1$) are also indicative of strong attractive forces between the polymer and the monomer.

Table 1. Changes in partial molar free energy, excess free energy, enthalpy, entropy and activity coefficient of sorption for system PVC/VCM/water at 8, 15, 22 and 30°C (classical partition).

a. 8°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma \times 10^{-3}$
60-80 M	-12.7	-37.5	-2.2	-2.7	8.5
100.120 M	-13.3	-38.8	-2.4	-2.8	6.5

b. 15°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma \times 10^{-3}$
60-120 M	-12.7	-37.7	-1.9	-2.5	13.4
100-120 M	-13.3	-38.7	-2.1	-2.7	8.6

c. 22°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma \times 10^{-3}$
60-80 M	-12.7	-37.8	-1.6	-2.3	19.0
100-120 M	-13.3	-39.0	-1.8	-2.5	13.6

d. 30°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma \times 10^{-3}$
60-80 M	-12.7	-38.0	-1.2	-2.2	29.7
100-120 M	-13.3	-39.4	-1.3	-2.3	23.9

These results can be explained on the basis of the active site hypothesis (Gilbert 1976), which states that at significantly low concentrations, the migrant is thermodynamically bound to structural irregularities in the polymer (active sites) which possess high binding energies for the

Table 2. Changes in partial molar free energy, excess free energy, enthalpy, entropy and activity coefficient of sorption for system PVC/VCM/olive oil at 8, 15, 22 and 30°C (classical partition).

a. 8°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-2}$
60-80 M	-7.4	-25.0	-0.35	-0.79	24.2
100-120 M	-7.1	-23.6	-0.51	-0.95	18.1

b. 15°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-2}$
60-80 M	-7.4	-25.0	-0.15	-0.74	27.4
100-120 M	-7.1	-23.6	-0.34	-0.93	19.8

c. 22°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-2}$
60-80 M	-7.4	-25.2	+0.06	-0.67	31.6
100-120 M	-7.1	-23.7	-0.14	-0.87	22.5

d. 30°C

PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-2}$
60-80 M	-7.4	-25.3	+0.31	-0.60	37.2
100-120 M	-7.1	-23.8	+0.09	-0.82	25.7

migrant. By increasing the surface area of the polymer (60-80 — 100-120M), a significant number of new active sites is uncovered which bind additional migrant molecules. This results in reduced values for ΔG_s, ΔG_{xs}. Thus surface affects the rate of sorption of the monomer.

Table 3. Changes in partial polar enthalpy (ΔH_s), partial molar entropy (ΔS_s), partial molar free energy (ΔG_s), excess molar free energy (ΔG_{xs}) and activity coefficient (γ) of sorption of vinylchloride by the three PVC resins at (a) 15 (b) 22 (c) 30 and (d) 40°C. (Inverse gas chromatography).

			a.15°C		
PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-3}$ 5.0
60-80 Mesh	-9.0	-22.8	-2.4	-3.0	5.0
100-120 Mesh	-7.9	-18.7	-2.6	-3.1	4.1
150-200 Mesh	-7.5	-16.4	-2.7	-3.3	3.0
			b.22°C		
PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-3}$
60-80 Mesh	-9.0	-23.5	-2.1	-2.8	8.1
100-120 Mesh	-7.9	-19.6	-2.3	-3.0	6.0
150-200 Mesh	-7.5	-17.1	-2.4	-3.2	4.5
			c.30°C		
PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole)	ΔG_s (Kcal/mole)	ΔG_{xs} Kcal/mole)	$\gamma x10^{-3}$
60-80 Mesh	-9.0	-24.2	-1.7	-2.6	13.3
100-120 Mesh	-7.9	-19.9	-1.9	-2.8	9.4
150-200 Mesh	-7.5	-17.6	-2.1	-3.0	6.5
			d.40°C		
PVC resin	ΔH_s (Kcal/mole)	ΔS_s (cal/mole	ΔG_s (Kcal/mole)	ΔG_{xs} (Kcal/mole)	$\gamma x10^{-3}$
60-80 Mesh	-9.0	-25.1	-1.2	-2.3	25.1
100-120 Mesh	-7.9	-20.5	-1.5	-2.7	13.9
150-200 Mesh	-7.5	-18.2	-1.8	-2.9	9.4

Increase in temperature on the other hand, results in increase of the kinetic energy of migrant molecules which in turn reduces thermodynamic binding of the migrant on active sites. Thus ΔG_s, ΔG_{xs} and γ increase.

For a given temperature and particle size e.g., 15°C and resin size 60-80 Mesh the values for ΔG_s were -2.4, -1.9 and -0.15kcal/mole for

systems PVC/VCM/air, PVC/VCM/water and PVC/VCM/olive oil, respectively. ΔG_{xs} and γ values follow the same pattern. These results indicate that sorption of VCM onto PVC is highly favored in the presence of air followed by water and lastly by a fatty phase. Differences in the above values can be explained in terms of the relative solubilities of VCM in the respective contacting phase. In the presence of oil, which is considered a "very good" solvent for the monomer, the tendency for sorption is significantly reduced. Relative differences in the above three values are an approximate measure of the tendency of VCM to desorb from the polymer into the surrounding medium depending on the nature of this medium.

In the presence of olive oil at 22°C and for resin particle size 60-80Mesh, ΔG_s becomes positive, an indication that desorption rather than sorption is thermodynamically favored at temperatures equal or higher than 22°C (e.g., 30°C etc.). However, in the same case for resin particle size 100-120Mesh, ΔG_s becomes slightly negative due to the uncovering of additional active sites which bind additional VCM molecules.

It can also be seen from Tables 1, 2 and 3 that the effect of temperature as well as nature of contacting phase on sorption is considerably more critical than the effect of resin particle size. Negative values of ΔH_s in all systems are indicative of the exothermic nature of the PVC/VCM interaction, while negative ΔS_s values are related to the high degree of order of the monomer molecules in the sorbed state. ΔS_s does not change considerably during sorption at temperatures below the glass transition temperature (T_g), indicating that there is probably no change in order in this temperature region, thus the probability for sorption of monomer molecules onto active sites does not change statistically.

Further support of the active site hypothesis is provided by chromatographic peak shapes shown in Fig. 1. For a given particle size and temperature e.g., 60-80Mesh and 15°C, the retention pattern of the monomer shifts from a more asymmetrical (tailing of peak) to a more symmetrical peak shape with increasing monomer concentration (a vs.b.). Analogous results are observed when increasing the particle size for a given temperature and monomer concentration (case c vs.a and d vs.b).

Asymmetric tailing of the peak is indicative of active site binding of the monomer while a symmetrical peak suggests that simple dissolution of VCM is the predominant mode of interaction of the migrant with the resin (Kiselev and Yashin 1969).

Finally it should be stressed that the two systems involved in the two methods employed, differ significantly in terms of the contacting phase (gas versus liquid) and therefore results from the two are not expected to be identical. Data however correlate reasonably well between the methods employed and support the active site hypothesis.

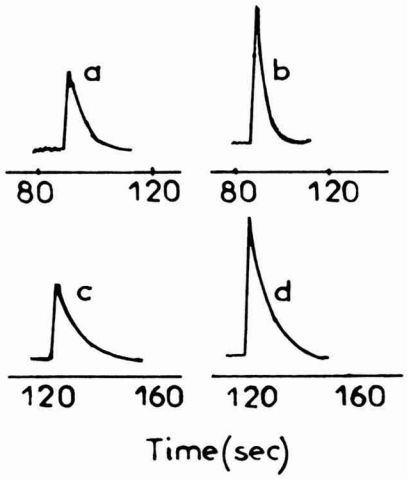

FIG. 1. EFFECT OF MONOMER CONCENTRATION AND RESIN PARTICLE SIZE ON CHROMATOGRAPHIC PEAK SHAPES (15°C)

(a) 60-80 Mesh 0.5 ppb $\frac{W}{W}$

(b) 60-80 Mesh 25 ppb "

(c) 150-200 Mesh 0.5 ppb $\frac{W}{W}$

(d) 150-200 Mesh 25 ppb "

REFERENCES

BELLEBONO, I. R., MARCANDALI, B. and SELLI, E. 1981. Migration of PVC monomer. Imballagio *32*(302), 177 and 241.

BEREZKIN, V. G., ALISHOYEV, V. R. and NEMIVOYSKAYA, I. B. 1977. *Gas Chromatography of Polymers*, p. 206, Elsevier, Amsterdam.

DANIELS, G. A. and PROCTOR, D. E. 1975. VCM extraction from PVC bottles. Modern Packaging April, 45.

DEMERTZIS, P. G. 1984. Doctorate thesis. Dept. of Chem. Univ. of Ioannina, Ioannina, Greece.

FIGE, K. 1972. Migration of additives from plastics films into edible oils and fat simulants. Food Cosmet. Toxicol. *10*(6), 815.

GILBERT, S. G. 1976. Migration of minor constituents from food packaging materials. J. Food Sci. *41*(4), 955.

GILBERT, S. G. 1984. Inverse gas chromatography. Adv. in Chromat. *23*, 199.

GILBERT, J., SHEPHERD, M., STARTIN, J. R. and WALLWORK, M. A. 1982. Identification by gas chromatography mass spectrometry of vinylchloride oligomers and other low molecular weight components in polyvinylchloride resins for food packaging applications. J. Chromatogr. *237*(2), 249.

HEFNER, R. E., WATANABE, P. E. and GEHRING, P. J. 1975. Preliminary studies of fate of inhaled vinylchloride monomer in rats. Ann. N.Y. Acad. Sci. *246*, 135.

KASHTOCK, M. E., GIACIN, J. R. and GILBERT, S. G. 1980. Migration of indirect food additives: A physical chemical approach. J. Food Sci. *45*(4), 1008.

KINIGAKIS, P. J. 1979. M.Sc. thesis, Dept. of Food Sci., Cook College, Rutgers Univ., New Brunswick N.J.

KISELEV, A. V. and YASHIN, H. J. 1969. *Adsorption Chromatography.* p. 105, Plenum Press, New York.

KNOX, J. H. and VASVARI, G. 1973. The performance of packings in high-speed liquid chromatography. III. Chemically bonded pellicular materials. J. Chromatogr. *83*, 181.

KONTOMINAS, M. G. 1980. Ph.D. thesis, Dept. of Food Sci., Cook College, Rutgers Univ. New Brunswick N.J.

KONTOMINAS, M. G., HATZIDIMITRIU, E. and VOUDOURIS, E. K. 1985. Migration of vinylchloride from rigid Polyvinylchloride bottles into various food simulating solvents. Accepted for publication. Sciences des Aliments.

KONTOMINAS, M. G., MILTZ, J. and GILBERT, S. G. 1982. Sorption-desorption of VCM in PVC/selected food simulants systems. J. Food Sci. *47*, 1208.

KONTOMINAS, M. G. and VOUDOURIS, E. K. 1982. Adsorption of VCM onto selected plasticized PVC resins: study of thermodynamic parameters by gas chromatography. Chim. Chron. (New Series), *11*, 59.

MALTONI, C. 1980. Studies update vinylchloride hazards. Chem. and Eng. News. April 7, 27.

MALTONI, C. and LEFEMINE, G. 1975. Carcinogenicity bioassays of vinylchloride. Current results. Ann. N.Y. Acad. Sci. *246*, 195.

MORANO, J. R., GIACIN, J. R. and GILBERT S. G. 1977. Sorption-desorption of vinylchloride in selected PVC-food simulating solvent systems. J. Food Sci. *42*, 230.

STROBEL, H. A. 1973. *Chemical Instrumentation.* p. 869, Addison-Wesley, Reading, MA.

THE EFFECT OF ULTRA-HIGH TEMPERATURE MILK PROCESSING ON YOGURT TEXTURE[1]

R.H. SCHMIDT, M.M. VARGAS, K.L. SMITH AND J.J. JEZESKI

Department of Food Science and Human Nutrition
University of Florida, Gainesville, Florida 32611

Received for Publication August 12, 1985

ABSTRACT

Skim milk fortified with 3.0% nonfat dry milk, heated by a conventional process (82C 20 min) and by ultra-high-temperature (UHT) processes (138C for 3 or 6 s), was inoculated with 0.5% each of Streptococcus thermophilus and Lactobacillus bulgaricus and incubated at 40C. The yogurt systems were stored at 4C and were evaluated for texture by stirred viscosity (consistency), by Instron penetration (penetration force, flow coefficient) and by sensory evaluation. Trends for penetration force, flow coefficient, and consistency were significantly correlated. Conventional yogurt had higher firmness and viscosity data but also had more syneresis than did UHT yogurt. Increased holding time in UHT processing significantly ($p<0.05$) increased yogurt firmness and consistency.

INTRODUCTION

Consumption trends for yogurt in the United States have shown steady increases over the last 20 years. While comprehensive reviews are available relating to the manufacture, technology and biochemistry of yogurt (Humphreys and Plunkett 1969; Robinson and Tamine 1975; Tamime and Deeth 1980), this commercial cultured dairy product is ill-defined and highly variable (Duitshaever *et al.* 1972; Kroger and Weaver 1973; Richmond *et al.* 1979).

An important processing variable in yogurt manufacture is the heat treatment given the milk. It is generally accepted that, in addition to

[1]*Florida Agricultural Experiment Station Journal Series No. 6471.*

Journal of Food Processing and Preservation 9 (1985) 235-240.

lowering microbial contamination levels, the degree of heat treatment given the milk critically affects yogurt texture. Conventional milk processing is usually a relatively high temperature, long time process with heating conditions approximately 80 to 85C for 20 to 30 min or 90 to 95C for 5 to 10 min (Davies *et al.* 1978; Humphreys and Plunkett 1969; Kalab *et al.* 1976; Robinson and Tamime 1975; Schmidt *et al.* 1980; Tamime and Deeth 1980). Ultra high temperature (UHT) processing of milk for yogurt manufacture may offer certain advantages to conventional batch-type heat processes in terms of more closely standardized process control, more controlled sanitation and potential energy and/or time savings (Labropoulas *et al.* 1981). In addition UHT milk processing may be stimulatory to growth and activity of yogurt starter cultures (Smith *et al.* 1982). A possible disadvantage of UHT compared to conventional milk processing may be less desirable yogurt texture. Lower gel firmness and viscosity and higher fluidity and spreadability have been reported for yogurt manufactured from UHT processed whole milk (149C for 3.3 s) compared to yogurt from conventionally processed milk (82C for 30 min) (Labropoulas *et al.* 1981; 1984). However, effects of UHT milk processing under different conditions have not been sufficiently investigated.

The objectives of this investigation were to examine and compare effects of UHT milk processing conditions on yogurt texture.

MATERIALS AND METHODS

Milk Processing and Yogurt Manufacture

Raw milk, obtained from the dairy herd at the University of Florida, was separated at 37C. The skim milk was fortified with 3.0% nonfat dry milk, warmed to 49C and homogenized (2500 psi stage 1; 1000 psi stage 2).Upon cooling to 4C, the fortified skim milk was stored 14 h prior to heating.

Conventional heating was done at 82C for 20 min with agitation. UHT processing was done in a continuous, indirect, tubular UHT system (Unitherm IV, Cherry-Burrell Corp., Cedar Rapids, IA). UHT heating conditions of 116, 127, and 149C for 3 s and 138C for 3 and 6 s were used.

Following heat treatment, the milk was cooled to 42C, transferred into sterile rectangular jars (300 ml) and inoculated with 0.5% (v/v) each of *Streptococcus thermophilus* S1 and *Lactobacillus bulgaricus* R1 (Microlife Technics, Inc., Sarasota, FL). The incubation samples were quiescently incubated at 40C to a pH of 4.3, whereupon, they were quiescently cooled in an ice bath and stored for 1 week at 4C prior to texture evaluation.

Texture Evaluation

Undisturbed yogurt samples were evaluated for gel strength or firmness by penetration on an Instron Model TM fitted with cylindrical disk probes (6, 8, 10, or 12 mm diameter). The Instron was operating at 2.0 cm/min to a 2.0 cm depth. Texture parameters compared were: initial breaking force (F) and the flow coefficient (K_f) calculated according to the following relationship (DeMan 1969):

$$F = K_f A$$

where F is initial penetration force (g); A is probe base area (cm^2) and K_f is the flow coefficient (g cm^{-2}). Data for F and A were plotted and fitted by linear regression and the slope (K_f) determined.

Consistency or "stirred viscosity," determined after yogurt samples were stirred manually with a spoon for 60 s, was determined on a Brookfield LVT synchroelectric viscometer on helipath at 3 rpm with T-bar spindle C. Four dial readings were taken at 30 s intervals and the mean of these readings is reported.

Sensory evaluation for body and texture was done by four experienced judges using the yogurt scorecard proposed by Duthie et al. (1977). Samples were coded and randomly presented to the judges. The rating scale used was as follows: 3 = pronounced defect, 2 = definite defect, 0 = no defect. Syneresis was evaluated by carefully inclining yogurt samples (approximately 90° angle) and removing the surface whey by aspiration after 3 and 7 days storage at 4C. Percent syneresis was calculated as follows:

$$\% \text{ syneresis} = \frac{\text{weight of whey (g)}}{\text{weight of yogurt (g)}} \times 100$$

Statistical Analyses

Replication of heat treatment trials has been summarized in Table 1. Data were subjected to analysis of variance and Duncan's multiple range test (Duncan 1972) was used for mean comparison.

RESULTS AND DISCUSSION

Yogurt manufactured from milk heated at 149C or at 116 and 127C in preliminary investigations was generally weak bodied, thus heating conditions of 138C for 3 and 6 s were used in subsequent experiments.

Effects of heat treatment of milk by conventional (82C 20 min) and UHT process (138C for 3 and 6 s) on texture, sensory rating and syneresis data for yogurt have been summarized in Table 2. Conventional batch heating

Table 1. Experimental replication for heat treatment effects on yogurt texture.

Replication	Heat Treatment[1]		
	82C 20 min	138C 3 s	138C 6 s
Runs	12	11	10
Samples	37	32	26

[1]Conventional heat treatment (82C 20 min) was batch process. UHT processing (138C, 3 and 6 s) was in an indirect tubular system.

Table 2. Heat treatment effect on yogurt texture, sensory rating and syneresis.

Heat Treatment	Penetration Data[1]		Viscosity[2]	Sensory Data	
	F(g)	K_f(g cm^{-2})		Rating[3]	Syneresis[4]
82C 20 min	10.6b	0.15f	43.6i	0.5	0.8
138C 3 s	4.7a	0.07d	21.0g	1.6	0.3
138C 6 s	7.4c	0.11e	29.5h	0.9	0.2

[1]Instron fitted with disk probes. F(g) is mean initial penetration force across all probe sizes; K_f is flow coefficient.
[2]Viscosity is 30 s intervals for stirred yogurt (Brookfield T-bar spindle C; helipath stand).
[3]Sensory rating: 0—no defect (weakness, 3—pronounced defect (weakness).
[4]Percent syneresis based on weight loss.
Means followed by the same letter are not different ($p > 0.05$).

has generally resulted in firmer yogurt as shown by significantly ($p < 0.05$) higher penetration force, flow coefficient and viscosity data and lower sensory weakness rating compared to yogurt from UHT processed milk. However, yogurt from UHT processed milk had lower syneresis than did yogurt from the conventional process. When comparing the two UHT processing conditions, it can be observed that increased heating time significantly ($p < 0.05$) increased yogurt firmness with higher penetration and viscosity data and lower sensory weakness rating. In fact, yogurt manufactured from milk processed at 138C for 6 s was rated acceptable.

Desired yogurt gel structure has optimal firmness but minimal syneresis. Heat treatment effects on this textural balance is complexly

related to the degree of casein micelle hydration and whey protein (primarily β-lactoglobulin) denaturation (Kalab 1976; Davies *et al.* 1978; Schmidt and Morris 1983). It is conceivable that conventional heating results in more whey protein denaturation as well as more casein micelle coalescence (less hydration) than does UHT processing under the condition used. Thus, yogurt from conventionally processed milk is firmer with more syneresis than yogurt from UHT processed milk. Attempts to evaluate differences in whey protein denaturation between conventional and UHT processed milk using polyacrylamide gel electrophoresis have been nonconclusive (Vargas 1982). Further research using electron microscopy may ellucidate subtle differences in casein micelle hydration and β-lactoglobulin interaction in yogurt gels and may substantiate differences in yogurt microstructure as affected by heat treatment of milk.

Data presented suggest that UHT milk processing for yogurt manufacture using moderate heating conditions is not as destructive to yogurt texture as previously reported using more severe UHT heating conditions (Labropoulas *et al.* 1981; 1984). In addition a technique using Instron penetration with different probe sizes for measuring yogurt firmness was evaluated. Further research is being conducted evaluating effects of UHT processing of different milk formulations (fat level, stabilizer addition etc.) on yogurt texture.

REFERENCES

DAVIES, F.L., SHANKAS, P.A., BROOKER, B.E., and HOBBS D.G. 1978. A heat-induced change in the ultrastructure of milk and its effect on gel formation in yoghurt. J. Dairy Res. *45*, 53.

DEMAN, J.M. 1969. Food texture measurements with the penetration method. J. Texture Studies. *1*, 114.

DUITSHAEVER, C.L., ARNOTT, D.R., and BULLOCK, D.H. 1972. Quality evaluation of yogurt produced commercially in Ontario. J. Milk Food Technol. *35*, 173.

DUNCAN, D.B. 1972. Multiple range and multiple 7 tests. Biometrics *11,* 1.

DUTHIE, A.H., NILSON, K.M., ATHERTON, H.V., and GARRETT, L.D. 1977. Proposed score card for yogurt. Cultured Dairy Prod. J. *12*(8), 10.

HUMPHREYS, C.L. and PLUNKETT, M. 1969. Yoghurt: A review of its manufacture. Dairy Sci. Abstr. *31*(11), 607.

KALAB, M., EMMONS, D.E., and SARGENT, A.G. 1976. Milk-gel structure. V. Microstructure of yoghurt as related to the heating of milk. Milchurssenschaft *31,* 402.

KROGER, M., and WEAVER J.C. 1973. Confusion about yogurt—compositional and otherwise. J. Milk Food Technol. *36,* 388.

LABROPOULAS, A.E., COLLINS, W.F., and STONE, W.K. 1984. Effects of ultra-high temperature and vat processes on heat-induced rheological properties of yogurt. J. Dairy Sci. *67,* 405.

LABROPOULOS, A.E., LOPEZ, A., and PALMER, J.K. 1981. Apparent viscosity of milk and cultured yogurt thermally treated by UHT and vat systems. J. Food Protection *44*(11), 874.

RICHMOND, M.L., CHANDON, R.C., and STINE, C.M. 1979. Yogurt—A compositional survey in the greater Lansing area. J. Food Protection. *42*(5), 424.

ROBINSON, R.K., and TAMIME, A.Y. 1975. Yogurt—A review of the product and its manufacture. J. Soc. Dairy Techol. *28*(3), 149.

SCHMIDT, R.H. and MORRIS, H.A. 1984. Gelation properties of milk proteins, soy proteins, and blended protein systems. Food Techol *38*, 85.

SCHMIDT, R.H., SISTRUNK, C.P., RICHTER, R.L., and CORNELL, J.A. 1980. Heat treatment and storage effects on texture characteristics of milk and yogurt systems fortified with oilseed proteins. J. Food Sci. *44*, 471.

SMITH, K.L., SCHMIDT, R.H., and ADAMS, J.P. 1982. Growth of yogurt cultures in UHT treated milk. J. Dairy Sci. *65* (supplement 1), 221.

TAMIME, A.Y. and DEETH, H.C. 1980. Yogurt: Technology and biochemistry. J. Food Protection *43*, 939.

VARGAS, M.M. 1982. The effect of ultra-high temperature milk processing in body and texture of yogurt. M.S. Thesis, University of Florida.

KINETICS OF HEAT COAGULATION OF EGG ALBUMIN DETERMINED BY WATER BINDING AND RHEOLOGICAL MEASUREMENTS

SETH M. GOLDSMITH and ROMEO T. TOLEDO

Department of Food Science
University of Georgia
Athens, Georgia 30602

Received for Publication September 6, 1985

ABSTRACT

Dynamic testing of heat coagulated egg albumin revealed that phase angle, a measure of the fluidity of a viscoelastic material, exhibits a first order change during heating in a similar manner as the changes in the spin-lattice relaxation time (T_1) of water protons measured by a pulsed nuclear magnetic resonance instrument. The absolute modulus, on the other hand, which is an index of the elastic properties exhibited a zero order change in a similar manner as the development of gel strength measured by constant rate penetrometry using an Instron universal testing instrument. Arrhenius activation energy of 29 kcal/mole for the phase angle and 38.9 kcal/mole for the absolute modulus indicate that the process of protein-water interaction to immobilize water within the gel and that of protein-protein interaction to form the solid gel network occur simultaneously during heating, but they proceed by different mechanisms with the latter process showing greater heat sensitivity.

INTRODUCTION

The application of heat to certain protein suspensions results in the formation of rigid viscoelastic gels. The gelling process, or coagulation, immobilize water and other components in a rigid structure and determine textural properties of the product.

A strong correlation has been shown to exist between water holding and structure formation in a variety of food gels. For example, Goldsmith and Toledo (1985) showed that proton spin-lattice relaxation time (T_1) in a gel, determined using pulsed-nuclear magnetic resonance spectrometry (NMR), is significantly correlated ($p < 0.01$) with gel strength measured

Journal of Food Processing and Preservation 9 (1985) 241-251. All Rights Reserved.

using constant rate penetrometry. Labuza and Busk (1979) analyzed water binding in starch, agar, carrageenan and gelatin gels and found that T_1, vapor pressure of water and suction potential of water in the gels, all measures of water binding in the gel network, were affected by the type of macromolecules that forms the gel.

The mechanism involved in the gelling of heated protein suspensions is of interest to food technologists since the final properties of the product is affected by the conditions that exist during the gelling process. Certain effects of heating are seen as changes in the individual proteins, while others are seen as collective or cooperative interactions between proteins, detected as changes in the system as an entity. Matsuda *et al.* (1981) used polyacrylamide gel electrophoresis to monitor the unaggregated portion of specific proteins in egg albumin heated at temperatures up to 94°C for up to 180 min. Each protein fraction exhibited a distinctive response to temperature and to time of heating. The strength of the gel is the result of the cooperative contributions of the protein fractions as shown by Johnson and Zabik (1981).

The firmness of heated egg albumin has previously been shown to be affected by heating temperature and time, protein concentration, pH and the presence of certain solutes (Seideman *et al.* 1963; Beveridge *et al.* 1980). Since each protein in egg albumin exhibits a different transition temperature (Johnson and Zabik 1981; Donovan *et al.* 1975; Wooton *et al.* 1981), the differences in gel strength with time and temperature of heating could be attributed to the incomplete transition of one or more of the proteins in the mixture. However, Goldsmith and Toledo (1985) showed that once the gel strength has reached a constant value at a given temperature, prolonged heating at the same temperature did not increase this value. Thus the influence of temperature on gel strength may be attributed to the mechanism with which the proteins aggregate during gel formation. Goldsmith and Toledo's (1985) data showed different reaction order for gelation measured by change in T_1 and by constant rate penetrometry. It was postulated that gel strength measured by constant rate penetrometry represents a gross property of the system and was insensitive to subtle changes in internal character of the gel. Thus the change in gel strength with isothermal heating time was zero order. On the other hand, T_1, a measure of the average translational mobility of water molecules within the confines of the gel network, exhibited a first order change. Both measures of protein gelation exhibited similar Arrhenius activation energies.

In order to better identify the underlying cause of this difference in reaction orders, and to relate these changes to the internal structure formed during gelation, the rheological properties of the gels were determined using a dynamic tester.

MATERIALS AND METHODS

Spray dried egg albumin ("High Whip", NEPCO, Social Circle, GA), which can be uniformly mixed and stored, was used to insure that experimental material characteristics are uniform in all tests. An adequate supply of the dried egg albumin was obtained, mixed thoroughly and stored in moisture-proof containers at 3 ± 2°C until used. A nominal 10% dispersion in distilled water was prepared followed by centrifugation and analysis of protein in the dispersed phase as described by Goldsmith and Toledo (1985). The dispersions contained 9.25% protein.

Heating

Egg albumin dispersion were heated in 10 × 75 mm glass test tubes for 0.5 to 1080 min (18 h) at 60°C, 65°C, 70°C, 75°C, 80°C and 90°C as described by Goldsmith and Toledo (1985). Time for tube center temperature to reach within 5% of water bath temperature was 24 s at 60°C and 7 s at 90°C. There were four tubes each for T_1, gel strength and dynamic tester measurements for each time × temperature treatment.

Determination of T_1

T_1 at room temperature of the cooked albumin gels was determined using a Praxis ED-102 pulsed-nuclear magnetic resonance (P-NMR) spectrometer operating at a nominal frequency of 10.72 MHz as described by Goldsmith and Toledo (1985). At least two values of T_1 were determined on each tube of heated albumin gel.

Dynamic Rheological Measurements

Viscoelastic rheological properties of the heat set gels were analyzed with a dynamic tester utilizing the direct measurement of stress and strain. This apparatus is slightly modified from what was described by Clark (1976) and Gross (1982). A much larger vertical mainframe was constructed, which allowed rheological measurements of heated gels while in the original tubes they were heated in. This permitted measurement of extremely soft and fragile gels which would have been destroyed by removal from the tubes. Measurements were made by preloading the surface of the gels with a cylindrical (5.75 mm) teflon

plunger attached to the dynamic force transducer. A constant static preload force of 1.25 N was applied to all samples before testing. The sinusoidal input signal of 240 Hz, at an amplitude of 0.15 V (RMS), was applied by means of a vibrator (Model AV-50, Alpha-M Corp., Dallas, TX), which supported and deformed the sample. Output signals measuring the dynamic force and sample acceleration were superimposed on a dual oscilloscope (Model 10-4510, Heath Company, Benton Harbor, MI). The resulting Lissajous ellipse was analyzed following the method described by Gross (1980). The absolute value of the complex dynamic modulus (absolute modulus), a measure of gel rigidity or elasticity, and the phase angle, which measures the internal viscosity of the gel, were calculated from the geometry of the ellipse. Dynamic rheological properties were measured on four replicate samples of each treatment for each of two experimental runs.

Statistical Analysis

The data collected in this study were analyzed using the Analysis of Variance and Regression procedures of the Statistical Analysis System (SAS, 1979) operating on the IBM 370 computer at the University of Georgia. A11 plots were produced by the SAS/GRAPH package (SAS, 1981) using a Versaplot plotter. Computer generated graphs were traced over for enhancement.

RESULTS AND DISCUSSION

Changes in T_1 as a function of heating time at constant temperature reproduced earlier data by Goldsmith and Toledo (1985). At all temperatures T_1 decreased rapidly at first, followed by gradually leveling off at an asymptotic value. The asymptotic value of T_1, and the time required to reach it decreased with increasing temperature. Table 1 summarizes the asymptotic T_1 values, the heating times required to reach these values at different temperatures, as well as asymptotic values for phase angle, absolute modulus and gel strength.

Figure 1 shows that the change in the phase angle of the heat coagulated gels is similar to that for T_1. A rapid initial decline occurred, followed by a leveling off at an asymptotic value. No curve is shown for phase angle at 60°C since solid gels were not produced (Baldwin 1977). The asymptotic value of phase angle decreased from 31.59 degrees to 65°C to 6.59 degrees at 80°C indicating the production of more elastic

Table 1. Time of transition and asymptotic values for T_1, phase angle, absolute modulus and gel strength for egg albumin heated at various temperatures

Heating Temperature (°C)	Transition Time (min)	Asymptotic Value			
		T_1 (ms)	Phase Angle (degree)	Absolute Modulus (kPa x 10^5)	Gel Strength[1] (g)
60	75	1009.7[a]	-	-	-
65	60	930.0[b]	31.59[a]	3.05[a]	13.9[a]
70	45	830.0[c]	14.12[b]	7.75[b]	80.9[b]
75	30	780.4[d]	10.56[c]	9.43[c]	97.3[c]
80	15	731.4[e]	6.59[d]	11.49[d]	131.4[d]
90	5	744.3[e]	6.71[d]	11.51[d]	125.2[d]

[1] Values obtained from Goldsmith and Toledo (1985).

(solid) and relatively less viscous (fluid) gels as the heating temperature was increased.

Figure 1 shows the effect of heating temperature and time on absolute modulus. The increase in rigidity of the heat coagulated gels based on the absolute modulus was linear with heating time at constant temperature until the gels were completely 'cooked", at which time the increase stopped abruptly and remained at a constant level. A similar trend was reported for gel strength by Goldsmith and Toledo (1985). The time required to reach this maximum value decreased with increasing temperature, but the constant values of gel strength and absolute modulus shown in Table 1 increased with increasing temperature. This result is similar to the findings of Beveridge *et al.* (1980) who noted an increase in shear force of heat coagulated egg albumin as heating temperature increased, although the time course of the heat coagulation process was not discussed.

Apparent rate constants for the changes in absolute modulus and gel strength with time at each temperature were determined from the slopes of the regression lines for the initial ascending region of the data plotted in Fig. 2 and 3. These zero order rate constants are contained in Table 2 along with the apparent activation energy (E_a) for elastic structure formation, which was determined using Arrhenius' equation. Absolute modulus and gel strength appear to measure similar physical properties of the heat set gels as shown by the proximity of the magnitude of activation energies; 38.934; and 43.676 kcal/mole for absolute modulus and gel strength, respectively. These two parameters, which describe gel rigidity and springiness, are highly correlated ($r = 0.9737$, $p < 0.0001$)

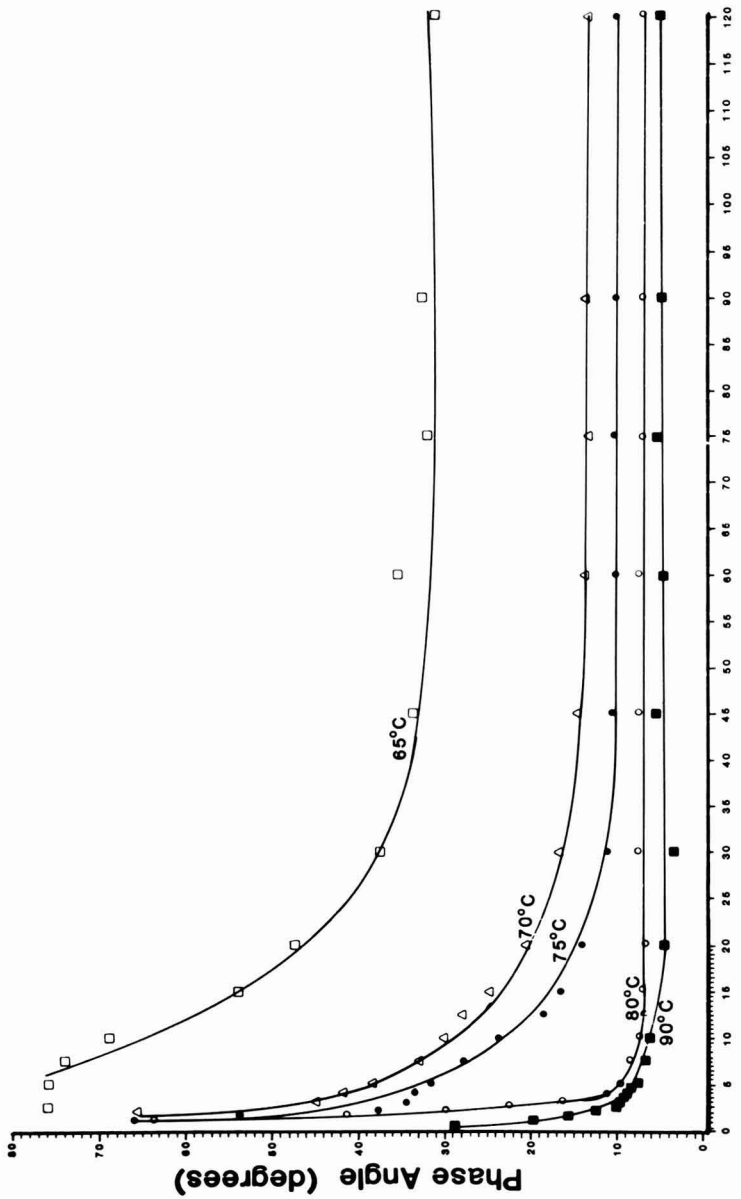

FIG. 1. CHANGE IN PHASE ANGLE AS A FUNCTION OF HEATING TIME AT INDICATED TEMPERATURES FOR 9.25% PROTEIN, EGG ALBUMIN DISPERSIONS.

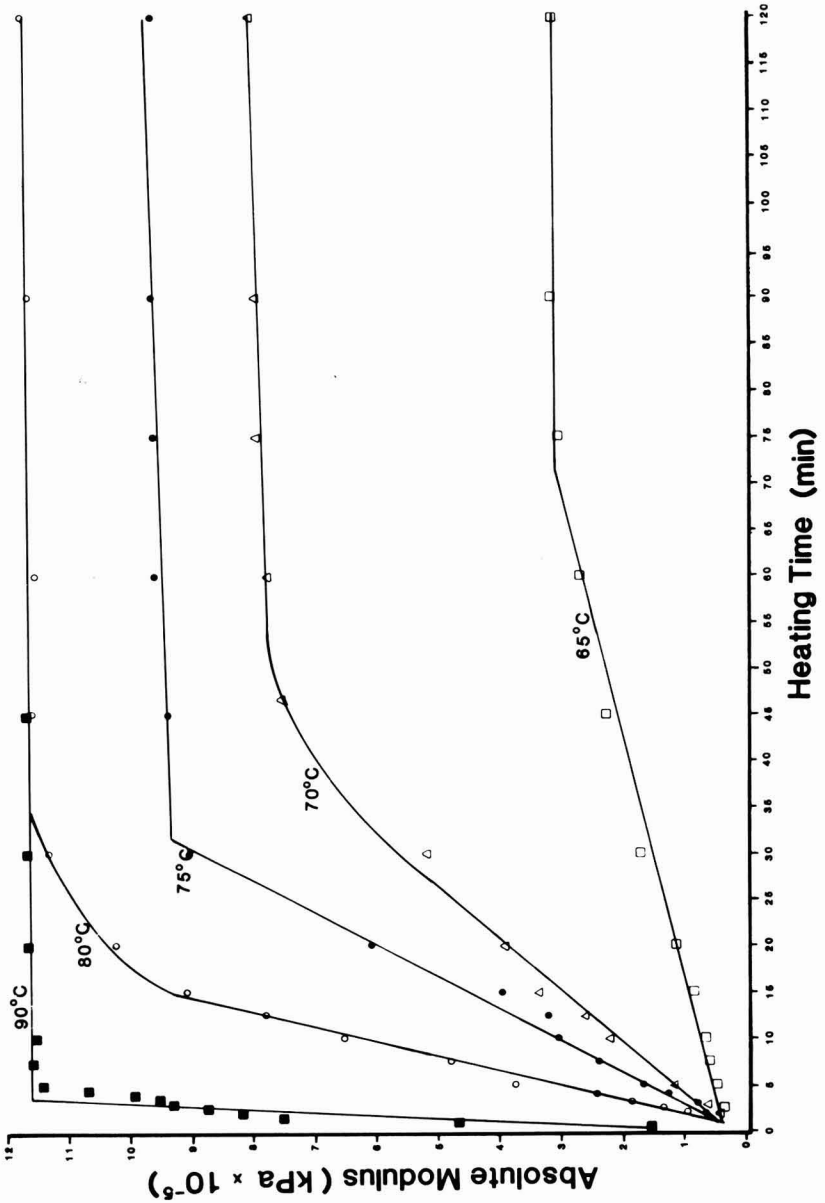

FIG. 2. CHANGE IN ABSOLUTE MODULUS AS A FUNCTION OF HEATING TIME AT INDICATED TEMPERATURES FOR 9.25% PROTEIN, EGG ALBUMIN DISPERSIONS.

Table 2. Rate constants for changes in T_1, phase angle, absolute modulus and gel strength

Heating Temperature (°C)	Rate Constant			
	T_1 (min^{-1})	Phase Angle (min^{-1})	Absolute Modulus (kPa x 10^5/min)	Gel Strength[1] (g/min)
60	-0.06472	-	-	-
65	-0.09965	-0.05167	0.0318	0.13914
70	-0.11364	-0.10405	0.1178	1.40232
75	-0.12299	-0.13509	0.2410	3.40495
80	-0.44661	-0.43312	0.5476	6.57480
90	-3.04516	-0.94011	1.9131	17.08750
Activation Energy (kcal/mole)	29.633	29.017	38.934	43.676

[1] Data from Goldsmith and Toledo (1985).

and are indicative of the relative degree of crosslinking existing in heat set gels (Van Kleef *et al.* 1978; Mitchell 1980).

The change in T_1 as a function of time at constant temperature was linearized by an equation of the form:

$$\ln (T_1 - T_A) = -k + \ln (T_o - T_A)$$

in which T_A is the asymptotic value of T_1 for a given temperature (Table 1) and k is the first order apparent rate constant describing the process and T_o is the value of T_1 for protons in the unheated protein solution. The change in phase angle as a function of isothermal heating time was modeled in a similar fashion using values of phase angle measured at discrete times and the asymptotic values of phase angle. The first order apparent rate constants for the changes in T_1 and phase angle at the temperatures tested are listed in Table 2. Again, the absolute value of the rate constants increased with increasing temperature. T_1 and phase angle appear to be related as shown by their similar temperature sensitivities; E_a = 29.63 kcal/mole for T_1 and E_a = 29.02 kcal/mole for phase angle; and their highly significant correlation coefficient ($r = 0.8555$, $p < 0.0001$).

T_1 provides a relative measure of the translational mobility of water molecules in food systems (Leung *et al.* 1976) and is a measure of the degree of interaction of water with the nonaqueous components of a gel system (Labuza and Busk 1979). In the heat coagulated egg albumin gels tested here, samples heated at increasing temperatures exhibited

significantly shorter values of T_1 ($p < 0.001$) and more rapid transitions. This is indicative of increased protein-water interaction at the time of heat coagulation, and decreased water mobility in the heated gels.

The phase angle of a viscoelastic material describes the degree of viscous character of the material; a viscous fluid has a phase angle of 90° while a perfectly elastic material has a phase angle of 0°. Viscoelastic materials, the class to which most solid foods belong, exhibit both viscous and elastic properties and have phase angles between 0° and 90° (Mitchell 1980).

The phase angle of the egg albumin gels tested here decreased with increasing temperature indicating the formation of a relatively less viscous and more elastic gel at higher temperatures. The significant correlation of phase angle with water mobility (T_1) suggests that the viscous or fluid properties of egg albumin gels are somehow related to the degree of protein-water interaction or to the degree of physical entrapment of bulk water in the crosslinked network of the heat coagulated protein matrix.

The absolute modulus, which is inversely correlated with T_1 ($r = -.7289$, $p < 0.001$) is a measure of solid or elastic character of the egg albumin gels. As heating temperature increased the complex elastic modulus increased significantly ($p < 0.001$) which indicates a greater number of crosslinks formed at higher temperatures. This increase in gel rigidity as not merely a factor of "doneness", because gels heated at 70°C for 60 min did not show any further increase in complex elastic modulus when heated at 90°C for an additional 15 min, an observation that was similar to that reported previously (Goldsmith and Toledo 1985) using constant rate penetrometry.

These results indicate that the temperature of heating has a major effect on the physical properties of the resulting egg albumin gels. The extent of network formation which contributes to the elastic properties and strength of the gel may be related to the number of covalent crosslinkages formed during heating. This type of structure forming process is described by a zero order model and is favored at higher temperatures of heating. The viscous or fluid properties of the viscoelastic gels formed seem to be related to the degree of protein-water interaction and to the mobility of water within the structure of the gel. The change in T_1 and phase angle were modelled using a first order apparent kinetic model and a higher magnitude of the asymptotic values indicating more fluidity in the heated gels were favored at lower temperatures. The higher heat sensitivity of the network forming process compared to water immobilization is reflected in its higher apparent activation energy.

REFERENCES

BALDWIN, R. E. 1977. Functional properties in foods. In *Egg Science and Technology,* (W. J. Stadelman and O. J. Cotterill, eds., pp. 246-277, AVI Publishing Company, Westport, CT.

BEVERIDGE, T., ARNTFIELDS, S. K. S. and CHUNG, J. K. 1980. Firmness of heat induced albumen coagulum. Poultry Sci. *59,* 1229.

CLARK, R.C. 1976. Sensory and rheological measurements of fresh peach texture. M.S. Thesis, University of Georgia, Athens, GA.

DONOVAN, J. W., MAPES, C. J., DAVIES, G. D. and GARIBOLDI, J. A. 1975. A differential scanning colormetric study of the stability of egg white to heat denaturation. J. Sci. Food Agric. *26*, 73.

FARRAR, T. C. and BECKER, E. D. 1971. *Pulse and Fourier Transform NMR.* p. 22. Academic Press, New York.

FERRY, J. D. 1970. *Viscoelastic Properties of Polymers.* 2nd ed. John Wiley & Sons, New York.

GOLDSMITH, S.M. and TOLEDO, R.T. 1985. Studies on egg albumin gelation using nuclear magnetic resonance. J. Food Sci. *50*, 59.

GROSS, M.O., 1980. Chemical sensory and rheological characterization of low methoxyl pectin gels. Ph.D Dissertation, University of Georgia, Athens, GA.

GROSS, M. O., RAO., V. N. M. and SMIT, C. J. B. 1982. Direct stress strain dynamic characteristics of low methoxyl pectin gels. J. Texture Studies *13,* 97.

HOLME, J. 1963. The thermal denaturation and aggregation of ovalbumin. J. Phys. Chem. *67*, 782.

JOHNSON, T. M. and ZABIK, M. E. 1981. Gelation properties of albumen proteins, singly and in combination. Poultry Sci. *60*, 2071.

LABUZA, T. P. and BUSK, G. C. 1979. An analysis of water binding in gels. J. Food Sci. *44*, 1379.

LEUNG, H. K., STEINBERG, M. P., NELSON, A. I. and WI, L. W. 1976. Water binding of macromolecules determined by pulsed NMR. J. Food Sci. *41*, 297.

MITCHELL, J. R. 1980. The rheology of gels. J. Texture Studies *11*, 315.

SAS. 1979. SAS User's Guide. Statistical Analysis Systems Inc., Cary, N.C.

SAS. 1981. SAS/Graph User's Guide. Statistical Analysis Systems Inc., Cary, N.C.

SEIDEMAN, W. E., COTTERILL, O. J. and FUNK, E. M. 1963. Factors affecting heat coagulation of egg white. Poultry Sci. *42*, 406.

VAN KLEEF, F. S. M., BOSKAMP, J. V. and VAN DEN TEMPEL, M. 1979. Determination of the number of cross-links in a protein gel from its mechanical and swelling properties. Biopolymers *17*, 225.

WOOTTON, M., HONG, N. T. and PHAMTHI, H. L. 1981. A study of egg white proteins during freezing using differential scanning calorimetry. J. Food Sci. *46*, 1336.

EVALUATION OF TIME-TEMPERATURE RELATED QUALITY CHANGES IN ICE CREAM DURING STORAGE

K.D. DOLAN, R.P. SINGH, and J.H. WELLS

Received for Publication September 13, 1985

ABSTRACT

The keeping quality of vanilla-flavored ice cream was investigated during 21 weeks of storage. Ice cream samples at 4 different temperature treatments were evaluated for 3 attributes by 14 judges using a deviation-from-reference scale. Sampling tools and presentation containers were designed to provide uniform samples and serving temperatures without melting. The ice cream kept at a variable storage treatment showed a trend toward becoming less firm and darker in yellow than the other samples from the 86th day of storage onward. Differences in creaminess, hardness, and vanilla flavor were noticed in ice cream after 170 days of storage. A commercial time-temperature indicator was used to monitor temperature exposure; but, since indicator response was slower than published, no correlations could be made with quality changes.

INTRODUCTION

Ice cream quality is based on many attributes, some of which may change during storage. Physical and chemical changes which occur in ice cream during storage are typically caused by oxidation of fats, sublimation of ice, and recrystallization of ice crystals. The physical characteristics of ice cream, a product with unstable texture, are particularly susceptible to fluctuating temperature. Any change in a specific product attribute should be less than the level considered unacceptable by a consumer.

The quality of a frozen food depends upon the cumulative effect of storage time and temperature. A longer storage time or a higher temperature may adversely affect quality. Londahl (1982) found that in an average of 45% of all in-transit spot checks conducted in 5 European countries, the product was above $-15°C$, while the recommended maximum temperature was $-18°C$. Improvements and increased

Authors' address: Dept. of Agr. Eng., Univ. of California, Davis, CA 95616

Journal of Food Processing and Preservation 9 (1985) 253-271. All Rights Reserved.

availability of commercial time-temperature indicators may provide a practical method of measuring the temperature exposure of ice cream, and other frozen foods during storage and handling.

Ice cream quality has been previously studied as a function of ingredients, such as whey solids (Guy 1980), corn syrup (Pearson and Ennis 1979), and carboxymethylcellulose (Moore and Shoemaker 1981). In general, however, limited information is available regarding the shelf-life of ice cream. This is evidenced in that neither Van Arsdel *et al.* (1969) nor Jul (1984) cite any stability data for the shelf-life of ice cream. The objective of this paper is to discuss methods to determine and evaluate time-temperature related quality changes of ice cream during frozen storage. This presentation is a portion of an investigation into the use of time-temperature indicators as frozen food quality change monitors (Singh *et al.* 1984).

MATERIALS AND METHODS

Vanilla Ice Cream

Six hundred containers of Dreyer's vanilla ice cream (1-qt (946 ml) cylindrical containers) packaged in 6-qt bundles were received 72 h before the first sensory session and were placed in temporary storage at −35°C. Since the product was commercially manufactured, the exact composition was unknown, but the listed ingredients were fresh cream, milk, skim milk, sugar, corn sweetener, vanilla extract, mono and diglycerides, cellulose gum, locust bean gum, guar gum, anatto seed color, and carrageenan. Additionally, it was assumed that the entire lot of product was of uniform composition and the same initial quality. Caution was taken to handle all product in a similar manner, as to balance any temperature exposures.

Storage Temperature Treatments

The day of the first sensory session, the ice cream was distributed to the storage treatment locations. Three constant temperature treatments of −18°C, −25°C and −35°C, and one variable temperature treatment were employed. The product at constant temperature was stored as 6-qt bundles in insulated containers to minimize temperature variations,

while the variable treatment product was stored as individual quarts uniformly spaced on stainless steel wire cooling trays on mobile carts. This arrangement allowed air flow on all sides of the quart container at variable treatment. The variable storage treatment consisted of $-25°C$ storage and 4 high temperature exposures at 6-week intervals. The high temperature exposures were step changes to 20°C for ½ h, followed by 5°C for 4 h. The variable treatment was chosen as a model of possible conditions in frozen food transport and handling. For the sensory testing, the $-35°C$ storage condition served as the reference treatment.

Time-Temperature Indicators

Time-temperature indicators respond to the combined effect of time and temperature. These types of devices can be classified according to the ability to respond to this combined effect, as either full-history, or partial-history time-temperature indicators (Wells and Singh 1985). A full-history time-temperature indicator responds over all temperature ranges, while a partial-history time-temperature indicator responds only after a threshold temperature is exceeded. The time-temperature indicator used in this investigation was the I-POINT Time/Temperature Monitor, a full-history device manufactured by I-POINT Technologies, (Malmo, Sweden). Two models of the indicator (models 1020 and 3015), each with a different response rate, were used in this study.

The I-POINT Time/Temperature Monitor consists of an inner transparent plastic pouch having two compartments, and an outer plastic casing with an adhesive backing. One compartment of the inner pouch contains a pH indicator and enzyme solution, the other a lipid substrate. The indicator is activated when the contents of the two compartments are mixed. Enzymatic hydrolysis of the lipid substrate follows, causing the solution to change pH as the reaction proceeds. The reaction is accelerated by increased temperature , and is irreversible. Response is measured as the change in color of the pH indicator compared to a standardized color scale on the cover of each indicator. The color scale is numbered 0, 1, 2, and 3 (green, yellow, brown, and red), and corresponds to 0%, 33%, 67%, and 100% full-scale readings, respectively.

The day before the first sensory evaluation each indicator was activated using a mechanical device supplied by the manufacturer. The activated indicators were attached to each bundle (or quart, for variable treatment) of ice cream, and the product was distributed proportionally to the different temperature treatments.

Sensory Testing Methods

Attribute Determination and Product Sampling. A preliminary descriptive analysis was conducted prior to the beginning of the storage study. Five individuals highly trained in sensory testing techniques and terminology examined ice cream which had been subjected to several freeze/thaw cycles. As a result of this preliminary session, three attributes, firmness, iciness, and darkness of yellow, were chosen for evaluation during the storage study. It was also noted during this session that the freeze/thaw cycle gave rise to growth of a layer of ice crystals from the bottom of the ice cream containers.

During the storage, study samples were prepared in a $-18°C$ environment to maintain the product's structural integrity. Three people "scooped" between 300 and 400 samples during three hours of preparation within a $-18°C$ walkin freezer. Before serving, the samples were equilibrated to $-12°C$. The $-12°C$ condition preserved the textural characteristics of the product, but prevented extreme sensory fatigue resulting from palate numbness.

Sample Evaluation. A panel of 14 trained judges (2 male and 12 female) participated in nine evaluation sessions held at 3-week intervals. Panelist training was conducted by research staff, and consisted of instructing each judge in the use of sensory evaluation facilities and procedures, the score sheet scaling method, and the particular attribute evaluation technique.

Ice cream samples were removed from $-12°C$ into insulated containers and delivered to the sensory lab 5 min before a scheduled testing. An identified reference and 4 samples (three treatment samples and one unidentified reference) were presented for each attribute. Each attribute was judged in terms of how much they deviated from the reference on a 10-cm unstructured anchored scale, marked with a middle point as the reference, as shown in Fig. 1. The attributes firmness and iciness were

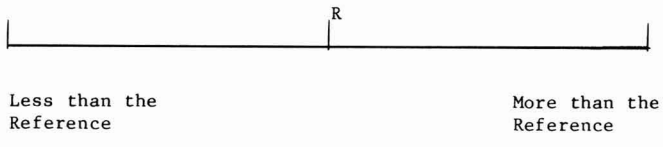

FIG. 1. DEVIATION-FROM-REFERENCE SCALE

evaluated under red light to mask appearance differences, while the attribute darkness of yellow was judged under white light. The judges evaluated duplicate samples for each attribute in individual separate booths.

Firmness. Firmness was described as the force required to penetrate the center of an ice cream sample with a spoon. The judges were instructed to penetrate a sample with a spoon, using approximately the same motion for all the samples. A tool (Fig. 2 and 2a) for extracting a 34-mm diameter ×

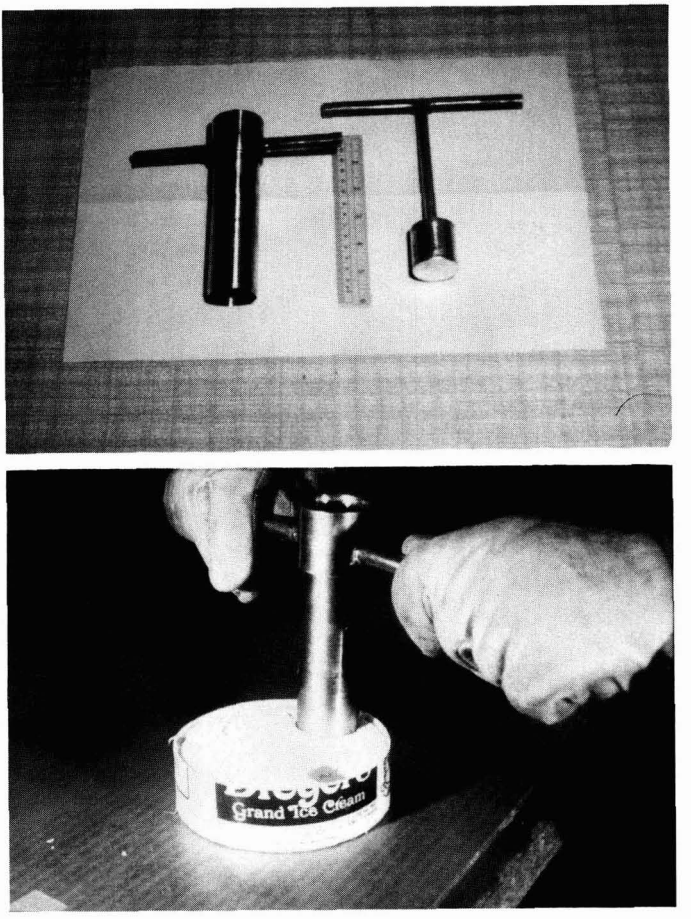

FIG. 2 and 2a. TOOL FOR EXTRACTING FIRMNESS SAMPLES OF ICE CREAM

25-mm long cylindrical "plug" of ice cream was constructed in 2 pieces, a stainless steel 35-mm internal diameter × 155-mm cylinder, and a close-fitting piston of aluminum, both with handles. The ice cream plug was pushed out of the cylinder into a 3-oz (89 ml) paper cup for presentation.

Effects of variable crystal formation along the vertical cross-section were examined by slicing quart containers in half, horizontally. Plugs from the top half were served for the first replication and plugs from the bottom half for the second. Uniform bisection of each quart was assured by using a mitre box (Fig. 3 and 3a) constructed for this purpose. The cups were placed in styrofoam holders (Fig. 4) designed for the study.

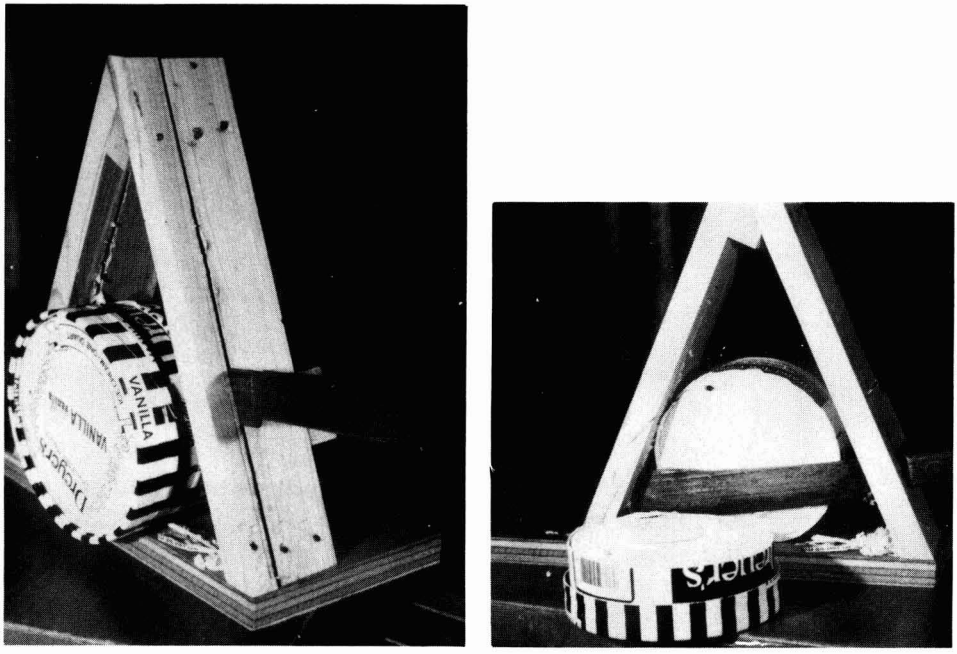

FIG. 3 and 3a. MITRE BOX FOR BISECTING QUARTS OF ICE CREAM

Iciness. Iciness was judged as the amount of crystals felt against the palate. The quart container was cut vertically and peeled back along the circumference to expose the side of the product. Samples representing the

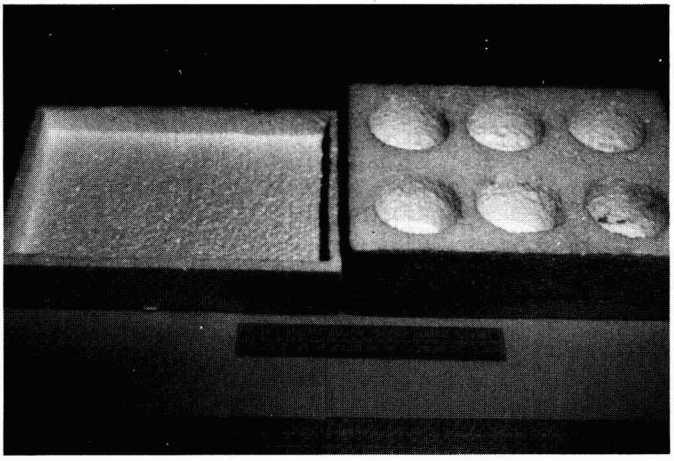

FIG. 4. STYROFOAM HOLDER FOR CUPS

vertical cross-section were obtained using a 2-cm diameter stainless steel scoop to scrape along the exposed side of the ice cream from top to bottom. Samples were served to the judges in 3-oz (89 ml) paper cups.

Darkness of Yellow. Darkness of yellow was evaluated as the relative intensity of the yellow color of a sample. A piston/cylinder sampling probe, with a 9-mm internal diameter and 101-mm length (Fig. 5 and 5a), was used to extract and place appearance samples in 10-mm diameter × 75-mm long disposable culture tubes. Each tube displayed a complete vertical cross-section of the ice cream. To prevent melting, the 5 tubes were presented in a 102-mm × 57-mm × 48-mm rigid foam block with a window for viewing two tubes (Fig. 6). The block was painted black and was presented on a cardboard stand which held the block at a 60° angle to the horizontal. The judges made a visual comparison by first placing the reference tube in one window slot and successively placing the other sample tubes in the adjacent slot.

Data Acquisition and Management

Temperatures of the stored ice cream were recorded at 20-min intervals throughout the duration of the investigation using T-type thermocouples

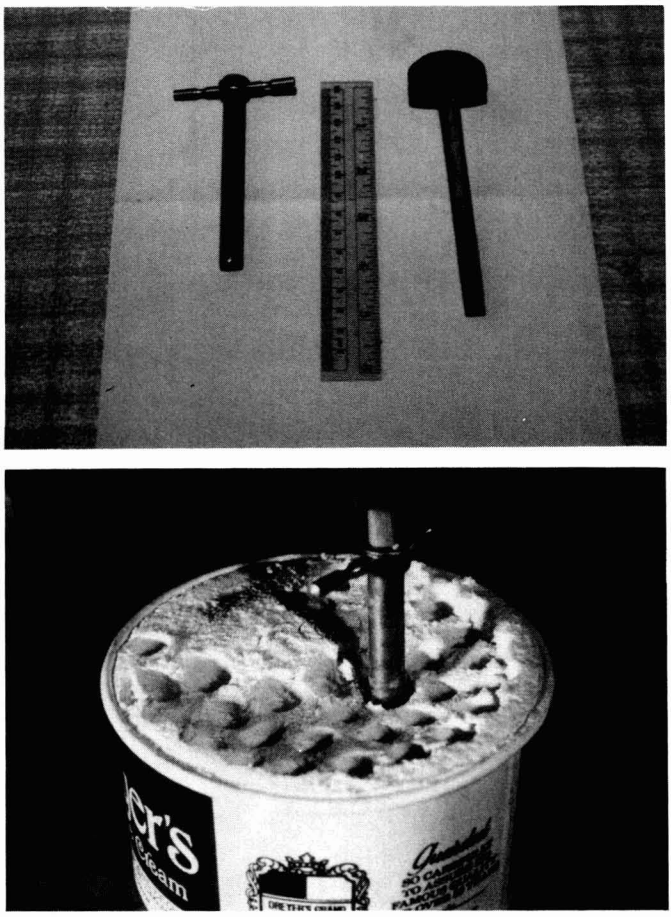

FIG. 5 and 5a. IMPLEMENT FOR EXTRACTING ICE CREAM DARKNESS OF YELLOW SAMPLES

and a digital data acquisition system. The daily average ice cream container surface temperature at $-25°C$ is shown in Fig. 7.

Simultaneous with product sampling for the sensory evaluation test, ten time-temperature indicators (five of each model) from all temperature treatment were inspected and recorded. It was hoped that any perceived time-temperature related quality change could be correlated with indicator response.

A system for managing the large amount of sensory data (approximately 800 responses) was developed using a desk top computer and a digitizing tablet. Random number labels to identify sensory

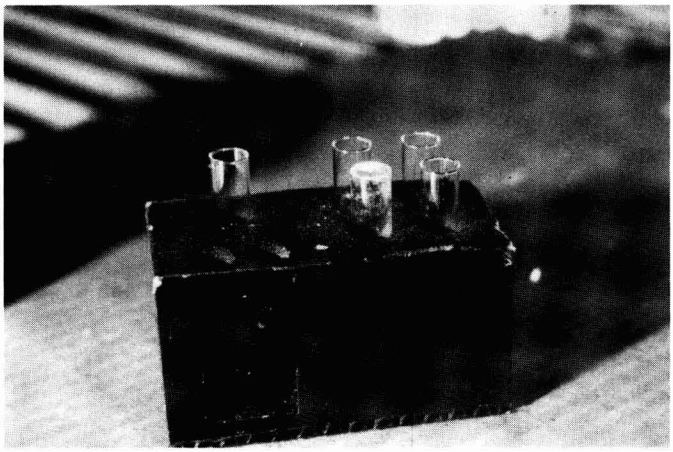

FIG. 6. FOAM BLOCK FOR ICE CREAM DARKNESS OF YELLOW SAMPLES

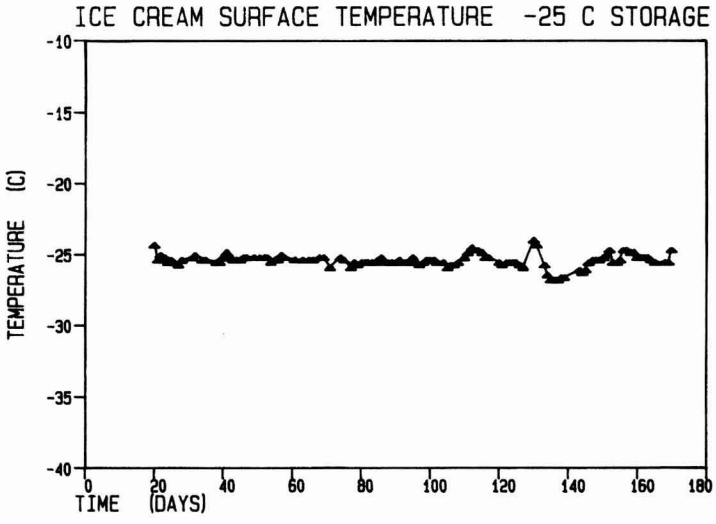

FIG. 7. DAILY AVERAGE ICE CREAM SURFACE TEMPERATURE AT −25°C STORAGE.

samples, and score sheets with corresponding numbers were printed by computer. The random numbers were assigned an identification code which indicated treatment, attribute, and replication. Later, the point marked by the judges on the 10-cm line of the score sheet was entered into the computer using the digitizing tablet. By entering the sample random number and judge code by computer keyboard, and the sensory response by digitizing tablet stylus, the data files were fully coded for analysis. These coded data files were transferred to a mainframe computer for statistical evaluation.

Data Analysis

Data analysis and statistical evaluations were conducted using the statistical software package SAS (Statistical Analysis System, SAS Institute, Cary, NC). The sensory data were analyzed using a complete 3-way analysis-of-variance model to determine significant effects. The means of all significant treatment effects were compared according to the multiple means comparison technique of the Scheffe minimum significant difference (Scheffe MSD) test at $\alpha = 5\%$. A pooled variance was used for the Scheffe MSD procedure.

RESULTS AND DISCUSSION

Sensory Procedures

Use of the previously described tools produced uniform sample sizes when samples were prepared in a $-18°C$ environment. Maintaining the contact surfaces of the metal utensils free form product residue aided in sample preparation. A limitation of the darkness-of-yellow test was the compression of the cross-section samples up to one-third of their original length. The amount of compression varied between sessions, but was virtually constant within a session. The height of the window in the foam block was adjusted accordingly, to cover the empty part of the culture tube and allow viewing of only the sample. Before the first session, the authors visually compared the darkness of yellow of compressed to uncompressed samples, and found no difference. The compression did not affect the surface texture seen through the culture tubes. The use of the foam block and insulated container held the sample temperature increase to less than 1°C. Condensation of moisture on the surface of the culture tubes was not apparent within the time for evaluation.

Results of Storage Study

Eight evaluation sessions were conducted for ice cream at 0, 21, 42, 65, 86, 107, 128, and 149 days after the beginning of storage. Three high-temperature treatments were held for the variable treatment at 24, 67, and 108 days after the beginning of storage. The summaries of the analysis of variance on the sensory data are given in Table 1, and the mean scores for each treatment and the corresponding MSD values between means are given in Table 2. Results are discussed separately for each attribute.

Table 1. Analysis of variance results - ice cream

				FIRMNESS		ICINESS		DARKNESS OF YELLOW	
Session	Day	Source[a]	d.f.[b]	S.S.[c]	sig.[d]	S.S.	sig.	S.S.	sig.
0	0	T	3	474.78	ns	1560.52	**	105.10	ns
		R	1	6.11	ns	0.79	ns	17.94	ns
		J	12	2369.78	ns	5897.41	***	3691.01	***
		T x R	3	1903.19	**	468.25	ns	18.30	ns
		T x J	36	5398.99	ns	7464.25	*	1312.09	**
		R x J	12	2956.65	ns	3923.72	**	221.72	ns
		Error	36	4994.68		3753.48		472.98	
1	21	T	3	138.35	ns	58.84	ns	161.20	*
		R	1	44.91	ns	90.28	ns	1.73	ns
		J	12	4083.85	***	1640.13	ns	734.28	***
		T x R	3	452.30	ns	96.73	ns	8.59	ns
		T x J	36	4296.15	ns	4771.08	ns	913.60	ns
		R x J	12	1905.32[e]	*	1410.20	ns	737.66	***
		Error	36	2356.87[f]		3637.15		551.70	
2	42	T	3	313.68	ns	161.23	ns	131.00	ns
		R	1	70.69	ns	0.04	ns	118.37	ns
		J	11	4196.99	**	1218.35	ns	1643.41	***
		T x R	3	217.17	ns	396.38	ns	61.92	ns
		T x J	33	2470.51	ns	1764.74	ns	402.01	ns
		R x J	11	1287.20	ns	690.74	ns	739.84	*
		Error	33	3332.77		3731.14		1025.48	
3	65	T	3	286.54	ns	505.54	ns	57.61	ns
		R	1	12.26	ns	342.77	*	2.87	ns
		J	11	1898.15	**	2797.33	**	898.45	*
		T x R	3	132.27	ns	529.63	ns	33.45	ns
		T x J	33	2607.82	ns	1459.78	ns	329.14	ns
		R x J	11	3600.82	***	2072.66	*	266.34	ns
		Error	33	1917.93		2406.90		979.40	

[a]Variance sources given as: T = Treatment; R = Replication; and J = Judge.
[b]Variance degrees of freedom (d.f.).
[c]Variance sum of squares (S.S.).
[d]Significance (sig.) level given s: ns = not significant
* = $p<0.05$
** = $p<0.01$
*** = $p<0.001$

Table 1 Continued. Analysis of variance results - ice cream

				FIRMNESS		ICINESS		DARKNESS OF YELLOW	
Session	Day	Source	d.f.	S.S.	sig.	S.S.	sig.	S.S.	sig.
4	86	T	3	1960.48	***	157.45	ns	165.07	*
		R	1	1.98	ns	14.96	ns	74.69	*
		J	9	3144.89	***	2509.52	***	515.81	**
		T x R	3	265.73	*	315.99	ns	42.42	ns
		T x J	27	2386.04	**	1169.09	ns	394.92	ns
		R x J	9	542.35	*	2399.09	***	92.76	ns
		Error	27	696.83		1029.37		371.72	
5	107	T	3	88.00	ns	744.18	***	125.60	*
		R	1	0.47	ns	106.72	ns	4.90	ns
		J	9	857.13	ns	416.12	ns	599.89	***
		T x R	3	865.15	**	383.51	*	77.15	ns
		T x J	27	1216.12	ns	1586.98	ns	629.71	*
		R x J	9	564.71	ns	2390.17	***	341.87	**
		Error	27	1200.17		911.80		270.45	
6	128	T	3	1136.03	***	24.81	ns	55.32	*
		R	1	41.56	ns	52.36	ns	3.17	ns
		J	8	2261.01	***	522.31	*	79.06	ns
		T x R	3	174.94	ns	120.85	ns	7.82	ns
		T x J	24	2542.18	*	936.21	ns	130.51	ns
		R x J	8	649.33	ns	326.92	ns	41.42	ns
		Error	24	1052.31		659.74		122.48	
7	149	T	3	2358.46	***	11.71	ns	106.62	*
		R	1	0.47	ns	526.13	*	0.16	ns
		J	7	1133.51	**	283.45	ns	378.61	***
		T x R	3	206.78	ns	278.89	ns	21.86	ns
		T x J	21	473.35	ns	2473.39	ns	473.10	*
		R x J	7	1434.02	ns	938.18	ns	73.34[g]	ns
		Error	21	791.97		1943.32		154.14[h]	

[e]Degrees of freedom = 11
[f]Degrees of freedom = 33
[g]degrees of freedom = 6
[h]degrees of freedom = 18

Firmness. Table 1 shows that the treatment × judge interaction was significant only at storage day 86 ($p<1\%$), and day 128 ($p<5\%$), indicating the attribute was generally evaluated in the same way by all the judges, and the samples were generally uniform within a treatment.

The treatment × replication interaction was significant at days 0, 86, and 107 only, and the replication effect was not significant in any of the sessions. Differential crystal growth was probably too subtle during the time of the study to produce a difference in firmness between the top and bottom part of the ice cream. The significance of the interaction was probably a result of uncontrolled factors during the evaluation.

Table 2. Mean scores and statistical comparison of means for sensory evaluations of ice cream

Session	Storage Day	Attribute	n	Storage Treatment -35°C	-25°C	Variable	-18°C	Means Comparison (α = 5%) Scheffe's MSD[a]
0	0	FIRM	26	50.39[b]	45.73	46.38	50.21	--
		ICY	26	46.61	55.02	47.49	44.91	--
		DARK	26	52.31	52.15	51.85	44.81	--
1	21	FIRM	25	48.53	48.83	48.54	51.36	--
		ICY	26	50.57	49.30	48.54	49.94	--
		DARK	26	50.18	51.11	49.33	52.69	3.30
2	42	FIRM	24	49.19	51.81	47.18	51.13	--
		ICY	24	46.78	50.19	49.12	49.62	--
		DARK	24	51.07	52.30	54.33	52.43	--
3	65	FIRM	24	51.47	51.00	48.22	47.46	--
		ICY	24	51.18	46.89	48.11	52.61	--
		DARK	24	48.92	49.77	50.77	50.79	--
4	86	FIRM	20	46.28	54.61	40.71	46.88	4.79
		ICY	20	46.59	50.34	49.14	49.53	--
		DARK	20	48.77	52.08	52.38	50.54	3.35
5	107	FIRM	20	46.81	49.68	48.85	48.19	--
		ICY	20	51.12	52.93	44.72	49.83	6.21
		DARK	20	50.96	49.74	53.22	51.12	3.19
6	128	FIRM	18	52.51	58.65	47.65	54.70	7.17
		ICY	18	51.31	52.39	52.52	52.88	--
		DARK	18	49.69	49.81	51.88	50.67	2.17
7	149	FIRM	16	50.97	53.60	37.93	50.44	7.64
		ICY	16	48.69	49.42	49.66	49.79	--
		DARK	15	50.37	52.38	54.15	51.15	3.31

[a]Minimum Significant Difference (MSD) between treatment means, according to Scheffe's multiple means comparison test (α = 5%).
[b]Mean attribute score.

The replication × judge interaction was significant at days 65 and 86, implying the judges' scoring was consistent for five of the seven sessions. The significant interactions for days 65 and 86 may have been due to initial product variability or to non uniformity of the product occurring during presentation at these sessions. For example, the first replication for one judge may have remained in the insulated containers for a longer time than the second.

Table 2 shows the sample held under variable temperature conditions was less firm at the highest significance ($p < 0.1\%$) than the other samples on days 86, 128, and 149. Furthermore, on day 149 it was noted during sample preparation that the appearance samples (cylindrical "plugs") for

the variable treatment were longer than any other. The reason for this difference was that the variable product was less firm than product stored at the other treatments and offered less resistance when the appearance probe was pressed into the ice cream container.

In Fig. 8, the mean sensory scores over time show that the product held under variable temperature conditions followed a decreasing firmness trend. One may expect an increasing firmness by the migration and later refreezing of water into the air space. However, the variable treatment was not sufficiently severe to induce melting in more than the surface of the product. Therefore, the effects of melting and separation of constituents were not pronounced.

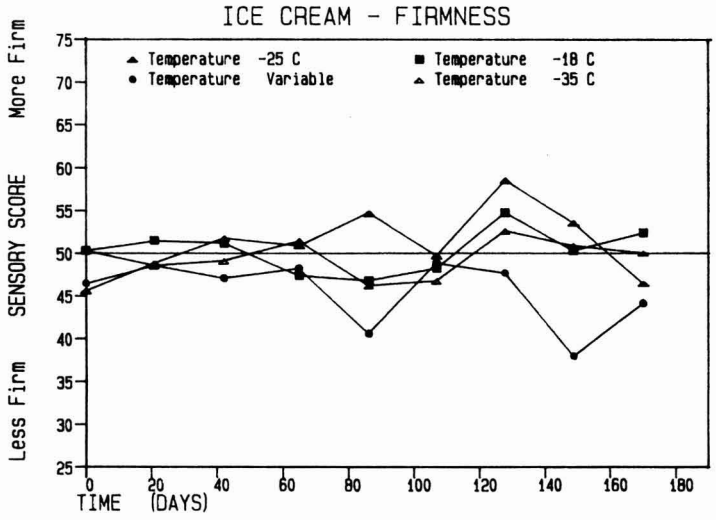

FIG. 8. SENSORY SCORES FOR ICE CREAM FIRMESS PLOTTED OVER TIME

Iciness. There was an effect of replication on days 65 and 149 and of replication × judge interaction on days 0, 65, 86, and 107 (Table 1). The inconsistent responses of the judges implied that the method of evaluation may have concealed differences in iciness until they were more prominent. Specifically, the judges pressed the sample against the palate with the tongue, rather than quickly biting through the sample with the teeth. The first method results in more melting of ice crystals than the second, and could disguise incipient differences. Moreover, the variable treatment was not designed to effect gross changes of quality,

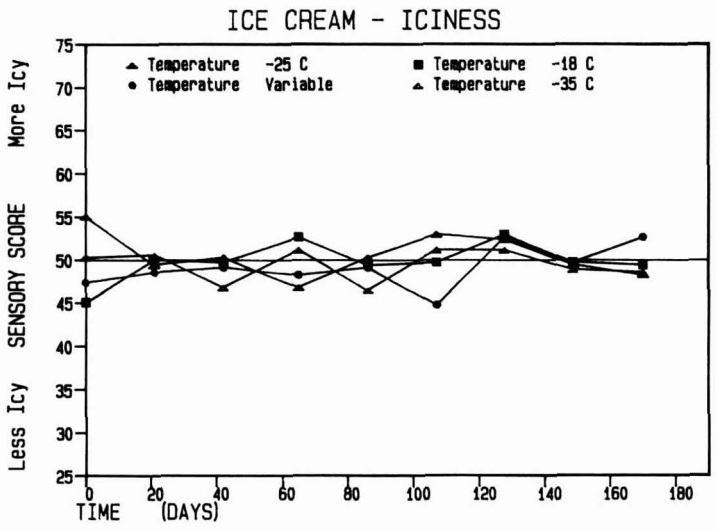

FIG. 9. SENSORY SCORES FOR ICE CREAM ICINESS PLOTTED OVER TIME

but rather was used to produce gradual differences typical in a product in transportation.

The treatments differed significantly (Table 1) on days 0 and 107 only. The plot of mean scores for each session and treatment (Fig. 9) shows this difference is not due to any trend, but most probably to uncontrolled factors, such as initial inexperience of the judges. No consistent significant differences between treatments were found for iciness.

Darkness of Yellow. The treatment × judge interaction (Table 1) was significant on days 0, 107, and 149. Moreover, there was a significant effect of replication on day 86, although samples for a treatment were taken from the same carton. There was a significant effect of replication × judge on days 21, 42, and 107.

The significant judge effect in all sessions except on day 128 implied the scale was used in different ways. Figure 10 shows mean scores for each session and treatment. Plots for all treatments are similar. However, beginning on day 86, the variable samples received consistently higher scores (Table 2 and Fig. 10), indicating this treatment caused a darker yellow color to appear. This trend is significant from day 86 through the last session.

The darker color was caused by a redistribution of water within the product as a result of moisture migration. Although the ice cream containers were vapor barriers, the separation of moisture from the

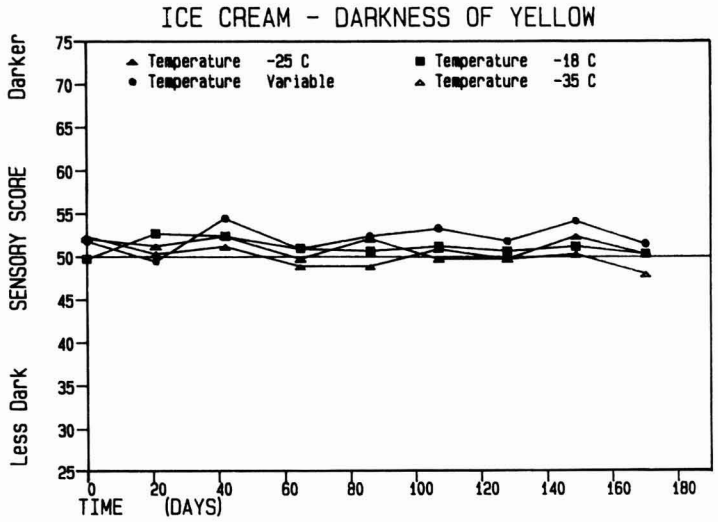

FIG. 10. SENSORY SCORE FOR ICE CREAM DARKNESS OF YELLOW PLOTTED OVER TIME

variable product surface during melting created a concentration gradient. As different levels of moisture were established, the wavelength of reflected light was shifted toward a more yellow color. The interactions were evident when the lids of the containers at variable treatment become "bonded" to the product surface by ice.

Final Descriptive Analysis

To determine all attributes which had changed during storage, a descriptive analysis session was held after 170 days of storage. The judges which had participated in the storage study received two samples, one reference, and the other a $-18°C$ sample. The panelists were given an aided recall list of descriptors, and were asked to mark all items describing differences between the two samples. The nature of this evaluation was qualitative as the judges were asked only to specify quality differences, and not rate the magnitudes of the perceived difference.

The specific terms included in the aided recall list were derived from notes taken during the preliminary evaluation and throughout the storage study. In addition, the judges were encouraged to mention any appropriate descriptor not included in the list. It was the intent of the

investigators to determine whether terms other than firmness, iciness, and darkness of yellow might identify and describe quality differences more appropriately.

The descriptors included in the aided recall list, and number of time each was indicated to describe the quality difference, are given in Table 3. The most frequently used descriptor was creaminess, used as a textural as well as a flavor term. Differences in vanilla flavor appear to be noticeable. Hardness or firmness by spoon and darkness of yellow were prominent, but iciness was not one of the important descriptors.

Table 3. Frequency of terms used in final descriptive analysis session-ice cream

NUMBER OF PARTICIPATING JUDGES: N = 9.

DESCRIPTORS	FREQ. OF USE	DESCRIPTORS	FREQ. OF USE
Appearance		Texture	
Crystallinity	5	Hardness (spoon)	6
Brightness	3	Smoothness	4
Glossiness	1	Iciness (bite)	4
Consistency	5	Iciness (tongue)	3
Darkness of Yellow	5	Firmness (spoon)	5
Graininess	2	Chewiness	2
		Graininess	3
Flavor		Creaminess	6
		Brittleness (spoon)	0
Flavor Intensity	5	Guminess	3
Sweetness	5	Chalkiness	1
Vanilla Flavor	6	Density	0
Creaminess	7		
Oxidized Flavor	3		
Rancidity	0		
Metallic	0		
Aftertaste	1		

NOTE: Samples compared had been stored 170 days at -35° and -18°C.

Time-Temperature Indicator Response

The I-POINT Time/Temperature Monitor models 1020 and 3015 stored at the $-35°C$, $-25°C$, and variable treatments yielded no response during 149 days of storage. Because of this, no correlation between sensory scores and indicator response could be determined. The literature supplied by the indicator manufacturer had reported that a 33% full-scale reading would occur between 114 and 126 days at $-18°C$ for model 3015.

Three out of the five model 3015 indicators stored at −18°C had responded to 33% full-scale at 149 days of storage. This response was somewhat slower than that reported by the manufacturer. The enzyme response mechanism was stated to remain viable for up to 1 year if the inactivated indicators were stored at 4°C. Unknown storage conditions of the indicators prior to this study may have altered the response rate.

CONCLUSIONS

For the duration of the study, only the variable storage temperature treatment affected ice cream characteristics from the 86th day of storage onward. Singh *et al.* (1984) present empirical evidence between the time until a "just noticeable difference" and the time until the beginning of consistently significant statistical differences. Based on these findings, one may expect a "just noticeable difference" on ice cream after two high-temperature cycles of a variable storage treatment. The samples at variable treatment showed a significant trend toward becoming less firm and darker in yellow over time. If consumer testing found these changes undesirable, the results would emphasize the importance of minimizing the exposure of ice cream to the variable environmental conditions examined in this study.

The response of the indicators used in this study was insufficient for significant correlations. To correlate quality change with indicator response, a longer study or a more rapidly responding indicator model would be necessary.

Based on the final descriptive analysis, techniques to quantify the sensory descriptors creaminess, hardness, and flavor should be considered for future studies of ice cream quality.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Ms. E.J. Gonnet and Dr. Carlos Zuritz in various phases of this study, and Dr. C.P. Dunn of the U.S. Army Natick Research & Development Center, Natick, Massachusetts, for consultation and guidance. The research was supported by U.S. Army Contract DAAK-60-30-C-0100.

Reference to brand names or manufacturers given in this presentation does not constitute an endorsement by the authors.

REFERENCES

GUY, E.J. 1980. Partial replacement of nonfat milk solids and cane sugar in ice cream with lactose hydrolyzed sweet whey solids. J. Food Sci. *45*(1), 129-133.

JUL, M. 1984. *The Quality of Frozen Foods*, Academic Press, New York.

LONDAHL, G. Market Needs for Temperature and Time-Temperature Monitoring Devices. Unpublished Report, 1983.

MOORE, L.J. and SHOEMAKER, C.F. 1981. Sensory textural properties of stabilized ice cream. J. Food Sci. *46*(2),399-402, 409.

PEARSON, A.M. and ENNIS, D.M. 1979. Sensory properties of high fructose corn syrup ice cream formulations. J. Food Sci. *44*(3), 810-812.

SINGH, R.P., WELLS, J.H., DOLAN, K.D., GONNET, E.J. and MUNOZ, A.M. October 1984. Critical evaluation of time-temperature indicators for monitoring quality changes in stored subsistence. Report prepared for U.S. Army Natick Research and Development Center, Natick, Mass.

VAN ARSDEL, W.B., COPLEY, M.J. and OLSON, R.L. 1969. *Quality and Stability of Frozen Foods,* Wiley-Interscience, New York.

WELLS, J.H. and SINGH, R.P. 1985. Performance evaluation of time-temperature indicators for frozen food transport. J. Food Sci. *50*(2), 369-371, 378.

STATISTICAL EVALUATION OF ARRHENIUS MODEL AND ITS APPLICABILITY IN PREDICTION OF FOOD QUALITY LOSSES

ELI COHEN

Institute of Quality Control &
Extension Services for the Food Industry
76 Mazeh Street
Tel Aviv, 65789
Israel

and

ISRAEL SAGUY[1]

Department of Food Sciences
Volcani Center
Agricultural Research Organization
P.O. Box 6
Bet Dagan 50-250
Israel

Received for Publication September 13, 1985

ABSTRACT

To minimize quality losses occurring during processing and storage and to predict shelf-life, quantitative kinetic models, expressing the functional relationship between composition and environmental factors on food quality, are required. The applicability of these models is based on the accuracy of the model and its parameters. In this paper, the calculation of the Arrhenius parameters and the accuracy of the derived model were compared, using three statistical methods, namely: linear least squares, nonlinear least squares and weighted nonlinear least squares. Results indicated that the traditional two-step linear method, was the least accurate and the derived energy of activation and the pre-exponential factor had the largest confidence interval. The latter was shown to have a profound effect on the precision of the calculated rate constant and the predicted shelf life. Based on previous reports that indexes of deterioration

[1]*Address Correspondence to: Israel Saguy, The Pillsbury Company, 311 Second Street S.E., Minneapolis, MN 55414.*

Journal of Food Processing and Preservation 9 (1985) 273-290. All Rights Reserved.

are log-normal distributed, the unweighted nonlinear least squares method was applied in a single-step on all the data points, following a logarithmic transformation. The overall better accuracy and superior performance of the nonlinear least squares method, suggests that this method should be utilized for routine kinetic data analysis.

INTRODUCTION

Foods are very sensitive and susceptible to quality losses due to chemical instability which depends both on compositional and environmental factors. To minimize quality losses occurring in processing and storage, to have a better understanding and an insight in these deleterious reactions, and to predict shelf life, kinetic models are utilized. The models express in a functional form the rate of the quality loss and its dependency on factors such as temperature, moisture content, water activity, concentration and others.

Compositional and/or environmental effects can be expressed by a functional relationship which applies only occasionally to several food systems and reactions. More often, food quality reactions are more complex and unique in their behavior, and the appropriate model must be derived for each product and food system individually.

Temperature is one of the main environmental factors which has a major impact and influence on quality loss rate. The most common and generally valid assumption is that temperature-dependency of food quality deterioration rate follows the Arrhenius model:

$$k = ko \exp(-Ea/RT) \tag{1}$$

where: k is the rate constant; ko is a constant, independent of temperature (also known as pre-exponential or frequency factor); Ea is the activation energy; R is the gas constant; and T is the absolute temperature.

The Arrhenius equation is frequently used as a theoretical basis for the development of a mathematical model which describes the temperature sensitivity of a food product and for shelf-life prediction. However, such prediction has limited practical use if the large statistical confidence interval and error of the predicted shelf life is considered (Labuza and Kamman 1983). Furthermore, the activation energy generally depends on composition factors such as water activity, moisture content, solid concentration, pH and others (Cohen and Saguy 1983; Connor *et al.* 1981; Goldman *et al.* 1983; Labuza 1972; Saguy 1979; Saguy *et al.* 1979; 1980).

Other models describing temperature effect were discussed (Labuza and Riboh 1982; Kirkwood 1977). However, the Arrhenius model provides the soundest approach for predicting the reaction rate and shelf life at temperatures different from those used in establishing the model. Table 1 summarizes a literature search which indicates the wide spread of the Arrhenius model.

Table 1. Literature Search (Key words and Abstracts) FSTA Files (1969-1984).

Topic	No. of citation
Arrhenius	172
Kinetic(s)	2037
Arrhenius & Kinetic(s)	66
Prediction(s)	3366
Arrhenius & Prediction(s)	36
Simulation(s)	2562
Arrhenius & Simulation(s)	15
Arrhenius & Kinetic(s) & Simulation(s)	12

The most common method to estimate the Arrhenius parameters is the "classic" successive ordinary linear least squares fit (Lund 1983). In this method the first regression is used to derive the rate constant which is then regressed versus the absolute of the 1/temperature to obtain the estimates for Ea and ko. The large confidence interval derived for Ea and ko (Arabshahi and Lund 1985; Labuza and Kamman 1983; Haralampu *et al.* 1985) is explained by the low number of temperatures (i.e., degrees of freedom), and unnecessary parameters estimated. The derived wide confidence interval reduces the applicability of the model and hampers its utilization. To avoid some of the aforementioned drawbacks nonlinear

least squares regression was suggested (Arabshahi 1982; Davies and Hudson 1981; Haralampu *et al.* 1985; Lund 1983; Nelson 1983). This approach increased the accuracy of the estimated Arrhenius parameters and ultimately improved the confidence interval of the predicted quality attribute.

This investigation was carried out with the overall goal of suggesting the most favorable method for deriving the Arrhenius parameters. Also, to compare the methods commonly utilized in the derivation of Ea and ko, and to establish their statistical confidence, limitation, drawbacks, implication, and applicability.

MATERIALS AND METHODS

The statistical analysis is illustrated on the kinetic data of nonenzymatic browning of nonhygroscopic whey (Labuza and Kamman 1983; Table 2), and thiamine retention in an intermediate moisture model system (Arabshahi and Lund 1985; Table 3). These data represent typical zero and first-order reaction kinetics, respectively. The original data (Labuza and Kamman 1983; Arabshahi and Lund 1985) which included duplicate determinations was randomly divided into two groups and named I and II. The first group was utilized to generate the kinetic model, the second group was used for checking the "goodness" of fit. Also, based on previous experience (Arabshahi and Lund 1985; Haralampu *et al.* 1985), to avoid time lags normally accounted at initial equilibration of the samples, zero time observations were omitted.

The methods to be discussed herewith, were used to estimate the activation energy (Ea), the pre-exponential factor (ko), to evaluate the accuracy and precision of the parameter estimates and to draw conclusions on the statistical accuracy and applicability of these methods.

Kinetics of nonenzymatic browning of nonhygroscopic whey and thiamine retention in an intermediate moisture model system containing propylene glycol can be described by a zero and first-order model (Labuza and Kamman 1983 and Arabshahi and Lund 1985), respectively:

$$dC/dt = k \tag{2a}$$

$$dC/dt = -kC \tag{2b}$$

where:

C is the browning index or the thiamine concentration at time t;
k is the rate constant; and
a, b denotes zero or first-order reaction.

Table 2. Browning data[1] for nonhygroscopic whey ($a_w = 0.44$) as a function of storage temperature

TEMP. (°C)	TIME (days)	Browning value (OD/g solid) X 10^2 Group I	Group II
25	30	4.3	4.1
	60	6.1	6.3
	90	7.4	7.6
	120	9.6	9.8
	150	11.8	12.0
	180	12.7	12.5
	210	14.5	14.8
35	10	5.0	5.2
	20	7.9	7.9
	30	10.5	10.6
	40	13.7	13.8
	50	16.3	16.5
	60	20.2	20.1
	70	23.2	23.4
	95	27.7	27.8
45	2	5.2	5.2
	4	7.1	7.0
	7	22.4	22.4
	11	25.2	25.3
	18	31.7	31.7
	28	44.4	44.2
	35	50.9	50.7

[1]Adopted from Labuza and Kamman (1983).

Integration of Eq. (2) yields:

$$C = Co + kt \tag{3a}$$

$$C = Co \exp(-kt) \tag{3b}$$

where Co is the initial browning index or thiamine concentration.

Table 3. Thiamin data[1] for an intermediate-moisture model system containing propylene glycol ($a_w = 0.75$ @ 20°C) as a function of storage temperature

TEMP. (°C)	TIME (days)	Thiamin concentration (μg/g solid) Group I	Group II
25	31	61.6	61.1
	62	60.2	60.3
	91	56.7	53.9
	122	47.9	46.9
	152	48.1	47.1
	197	42.7	39.8
	257	32.2	34.4
35	31	50.5	48.9
	62	43.8	42.7
	91	39.2	38.7
	122	33.0	32.4
	152	24.1	23.5
45	31	31.1	32.9
	62	17.2	19.0
	91	7.0	7.1
55	3	55.5	56.2
	7.25	35.2	33.8
	11.1	24.7	25.3
	19	9.1	9.9

[1]Adopted from Arabshahi and Lund (1985).

Substituting the Arrhenius model in Eq. (3) results:

$$C = Co + ko\, t \exp(-Ea/RT) \tag{4a}$$

$$C = Co \exp[-ko\, t \exp(-Ea/RT)] \tag{4b}$$

Three least squares methods were considered for deriving the Arrhenius model parameters. The following methods were utilized:

Method 1: Two-step Linear Least Squares

The most common method to estimate the Arrhenius parameters is the "classic" successive two-steps ordinary linear least squares fit. In this method the first regression of C vs. t, is done at each temperature, to estimate the rate constant k. The second step is to regress ln(k) vs. 1/T to obtain the estimates of ln(ko) and Ea/R.

Method 2: Nonlinear Least Squares

The nonlinear regression performs a single regression on all of the data points (i = 1, . . . ,n), to estimate Ea/R and ln(ko) without calculating the rates at each temperature.

It was shown previously that most measured indexes of deterioration are log-normal distributed (Davies and Hudson 1981; Haralampu 1984; Nelson 1983). Hence, for the deterioration of a single reactant following a zero or a first-order model (Eq. 4), may be rewritten (Nelson 1983):

$$\ln(C) = \ln(Co) + \ln\{1 + t\exp[\alpha - (Ea/R)(1/T - \beta)]\} \quad (5a)$$

$$\ln(C) = \ln(Co) - t\exp[\delta - (Ea/R)(1/T - \beta)] \quad (5b)$$

where:

$$\alpha = \ln(ko/Co) - \beta\,(Ea/R)$$

$$\beta = [\Sigma(1/T)\,w_i]/(\Sigma w_i) \qquad i = 1, \ldots, n$$

and

$$\delta = \ln(ko) - \beta\,(Ea/R)$$

For clarity of the presentation, the temperature subscript, i (i.e., i = 1 , . . m; m = number of temperatures tested) was omitted throughout the manuscript.

For unweighted regression $w_i = 1$ and for weighted nonlinear least squares (see method 3 below) w_i is the inverse of the variance estimated by Eq. (7) (Nelson 1983).

It is worth noting that for the unweighted least squares ($w_i = 1$) the origin of 1/T was moved to the unweighted mean, β. This was required since the parameters are highly colinear and are not easily regressed

directly (Haralampu *et al.* 1985; Nelson 1983). The latter transformation obviates in most cases the severe numerical difficulties in some nonlinear softwares.

Finally, to avoid bias in the determination, Co was derived as a parameter (Haralampu *et al.* 1985). The COMPLEX method (Saguy 1983) was used to derive Co, Ea/R and ko.

Method 3: Weighted Nonlinear Least Squares

Davies and Budget (1980) postulated that the errors in the index of deterioration originate from three sources. The variance of the errors can therefore be described as:

$$\text{Variance of } \log(C) = \sigma_0^2 + (Co/C - 1)\,\sigma_1^2 + (Co/C)\,\sigma_2^2 \quad (6)$$

where: σ_0 is the standard error that is proportional to the concentration (e.g., dilution error); σ_1 is the standard error proportional to the amount of deterioration (e.g., temperature variations); and σ_2 is the standard error which is proportional to the measurement method.

The overall error in the measurement of the index of deterioration has a log normal distribution (Davies and Hudson 1981) with a zero mean and variance of σ_t^2.

Based on the above assumptions, Nelson (1983) developed the following relationship:

$$\sigma_t^2 = \ln[(1 + \tfrac{1}{2}\sqrt{(1 + 4\epsilon)}] \quad (7)$$

where: ϵ is the right-hand-side of Eq. (6).

A FORTRAN program which initially fit the data using unweighted least squares (Eq. 5), and then refits the model using weighted least squares where the appropriate weights are derived from eq. (7) was developed by Nelson (1983). This program was adopted for an IBM PC and utilized to carry out the calculations

As in the previous method, to avoid bias in the determination, Co was derived as a parameter.

It is worth noting that all the methods used for the nonlinear least squares (BMDPAR, Dixon 1983; Nelson 1983) are derivative free codes, thus the need for the parameter derivatives normally required was obviated. However, if these computer programs are not available, the appropriate parameters derivatives may be found in the literature (Arabshahi and Lund 1985).

Statistical Evaluation

Statistical software BMDP1R and BMDPAR (Dixon 1983) were used for the linear and the nonlinear least squares, respectively. The joint confidence region of the parameters (Ea/R and ln ko) was established following well documented methods (Draper and Smith 1981; Hunter 1981). The statistical tests may be performed on SAS (SAS, 1982).

RESULTS AND DISCUSSION

To evaluate the accuracy of the regression methods used in this study, two basic criteria were used, namely: the accuracy and precision of the parameters estimates, and the accuracy and precision of the quality losses expressed by their half-lives.

The Arrhenius parameters and the initial concentration derived using the three regression methods are summarized in Tables 4 and 5 for a zero and first-order kinetics, respectively. The results showed no substantial differences among the derived values of Ea and ln(ko) when Methods 1 and 2 were applied. Nevertheless, the error mean squares (EMS) was significantly larger in method 1 for all the cases tested. The relatively high EMS values of method 1 is however not surprising, as the number of

Table 4. Effect of the regression method on the Arrhenius parameters derived for browning of nonhygroscopic whey

Regression method	df[a]	Ea/R (K)	ln(ko)	Co ODX100/g	ß X1000	EMS[b] Group I[c]	EMS[b] Group II[d]
Method 1	1	14,885	47.08	1.75	-	1.12	1.92
Method 2	19	15,244	48.39	1.79	3.249	1.19	1.19
Method 3	19	17,115	54.18	4.80	3.259	1.30	1.22

[a] - Degrees of freedom
[b] - Error mean squares
[c] - Calculated by applying the individual rates derived for each temperature
[d] - Calculated by applying the Arrhenius model to derive the appropriate rates

Table 5. Effect of the regression method on the Arrhenius parameters derived for thiamin retention in an intermediate-moisture model system containing propylene glycol

Regression method	df[a]	Ea/R (K)	ln(ko)	Co µg/g	ß X1000	EMS[b] Group I	EMS[b] Group II
						[c]	[d]
Method 1	2	12,162	34.68	71.00	-	1.48	1.80
Method 2	16	13,141	37.67	68.01	3.249	1.15	1.16
Method 3	16	13,543	38.86	61.89	3.233	1.15	1.14

[a] - Degrees of freedom
[b] - Error mean squares
[c] - Calculated by applying the individual rates derived for each temperature
[d] - Calculated by applying the Arrhenius model to derive the appropriate rates

data points is quite limited for each temperature, thus any discrepancy from the theoretical regression model would have a vast impact on the EMS. The latter can be demonstrated in the case of the nonenzymatic browning tested. The individual EMS at 25, 35 and 45°C where 0.39, 0.78 and 8.86, respectively. Obviously, the extremely high EMS value corresponding to 45°C would have required testing for outliers, and discarding the data points that carry either experimental errors or others extraneous effects. The decision when to discard and omit data should follow proven statistical procedures (e.g., Arabshahi and Lund 1985; Draper and Smith 1981). Yet, in this particular case, to demonstrate the differences between the different regression methods, all the data was included.

The values of the Arrhenius parameters derived for group I and II were very close, for all the methods tested. This verification indicated that the values derived were representing the actual reaction kinetics, and therefore may be used for prediction.

When method 3 was applied the values derived for the initial concentration was completely different from those obtained with Methods 1 and 2. Also, the derived value was in disagreement with the experimental values reported (Arabshahi and Lund 1985; Labuza and Kamman 1983). Yet, the appropriate EMS values for method 3 were very close to those derived by method 2 (Tables 4 and 5), hence a rigorous analysis was required to justify a clear choice between these two methods.

As method 3 is much more complicated than method 2 for computation, its application should be further weighed by the distribution of the residuals. If method 2 yields a randomly unskewed distribution of the residuals about zero and no pattern may be observed, this indicates that method 2 is appropriate and that method 3 may not be needed. When this approach was implemented for both zero and first-order kinetics analyzed, the distribution of the residuals (Fig. 1 and 2) fulfilled all the aforementioned requirements. Although one data point in Fig. 1 was far from the expected mean of the error (i.e., zero), this data was not discarded and justified by the explanation given above.

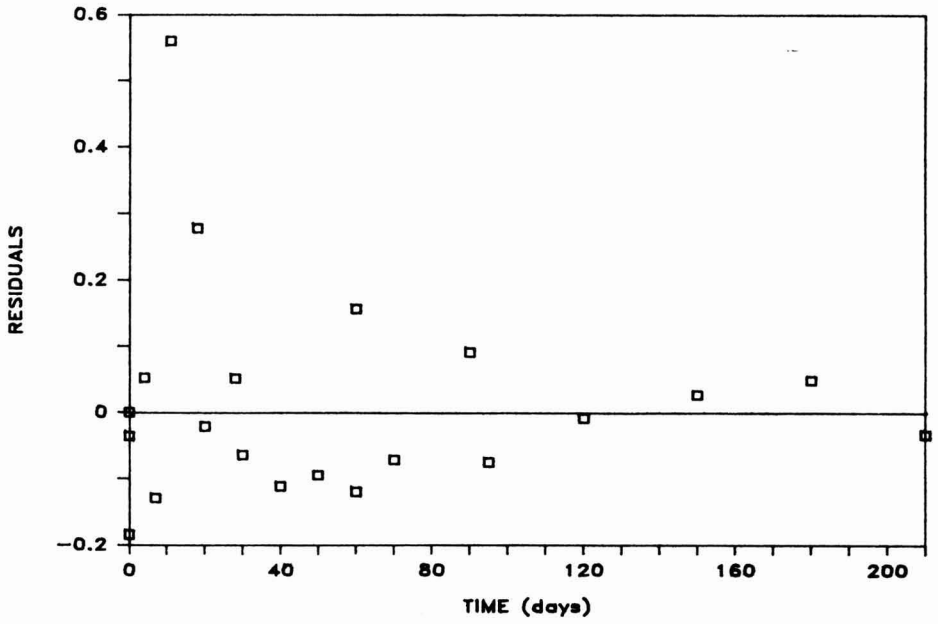

FIG. 1. PLOT OF THE RESIDUALS OF NONENZYMATIC BROWNING DERIVED FROM UTILIZING NONLINEAR LEAST SQUARES REGRESSION (METHOD 2)

Similar normal distribution of the residuals was reported for other cases (Haralampu et al. 1985). Therefore, it was concluded that weighted regression (although has its own merits; e.g., Arabshahi and Lund 1985), may be avoided due to the fact that the logarithmic transformation incorporated in method 2 obviates the need for further variance stabilization. The latter effect of the logarithmic transformation was discussed in detail elsewhere (Haralampu et al. 1985).

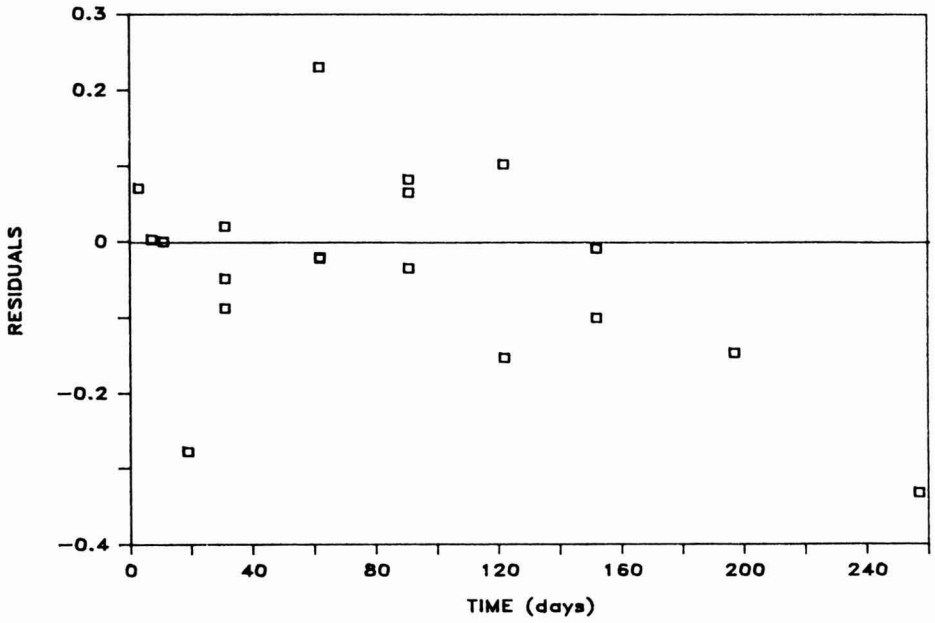

FIG. 2. PLOT OF THE RESIDUALS OF THIAMIN RETENTION DATA DERIVED FROM UTILIZING NONLINEAR LEAST SQUARES REGRESSION (METHOD 2)

It is worth noting that method 3 yielded completely different values, not only for the energy of activation and the pre-exponential factor, but also for the initial concentration. Also, the values of the latter were substantially different from the reported experimental data (Arabshahi and Lund 1985; Labuza and Kamman 1983). Hence, it was concluded that method 3 should be used only for those cases where the plot of the residuals shows some nonnormal distribution and/or skewness. No further analyses were carried out for method 3.

In this work, Co was considered to be a parameter that was estimated by the nonlinear regression methods. This approach was found to be more accurate than defining the initial concentration as 100% (Davies and Hudson 1981; Nelson 1983). Also, by adopting this approach, actual concentration data was used rather than the retention (or percent). The latter avoids the uncertainty introduced by dividing all concentration values by the initial concentration. The uncertainty associated with the initial concentration is at least the same order of magnitude as the uncertainty in any other concentration (Arabshahi and Lund 1985).

The Arrhenius parameters estimates should be judged on the size of the joint confidence region at 90% (i.e., $0.95^2 = \sim 90\%$). The latter is the

ellipsoid in which the true parameters probably exist together at a specified confidence level. The extremes of the 90% confidence ellipsoid region are not corresponding to the ends of the 95% confidence intervals (derived from a t-test) for the individual parameters. Since Ea and ln(ko) are so highly correlated, the ellipsoid is by far more accurate representation of the confidence region (Draper and Smith 1981; Hunter 1981). The region is constructed by considering both the variance and covariance of the parameters estimates, and by assuming that the estimates are from a bivariate normal distribution. Figures 3 and 4 depict the joint confidence region for the parameter estimates derived from method 1.

It is important to emphasize that the joint confidence region should be used rather than the individual confidence interval due to the high correlation observed between Ea/R and ln(ko).

The confidence contours for the nonlinear regression (method 2) would create some sort of a deformed ellipsoid. However, the complexity of the computation hampers its application as a routine statistical test. Furthermore, based on our knowledge, only one statistical package (i.e., TROLL; Haralampu *et al.* 1985) provides this test as a standard routine.

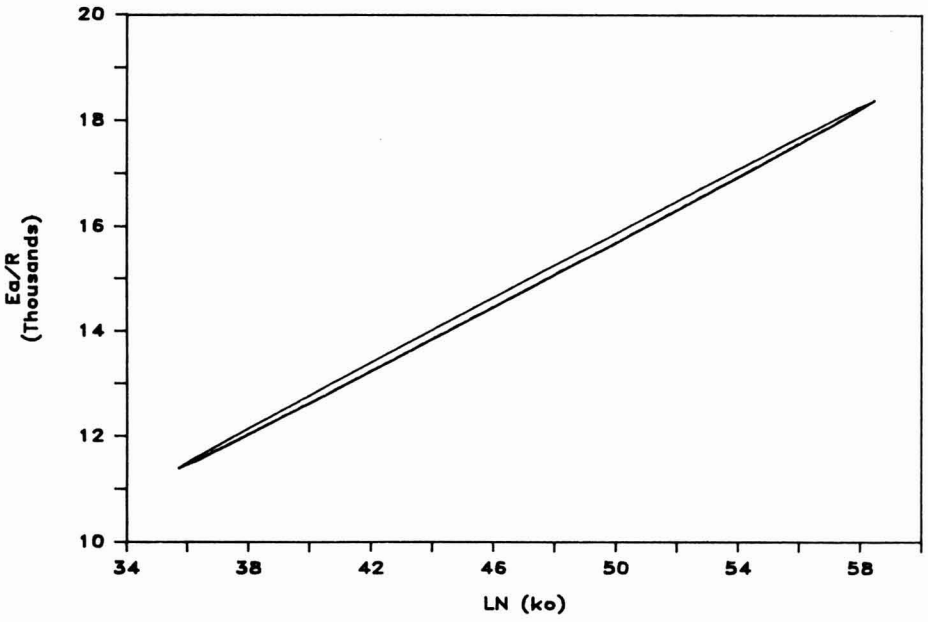

FIG. 3. JOINT CONFIDENCE REGION (90%) FOR Ea AND ko DERIVED BY TWO-STEPS LINEAR LEAST SQUARES (METHOD 1), FOR NONENZYMATIC BROWNING

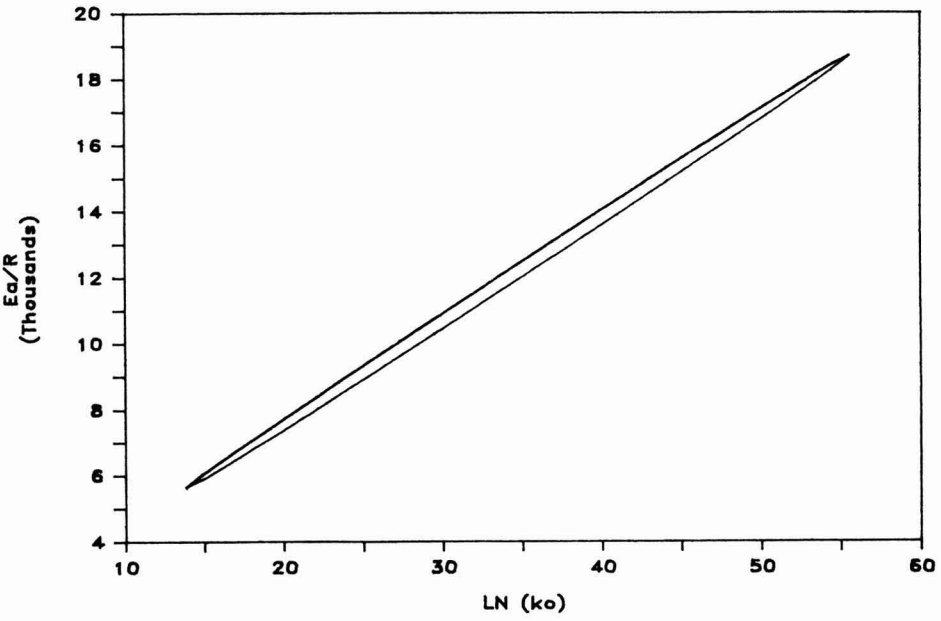

FIG. 4. JOINT CONFIDENCE REGION (90%) FOR Ea AND ko DERIVED BY TWO-STEPS LINEAR LEAST SQUARES (METHOD 1), FOR THIAMIN RETENTION

Yet, for this case, the appropriate extreme points on the confidence region were derived by a FORTRAN program which was written following the technique recommended by Draper and Smith (1981). The confidence contour, S, was approximated as follows:

$$S = SS[Co, \ln(ko), Ea/R][1 + n\,F(p, n-p, 1-q)/(n-p)] \quad (8)$$

and

$$SS[Co, \ln(ko), Ea/R] = \Sigma[\ln(C_i) - f]^2 \quad i = 1, \ldots, n \quad (9)$$

where:

f is the fitted nonlinear model defined in Eq. (5);
SS[Co, ln (ko), Ea/R] is the nonlinear least squares estimate of the fitted model;
n is the number of data points;
p is the number of parameters derived from the nonlinear least squares (i.e., p = 3);
100 (1 − q) % is the confidence contour (i.e., q = 0.1); and
F is the F-statistics from the F-distribution.

The extreme values of Ea/R and ln(ko) were derived using the COMPLEX method (i.e., nonlinear optimization; Saguy 1983). The appropriate values derived are summarized in Table 6. For most practical purposes the confidence region may be evaluated by linearization of the model. The latter is a standard option of most statistical software (Dixon 1983; SAS 1982).

The accuracy of a rate constant for the prediction was estimated by first locating the extremes associated with the boundary of the confidence ellipsoid (Fig. 3 and 4) for Ea and ln(ko). In method 2 the appropriate values were derived by the procedure outlined previously. The extreme values (denoted as low and high), and the average of Ea and ln(ko) as derived from the regression procedure (denoted mid.) were used to calculate the appropriate half-life and the rate constant. These values are summarized in Table 6. For a first-order reaction, the half-life time is independent of the concentration (i.e., $t_{1/2} = \ln(2.)/k$). In the case of a zero-order reaction, the half-life time was defined as the time required to reach an optical density of 0.2/g solids. This definition is obviously arbitrarily, and should be redefined for each case and system studied. Nevertheless, for comparison purposes, this value was quite appropriate.

Method 1 gave a much larger confidence region for Ea/R and ko. This large region resulted in a very wide span in the calculated values of Ea/R and k. Method 2 resulted in a much smaller confidence region and a better estimation of the half-life and the rate constant. The comparison also indicated that the traditional method for deriving the Arrhenius parameters agreed only partially with the values derived from method 2. Also, the half-life and k values were quite similar only for 35°C (i.e., the mid of the temperature range studied). This discrepancy not only projects the need for special attention when kinetics data is compared but also depicts the confidence of the determination. Hence, it may be difficult to connect energy of activation and entropy or any other thermodynamic quantities.

The confidence region of method 2 is much narrower when compared to the one derived from method 1. Yet, even in this improved case, prediction at temperature far from the average range used for deriving the kinetic model requires special caution. Since small errors in conducting the tests may be magnified if extrapolation is utilized. Hence, in this case, precaution should be reemphasized.

It is worth noting that method 2 is highly sensitive to the computation method. As the derivation of the parameters is based on nonlinear least squares, the procedures applied in the minimization of the sum of squares of the residuals, are very sensitive to the initial guess of the parameters and the criteria used for convergence. It is therefore strongly recommended that the computation should start at different initial

Table 6. Effect of the regression method on the half-life time and reaction rate constant for different reactions and temperatures (units of the zero and first-order rate constants are given in the text).

	ko	Ea/R	Half-life (days)			Reaction rate constant		
			25 C	35 C	45 C	25 C	35 C	45 C
Zero-order reaction								
Method 1								
Low	35.70	11384	223.7	64.7	20.2	0.082	0.282	0.902
Mid.	47.09	14888	322.4	63.7	13.9	0.057	0.287	1.311
High	58.46	18387	464.9	62.7	9.6	0.039	0.291	1.902
Method 2								
Low	45.97	14509	275.9	56.8	12.9	0.066	0.321	1.411
Mid.	48.39	15244	289.0	54.9	11.6	0.063	0.332	1.573
High	50.79	15969	298.6	52.4	10.3	0.061	0.347	1.774
First-order reaction								
Method 1								
Low	13.79	5637	117.2	63.4	35.7	0.006	0.011	0.019
Mid.	34.68	12162	318.2	84.6	24.4	0.002	0.008	0.028
High	55.59	18688	862.2	112.5	16.7	0.001	0.006	0.041
Method 2								
Low	34.95	12255	332.8	87.6	25.1	0.002	0.008	0.028
Mid.	37.67	13141	428.7	102.4	26.8	0.002	0.007	0.026
High	40.39	14032	561.6	121.7	29.1	0.001	0.006	0.024

guesses before any conclusion on the derived value is to be made. Also, the high correlation between the Arrhenius parameters (e.g., Fig. 3 and 4), may indicate the need for reparameterization. The latter may project the inadequacy of the Arrhenius model. Nevertheless, until a better model which is backed by kinetic theory is established, applying empirical models is not recommended.

In conclusion, the traditional analysis (method 1) gave the least accurate estimates for the Arrhenius parameters. This inaccuracy is due probably to the need to estimate many intermediate values and by not considering the data as a whole set. Also, method 1 estimates unnecessary parameters and carry out regressions on regression parameters. Method 2 (i.e., nonlinear least squares) proved to be superior, as it gave unbiased and precise estimation of the parameters. It is undoubtfully the method of choice, and should be applied in kinetic studies. Yet, even this method has limitations mainly due to the computation complexity. Method 3 was much more difficult to apply and the values derived were different from those of methods 1 and 2, and thus should be used only for cases which do not fulfill the assumption of normality.

ACKNOWLEDGMENT

We wish to thank Dr. Peter R. Nelson, Searle, Research and Development for the FORTRAN program of weighted nonlinear least squares. Also, we would like to thank Mr. Steve Fahrenholtz, The Pillsbury Company for the useful statistical discussions.

REFERENCES

ARABSHAHI, A. 1982. Effect and interaction of environmental and composition variables on the stability of thiamin in intermediate moisture model systems. Ph. D. Thesis, Univ. of Wisconsin, Madison, Wisconsin.

ARABSHAHI, A. and LUND, D. 1985. Consideration in calculating kinetic parameters from experimental data. J. Food Proc. Eng. *7*, 209.

COHEN, E. and SAGUY, I. 1983. The effect of water activity and moisture content on the stability of beet powder pigments. J. Food Sci. *48*, 703.

CONNORS, K. A., AMIDON, G. L. and KENNON, L. 1981. *Chemical Stability of Pharmaceuticals.* John Wiley & Sons, New York.

DAVIES, O. L. and BUDGETT, D. A. 1980. Accelerated storage tests on pharmaceutical products: effect of error structure of assay and errors in recorded temperature. J. Pharm. Pharmacol. *32*, 155.

DAVIES, O. L. and HUDSON, H. E. 1981. Stability of drugs: Accelerated storage tests. In: *Statistics in the Pharmaceutical Industry.* (C. R. Buncher and J. Y. Tsay, eds.), Marcel Dekker, New York.

DIXON, W. J. (ed.) 1983. *BMDP-Statistical Software.* University of California Press, Berkeley, CA.

DRAPER, N. and SMITH, H. 1981. *Applied Regression Analysis*, 2nd ed., John Wiley & Sons, New York.

GOLDMAN, M., HOREV, B. and SAGUY, I. 1983. Decolorization of beta-carotene in model systems simulating dehydrated foods. Mechanism and principles. J. Food Sci. *48*, 751.

HARALAMPU, S. G., SAGUY, I. and KAREL, M. 1985. Estimation of Arrhenius parameters using three least squares methods. J. Food Proc. Preserv. *9*, 129.

HUNTER, J. S. 1981. Calibration and the straight line: Current statistical practices. J. Assoc. Off. Anal. Chem. *64*, 574.

KIRKWOOD, T. B. L. 1977. Predicting the stability of biological standards and products. Biometrics *33*, 736.

LABUZA, T. P. and RIBOH, D. 1982. Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. Food Technol. *36*(10), 66.

LABUZA, T. P. 1972. Nutrient losses during and storage of dehydrated foods, CRC Crit. Rev. Food Technol. *3*(2), 217.

LABUZA, T. P. and KAMMAN, J. F. 1983. Reaction kinetics and accelerated tests simulation as a function of temperature. In *Computer-Aided Techniques in Food Technology.* (I. Saguy, ed.) Marcel Dekker, New York.

LUND, D. B. 1983. Consideration in modeling food processing. Food Technol. *37*(1), 92.

NELSON, P. R. 1983. Stability prediction using Arrhenius model. Com. Programs in Biomedicine *16*, 55.

SAGUY, I. 1979. Thermal stability of red beet pigments (betanine and vulgaxanthine I): Influence of pH and temperature. J. Food Sci. *44*, 1554.

SAGUY, I. 1983. Optimization of dynamic systems using the maximum principle. In *Computer-Aided Techniques of Food Technology.* p. 343. Marcel Dekker, New York.

SAGUY, I., KOPELMAN, I. J. and MIZRAHI, S. 1979. Simulation of ascorbic acid stability during heat processing and concentration of grapefruit juice. J. Food Proc. Eng. *2*, 213.

SAGUY, I., MIZRAHI, S., VILLOTA, R. and KAREL, M. 1978. Accelerated method for determining the kinetic model of quality deterioration during dehydration. J. Food Sci. *43*, 1861.

SAS, 1982. The NLIN Procedure. *SAS Users's Guide: Statistics.* Sas Inst. Cary, NC.

AUTHOR INDEX

SUBJECT INDEX

F N P

JOURNALS AND BOOKS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD SERVICE SYSTEMS, G. E. Livingston and C. M. Chang

JOURNAL OF FOOD BIOCHEMISTRY, H. O. Hultin, N. F. Haard and J. R. Whitaker

JOURNAL OF FOOD PROCESS ENGINEERING, D. R. Heldman

JOURNAL OF FOOD PROCESSING AND PRESERVATION, T. P. Labuza

JOURNAL OF FOOD QUALITY, M. P. De Figueiredo

JOURNAL OF FOOD SAFETY, M. Solberg and J. D. Rosen

JOURNAL OF TEXTURE STUDIES, M. C. Bourne and P. Sherman

JOURNAL OF NUTRITION, GROWTH AND CANCER, G. P. Tryfiates

Books

NEW DIRECTIONS FOR PRODUCT TESTING AND SENSORY ANALYSIS OF FOODS, H. R. Moskowitz

PRODUCT TESTING AND SENSORY EVALUATION OF FOODS, H. R. Moskowitz

ENVIRONMENTAL ASPECTS OF CANCER: ROLE OF MACRO AND MICRO COMPONENTS OF FOODS, E. L. Wynder *et al.*

FOOD PRODUCT DEVELOPMENT IN IMPLEMENTING DIETARY GUIDELINES, G. E. Livingston, R. J. Moshy, and C. M. Chang

SHELF-LIFE DATING OF FOODS, T. P. Labuza

RECENT ADVANCES IN OBESITY RESEARCH, VOL. III, P. Bjorntorp, M. Cairella, and A. N. Howard

RECENT ADVANCES IN OBESITY RESEARCH, VOL. II, G. A. Bray

RECENT ADVANCES IN OBESITY RESEARCH, VOL. I, A. N. Howard

ANTINUTRIENTS AND NATURAL TOXICANTS IN FOOD, R. L. Ory

UTILIZATION OF PROTEIN RESOURCES, D. W. Stanley, E. D. Murray and D. H. Lees

FOOD INDUSTRY ENERGY ALTERNATIVES, R. P. Ouellette, N. W. Lord and P. E. Cheremisinoff

VITAMIN B_6: METABOLISM AND ROLE IN GROWTH, G. P. Tryfiates

HUMAN NUTRITION, 3RD ED., F. R. Mottram

FOOD POISONING AND FOOD HYGIENE, 4TH ED., B. C. Hobbs and R. J. Gilbert

POSTHARVEST BIOLOGY AND BIOTECHNOLOGY, H. O. Hultin and M. Milner

THE SCIENCE OF MEAT AND MEAT PRODUCTS, 2ND ED., J. F. Price and B. S. Schweigert

U.S. Postal Service

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

Required by 39 U.S.C. 3685)

1A. TITLE OF PUBLICATION	1B. PUBLICATION NO.	2. DATE OF FILING
JOURNAL OF FOOD PROCESSING & PRESERVATION	4 5 6 4 7 0	Oct. 1, 1985
3. FREQUENCY OF ISSUE	**3A. NO. OF ISSUES PUBLISHED ANNUALLY**	**3B. ANNUAL SUBSCRIPTION PRICE**
Quarterly	4	$75.00

4. COMPLETE MAILING ADDRESS OF KNOWN OFFICE OF PUBLICATION *(Street, City, County, State and ZIP Code) (Not printers)*

155 Post Road E., Suite 6, POB 71, Westport, Fairfield, CT 06881

5. COMPLETE MAILING ADDRESS OF THE HEADQUARTERS OF GENERAL BUSINESS OFFICES OF THE PUBLISHER *(Not printer)*

155 Post Road E., Suite 6, POB 71, Westport, CT 06881

6. FULL NAMES AND COMPLETE MAILING ADDRESS OF PUBLISHER, EDITOR, AND MANAGING EDITOR *(This item MUST NOT be blank)*

PUBLISHER *(Name and Complete Mailing Address)*

Food & Nutrition Press, Inc., 155 Post Rd.E., #6, POB 71, Westport, CT 06881

EDITOR *(Name and Complete Mailing Address)*

Dr. Daryl B. Lund, Univ. of Wisconsin, Dept. of Food Science, 1605 Linden Drive, Madison, WI 53706

MANAGING EDITOR *(Name and Complete Mailing Address)*

7. OWNER *(If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Item must be completed.)*

FULL NAME	COMPLETE MAILING ADDRESS
Food & Nutrition Press, Inc.	155 Post Rd.E., #6, POB 71, Westport, CT 06881
John J. O'Neil	53 Stonehouse Rd., Trumbull, CT 06611
Michael J. Tully	3 North Slope, Union Gap Village, Clinton, NJ 08809
Kathryn O. & Christopher R. Ziko	24 Ingalls Ave., Norwalk, CT 06854
John J. O'Neil, Jr.	145 Dupont Place, Bridgeport, CT 06610

8. KNOWN BONDHOLDERS, MORTGAGEES, AND OTHER SECURITY HOLDERS OWNING OR HOLDING 1 PERCENT OR MORE OF TOTAL AMOUNT OF BONDS, MORTGAGES OR OTHER SECURITIES *(If there are none, so state)*

FULL NAME	COMPLETE MAILING ADDRESS
None	

9. FOR COMPLETION BY NONPROFIT ORGANIZATIONS AUTHORIZED TO MAIL AT SPECIAL RATES *(Section 423.12 DMM only)*
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes *(Check one)*

☐ (1) HAS NOT CHANGED DURING PRECEDING 12 MONTHS ☐ (2) HAS CHANGED DURING PRECEDING 12 MONTHS *(If changed, publisher must submit explanation of change with this statement.)*

10. EXTENT AND NATURE OF CIRCULATION	AVERAGE NO. COPIES EACH ISSUE DURING PRECEDING 12 MONTHS	ACTUAL NO. COPIES OF SINGLE ISSUE PUBLISHED NEAREST TO FILING DATE
A. TOTAL NO. COPIES *(Net Press Run)*	500	500
B. PAID CIRCULATION 1. Sales through dealers and carriers, street vendors and counter sales	0	0
2. Mail Subscription	328	330
C. TOTAL PAID CIRCULATION *(Sum of 10B1 and 10B2)*	328	330
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	36	35
E. TOTAL DISTRIBUTION *(Sum of C and D)*	364	365
F. COPIES NOT DISTRIBUTED 1. Office use, left over, unaccounted, spoiled after printing	136	135
2. Return from News Agents	0	0
G. TOTAL *(Sum of E, F1 and 2--should equal net press run shown in A)*	500	500

11. I certify that the statements made by me above are correct and complete

SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER

John J. O'Neil, President

PS Form 3526 July 1982

(See instruction on reverse)

NEW DIRECTIONS FOR PRODUCT TESTING AND SENSORY ANALYSIS OF FOODS

By **Howard R. Moskowitz, Ph.D.,** Moskowitz/Jacobs, Inc., Valhalla, New York

In his newest, just published book, Dr. Howard Moskowitz discusses the procedures currently used by marketing researchers and sensory analysts that will continue to be used for the next several

ANOTHER IMPORTANT BOOK

PRODUCT TESTING AND SENSORY EVALUATION OF FOODS: MARKETING AND R&D APPROACHES

By **Howard R. Moskowitz; Ph.D.,** Moskowitz/Jacobs, Inc., Valhalla, New York

This book fills a gap in our knowledge of how to properly conduct product tests. The past two decades have seen rapidly growing interest in product testing by marketers and by R&D. The novice and the professional can go to different sources to learn about product testing, but it requires the practitioner to add the essence of experience. Only through experience can the novice really learn how best to assess consumer reactions to foods.

The spectrum of product testing for both R&D and marketing is presented. Moreover, it presents much of the material in case-history form. The reader can see from these case histories the problem definition, the approach, and the outcome in clear register. The case-history approach to product testing does not set each fact down in black and white. Rather, it presents to the reader one approach to solving the problem. In that spirit, this book also presents one approach to product testing, gleaned from a decade and a half of the author's experience in a variety of contexts.

This book will be invaluable for food scientists and technologists, home economists and dietitians, marketers, students, and everyone interested in the sensory evaluation of food.

Testing; 6. Hedonic Scaling — Likes and Dislikes; 7. Food Product Optimization; 8. Product Measurement and Marketing Research; 9. Food Product Testing — Laboratory Law and Lore; 10. Correlating Sensory Ratings and Objective Measures of Foods; 11. Quality Control from the Sensory Point of View; Index.

Price $73.00 - 1983 - 605 Pages - ISBN 0-917678-16-8
See Special Price Offer on Order form

HOW TO ORDER

TO: Food & Nutrition Press, Inc., 155 Post Road East, Suite 6, P.O. Box 71, Westport, CT 06881 USA Tel: 203-227-6596; 203-227-8779

Please send me item(s) checked below.

- ☐ New Directions for Product Testing and Sensory Analysis of Foods $60.00
- ☐ Product Testing and Sensory Evaluation of Foods: Market and R & D Approaches $73.00
- ☐ Both Books: (1) New Directions . . . and (2) Product Testing . . . at special price $110.00
- ☐ Complete Brochure .....

Name _____ Title _____ Check enclosed $_____ Bill Co. PO NO. _____

Signature (For Charge Card) _____ American Express No. _____ Exp. date _____

Organization _____ Tel. _____ Master Charge No. _____ Exp. date _____

Address _____ VISA No. _____ Exp. date _____

City _____ State _____ Zip _____ Connecticut residents please add 7½% Sales Tax

years and possibly the next decade.

These procedures include coverage of product testing, package evaluation, pricing and concept optimization. As was done so successfully in the author's well-received current book: *Product Testing and Sensory Evaluation of Foods: Marketing and R&D Approaches,* case histories are used to demonstrate the new approaches and new technology in product testing and sensory analysis of foods.

Special emphasis is given to the marketing and business orientation aspects of product testing.

The book is divided into three parts comprising 17 chapters. There is a special bibliographic section, and numerous tables and illustrations are used throughout the book.

Food scientists and technologists, marketers, home economists, dietitians, students and those interested in product testing and sensory analysis of foods will find this book invaluable.

Price $60.00 – 1985 – 370 Pages – ISBN 0-917678-18-4

See Special Price Offer on Order Form

over

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper. Type tables neatly and correctly as tables are considered art and are not typeset.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Dr. D. B. Lund, Editor, Journal of Food Processing and Preservation, University of Wisconsin, Department of Food Science, 1605 Linden Drive, Madison, Wisconsin 53706 USA.

CONTENTS