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CONTENTS

MATHEMATICAL MODELING OF MICROWAVE HEATING BY THE METHOD OF DIMENSIONAL ANALYSIS

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ABSTRACT

Dimensional analysis was used to develop a predictive mathematical model for microwave heating of water. Water properties (density, specific heat, thermal conductivity and dielectric loss factor), power output and frequency of microwave, and geometric parameters were included in the dimensionless terms. Two household microwave cavities (donated by Tappan and Litton) were used. Time-temperature data were collected using a Luxtron fluoroptic temperature sensor (model 750) equipped with fiber optic probes. The predictive model, which was based on data taken in a Litton microwave cavity, was used to predict heating time of water in a Tappan microwave cavity. The results showed good agreement between the measured and the predicted time duration, with a high correlation coefficient ($R^2 = .99$).

INTRODUCTION

Although microwave thermal processing could replace conventional processing systems in many cases, there are few applications of the technology. Mudgett (1986) stated that the reasons for the difficulties encountered in microwave food processing are the lack of understanding of the interaction between food materials and microwave energy, and the lack of quantitative and predictive models relating the electrical properties of foods to transient time-temperature profiles that determine microbial safety and product quality.

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Time-temperature profiles within the products are influenced by both internal heat generation due to absorption of electrical energy from the microwave field, and heat transfer by conduction, convection and evaporation (Mudgett 1982). Microwave heating is complicated and is not easily modeled because the rate of energy absorption and energy distribution within the product is governed by the physical, thermal and electrical properties of the products and the variation with temperature during radiation.

The finite element method and related modifications based on conventional heat transfer equations have been used in modeling microwave heating of selected products (Ohlson and Bengtsson 1971; Nykvist and Decareau 1976; and Taoukis *et al.* 1987); however, no studies have been reported using the dimensional analysis technique.

This study focuses on investigating the possibility of using dimensional analysis in developing a mathematical model to characterize the microwave heating of food products.

BACKGROUND AND LITERATURE REVIEW

Microwave heating of foods results from the coupling of electrical energy from an electromagnetic field in a microwave cavity and its dissipation within the product. The mechanism is related to the dielectric properties and electrical transmission properties of the food material. The dielectric properties of a material are given by

$$\kappa^* = \kappa' - j\kappa'' \tag{1}$$

and

$$\tan \delta = \frac{\kappa''}{\kappa'} \tag{2}$$

where these and all variables used in this paper are defined in the nomenclature.

The parameter, κ^*, may be defined as the ratio of the equivalent capacitance, C, of a dielectric material to the capacitance C_o of the same capacitor of free space or vacuum (Mohsenin 1984). κ' is related to the amount of energy that can be stored in a material in the form of electric fields, while κ'' is related to the amount of energy a dielectric material can dissipate in the form of heat when the material is exposed to alternating electric fields.

The amount of power absorbed by a nonconducting food product is (Goldblith 1966)

$$P' = \omega \varepsilon_o E^2 \kappa'' \tag{3}$$

The increase in temperature of a material per unit time is given by

$$\frac{dT}{dt} = \frac{P'}{\rho C_p} \tag{4}$$

Transmission properties of basic interest are penetration depth and intrinsic impedance. These determine the ability of a product to attenuate absorbed energy and to coupling energy from the magnetron, respectively.

The energy coupling by foods is related to their intrinsic impedance in relative free space in the cavity, and is defined as (Von Hippel 1954):

$$\eta = \eta_o \left(\frac{1}{\kappa^*} \right)^{\frac{1}{2}} \tag{5}$$

The impedance mismatch between the product and free space determines the fraction of energy transmitted and reflected at the product surfaces. The greater the mismatch, the more incident energy is reflected from the air-food interface, and the less transmitted to the product.

The concept of intrinsic impedance provides a rational basis for "anomalous" heating effects in composite food systems with dissimilar phase properties; however, it is not clear how this property is related quantitatively to energy coupling by foods of widely varying size, geometry and chemical composition (Decareau 1985). Empirical models for energy coupling as a function of load volume may be expressed as (Decareau 1985)

$$P_o = \frac{P_m V^a}{K_m + V^a} \tag{6}$$

or

$$P_o = P_m (1 - e^{-bV}) \tag{7}$$

The models were developed based on calorimetric measurements of materials with properties similar to those of water, such as aqueous ions, oil and glass beads.

Microwave energy absorbed within a product is determined by an attenuation factor that is related to the product's dielectric properties. The attenuation factor (μ) determines the absorption of energy as a function of depth from the surface, as described by Lambert's law (Von Hippel 1954).

$$P_y = P_o e^{-2\mu y} \tag{8}$$

It is inversely related to the material's penetration depth (Z) which is the depth from the surface at which $1/e$ of the power at the surface is not absorbed and is given by the equation (Von Hippel 1954):

$$Z = \frac{1}{\mu} = \frac{\lambda}{2\pi} \sqrt{\frac{2}{\kappa'\left((1 + \tan \delta^2)^{\frac{1}{2}} - 1\right)}} \tag{9}$$

Mudgett (in Decareau 1985) illustrated, using compiled data obtained at 915 and 2450 MHz, that penetration depths increase as moisture content of the product and processing frequency are decreased. Therefore, nonuniform temperature profiles will occur in processing large, high moisture products at high frequency.

Thermal properties of foods also affect their heating characteristics in microwave processing. The basic thermal property is thermal diffusivity, defined by

$$\alpha = \frac{k}{\rho C_p} \tag{10}$$

The thermal diffusivity of a material is dependent on both its moisture content and temperature as well as composition and porosity (Singh 1982). Many products are nonhomogeneous, thus the thermal diffusivity may vary from one location to another within the same product. Singh (1982) cited the following models for estimating thermal diffusivity as a function of moisture content and temperature

$$\alpha_t = 0.088 \times 10^{-6} + (\alpha_w - 0.088 \times 10^{-6})W \tag{11}$$

$$\alpha_t = (0.057363W + 0.000288(T + 273)) \times 10^{-6} \tag{12}$$

Heat transfer in microwave processes can be represented by the same equations as those in conventional heating, with the addition of an internal heat generation term resulting from the coupling of electrical energy. Heat transfer by internal conduction, surface convection and moisture evaporation are all involved in the process. Modeling a microwave heating process using these equations requires an assumption of product homogeneity. Time-temperature profiles cannot be derived using these equations because the internal heat generation term is nonlinear and varies, due to the changing of dielectric behavior with time and temperature during the heating cycle. Heat transfer at the product surfaces is dependent on ambient air temperature, water vapor pressure at the surface of the product, water vapor pressure in the air and the air flow pattern in the microwave cavity.

The rate of internal heat generation may be estimated in finite difference analysis by using the average power absorbed in each differential element of

volume within the product during each time interval. Temperature and physical properties of the material within the volume element at each time interval must be known.

Discussion of Three Models Developed for Microwave Heating Analysis

Ohlson and Bengtsson (1971) developed a computer program to study combined dielectric and conductive heating of meats. They compared simulated and experimentally determined heating profiles in rectangular slabs of foods and food substitutes. The heat transfer at the surface was calculated according to the Plank (1959) method using a previous program developed by P. Leth-Moller of The Danish Meat Research Institute, Roskilde.

The authors made the following assumptions:

(1) The field is a plane wave propagating perpendicularly to the surface of the sample being heated.
(2) The electric field E decreased exponentially from the surface into the material according to the expression:

$$E = E_o e^{-\mu y} \tag{13}$$

They reported good agreement between experimental and simulated values. However, the model was limited to one dimensional heating. Uneven field distribution or standing wave patterns were not taken into account. The model was not valid for temperatures of 100C or greater, because the heat of evaporation was ignored.

Nykvist and Decareau (1976) investigated microwave and conventional heating of cylindrically shaped beef roasts, excluding end heating effects. They developed a computer program to simulate the process and reported good agreement between the model and experimental data. The cooking processes were carried out in a specially constructed oven with certain levels of energy sources (microwave power, steam and radiant heat) applied and automatically controlled by computer. The oven was thermostatically controlled within ±5C of the desired temperature.

Conductive losses and losses due to polarization were ignored, with the two dimensions of the problem radial and axial being considered individually. Experimental and computed time-temperature profiles showed good agreement for both cooking conditions of 300 W at 2450 MHz for 90 min and 285 W at 915 MHz for 50 min. At 600 W and 2450 MHz (similar to industrial use), substantial over-cooking and uneven cooking resulted, because the rate of energy input at this power level was much greater than the rate of thermal conduction. The authors concluded that roast geometry had a very strong influence on the diametrical temperature profile.

The thawing process is complex due to unsteady state heat transfer involving a phase change. The freezing and thawing of food takes place over a range of temperatures, rather than at a specific temperature. Thermal properties of foods subjected to freezing or thawing vary considerably with temperature. Irregular shape and heterogeneity of foods also complicate analysis. Taoukis *et al.* (1987) recently developed a model to predict the microwave thawing of frozen cylindrically-shaped beef using the Modified Isotherm Migration Method (MIMM). They reported good agreement between model results and experimental data.

The system was connected to a balance to monitor water loss. Microwave power, oven temperature and air flow rate were controlled in a specially constructed oven. Thermocouples were connected to a Honeywell recording potentiometer through a specially constructed oven door. A Luxtron fluorometric probe was inserted through the top of the oven to measure the sample's surface temperature. The surface temperature at each time step was calculated to determine the thawing time, defined as the time required for the thawing front to reach the center. The results showed good agreement between measured and predicted temperatures. The model is applicable to products with a narrow thawing temperature range.

THEORETICAL DEVELOPMENT

Dimensional analysis was used to develop meaningful dimensionless terms for a mathematical model to describe the microwave heating process. The pertinent variables are listed in Table 1. The time required to heat the material can be correlated to the other pertinent variables in the form

$$t = F\ (T_i, T - T_i, C_p, k, \rho, \kappa'', f, P, R, y, x, H) \tag{14}$$

Since Eq. 14 is differentiable over the range of variables considered, t can be expressed as:

$$t = C\ T_i^{n_1}(T - T_i)^{n_2} C_p^{n_3} \dots H^{n_{13}} \tag{15}$$

where the n_i are experimentally determined constants, and C is a coefficient that may be a function of the variables.

According to the Buckingham Pi Theorem, the number of dimensionless and independent terms required to express a relationship among variables of any system is equal to the number of variables involved minus the number of basic dimensions used to measure the variables. In this study there are 4 basic dimensions (mass, dimension M; length, dimension L; time, dimension T and temperature, dimension θ) and 13 variables. Therefore, there are 9 (13-4) in-

TABLE 1.
PERTINENT VARIABLES IN THEORETICAL DEVELOPMENT

Quantity	Symbol	Unit	Dimensions
Local temperature	T	C	θ
Initial temperature	T_i	C	θ
Specific heat	C_p	kJ kg^{-1}C^{-1}	$L^2T^{-2}\theta^{-1}$
Thermal conductivity	k	kJ s^{-1}m^{-1}C^{-1}	$MLT^{-3}\theta^{-1}$
Density	ρ	kg m^{-3}	ML^{-3}
Dielectric loss factor	κ''	dimensionless	1
Frequency	f	Hz	T^{-1}
Power supplied	P	kW	ML^2T^{-3}
Radius of container	R	m	L
Depth from the surface	y	m	L
Radial distance	x	m	L
Total height of sample	H	m	L
Duration of exposure	t	s	T

dependent dimensionless terms necessary to describe the microwave heating of a particular material.

Table 2 lists the independent dimensionless Pi terms for this study. There are other possible sets of dimensionless terms.

Equation 14 may be reduced to

$$\pi_1 = F(\pi_2, \pi_3 \ldots \pi_9) \tag{16}$$

and written in the general form

$$\pi_1 = C\pi_2^{c_2}\pi_3^{c_3} \ldots \pi_9^{c_9} \tag{17}$$

Equation 17 may be linearized as

$$\ln \pi_1 = \ln C + c_2 \ln \pi_2 + c_3 \ln \pi_3 + \ldots + c_9 \ln \pi_9 \tag{18}$$

TABLE 2.
LIST OF INDEPENDENT DIMENSIONLESS TERMS

Pi Term	Formula	Description
π_1	$f t$	Product of frequency and heating time
π_2	$\dfrac{T - T_i}{T_i}$	Ratio of temperature increase to initial temperature
π_3	$\dfrac{\rho y^3 f^3}{P}$	Ratio of kinetic energy transfer from the surface to the power supplied
π_4	$\dfrac{\rho C_p f\, y^3 (T - T_i)}{P}$	Ratio of the absorbed energy to the power supplied
π_5	$\dfrac{x}{R}$	Ratio of radial distance to radius of cylinder
π_6	$\dfrac{(T - T_i)k}{f^3 \rho\, y^4}$	Heat conduction per unit mass of product
π_7	κ''	Dielectric loss factor
π_8	$\dfrac{y}{H}$	Ratio of depth to height of product
π_9	$\dfrac{y}{x}$	Ratio of depth to radial distance

The constant and coefficients in Eq. 18 are determined by stepwise multiple regression.

A component equation is obtained by substituting mean values of the other π terms into the general equation while retaining one π term as a variable. The number of component equations is equal to the number of selected π terms included in the general equation.

MATERIALS AND METHODS

Distilled water was used as a testing material because its physical, thermal and dielectric properties are known. The specific heat, density and thermal conductivity of water were obtained from Holman (1976) and the dielectric loss factor of water at 3000 MHz was extracted from Mohsenin (1984).

Distilled water (200 g) at room temperature (23C-26C) was placed in a cylindrically shaped glass dish (10 cm in diameter and 5 cm in height). The dish was always placed at a premarked spot at the center of a Tappan microwave

cavity (36cm × 38cm × 17.5cm). The dish was then covered with a 4cm thick plastic temperature probe ''guide'' containing 13 predrilled holes for probe insertion and 8 slots for releasing pressure due to evaporation, and maintaining a constant pressure inside the dish during microwave exposure. The 13 holes are distributed in such a way that each is located in an equal volume segment of the sample (Fig. 1).

A Luxtron (Model 750) fluoroptic temperature sensor with four fiber optic probes was used to measure temperatures during microwave heating. Measured temperatures were transferred to a computer via an RS 232 serial port. Four temperatures were measured and recorded simultaneously at intervals of 4 s to obtain time-temperature data. These probes were inserted through small holes drilled from the back of the microwave and through the plastic probe guide to the selected locations in the sample. All four probes were located at the same depth and same radial distance. Thus, four replicate data were obtained if it is assumed that the electric field intensity is uniform at the surface. Figure 2 shows the experimental setup. The microwave oven used operates at a frequency of 2450 MHz, with 650 watts of output at power level 10.
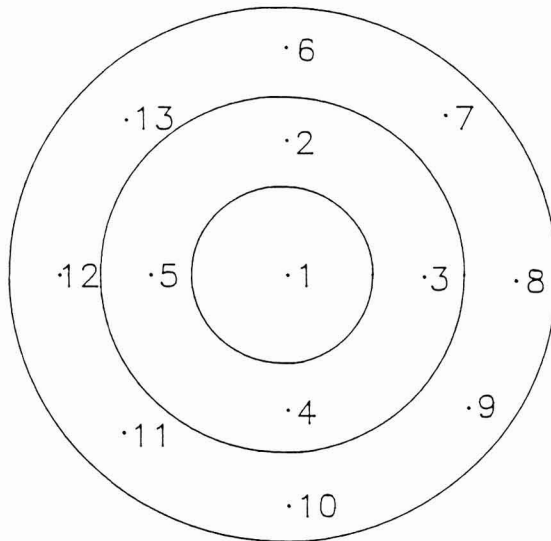
FIG. 1. TOP VIEW OF PLASTIC LID USED TO GUIDE TEMPERATURE PROBES
INTO PRE-DETERMINED LOCATIONS

Microwave cavity (36cm x 38cm x 27.5cm)
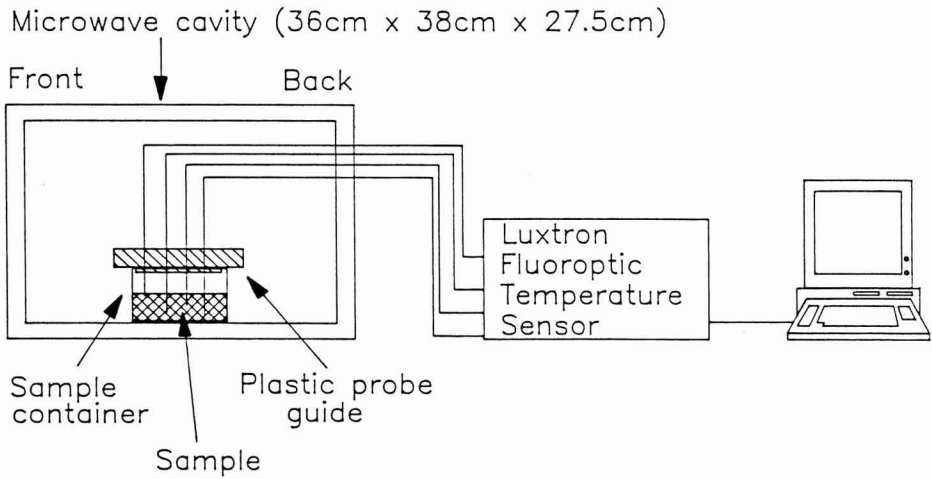
FIG. 2. SCHEMATIC OF EXPERIMENTAL SET-UP

TABLE 3.
EXPERIMENTAL DESIGN

Test No.	Power (kW)	Time Cycle (s)	Depth (m)	Radial Distance (m)
1	.520	100	.013	.025
2	.325	100	.013	.025
3	.520	100	.026	.025
4	.325	100	.026	.025
5	.520	100	.013	.007
6	.325	100	.013	.007
7	.520	100	.026	.007
8	.325	100	.026	.007

The experimental design for this study is listed in Table 3. The time-temperature data were recorded at selected locations except: (1) at the surface, to avoid the effect of water evaporation and convective heat transfer, (2) at the cylindrical wall to avoid edge effects, and (3) at the central point to avoid core heating effects. The water loss due to evaporation was small (less than .005%) when power levels between 5 and 8 were used. After each test, the microwave cavity and probes were cooled to room temperature.

To determine the validity of the model (which was based on data collected in a Tappan microwave cavity), the model was used to predict the results of an experiment carried out in a Litton microwave cavity (2450 MHz, 500 watts at power level 10), using the same procedure and experimental design. The power outputs at levels 10 and 7 respectively, were .50 and .35 kW, which were close to the .52 and .325 kW of the Tappan oven. The Litton cavity is smaller (30cm × 36cm × 19cm), therefore it is conceivable that the effect of cavity size may influence the comparative result. Also, the variation in power outputs may result in different electric field intensities for the two cavities and may give rise to differences in the temperatures profiles.

RESULTS AND DISCUSSION

The maximum temperature rise observed in 100 s of exposure was 76C, hence the physical and thermal properties may be assumed constant at the average of the initial and final temperatures. The values are:

Density, ρ	987.6 kg m^{-3}
Specific heat, C_p	4.175 kJ kg^{-1}C^{-1}
Thermal conductivity, k	6.45 x 10^{-4} kW m^{-1}C^{-1}

The dielectric loss factor (κ'') of water is highly dependent on temperature. Based on the data of Von Hippel (1954) at 3000 MHz, κ'' may be expressed as a function of temperature as

$$\kappa'' = 20.95e^{-0.0225T} \tag{19}$$

TABLE 4.
INITIAL AND FINAL TEMPERATURES AFTER 100 s

Test no.	Litton			Tappan		
	T_i	T_f	$T_f - T_i$	T_i	T_f	$T_f - T_i$
1	24.9	75.8	50.9	22.5	70.9	48.9
2	25.4	61.0	35.6	22.7	56.0	33.3
3	25.3	67.7	42.4	22.7	63.3	40.6
4	25.4	53.1	27.7	22.6	47.5	24.9
5	25.3	75.3	50.0	25.9	76.4	50.5
6	25.0	60.6	35.6	25.6	61.6	36.0
7	25.4	65.4	40.0	26.3	67.5	41.2
8	25.5	51.0	25.5	26.0	51.9	25.9

The initial and final temperatures observed in the experiments in the two cavities are given in Table 4. The final temperature is a function of the initial temperature, location within the sample and microwave power level.

The nine π terms were calculated and transformed into logarithmic numbers using a computer program written in Advanced Basic. Litton data and Tappan data were separately analyzed using the SAS statistical software program by stepwise multiple linear regression at the 0.15 significance level. The final models of the data sets are:

Litton:

$$\pi_1 = 11.252 \, \pi_2^{-.1306} \pi_4^{1.0277} \pi_5^{-.0157} \pi_7^{-.215} \pi_8^{-2.6098} \tag{20}$$

$R^2 = .9866$ (significance at P <.001)

Tappan:

$$\pi_1 = 1.10x\,10^{-5}\,\pi_2^{.779}\pi_4^{.862}\pi_6^{-1.0444}\pi_7^{-.995}\pi_8^{-6.273} \tag{21}$$

$R^2 = .9438$ (significance at $P < .001$)

The significance of exponent values in both models is at a probability level less than .005, but the model obtained from the Litton data gave a higher correlation coefficient than Tappan's. In this study, the smaller microwave cavity (Litton) appeared to have a more uniform electric field intensity than the larger cavity. The microwave beams passing through a waveguide are distributed by a special metal stirrer, revolving constantly, to all areas of the oven cavity. Some waves go directly to the material while others are reflected off the metal oven walls and oven bottom then are directed to the material. With the smaller oven cavity, the waves may reflect more frequently than the larger one and give rise to more uniform heating. However, other factors relating to the microwave design may explain the difference.

Comparing the two models, it is obvious that π_2, π_4, π_7, and π_8 are important terms which should be included. The difference between the two models is that the Tappan model contains π_6 while the Litton contains π_5. Because water has low thermal conductivity (k), π_6 which contains k may be considered unimportant. For other materials π_6 may be necessary. π_5, containing the radial distance effect, was found to have a small effect on the time-temperature profiles. However, π_5 is included in the model in order to make the model statistically reliable in terms of the C(p). This criterion is used to indicate the best regression model, and provides a useful parameter in addition to the correlation coefficient value. When the C(p) value is nearest the number of predictors (in this case the exponents of the model) the prediction is least biased. The Litton model not only has a higher correlation coefficient but is also less complicated than Tappan's. The Litton model was, therefore, used to predict the time required to heat water in the Tappan microwave.

The predicted times were calculated using the average temperature from four replicates. The measured and predicted times are presented in Fig. 3 and 4. The variations in predicted versus measured times may be a result of the greater variation in temperature data taken in the Tappan cavity. The assumption of a uniform electric field strength may also be invalid. Most of the data points from all 8 cases in the experimental design fall along the prediction (Fig. 3) line; the predictive equation shows excellent agreement with the average of the eight cases, with a high correlation coefficient ($R^2 = .99$) between the measured and the predicted values, at a significant level of $P < .001$. It can be concluded that the Litton model is applicable to other microwave cavities when used to analyze the material for which the model was derived.
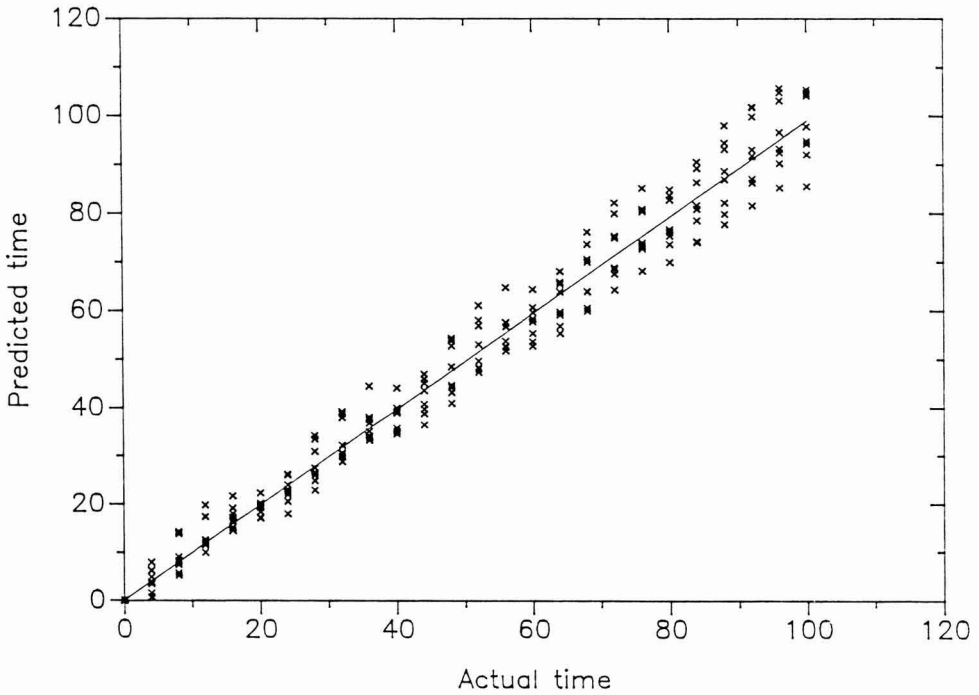
FIG. 3. PREDICTED VERSUS MEASURED PROCESSING TIME

The Litton model can be broken down into component equations by substituting the means of all π terms except one into the general model. The component equations are as follows:

$$\pi_1 = 9.346x\,10^{10}\pi_2^{-.1306} \tag{22}$$

$$\pi_1 = 23.88\pi_4^{1.0277} \tag{23}$$

$$\pi_1 = 9.78x\,10^{10}\pi_5^{-.0157} \tag{24}$$

$$\pi_1 = 1.534x\,10^{11}\pi_7^{-.215} \tag{25}$$

$$\pi_1 = 3.33x\,10^{10}\pi_8^{-2.6098} \tag{26}$$

Figures 5 through 9 show the plots of the component equations above. Several points are noteworthy from the graphs. Figure 5 shows that π_2 (the temperature
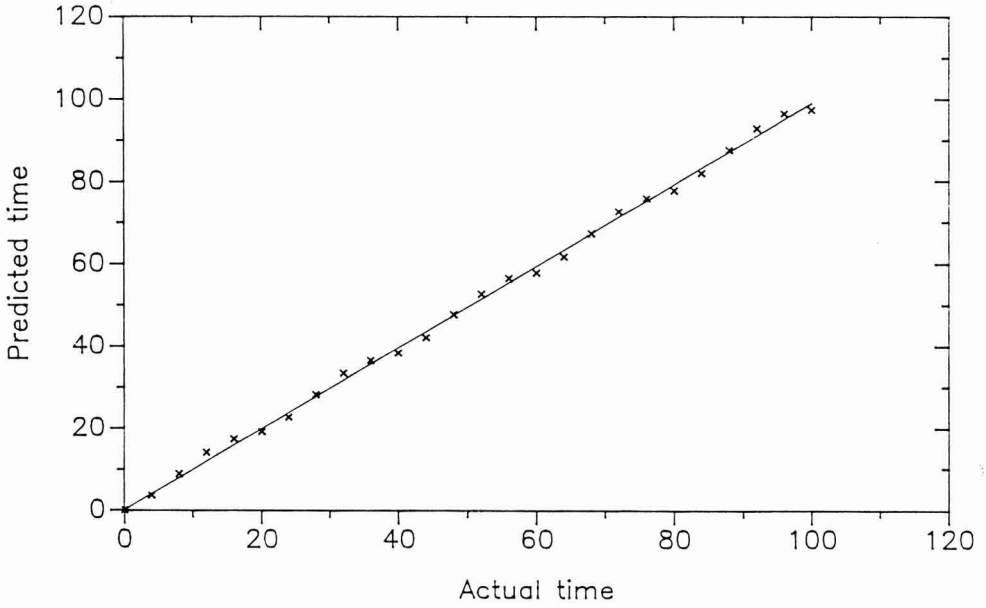
FIG. 4. PREDICTED PROCESS TIME VERSUS AVERAGE OF RECORDED DATA
FOR EIGHT CASES
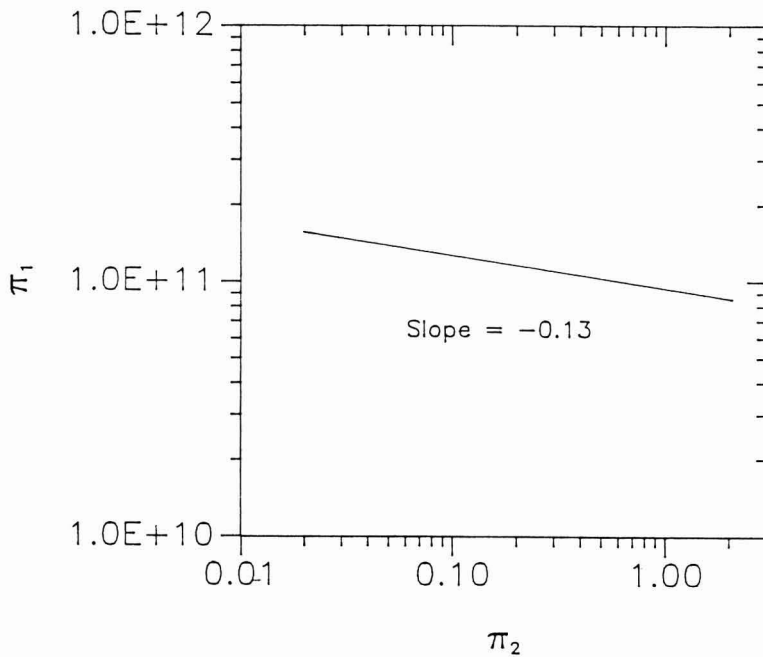
FIG. 5. SENSITIVITY of π_1 TO CHANGES IN π_2

term) is inversely related to π_1 (the time parameter), implying that as heating time increases, there is a slight decrease in the temperature of the sample. The reason for this apparent contradiction is that the exponent on π_2 is influenced by the π_4 term which also contains the temperature parameter. As Fig. 6 shows, the slope of the relationship between π_4 and π_1 (which is a measure of the amount of absorbed energy) is positive. The interaction of the two terms provides the expected relationship between time and temperature: the temperature rises as duration of exposure increases.

The dimensionless term π_5 is quite insensitive to π_1 (Fig. 7), implying that the radial distance has a small effect on the temperature profile. As a result, even when π_5 is deleted from the model, a fairly high correlation coefficient (R^2 of .986) is still obtained. As is to be expected, the dielectric loss factor (π_7) decreases with increases in temperature (Fig. 8). The most sensitive parameter is π_8 which accounts for the depth in the sample from which the temperature is recorded, and provides a measure of the resistance to energy transport (Fig. 9).

CONCLUSIONS

It has been demonstrated that dimensional analysis can be used in developing a predictive mathematical model for microwave heating analysis. The model
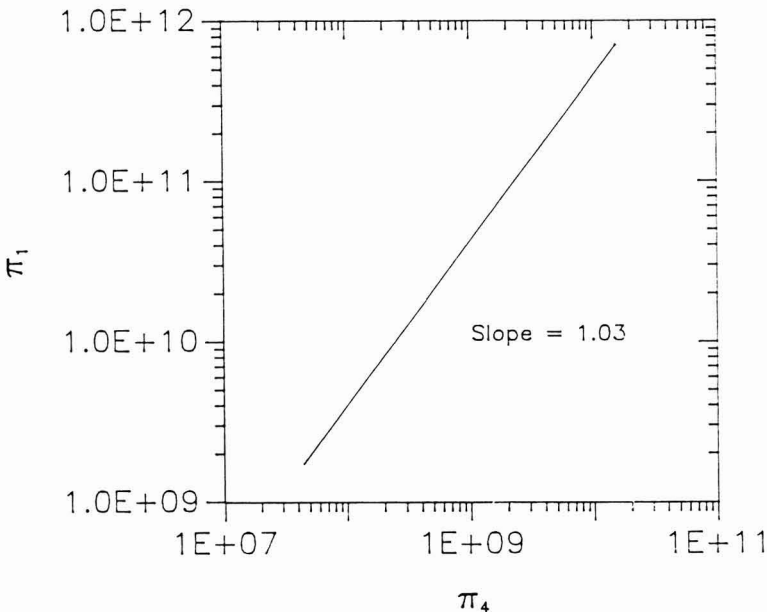
FIG. 6. SENSITIVITY OF π_1 TO CHANGES IN π_4
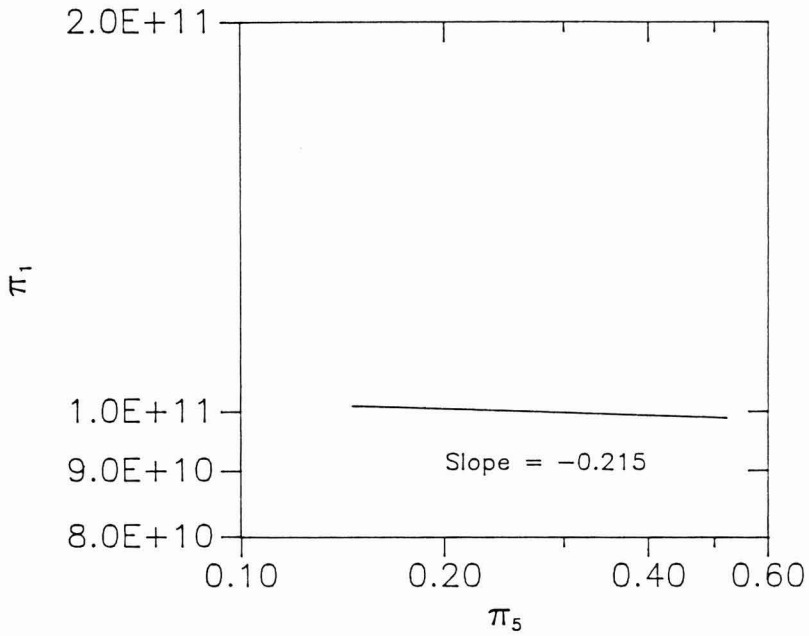
FIG. 7. SENSITIVITY OF π_1 TO CHANGES IN π_5
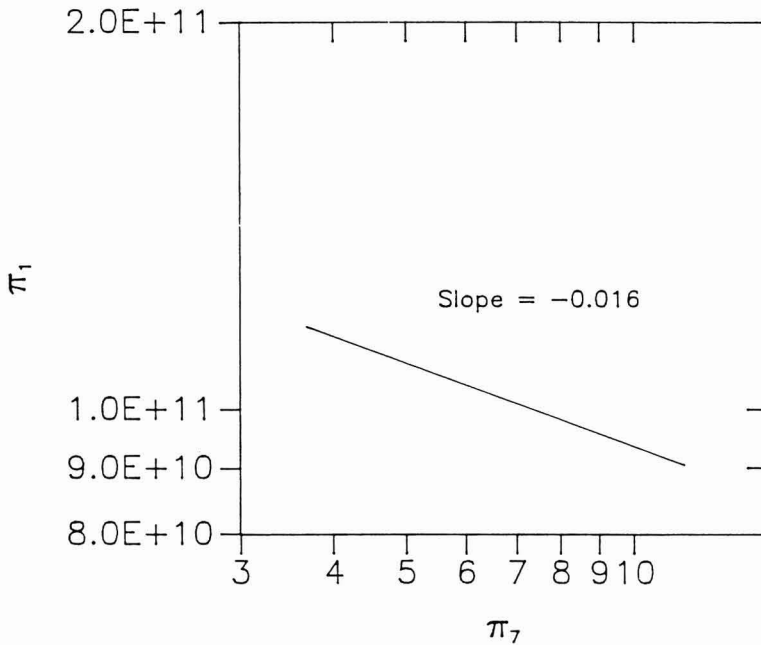
FIG. 8. SENSITIVITY OF π_1 TO CHANGES IN π_7
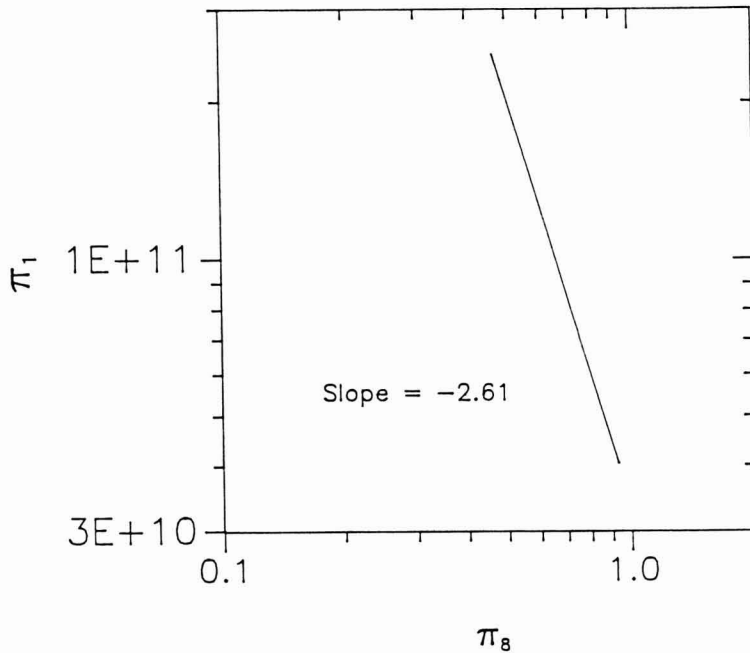
FIG. 9. SENSITIVITY OF π_1 TO CHANGES IN π_8

developed in this study shows very good agreement to experimental data. However, use of the model may be limited to water and other materials which are similar to water in terms of physical, thermal and electromagnetic properties. Further validation and/or modification of this model for other materials such as low moisture food products is highly recommended.

There are several difficulties encountered in dealing with real food materials. First, food products are normally multi-component. Second, their interaction with water makes the analyses more complicated; therefore, the moisture content of the sample, which was not included in this initial model, will have to be incorporated in subsequent analyses. The second problem encountered in the analysis of food materials is that the dielectric properties of foods are not easy to determine. Third, physical changes such as protein denaturation and starch gelatinization may occur during the microwave exposure and these changes can affect the absorption of electromagnetic energy. These factors also need to be considered in subsequent models.

In order to use the model to predict the process time required to achieve microbial safety and product quality, the location of the coldest point inside the

material and the maximum tolerant temperature for the particular material should be known. It is also desirable to predict the process time required to obtain a desired product mass-averaged temperature, as a function of product geometry, thermal, physical and electromagnetic properties, and process parameters. These factors are currently under study.

NOMENCLATURE

a	Empirical coupling constant, dimensionless
b	Empirical coupling constant, dimensionless
C	Empirical constant, dimensionless
C'	Empirical constant, dimensionless
c_i	Empirical constant, dimensionless
C_P	Specific heat, kJ kg^{-1}C^{-1}
E	Electric field intensity coupled by matched load, V m^{-1}
E_o	Electric field intensity coupled by unmatched load, V m^{-1}
f	Microwave frequency, Hz
H	Height of the tested material, m
k	Thermal conductivity, kW m^{-1}C^{-1}
k_m	Empirical coupling constant, dimensionless
P	Power output from the microwave, kW
P'	Power per unit volume, W m^{-3}
P_m	Power coupled by matched load, W
P_o	Power coupled by unmatched load, W
P_y	Power transmitted at depth y from the surface, W
R	Radius, m
T	Temperature, C
T_i	Initial Temperature, C
T_f	Final temperature, C
t	Time, s
V	Volume, m^3
W	Moisture content, % by weight
x	Radial distance from the edge, m
y	Depth from the surface, m
Z	Penetration depth, m

GREEK LETTERS

α	Thermal diffusivity, m^{2}s^{-1}
δ	Dielectric loss angle, radians

ϵ_o Dielectric constant of free space (8.854×10^{-12}), $F\ m^{-1}$

η Intrinsic impedance of dielectric, ohm

η_o Intrinsic impedance of free space, ohm

κ^* Relative complex permittivity, dimensionless

κ' Relative dielectric constant, dimensionless

κ'' Relative dielectric loss factor, dimensionless

λ Wavelength in free space, m

μ Attenuation factor, m^{-1}

ω Angular frequency, $rad\ s^{-1}$

π Dimensionless term, dimensionless

ρ Product density, $kg\ m^{-3}$

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FISH GEL FORMATION WITHOUT ADDED SALT: IMPROVEMENT VIA MIXED SPECIES

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ABSTRACT

A series of experiments were conducted to determine the effect on gel characteristics, in the absence of NaCl, of mixing a species known to make good salt-free gels (red hake) with a species which does not possess this property to the same extent (winter flounder). Results indicated that a small amount (1%) of red hake tissue added to flounder mince before washing can significantly modify the properties of the gels produced without added NaCl. Generally, there were greater effects on the eleastic properties of the gel as measured by fold test and Instron percent recoveries, than on hardness. Differences in response to hardness values suggest the possibility of selective modification to give a wider range of physical properties than is now available.

INTRODUCTION

Much interest has recently been focussed in the United States on the traditional Japanese process of making fish gels and, as a result, a significant industry has developed, and is continuing to develop. Much of the impetus for this use of fish proteins derives from their ability to form highly elastic gels, making it suitable for the manufacture of a variety of products.

In a recent paper, we reported that it was possible to produce a good fish protein gel without the use of the 2.5 to 3% added NaCl that is generally thought to be required (Hennigar *et al.* 1987). This property appears to be unique to fish proteins since good gels cannot be formed in the absence of salt with mammalian

species. Given current interest by American consumers in health and nutrition, we felt there should be considerable interest in the ability to form good protein gels, and ultimately products, from muscle tissue in the absence of salt. Consequently, we have investigated the process further.

Our earlier observations demonstrated that there was a species difference in the ability to form good gels in the absence of salt. In this study we address the question of whether a species that makes good gels in the absence of salt (red hake) can be used to improve the gel-forming properties, in the absence of salt, in a species which does not possess this property to the same extent (winter flounder).

MATERIALS AND METHODS

Sample Acquisition

Red Hake (*Urophycis chuss*) and black-backed flounder (*Pseudopleuronectes americanus*) were purchased from day boats operating out of Gloucester, Massachusetts during the period October through December, 1986. The whole fish were transported on ice to the University of Massachusetts at Amherst where they were hand-skinned and filleted on the same day. Fillets were placed in polyethylene bags and held on ice in a 4°C refrigerator overnight after which they were processed.

Washing and De-watering

The boneless fillets were ground through the 1/4 in. plate of a Toledo table model grinder. Mixing of species was done immediately after grinding by vigorously mixing by hand for 1 min. In those cases where a slurry was used, the appropriate amount of red hake was blended with 100 mL of distilled water for 1 min. The slurry obtained was then added to the flounder mince which was already being mixed in the bowl of a Hobart Model N-50 mixer set on high. The final ratio of added water to mince was 1:15. Mixing was continued for 1 min. An equivalent amount of distilled water was mixed into red hake and flounder controls prior to further processing. Timing of minces held before washing was begun when mixing was complete. Washing and de-watering of minces was done according to the methods outlined by Vareltzis and Buck (1987).

The de-watered mince was removed from the press and made into gels following a 2 h chill period at 2°C. Cryoprotectants were not added since no storage was involved.

Gel Production

Gels were made in 220g batches using a Cuisinart Food Processor (Model DLC-7PRO) for chopping. The well-chilled mince was placed in the bowl of

the food processor which had been previously chilled to $-25°C$. It was chopped at high speed for 2 min. This procedure ensured that the temperature of the fish paste was kept below 15°C at all times. Sodium chloride was not added to any of the gels in this process.

The resulting pastes were immediately transferred to a hand sausage stuffer (The Sausage Maker, Buffalo, N.Y.) and stuffed into nylon cylinders (2.1 cm × 13 cm). The ends were stoppered with cork and the cylinders were immersed first into a 40°C water bath for 30 min and then into a 90°C water bath for 20 min. The cylinders were removed from the second water bath and cooled by placing in a mixture of ice and water for 20 min. The gels were then removed from the cylinders, patted dry with paper toweling and wrapped in aluminum foil for overnight storage in a 4°C refrigerator, prior to subsequent testing.

Physico-chemical Measurements

The pH values of the initial mince, the de-watered mince, and the gel were determined by blending in a laboratory blender at high speed for 1 min a 10-g sample with 90 mL of distilled water. An electrode was placed into the resultant slurry, and the meter was allowed to equilibrate for 20 s. Duplicate samples were read.

Triplicate moisture determinations were made on initial minces and gels using a modification of the microwave method described by Pettinati (1975). The modification consisted of using Petri plates instead of jars, a 750w oven (Sharp, Carousel) rather than a 1000w oven, and a 3 min drying time rather than 2 min. Comparison of the modified procedure with the standard method (AOAC, 1980) yielded equivalent results. The moisture content was calculated from weight loss as a percent of the original sample weight.

The mechanical properties of the gels were determined by three different procedures.

The fold test, as reported by Kudo et al. (1973) and described by Hennigar et al. (1987) was performed on 3 mm thick slices of gel which were folded first in half and then in quarters, if possible. A score of 5 (AA grade) was awarded to a gel slice that could be folded into quarters without cracking at the fold lines. No cracks on folding in half was equal to a 4 (A grade). Some cracking when folded in half was equal to a 3 (B grade). Breaking in half when folded in half was equal to a 2 (C grade) and breaking of the slice into fragments from finger pressure was equal to a 1 (D grade). Values reported are the average of four slices per gel.

The shear force of a gel was determined with a Warner-Bratzler Shear (Model 2000) equipped with a dial calibrated in 0.02 lb increments to 10 lb. Gel cylinders 2.1 cm in diameter were sheared perpendicular to their long axes. Values reported indicate maximum force required to shear the gel and are the average of five measurements per sample.

Instron recovery and deformation values were determined with an Instron Universal Testing Machine (Model TM) using procedures described by Johnson *et al.* (1980). Duplicate specimens 1.5 cm long by 2.1 cm in diam were taken from each gel and subjected to 60% deformation since beyond this level specimens fractured.

Statistical Analysis

Multiple comparisons were performed on the data using the Statistical Package for the Social Sciences (SPSS) (Nie *et al.* 1975).

RESULTS

The effects on chemical and physical properties of flounder gels containing 25% red hake added before or after washing and held 0-30 min prior to chopping are shown in Table 1. The pH and moisture values of the initial minces were in expected ranges although the pH of the mixed species was lower than expected based on the data for the individual species. The moisture contents of the control gels were significantly different from each other and reflected the species difference in initial mince moisture contents. The moistures of mixed species gels were additive based on the proportions of the two species.

Over twice as much force was required to shear flounder gels as compared to red hake gels indicating greater hardness or rigidity. These findings were supported by the Instron percent deformation values which were proportionately similar. The rigidity of the mixed gels was greater than could be accounted for from additive effects alone, i.e., rigidity of the mixed gels were more like those of the flounder than would have been predicted, especially the sample which was mixed before washing and held 30 min before further processing.

Results that are more a reflection of elasticity (fold test and percent recovery) than hardness also did not show simple additive effects. However, in this case the results were skewed towards the characteristics of red hake. From the standpoint of elasticity, which is one of the more important gel criteria, best results were obtained by washing mixed minces immediately after mixing (no holding) or mixing species after separate washings.

Our original hypothesis (see Discussion) was based on the proposition that perhaps an enzyme-catalyzed reaction was involved in the ability of the fish proteins to form good gels in the absence of NaCl. This proposition is consistent with the data in Table 1 which indicate that adding muscle tissue from a fish with the ability to form good fish gels in the absence of NaCl (red hake) to that of a species that does not have this property (winter flounder) improved the gelforming properties of the latter.

TABLE 1.
AVERAGE pH, MOISTURE, AND MECHANICAL PROPERTIES OF CONTROLS AND
FLOUNDER GELS CONTAINING 25% RED HAKE ADDED BEFORE OR AFTER
WASHING AND HELD PRIOR TO CHOPPING

	INITIAL MINCES		GELS				
TREATMENTS	pH	% MOISTURE	% MOISTURE	SHEAR FORCE	% DEFORMATION	FOLD TEST	% RECOVERY
Red Hake	7.05^a	84.6^a	80.8^a	1.24^a	11.8^a	4.0^a	66.3^a
Flounder	6.83^b	78.1^b	74.0^b	3.28^b	29.0^b	2.0^b	50.0^b
Fl + RH$_{25}$ (0 min)[1]	6.79^b	80.2^c	76.3^c	2.77^c	20.5^c	4.0^a	70.3^c
Fl + RH$_{25}$ (30 min)	--	--	75.3^{bc}	3.05^b	24.8^d	3.5^{ab}	58.9^d
Fl + RH$_{25}$ PW (0 min)[2]	--	--	75.5^{bc}	2.44^d	22.5^e	4.0^a	68.5^e

[1]Fl = flounder; RH = red hake. The subscript on RH indicates percent of red hake added by hand
mixing. Times in parentheses refer to time mixed minces were held prior to washing.

[2]PW (post wash) indicates that species were mixed after each was separately washed and dewatered
rather than before.

Note: Values in columns not sharing a common superscript letter are significantly different
(P<0.05).

The effect of adding 5% minced red hake to minced flounder while holding the samples before washing for various periods of time was examined (Table 2). If an enzyme was involved, one would expect to see a change with time of holding.

Decreases in gel moisture content were reflected in an increase in gel hardness as measured by shear force and percent deformation, especially after a period of holding for 30 min. It has been reported that water content influences measurements of hardness more than those of elasticity; the latter is more affected by protein quality (Hamann and Lanier 1987). Although fold test scores and percent recoveries for the gels prepared from mixed species were not significantly improved, there was a trend towards improvement at 0 time. Five percent added red hake significantly decreased the hardness of the mixed gel with no holding time. However, hardness increased with increasing time of holding before washing. Changes in percent deformation were similar to those for shear force values. There was also a decrease in the moisture content of the gels with increased time of holding. Since moisture content is a major determinant of hardness measurements in fish protein gels (Hamann and Lanier 1987), it is not surprising that there is an increase in both shear force and percent deformation with holding time. It is possible that the decrease in moisture is due to changes in proteins occurring during the holding period which affect their water binding capacity.

As the concentration of red hake added to the mixture is reduced, mixing becomes a very critical process. This is particularly true since much of the enzymic activity in red hake muscle has been demonstrated to be associated with particulate fractions (Phillippy 1984). Thus, to improve mixing in subsequent experiments, the slurry procedure, described earlier, was employed to facilitate rapid distribution of the small amount of red hake throughout the flounder mince.

The effect of adding red hake without a holding period at 5%, 2% and 1% of the mixed gels was studied (Table 3). The elasticity of the test gels as measured by percent recoveries was improved at all levels of added red hake. Direct comparison of the different experiments performed in these studies are difficult because of the variations that were observed in the control samples, i.e., pure flounder or red hake. Nevertheless, it appeared that for 5% red hake samples, slurry mixing (Table 3) produced a superior gel from the standpoint of the fold test score when compared with the hand-mixed 5% sample (Table 2). The hardness of the mixed gels as measured by shear force was reduced by all the levels of red hake. There was, however, little effect of the added red hake on percent deformation.

Since there appeared to be a positive effect on the elastic properties of the mixed fish gels with levels of red hake as low as 1%, the effect of this level of red hake at various holding times was examined (Table 4). Significant changes were observed in the mixed gels with 1% added red hake muscle tissue with the greatest effects at no holding time. There was a tendency for the properties of

TABLE 2.
AVERAGE pH, MOISTURE, AND MECHANICAL PROPERTIES OF CONTROLS AND
FLOUNDER GELS CONTAINING 5% RED HAKE ADDED AS MINCES AND HELD
VARIOUS TIMES BEFORE WASHING

	INITIAL MINCES		GELS				
TREATMENTS	pH	% MOISTURE	% MOISTURE	SHEAR FORCE	% DEFORMATION	FOLD TEST	% RECOVERY
Red Hake	6.94^a	80.8^a	78.5^a	1.69^a	10.0^a	4.0^a	71.0^a
Flounder	6.67^b	79.8^a	76.1^a	2.42^b	18.0^b	2.5^b	62.0^b
Fl + RH$_5$ (0 min)[1]	6.76^b	79.0^a	76.2^a	2.08^c	12.0^c	3.0^{ab}	67.0^c
Fl + RH$_5$ (30 min)	--	--	73.5^b	2.42^b	16.0^b	2.8^{ab}	66.0^c
Fl + RH$_5$ (60 min)	--	--	69.9^c	2.44^b	17.0^b	2.5^b	64.0^b

[1]Fl = flounder; RH = red hake. The subscript on RH indicates percent of red hake added by hand mixing. Times in parentheses refer to time mixed minces were held prior to washing.

Note: Values in columns not sharing a common superscript letter are significantly different (P<0.05).

TABLE 3.
AVERAGE pH, MOISTURE, AND MECHANICAL PROPERTIES OF CONTROL AND
MIXED SPECIES GELS CONTAINING 1, 2, or 5% RED HAKE WITHOUT HOLDING

TREATMENTS	INITIAL MINCES		GELS				
	pH	% MOISTURE	% MOISTURE	SHEAR FORCE	% DEFORMATION	FOLD TEST	% RECOVERY
Red Hake	6.92^a	83.0^a	81.6^a	1.69^a	5.3^a	5.0^a	81.0^a
Flounder	6.70^b	79.8^b	75.5^b	2.15^b	8.3^b	3.3^b	64.5^b
Fl + RH_1 (0 min)[1]	--	--	80.3^{ac}	1.92^c	8.5^b	3.8^{ab}	69.0^c
Fl + RH_2 (0 min)	--	--	79.3^c	1.97^c	8.7^b	3.8^{ab}	69.6^c
Fl + RH_5 (0 min)	--	--	79.8^c	1.89^c	7.7^b	4.5^{ab}	70.3^c

[1]Fl = flounder; RH = red hake. The subscript of RH indicates the percent of red hake added (as slurry) to the mixture. Samples were processed with no holding period.

Note: Values in columns not sharing a common superscript letter are significantly different ($p < 0.05$).

TABLE 4.
AVERAGE pH, MOISTURE, AND MECHANICAL PROPERTIES OF CONTROLS AND
FLOUNDER GELS CONTAINING 1% RED HAKE ADDED AS MINCES AND HELD
VARIOUS TIMES BEFORE WASHING

| | INITIAL MINCES | | GELS | | | | |
	pH	% MOISTURE	% MOISTURE	SHEAR FORCE	% DEFORMATION	FOLD TEST	% RECOVERY
TREATMENTS							
Red Hake	6.97^a	84.2^a	82.8^a	1.05^a	3.9^a	4.8^a	71.0^a
Flounder	6.70^b	79.2^b	73.9^b	2.39^b	12.2^b	3.0^b	67.0^b
Fl + RH$_1$ (0 min)[1]	--	--	81.9^{ac}	1.86^c	7.3^c	4.5^a	74.0^c
Fl + RH$_1$ (30 min)	--	--	80.8^c	1.95^c	10.0^d	4.0^{ab}	71.0^a
Fl + RH$_1$ (60 min)	--	--	81.2^c	2.10^d	11.3^b	3.5^{ab}	70.0^a

[1]Fl = flounder; RH = red hake. The subscript on RH indicates the percent of red hake (added as slurry). Times in parentheses refer to time mixed minces were held prior to washing.

Note: Values in columns not sharing a common superscript letter are significantly different (P<0.05).

the mixed gels to revert to values closer to that of the flounder gels with increased time of holding of the mixed flounder and red hake minces before washing. This meant a decrease in the elasticity (fold test and percent recovery) and an increase in the hardness (shear force and percent deformation) with time of holding. These results indicate that if this is an enzymic effect, it occurs relatively quickly since the major changes are observed with the "0 min holding time". However, in the samples subjected to the "0 min holding time", there is still a significant amount of time in which the flounder and red hake are in contact before the first wash is complete. Indeed, it may be that the factor(s) in the red hake which is responsible for inducing the change in the flounder gels is not sufficiently removed after the first wash.

Finally, the effect of holding red hake muscle after mincing and before further processing was determined (Table 5). There was some increase in hardness with holding, as measured by percent deformation, with little change in elasticity. It would seem, therefore, that the results obtained in Table 4 were due to an interaction of the red hake mince with that of the flounder, i.e., the changes observed in Table 5 with red hake alone were insufficient to explain the results observed in the mixed gels with 1% red hake.

DISCUSSION

We previously reported (Hennigar *et al.* 1987) that red hake (*Urophycis chuss*), cod (*Gadus morhua*), and winter flounder (*Pseudopleuronectes americanus*) formed excellent gels with or without washing when 2.5% NaCl was added to the washed minces prior to heating. We reported, moreover, that fish gels could be formed in the absence of added salt if the muscle tissue was washed prior to the production of the gels. Also, the ability to form good gels (as measured by the fold test) in the absence of salt was species-dependent since red hake formed the best gels (score of 5) while cod formed gels with a fold score of 4 and flounder of 3. The ability to form these gels was unrelated to the amount of contractile protein present.

Although we do not have an explanation, on a molecular basis, as to why these fish proteins can form good gels in the absence of salt, it seemed there was a relationship between the ability to form good gels in the absence of salt and the ability to decompose trimethylamine-N-oxide (TMAO) to dimethylamine and formaldehyde. Red hake has a very active system for decomposing TMAO while that of cod is considerably less; flounder, a nongadoid species, does not break down TMAO to any significant extent (Lundstrom *et al.* 1982). Also, it has been recently demonstrated in our laboratory (Aug 1987) that formaldehyde is capable of interacting with side chains on the myosin molecule making the myosin more susceptible to denaturation under some conditions without inducing a significant amount of covalent crosslinking between the molecules. Wicker *et*

TABLE 5.
AVERAGE pH, MOISTURE, AND MECHANICAL PROPERTIES OF RED HAKE GELS
MADE FROM MINCES HELD 0, 30, or 60 MIN BEFORE WASHING

TREATMENTS	INITIAL MINCES		GELS				
	pH	% MOISTURE	% MOISTURE	SHEAR FORCE	% DEFORMATION	FOLD TEST	% RECOVERY
Red Hake (0 min)	7.25	83.5	82.8[a]	1.41[a]	3.2[a]	5.0[a]	75.0[a]
Red Hake (30 min)	--	--	82.9[a]	1.61[a]	3.9[b]	4.8[a]	74.0[a]
Red Hake (60 min)	--	--	82.3[a]	1.58[a]	3.8[b]	4.8[a]	71.0[b]

Note: Values in columns not sharing a common superscript letter are significantly different ($p < 0.05$).

al. (1986) obtained results during a study of the relation of thermal transitions in myosin to gel rigidity that indicated an increase in effective hydrophobicity occurred just prior to the onset of gelation. This parameter may be equated to an increase in denatured protein. Myosin is the major protein which is involved in gelation of fish muscle proteins (Akahane *et al.* 1984).

The results of the experiments reported here indicate that a small amount of red hake tissue can significantly modify the properties of the gels produced without salt from flounder muscle proteins. This means either that a small amount of protein from the red hake muscle had a predominant influence on the properties of the gel in the presence of a large amount of the flounder muscle proteins, or there is some other factor(s) in the minced red hake muscle which is capable of modifying the properties of the proteins of the flounder muscle. It is difficult to conceive of as little as 1% of the proteins being capable of conferring physical attributes on the gel as a whole; therefore, the latter explanation seems more reasonable.

The previously noted relation between the ability to confer the property of improved gelation and the ability of the tissue to decompose TMAO raises the possibility that the latter process may be responsible for the former. This poses the question of whether the TMAO degradation system of red hake muscle is sufficient at 1% added red hake tissue to modify the properties of the other 99% of the gel proteins. Red hake muscle tissue contains a level of TMAO degrading enzyme in its microsomal fraction more than sufficient to break down the TMAO of stored red hake muscle (Parkin and Hultin 1982). This estimate was based on recovered microsomal protein only and thus is an underestimation. In addition, the calculation did not take into account the presence of soluble activity that had also been shown to be present in the muscle tissue of red hake (Phillippy 1984). The conclusion is that red hake muscle tissue contains a high level of TMAO degrading enzyme and that the level of enzyme is not the rate-limiting factor in TMAO breakdown.

The concentration of TMAO is not rate-limiting since we have found that the K_M values for TMAO in the microsomal enzyme system is about 3 mM for the aerobic system of Fe and reducing agent such as ascorbate or cysteine, and about 20 mM for anaerobic decomposition by flavin mononucleotide (FMN) and NADH (Parkin and Hultin 1986). TMAO concentrations for most saltwater fish are well above these values (Hebard *et al.* 1982).

Most likely, the rate-limiting factor that controls the breakdown of TMAO in red hake muscle is the concentration of cofactors of the reaction, either NADH and flavin or iron and reducing agent such as ascorbate or cysteine (Landolt and Hultin 1982; Banda and Hultin 1983; Parkin and Hultin 1986). These critical cofactors occur at roughly the same concentration in all fish muscle tissue, as they do amongst the tissues of most higher organisms (Long 1961). What this means is that the rate-limiting components for TMAO breakdown in red hake

muscle are present at comparable concentrations in most saltwater fish species. It could be predicted, therefore, that it should be possible to add tissue from a catalytically active species to a nonactive species without diminishing the enzymic reaction rate of TMAO decomposition until the point is reached where the cofactors no longer are rate-limiting, i.e., the point where the enzyme becomes rate-limiting. That this indeed might be the case was demonstrated by mixing the muscle of active and nonactive species at a ratio of 1:4 active to inactive species and observing undiminished production of DMA from TMAO (Dingle *et al.* 1977). Although we have not made the analyses for flounder muscle, the cofactor concentrations involved in TMAO degradation reactions found in red hake flesh are in some cases below the K_m values for the reaction. In other cases, there is no saturation of the reaction but instead, there is a continuous increase of activity as the cofactor concentration is increased until it is well above the concentration that might be expected to be found in fish muscle tissue (Parkin and Hultin 1982; 1986; Phillippy 1984). Thus, TMAO breakdown is highly dependent on cofactor concentrations. Thus, if flounder muscle behaved in a similar way to red hake, one would not expect major decreases in cofactor concentration over the period of time during which the red hake muscle was mixed with flounder prior to removal of the cofactors by washing and dewatering.

In practical terms, the amount of active species, i.e., red hake, that must be added to the non-active species may be dependent on the efficiency of mixing as much as the absolute TMAO degrading activity in the flesh. Since a significant portion of the degrading activity is associated with the particulate fraction, it would make it particularly difficult to distribute this activity equally throughout the flesh. Our work reported here indicates that adding the mince as a slurry gave some improvement in final gel properties as measured by the standard commercial fold test.

Modification of the flounder muscle proteins by the addition of minced red hake muscle affects different physical properties in different ways. Generally, there were greater effects on the elastic properties of the gel as measured by the fold test and per cent recoveries than there were on gel hardness. An increase in elastic properties of the gel is generally considered to indicate improved quality of the gel (Hamann and Lanier 1987). The difference in response to the hardness values indicates the potential for selectively modifying fish gels to give a wider range of physical properties than is now available.

The results reported in this paper do not shed any light on the fundamental question of why and how good gels can be formed from fish muscle proteins in the absence of salt. The effect on gel formation of adding an active TMAO degrading species to that of a nonactive species in the presence of salt should also be examined since if a change in the property of fish gels formed without salt occurs via a modification of the proteins, it could be expected that gels prepared in the presence of salt could also be affected by such a system.

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SOFTENING OF FISH BONE. II. EFFECT OF ACETIC ACID ON SOFTENING RATE AND SOLUBILIZATION RATE OF ORGANIC MATTER FROM FISH BONE

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ABSTRACT

The rates of fish bone softening and solubilization of organic matter from it were determined in dilute acetic acid of concentration ranging from 0.0% to 3.0% at temperatures ranging from 80°C to 140°C. The first-order constant of softening, $k_h(min^{-1})$, was described by the following experimental equation.

$$k_h = (8.8 \times 10^8 + 2.8 \times 10^9 \sqrt{C_a})exp(-93000/(RT))$$

where T is absolute temperature, °K, C_a is acetic acid concentration, %, and R is the gas constant, $8.314J\ K^{-1}mol^{-1}$. The hardness of the bones was approximately proportional to the fourth power of the normalized weight of residual organic matter (the ratio of organic matter in the cooked bone to that in the raw bone). On the other hand, the dissolution of ash from the fish bone was hardly observed.

INTRODUCTION

Recently many people were taking thought that fish is good for keeping themselves in good condition. It is very difficult to take the bones out of fish, especially small fish. If it were possible to eat bone and all, the utilization of fish would be expanded as food rather than as feed. A previous paper has dealt with thermal softening of fish bone in water (Ishikawa *et al.* 1987). One of the traditional

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Japanese ways of softening bone is to soak a fish like sardine in vinegar. Watanabe *et al.* (1985) observed the accelerating effect of acetic acid on softening in cooking of fish bone.

Ishikawa *et al.* (1987) has shown that softening of fish bone and solubilization of organic matter in it are interrelated. On the other hand, it has been recorded that the mechanical strength of bones of mammals correlates to their ash contents (Bell *et al.* 1947; Weir *et al.* 1949; Ascenzi *et al.* 1972) and that calcium salts can be satisfactorily dissolved out from fish bone tissue in 2%-6% hydrochloric acid at 0°C-15°C for production of ossein (Hinterwaldner 1977).

The purposes of the present work are to know which of the reactions, dissolution of ash and solubilization of organic matter, causes the softening of bone as well as to describe quantitatively the effect of acetic acid concentration.

MATERIALS AND METHODS

Materials, apparatus, and methods, as well as the terms used to describe the results, were the same as those in the previous paper (Ishikawa *et al.* 1987), except that fish bones were heated in aqueous solutions of acetic acid.

Changes of hardness of fish bone in 0.0%, 0.040%, 0.20%, 0.75%, 1.5%, 3.0% acetic acid solutions were determined at temperatures at 80°C, 100°C, 120°C and 140°C. Changes of hardness, organic matter and ash in 3.0% acetic acid solution were determined in other experiments to examine the correlation between organic matter or ash and hardness.

The spines of mackerels landed at Hachinohe, Japan in October 1985 were used as bone samples. The mackerels were stored at $-20°C$ until they were thawed at room temperature before use.

Spines of known weight (about ten pieces) were put in a stainless tube to which dilute acetic acid was added. The sealed tube was heated for a certain period of time. After the time, the tube was immediately cooled in tap water, the content was washed with distilled water and subjected to measurement of hardness, organic matter and ash. The definition and measurements of hardness were the same as those described by Watanabe *et al.* (1985) and by Ishikawa *et al.* (1987). The hardness was defined as the cutting force, $F(N)$, divided by the cutting width, $a(m)$. The cutting force was defined as the maximum load applied on the razor blade when the sample had been cut. After the measurement of hardness, all spines cooked at each experimental condition were collected in a small aluminum cup and oven-dried at 105°C for 8 h and weighed. Then the dried matter was ignited at 600°C for 8 h and ash left behind was weighed. The weight was called residual ash. The weight of dry matter minus ash was defined as residual organic matter, and the normalized weight of residual organic matter, η, was defined as the residual organic matter after cooking divided by the organic matter in the raw bone.

RESULTS AND DISCUSSION

Softening Rate and Solubilization Rate

After the fish bones were heated in acetic acid solution of various concentrations at different temperatures during various periods of time, their hardness and contents of organic matter and of ash were determined. The results are shown in Fig. 1 to 5. The figures show that softening of fish bone as well as solubilization of organic matter, proceeds as a first-order reaction and that there is a regular increase in the softening rate as the acid concentration increases. The first-order reaction rate constants for softening and for solubilization of organic matter, k_h (s^{-1}) and k_o (s^{-1}), were obtained from the slopes of the straight lines drawn by the linear regression method applied to the points in the figures. On the other hand, ash content hardly decreased even in cooking in hot acetic acid solution as shown in Table 1.
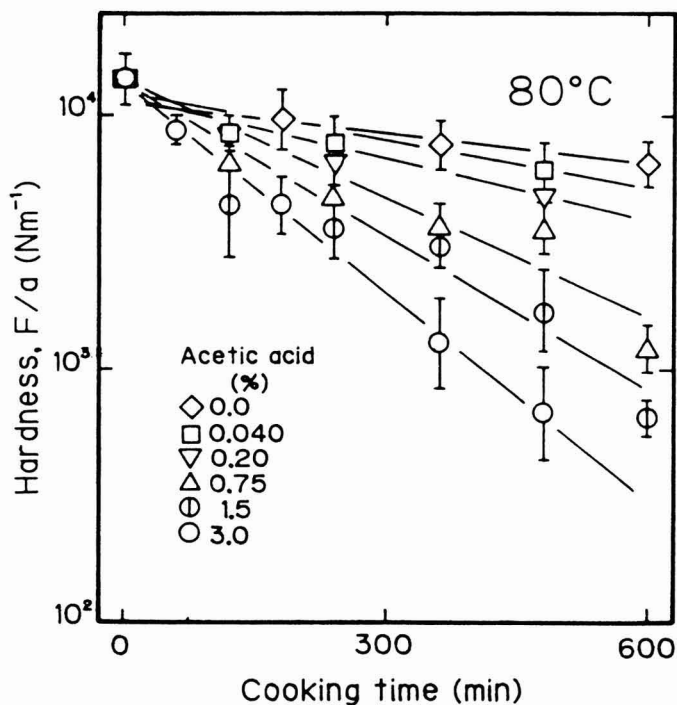

FIG. 1. SOFTENING OF MACKEREL SPINE IN ACETIC ACID SOLUTION OF VARIOUS CONCENTRATIONS AT 80°C The vertical bars on the data points are standard deviations.

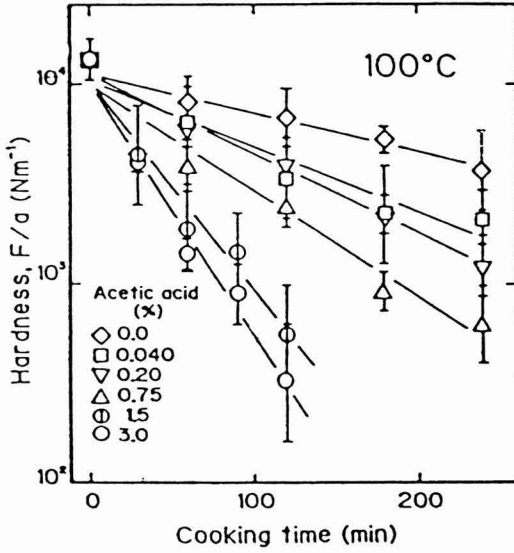M. ISHIKAWA, S. MORI, H. WATANABE and Y. SAKAI

FIG. 2. SOFTENING OF MACKEREL SPINE IN ACETIC ACID SOLUTION OF VARIOUS
CONCENTRATIONS AT 100°C The vertical bars on the data points are standard deviations.
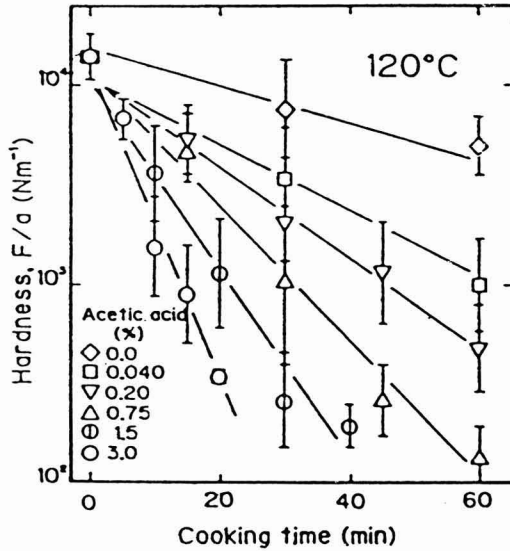

FIG. 3. SOFTENING OF MACKEREL SPINE IN ACETIC ACID SOLUTION OF VARIOUS
CONCENTRATIONS AT 120°C The vertical bars on the data points are standard deviations.
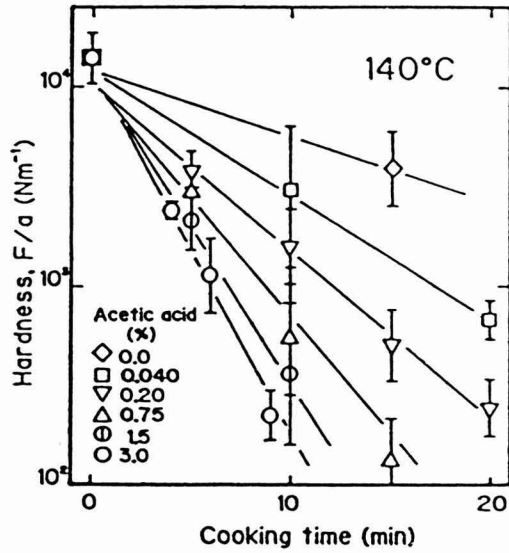
FIG. 4. SOFTENING OF MACKEREL SPINE IN ACETIC ACID SOLUTION OF VARIOUS CONCENTRATIONS AT 140°C The vertical bars on the data points are standard deviations.
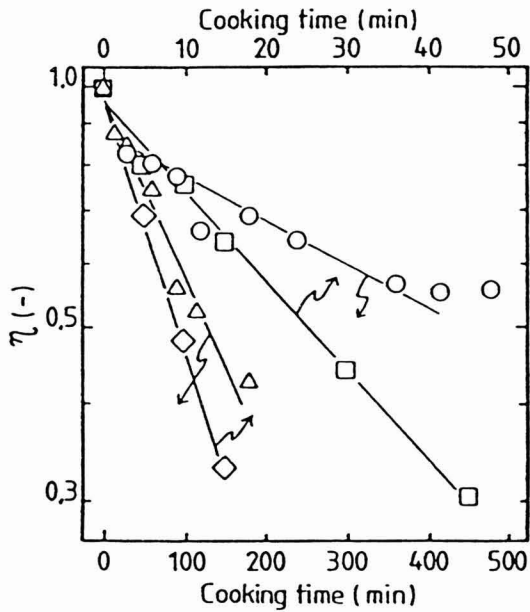

FIG. 5. SOLUBILIZATION OF ORGANIC MATTER FROM FISH BONES IN 3.0% ACETIC ACID SOLUTION AT VARIOUS TEMPERATURES

TABLE 1.
THE PERCENTAGES OF ASH REMAINED AFTER COOKING IN 3.0% ACETIC
ACID SOLUTION

Temp.($^{\circ}$C)	Cooking time(min)											
	5	10	15	30	60	90	120	180	240	360	420	480
80				86	87	89	78	89	88	90	95	101
100			96	97	97	88	95	97	93			
120	89	91	85	85								
140	93	91	89									

When the logarithms of rate constants, k_h and k_o, were plotted against the reciprocal of the absolute temperature, Fig. 6 was obtained. If the best fit line is drawn visually through a group of points at each concentration of acetic acid, parallel straight lines were obtained at successive intervals. This shows that the effect of temperature on softening, as well as on solubilization of organic matter, follows the Arrhenius law. The activation energies in both reactions are the same and the value is 93 kJmol^{-1}.
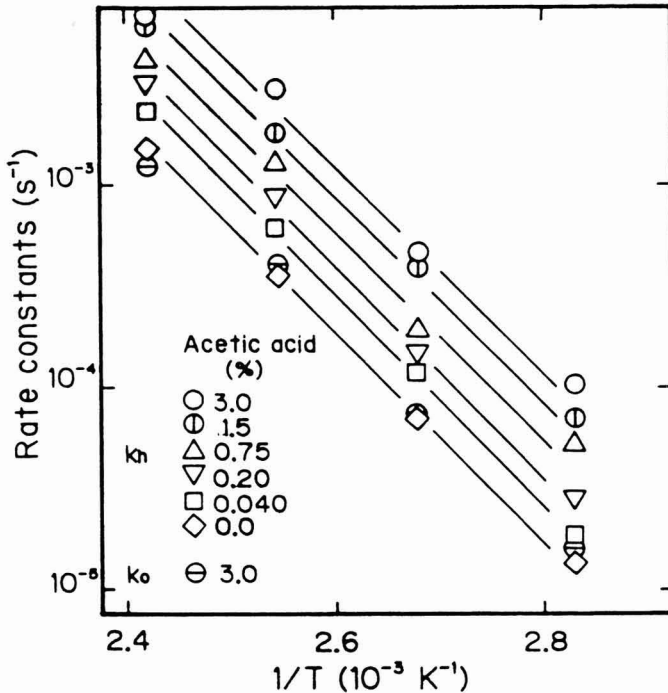

FIG. 6. EFFECT OF TEMPERATURE ON SOFTENING RATE OF MACKEREL SPINE AND SOLUBILIZATION RATE OF ORGANIC MATTER FROM MACKEREL SPINE IN VARIOUS CONCENTRATIONS OF ACETIC ACID SOLUTION

When the frequency factor in the Arrhenius equation describing k_h at each acetic acid concentration was plotted against the square root of the acetic acid concentration, C_a(0.0%-3.0% w/w), a straight line was obtained, as shown in Fig. 7. The straight line indicates that the frequency factor, A_h, is described by the empirical Eq. (1).

$$A_h = 8.8 \times 10^8 + 2.8 \times 10^9 \sqrt{C_a}$$

(1)

Consequently the softening rate constant of fish bone in cooking in hot acetic acid solution is described by the empirical Eq. (2).

$$k_h = (8.8 \times 10^8 + 2.8 \times 10^9 \sqrt{C_a})\exp(-93000/(RT))$$

(2)

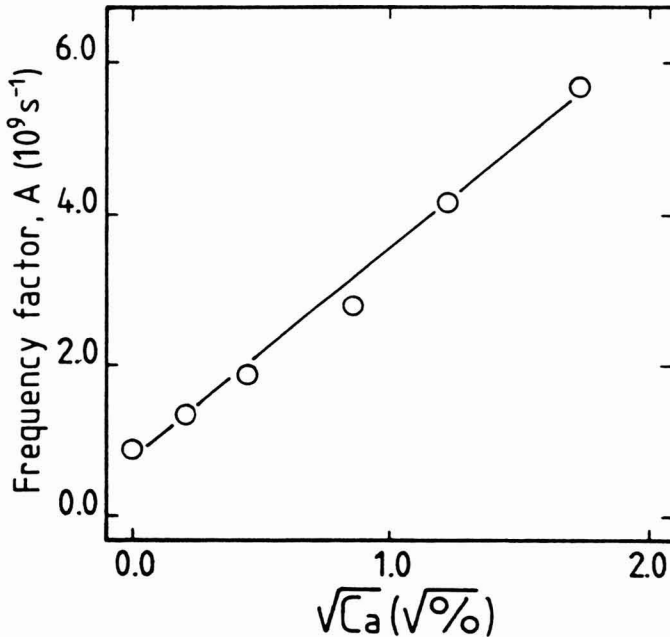where T is absolute temperature, (353K-413K), R the gas constant, 8.314 $JK^{-1}mol^{-1}$.

FIG. 7. EFFECT OF ACETIC ACID CONCENTRATION ON FREQUENCY FACTOR OF SOFTENING RATE CONSTANT OF MACKEREL SPINE

Some previous papers have recorded the kinetic parameters for the thermal softening as follows: activation energies for fish bone and some vegetables are 93.2 kJmol^{-1} (Watanabe *et al.* 1985)[1] and 77.7kJmol^{-1} − 118 kJmol^{-1} (Rao and Lund 1986).

It is interesting that acetic acid affects only the frequency factor and does not affect the apparent activation energy. Similar phenomenon was observed in the hydrolysis of cellulose and hemicellulose, and in the decomposition of mono-saccharide (Kobayashi and Sakai 1968).

Relationship Between Hardness and Organic Matter

The softening rate, r_h (Nm^{-1}s^{-1}), and the solubilization rate, r_o (s^{-1}), can be described as follows:

$$r_h = [A_h \exp\{-E_h/(RT)\}] \times (F/a)$$
(3)

$$r_o = [A_o \exp\{-E_o/(RT)\}] \times (\eta)$$
(4)

where E is an apparent activiation energy, A is a frequency factor, R is the gas constant, and subscripts "h" and "o" denote softening of fish bone and solu-bilization of organic matter, respectively. Integration of (3) and (4) with respect to time, followed by elimination of time gives the relation between F/a and η as follows:

$$F/a = (F/a)\big|_{t=o} \eta^{f(T)}$$
(5)

where

$$f(T) = (A_h/A_o)\exp\{-(E_h-E_o)/RT\}$$
(6)

Since E_h nearly equals E_o in size, as shown in Fig. 6, Eq. (5) becomes

$$F/a = (F/a)\big|_{t=o} \eta^{(A_h/A_o)}$$
(7)

Equation (7) suggests that a linear relationship exists between log(F/a) and log (η) independently of the temperature. The correlation between the hardness and

[1]The values of frequency factors in Watanabe *et al.* (1985) should be multiplied by 4.88×10^{11}.

the normalized weight of residual organic matter is depicted in Fig. 8. The straight line in Fig. 8 is expressed by

$$F/a = 1.4 \times 10^4 \eta^{4.1}$$

(8)

Water cooking of fish bone in the previous work (Ishikawa *et al.* 1987) led to the same value of 4.1 for the index A_h/A_o as in the present work.

CONCLUSIONS

The conclusions to be drawn from the present results are as follows. (1) The empirical Eq. (3) described the first-order reaction rate constant of fish bone softening as a function of acetic acid concentration (0.0%-3.0%) and temperature (80°C-140°C). (2) There was a regular decrease in the hardness of fish bone as the normalized weight of residual organic matter decreases, as shown in Eq. (5). (3) Acetic acid cooking hardly dissolved ash from fish bone.
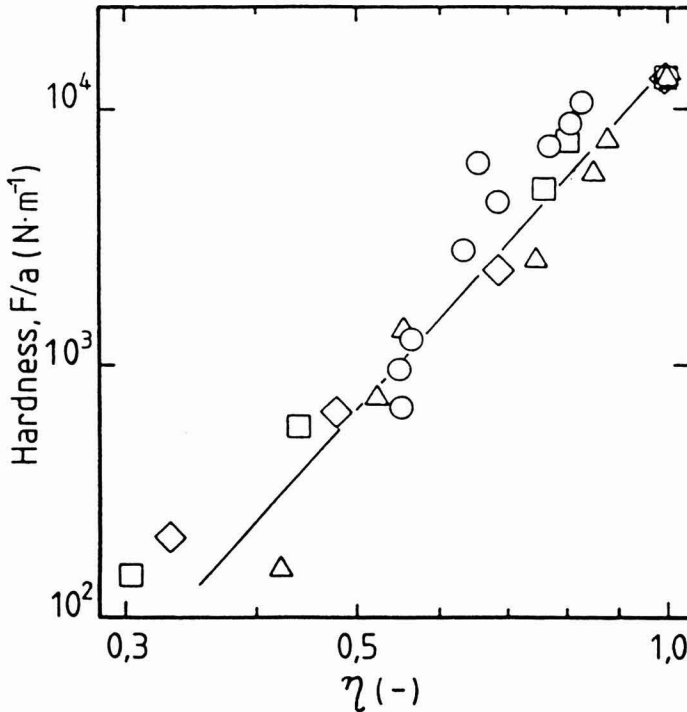

FIG. 8. CORRELATION BETWEEN HARDNESS, F/a, AND NORMALIZED WEIGHT OF RESIDUAL ORGANIC MATTER, η, IN 3.0% ACETIC ACID SOLUTION ○:80°C, △:100°C, □:120°C, ◇:140°C.

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PROPERTIES OF STEAM BLANCHED MAIZE FLOUR AS A CONSTITUENT OF WEANING FOOD

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ABSTRACT

Pregelatinized maize flour, produced from steamed grains and fortified with 30% soybean flour, was evaluated for its reconstitution ability, water hydration capacity and nutritive quality. The mixture, which was found to reconstitute easily on mixing with hot water, had water hydration capacity of 1.05 mL/g, a 14.5% protein and an 85% pepsin digestibility. The properties of the mixture indicated that blanching could be applicable in the preparation of pregelatinized maize flour for the manufacture of instant weaning food.

INTRODUCTION

Protein-Energy Malnutrition (PEM) is a major contributory factor to the high infant mortality rate in most developing countries and is attributed to the poor availability of nutritionally adequate weaning foods (Brown 1978 and Jansen 1982). For instance, ogi, a traditional weaning food in Nigeria, is prepared by wet-milling and wet-sieving of maize, sorghum or millet (Akinrele 1970). The product (ogi) is of poor biological value as it does not support growth in rats and is implicated in the development of kwashiorkor, a nutritional disease in children weaned mainly on ogi porridge (Collis *et al.* 1962; Eka 1978 and Ekpenyong 1980). Although attempts have been made at producing instant weaning foods using drum drying, extrusion or spray drying techniques (Milner 1969; Cupte 1972; Banigo *et al.* 1974; Anon 1977; Adeniji and Potter 1978 and Cheryan

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et al. 1979), the commercialization rate of these technologies in developing countries had been very low due to capitalization cost which eventually results in a cost of such manufactured foods exceeding the means of those in need of the products (Milner 1969). One of such instant products in Nigeria is Cerelac, a commercial weaning food manufactured by roller drying of maize or wheat and skim-milk powder which is fortified with vitamins and minerals.

In spite of this development, nursing mothers in the low socio-economic group, especially those in the rural areas, continue to depend upon traditional weaning foods which are of low nutritional value (Jansen 1982). Therefore the use of traditional technologies to develop instant and nutritious weaning foods has been encouraged (Malleshi *et al.* 1986). Furthermore the use of soybean flour in improving the nutritive value of infant and adult foods has been widely reported (Akinrele and Edwards 1971; Eka 1978; Ekpenyong 1980; Kapoor and Gupta 1981; Kaur and Gupta 1982 and Plahar and Leung 1985). Meanwhile a simplified approach such as dry-milling for the manufacture of a high protein, low-cost, instant, weaning food has been suggested (Gupte 1972 and Odum *et al.* 1981). However, the technique selected in producing a pregelatinized flour would impact the cost of such instant weaning foods. In this paper the use of a steamer (blancher) in producing pregelatinized maize flour is investigated while some physicochemical and nutritional properties of an instant maize weaning food are presented.

MATERIALS AND METHODS

White maize (*Zea mays L.*) was purchased from a market in Ile-Ife, Nigeria and soybeans were obtained from the Institute of Agricultural Research and Training (I.A.R. & T), Ibadan, Nigeria. Soy-ogi and maize Cerelac were obtained from Federal Institute of Industrial Research, Oshodi, Lagos and Food Specialities (Nigeria) Ltd., Agbara Factory, respectively.

Preparation of Ogi, Pregelatinized Maize Flour, Soybean Flour and "Weaning Food"

Ogi was prepared from maize grains by the traditional wet-milling process (Adeyemi *et al.* 1987). Four samples of pregelatinized maize flour were prepared from maize grains. For its (pregelatinized maize flour) preparation, 2 Kg each of maize grains were weighed into four separate containers and steeped overnight in 4L of warm water of initial temperature of 70°C. After steeping, each sample was drained and three of the samples were spread in different perforated trays placed inside a Barlett steamer (G. F. E. Barlett & Sons Ltd.) and steamed at atmospheric pressure for 20, 40 or 60 min. The fourth sample was steamed in an autoclave at 15 PSI (121°C) for 10 min. Each steamed sample was dried at

45°C in a cabinet dryer to a moisture content of between 12 and 14%, cooled, conditioned, milled on a Premier mill (attrition mill) and sieved to obtain flour fraction of <600 μ (Fig. 1). The yield of this fraction varied between 50-55% of the original weight of grains. Ungelatinized maize flour was also produced from the maize grains. Soybean flour was prepared by heating soybeans in an

Maize grains
↓
Cleaning
↓
Steeping in water
(70°C) overnight
↓
Steaming
↓
Drying in a cabinet
dryer at 45°C
↓
Cooling
↓
Conditioning
Conditioned by adding 3%
water on weight basis
↓
Milling on Premier Mill
(attrition mill)
↓
Sieving on Endecott test
sieve shaker using 600 μm sieve
(B. S. 410)
→ Overtails
(bran, germ + grits)
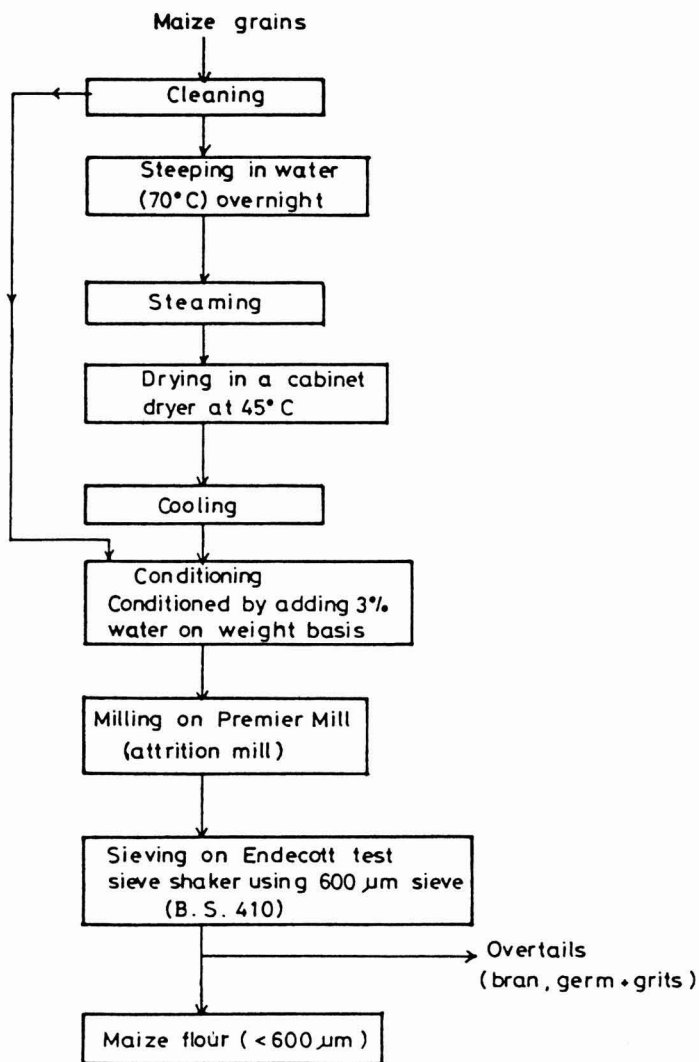↓
Maize flour (<600 μm)

FIG. 1. PREPARATION OF UNGELATINIZED AND PREGELATINIZED MAIZE FLOUR
SAMPLES

air oven at 110°C for 1 h, soaking in water to remove the seed coat, autoclaving at 15 PSI for 2 min, drying at 45°C, milling and sieving to obtain (whole) soybean flour. Pregelatinized maize flour prepared from grains steamed at 15 PSI was blended with soybean flour at a ratio of 70:30 (w/w) and this is referred to as "weaning food" in this paper.

Amylograph Pasting Viscosity, Water Hydration Capacity, Reconstitution and Consistency Measurements

Amylograms of maize flour samples, ogi, soy-ogi, Cerelac and the "weaning food" were recorded on a Brabender amylograph. The slurry, prepared at 10% concentration (w/w), was heated to a maximum of 92°C, held at this temperature for 15 min and cooled to 50°C (Adeyemi 1983). The degree of gelatinization was indicated by the peak viscosity and viscosity on cooling to 50°C (Ferrel and Pence 1964). Water hydration capacity (WHC) was determined on 5g sample using the method of Quinn and Paton (1979). Reconstitution was subjectively evaluated by preparing pap from each sample with boiling water and examining for lump formation and the ease of preparation. For consistency measurement, each sample was prepared in hot water with continuous stirring at different solid to water ratio (w/v, dry basis): ogi and soy-ogi (10%), maize flour samples and Cerelac (20%) and the "weaning food" (25%). Consistency was evaluated on an Adams consistometer as described previously (Adeyemi et al. 1987).

Nutritive Value Composition

Proximate analyses were carried out for moisture, protein, ash and fat contents (AOAC 1975) while pepsin digestibility was determined as described by Edwin et al. (1984). Each analysis was in triplicate and the means were separated by Duncan Multiple Range test (Duncan 1955).

RESULTS AND DISCUSSION

Brabender Amylograph Pasting Viscosity

There was a reduction in peak viscosity and viscosity at 50°C with increase in steaming time (Fig. 2). It is apparent that steaming for up to 1 h in the Barlett steamer did not fully gelatinize the maize flour. However, it is likely that the thickness of maize grains in the bed of the steamer affected the efficiency of heat treatment. This needs further evaluation. It is apparent that a Barlett steamer could find useful applications in developing countries, especially in the rural
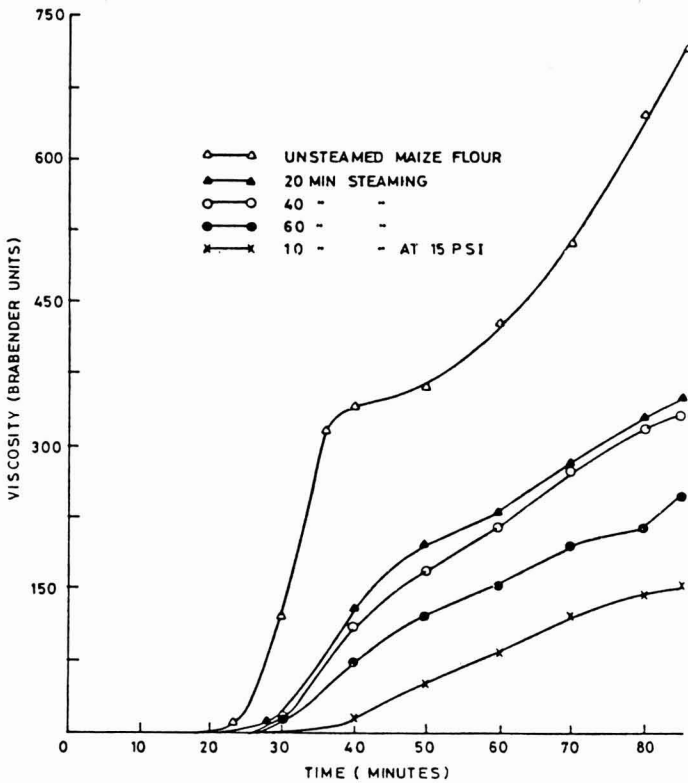
FIG. 2. BRABENDER AMYLOGRAPH PASTING VISCOSITIES OF MAIZE FLOUR
SAMPLES

areas since the built-in boiler is gas fired and thus the steamer does not rely on
electricity for its functioning. Such a steamer would be useful in the production
of pregelatinized maize flour for the manufacture of weaning foods at a regional
or local level and this may reduce overhead cost and thereby ensure availability
at reasonable prices (Mallashi *et al.* 1986). However, a comparative cost ad-
vantage in the use of a steamer for producing pregelatinized maize flour over
other techniques such as drum-drying needs to be ascertained. Ogi and soy-ogi
were not pregelatinized as reflected by Peak viscosities of 1,100 and 680 B.U.
respectively, (Fig. 3) reflecting high levels of raw starch in the samples (Banigo
et al. 1974). The amylograms of Cerelac and the "weaning food" (Fig. 3)
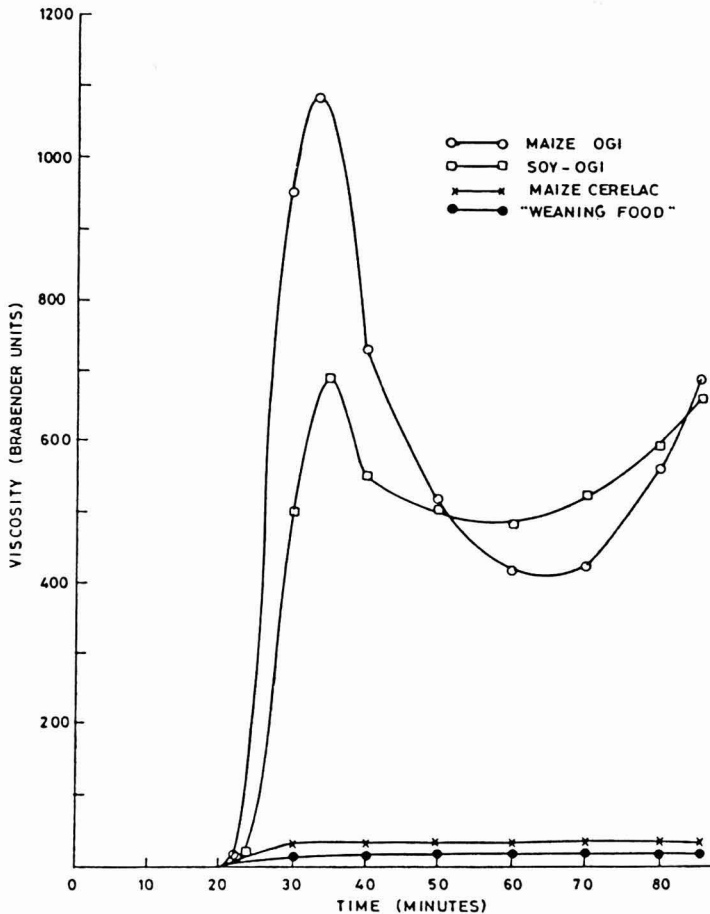indicated that the samples were fully gelatinized.

FIG. 3 BRABENDER AMYLOGRAPH PASTING VISCOSITIES OF OGI, SOY-OGI, CERELAC AND "WEANING FOOD"

Water Hydration Capacity, Reconstitution and Consistency

There was an increase in water hydration capacity with period of heat treatment (Table 1). For instance the ungelatinized maize flour sample and ogi absorbed the least amount of water (0.95 mL/g) while Cerelac recorded the highest value of 3.15 mL/g. The higher the extent of heat treatment the higher the water hydration capacity probably due to a higher starch damage during processing (Chilton and Collison 1974). Ogi and soy-ogi were found to reconstitute into pap after boiling the slurry for about 5-10 min while Cerelac and the "weaning food" reconstituted easily just with stirring in warm water. The former (Ogi and

TABLE 1.

WATER HYDRATION CAPACITY, RECONSTITUTION AND CONSISTENCY OF
MAIZE FLOUR SAMPLES, OGI, SOY-OGI, CERELAC AND "WEANING FOOD"

Sample	Water hydration capacity (ml/g)	Reconstitution	Adams Consistometer value
A	0.95	Reconstituted with hot water	5.6
B	1.05	"	3.0
C	1.25	"	4.9
D	1.45	"	7.8
E	1.75	"	13.3
Ogi	0.95	Formed pap on boiling with water for 5-10 min on a hot plate.	13.4
Soy-ogi	0.83	"	10.4
Cerelac	3.15	Reconstituted easily with warm water	6.1
"Weaning food"	1.05	"	8.6

A = Ungelatinized maize flour
B, C, D & E = Flour samples from maize grains steamed for 20, 40 or 60 min, and at 15 PSI for
10 min, respectively.

Soy-ogi) did not reconstitute easily because of the presence of raw starch as reflected by their amylograms and values for water hydration capacity (Fig. 3 and Table 1). At 10% concentration (dry basis), ogi and soy-ogi had consistency values of 13.4 and 10.4 respectively, (Table 1) while corresponding values were 6.1 and 8.6 for Cerelac and the "weaning food" when prepared at 20 and 25% concentration, respectively. It was observed that when prepared at concentrations above 10%, ogi and soy-ogi produced thick gels which did not flow on the consistometer plate while the slurries obtained from maize flour samples, Cerelac or the "weaning food" at concentrations below 20 or 25% was too thin to be measured on the consistometer. This difference could be due to the fact that the gelling ability of the latter samples had been reduced as a result of heat treatment.

Proximate Composition and Pepsin Digestibility

Maize ogi recorded a slightly lower protein content of 7.06% compared to a value of 8.65% obtained for maize flour (Table 2). It is apparent that the wet-milling process of ogi manufacture leads to a protein loss of about 36% while corresponding value for maize flour was 21.6% thus indicating that dry-milling might conserve protein better than the wet-milling process (Adeyemi 1983). Losses of nutrients during ogi manufacture accounted for low ash content of ogi (Akingbala *et al.* 1981). Fortification of pregelatinized maize flour at 30% with undefatted soybean flour gave a protein content of 14.5% (Table 2) which was close to a value of 15% reported by Cheryan *et al.* (1979) at 20% fortification. Protein quality has been shown to be optimal at 30% level of soybean fortification. However, organoleptic properties and storage stability are adversely affected (Cheryan *et al.* 1979 and Plahar and Leung 1985). It is desirable that for future studies defatted soybean flour, soy concentrate or isolate could be used at a much lower level to give comparable protein content.

Soybean flour had a higher pepsin digestibility value of 86.11% compared with the value for whole maize, maize flour or ogi samples (Table 3). Pepsin digestibility was found to increase with increasing steaming time thus confirming that heat treatment (precooking) improves digestibility (Edwin *et al.* 1984). It is also apparent that fermentation improves digestibility (Edwin *et al.* 1984) since values of 43.7 and 70.82% were obtained for maize flour and ogi samples respectively, (Table 3). However the value obtained for the fully gelatinized maize flour (sample E) was higher (82%) than that of ogi (71%) which would appear to show that gelatinization improved protein digestibility more than fermentation. A digestibility value of 78.63% (Table 3) for soy-ogi is comparable to 77.22% reported earlier (Ekpenyong 1980). A value of 85.03% was obtained for the "weaning food" while the value of 91.55% for Cerelac could be due to its source of protein (milk) which is more digestible than plant protein (Bender and Doell 1957).

TABLE 2.
PROXIMATE COMPOSITION* OF MAIZE, PREGELATINIZED MAIZE FLOUR, OGI,
SOY-OGI, CERELAC AND "WEANING FOOD"

Sample	Moisture %	Protein %	Fat %	Ash %	Carbohydrate** %
Maize grain	9.42ac	11.03a	7.50a	1.70a	79.77a+
Maize flour	11.99b	8.65b	4.50b	1.21a	85.65b
Ogi	10.42bc	7.06bc	5.75b	0.42b	86.77b
Soy-ogi	8.02a	11.70a	5.80b	2.00ac	80.50a
Maize cerelac	3.81d	16.09d	5.65b	2.92c	75.34c
"Weaning food"	10.97bc	14.50d	13.10c	1.68a	70.72d

*Data expressed on dry basis except moisture
**Calculated by difference
+ Means with same letters are not significantly different (P>0.05).

TABLE 3.
PEPSIN DIGESTIBILITY OF MAIZE GRAIN AND FLOUR, OGI, SOY-OGI, CERELAC
AND "WEANING FOOD"

Sample	Pepsin digestibility %
Maize	67.63a*
Soy-flour	86.11b
A	43.70c
B	61.92d
C	76.91e
D	70.97f
E	82.54g
Ogi	70.82f
Soy-ogi	78.63e
Maize cerelac	91.55h
"Weaning food"	85.03b

A = Ungelatinized maize flour
B, C, D & E = Flour samples from maize grains steamed for 20, 40 or 60 min, and at 15 PSI for
    10 min, respectively.
*Means with same letters are not significantly different (P>0.05).

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THE INFLUENCE OF SOLID SURFACE ENERGETICS ON MACROMOLECULAR ADSORPTION FROM MILK[1]

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ABSTRACT

Four materials characterized by their differing values of solid surface tension were implanted into different locations within the tube wall of a shell and tube heat exchanger. The implants were subjected to milk flowing at temperatures from the ambient to the ultra-high temperature (UHT) range (150°C) for contact times varying from 1 to 30 min. After contact the implanted materials were removed for surface film analysis by ellipsometry and internal reflectance infrared spectroscopy. Deposition rates of the molecular subfractions first adsorbed appear to be strongly dependent upon physical properties of the bare surface. Macromolecular deposition rates were described with Arrhenius kinetic expressions in which E_a, the apparent activation energy required for the transformation, is dependent on the surface temperature and the deviation of the solid surface tension from some value evoking minimal biological adhesion.

INTRODUCTION

Many seemingly diverse fields collapse into a single field when attention is focused at the interface. Thrombus formation, dental plaque attachment, and fouling of heat exchange surfaces by sea water, dairy fluids, or any fluid food may be characterized by fundamentally similar events.

The parameters developed from an evaluation of surface chemistry (energetics) have proven to be most useful descriptors of the surface chemical identity of

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many polymeric materials dealt with in biomedical devices. Baier (1970), and Baier and DePalma (1971) have correlated an empirical parameter of surface free energy, the critical surface tension, with biological compatibility in general and with blood compatibility in particular. A particular range of critical surface tension values between 20 and 30 dynes/cm was identified as evoking a bio-compatible response, *i.e.*, materials exhibiting a critical surface tension value in this range experience minimal biological adhesion. It has been suggested that the surface chemistry of a material most influences the initial adsorptive events such as the distortion of adsorbed molecules, and consequently determines the surface biocompatibility.

In addition to surface studies, protein adsorption at solid surfaces has been described with relation to adsorption kinetics, surface- and bulk-protein equilibria and site competition among bulk proteins. There is general agreement that protein adsorption is rapid, and the rate is transport controlled when the existing fractional surface coverage is low (<30%)(Brash 1985). Adsorption subsequently enters a kinetically controlled regime.

For obvious reasons, the great majority of investigations of adsorptive phenomena in clinical research have been studied at or near physiologic temperatures; the effect of temperature on adsorptive events has not been seriously addressed. The situation is different in cases concerning adsorptive phenomena of fluid food components. However, although temperature effects have historically been studied and considered significant in fouling of heat exchange surfaces, the whole process of fouling has in general been treated as a totally separate field: a heat transfer problem and not a surface chemical problem. Indeed, not until the Tylösand meeting in 1981 had fouling been recognized as a problem associated with surface chemistry (Hallström *et al.* 1981). Clearly, approaches used by researchers in clinical situations might prove useful if carefully adapted to the engineering problem of deposition onto heat exchange surfaces.

Observations related to protein adsorption from fluid food are conflicting. The following is a summary of observations which have resulted from food engineering investigations concerning deposition of whole milk components flowing through varying temperature regimes in a heat exchanger (McGuire 1987):

(1) the whey proteins (α-lactalbumin and β-lactoglobulin) are very heat-sensitive relative to the caseins and are rapidly, irreversibly adsorbed (along with albumin and the γ-globulins);

(2) the caseins (α_{s1}, α_{s2}, β, and κ) which are organized into micellar structures, are extremely stable but may become part of the fouled layer by entrapment or, with exposure to high temperatures for long periods of time, micellar components may irreversibly adsorb;

(3) mineral deposition accompanies protein deposition, primarily in the form of $Ca_3(PO_4)_2$, although it is difficult to distinguish between the 1.5 and 1.67

molar ratio in the various calcium phosphate deposits (raw milk exhibits a high degree of supersaturation with regard to all solid phases known for calcium phosphate);

(4) the whey proteins remaining in solution may undergo extensive denaturation and agglomeration as a function of time and temperature (*via* disulfide linkages and noncovalent bonding) with each other, casein micelles and fat globule membranes;

(5) large, proteinacious aggregates are likely adsorbed to a lesser extent due to increased resistance encountered upon transport to the interface; and

(6) the overall process of dairy fluid component adsorption in turbulent flow conditions is considered to be kinetically controlled, as opposed to transport or diffusion controlled.

Arnebrant and Nylander (1986) have used ellipsometry to study the adsorptive behavior of β-lactoglobulin and κ-casein onto hydrophilic and hydrophobic surfaces. They observed that if β-lactoglobulin is adsorbed first, κ-casein does not replace it but adsorbs at the already adsorbed β-lactoglobulin layer. If κ-casein is adsorbed first, the resulting surface appeared "passive" to further adsorption. However, during a study in which heat exchange surfaces were submitted to adsorption of κ-casein prior to pasteurization of milk, no significant reduction of fouling was detected by these investigators.

An understanding of the role of solid surface tension in adsorption of fluid food components, especially at elevated temperatures, has the potential to provide direction in development of surfaces which reduce (or eliminate) fouling by organic material. At the very least, enhanced understanding of the pertinent surface chemistry may greatly enhance understanding of competitive protein adsorption. Consequently, problems associated with fouling, spore adhesion on packaging materials, foreign protein adhesion on immobilized enzyme carriers, and any bioparticle-surface interaction will be more clearly defined.

MATERIALS AND METHODS

McGuire *et al.* (1985) developed methodology to characterize films formed during the first moments of contact between milk and various solid surfaces. The use of ellipsometry and internal reflectance infrared (IR) spectroscopy was proposed to aid in the analysis of the films deposited (*i.e.*, mass retained) on specially designed plates implanted within the conduit wall of a tubular flow heat exchange unit.

The flow unit featured a tube bundle, four parts of which were connected by three tube couplings. Each coupling was constructed to house a removable implant (flat plate), the surface of which contacted the fluid while maintaining a nearly circular cross section (less than 1.5% of the original cross sectional area

is obstructed). Clean plates were characterized with respect to surface physical properties, implanted into the unit, contacted with milk at some temperature for some time, removed and analyzed by ellipsometry and internal reflectance IR spectroscopy.

Surface Characterization

Four surfaces, those of Teflon® (TFE), polished #304 stainless steel (ss), rough #304 ss, and an aluminosilicate (coating applied to #303 ss) had their composite surface tensions, composed of dispersive and polar force contributions, determined by contact angle methods described in a previous paper (McGuire and Swartzel 1987). The surface properties, *i.e.*, solid surface tensions and roughness indices, are shown in Table 1.

Ellipsometry

The ellipsometer is used to determine the thickness and refractive index of thin films by measuring changes in the state of polarized laser light reflected from the sample surface. The technique may be applied to any substrate-film combination that provides reasonably specular reflection of the incident light beam. As a surface becomes increasingly rough and heterogeneous, the criterion of sufficiently specular becomes difficult to satisfy. To combat this problem, increased intensity of the incident light beam is a viable option. For all materials considered in this study, a 3mW HeNe laser was used and directed to the sample at an incident angle of 70°.

It is not appropriate to review the theory of ellipsometry here. Reviews of varying detail are available elsewhere (McCrackin *et al.* 1963; Archer 1968; Azzam and Bishara 1977). It is necessary to note, however, that the ellipsometric parameters used to calculate film thickness are themselves cyclic functions of film thickness. That is, there is a period associated with any ellipsometrically

TABLE 1.
SOLID SURFACE TENSION AND ROUGHNESS INDEX OF EACH MATERIAL
UNDER STUDY

Material	Surface Tension (γ_s), dynes/cm	Roughness Index (R_a), μin
TFE	22.1	76
aluminosilicate	38.6	91
polished ss	46.4	1.5
rough ss	51.4	16

determined film thickness. Unfortunately, this period is of the same order of magnitude as some of the dimensions characteristic of colloidal particles in milk (*e.g.*, the micelles). In studying adsorption from a system as complex as whole milk, it is difficult to unambiguously report film thicknesses without the aid of a second method of evaluation.

Fortunately, the refractive index of the film is a directly calculable (and useful) optical property. Refractive indices for all films generated were determined ellipsometrically.

Internal Reflectance Infrared Spectroscopy

The films generated were analyzed using an attenuated total reflectance (ATR) technique, in which the implants (with an adsorbed film on one surface) were clamped to the IR transmitting crystal, "sandwiching" the adsorbed layer between the implant and the crystal. The IR spectra display absorption bands characteristic for protein, carbohydrate and lipid. Quantitative information can be obtained if the absorption band area for each subfraction is correlated to quantity adsorbed. Considering protein, within the peptide bond, the Amide I ($C = O$ stretch) and Amide II (mostly N-H bending) bands are due to IR absorption at frequencies of 1650 and 1550 cm^{-1} (in the solid state), respectively. Since great amounts of these bonds are present in protein and they have much less representation in other molecules in milk, it was suspected that the total area comprising the Amide I and II absorption bands could be related to the amount of protein adsorbed. Therefore, known amounts of protein (egg albumin) were deposited onto each material by dropping μL quantities of an aqueous solution of albumin onto the surfaces and allowing these to dry overnight in a dessicator. The Amide I and II bands on each spectrum were numerically integrated and calibration curves were generated relating surface coverage of protein to absorption band area. No attempt was made to separate and quantify the particular proteins involved in fouling; such information is available elsewhere (Maas *et al.* 1985; Nordman-Montelius and von Bockelmann 1985; Tissier *et al.* 1985).

Similarly, for lipid adsorption, known amounts of tripalmitin were transferred to the surfaces from dilute aqueous solution and related to IR absorption bands at 2920 and 2850 cm^{-1} (C-H stretching of methyl and ethyl groups), and 1740 cm^{-1} (C-O stretch of the ester bond). Bands related to C-H deformation within methyl and ethyl groups exist at 1450 and 1380 cm^{-1} as well, but these absorption bands were not candidates for integration as they were effectively masked by the always present C-F absorption observed with the TFE surface; moreover, CH_3 deformation for a variety of compounds is detected at 1380 cm^{-1}, suggesting that the bands in this region are less useful than those at 2920, 2850, and 1740 cm^{-1}. Lipid adsorption was actually correlated with the total area due to absorption at 2920, 2850 and 1740 cm^{-1}, and this correlation was most often used.

However, lipid adsorption was also related to the band area at 2920 and 2850 cm^{-1} alone, for use in cases where the 1740 cm^{-1} band was masked by heavy protein adsorption.

The presence of carbohydrate is accompanied by IR absorption at 1060 cm^{-1}, where C-O stretching associated with the glycosidic linkage is detected. The mass of β-D-lactose adsorbed was related to the area of this band; however, the particular relationship obtained was a function of not only band area at 1060 cm^{-1} but also of proteinaceous material present. Determinations of carbohydrate loading were consequently subject to greater error than determinations of lipid and protein surface loading. It should be noted that mineral deposition plays an important but somewhat obscure role in the overall fouling process. Mineral deposition was not taken into account here; only the deposition of carbohydrate, lipid, and especially proteinaceous components were considered.

Tests were carried out in which the implanted materials were subjected to milk flowing at temperatures from 10°C to nearly 160°C for contact times ranging from 1 to 30 min, and to temperature driving forces (ΔT's, where ΔT is the difference between the bulk fluid temperature and the surface temperature) from 0°C to slightly greater than 100°C.

RESULTS AND DISCUSSION

The Influence of ΔT, Surface Temperature (T$_s$, and γ$_s$)

Although the characterization of the effect of ΔT on milk fouling rates has proven to be difficult, in general the evidence derived from food engineering literature indicates that increased ΔT promotes increased heat exchanger fouling by dairy fluids (Burton 1968; Maas *et al.* 1985). Results of this investigation suggest that ΔT is not significant.

As expected, deposition rates increased with increasing surface temperature as shown in Fig. 1, where in this case the rates were measured on polished stainless steel near the entrance of the heat exchanger. Figure 1 is representative of families of curves which display identical trends; however, increasing temperature had little effect on deposition rate as the surface tension of the contact material approached the value characterized by minimal biological adhesion. This is readily observable in deposition rate curves plotted for aluminosilicate surfaces submitted to conditions identical to those resulting in Fig. 1; these curves are shown in Fig. 2. It is interesting to note that the amount of adsorbed protein on the aluminosilicate is an order of magnitude lower than that on polished stainless steel.

Figure 3 is representative of the influence of solid surface tension on deposition rate. These results suggest that an intermediate range of surface tension values would correlate best with minimal biological response. At decreasing tempera-

tures, the effect of surface tension on adsorption rate is present but somewhat less pronounced; these curves are plotted in Fig. 4. With respect to Fig. 1–4, it must be noted that "mass adsorbed" refers to the mass retained on each surface after rinsing *in-situ*.
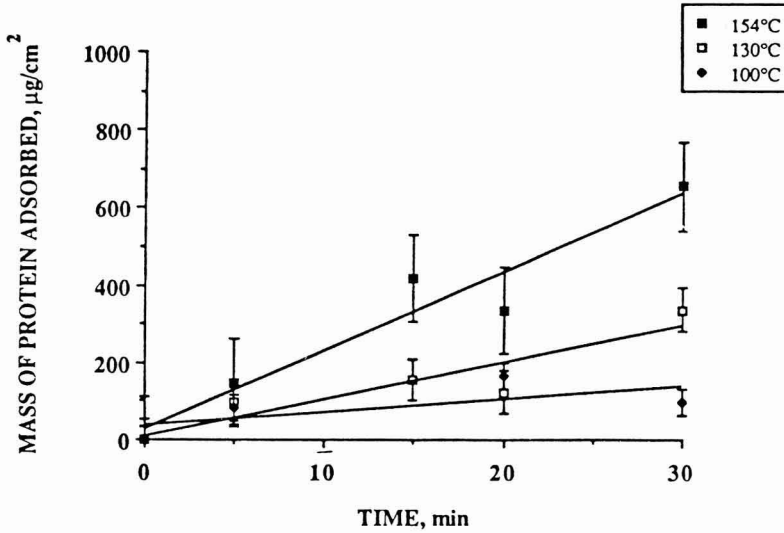
FIG. 1. INCREASE IN PROTEIN DEPOSITION RATE (ON POLISHED STAINLESS STEEL) WITH SURFACE TEMPERATURE
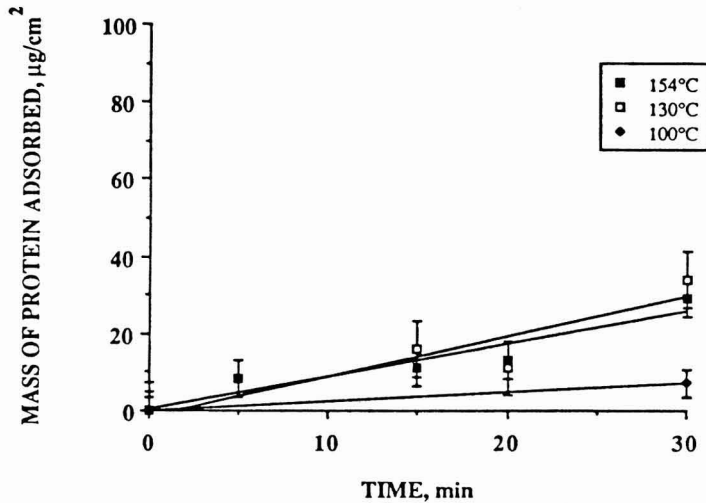
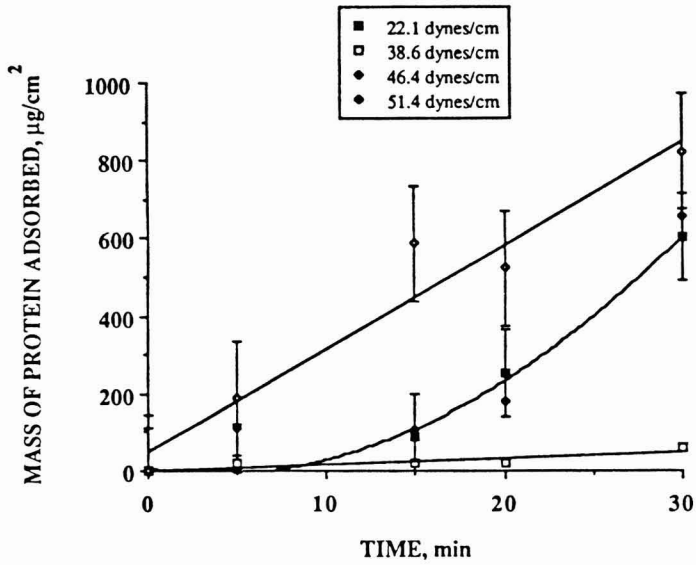FIG. 2. INCREASE IN PROTEIN DEPOSITION RATE (ON ALUMINOSILICATE) WITH SURFACE TEMPERATURE

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FIG. 3. INFLUENCE OF SOLID SURFACE TENSION ON PROTEIN DEPOSITION RATE AT
A SURFACE TEMPERATURE OF 158°C
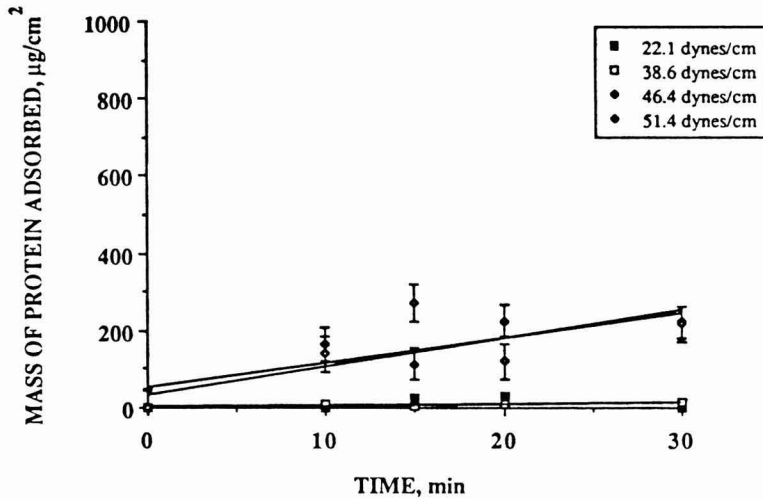
FIG. 4. INFLUENCE OF SOLID SURFACE TENSION ON PROTEIN DEPOSITION RATE AT
A SURFACE TEMPERATURE OF 10°C

Quantitative Representation of Observations

The net amount of macromolecular deposition was observed to follow an Arrhenius type expression of the general form:

$$\partial M/\partial t = f(t) \exp[-E_a/RT_s f(\gamma_s, \gamma_{s,min})] \tag{1}.$$

where M represents mass ($\mu g/cm^2$) of protein, lipid, protein and lipid, or protein, lipid and carbohydrate adsorbed at any time t (min) onto a material with a surface temperature T_s (K) and surface tension γ_s (dynes/cm); $\gamma_{s,min}$ (dynes/cm) is a value of solid surface tension which, depending upon surface temperature and class of molecule adsorbed, represents a material evoking minimal biological adhesion; R is the gas constant (J/(mole)K); and E_a (J/mole) represents an apparent activation energy required for transformation of an adsorbed species to an irreversibly adsorbed species (a function of T_s and γ_s). The exponential term must be dimensionless; consequently, the function $f(\gamma_s, \gamma_{s,min})$ must be dimensionless. Deposition was observed to increase as the deviation of γ_s from $\gamma_{s,min}$ increased. For all equations developed in this work, $f(\gamma_s, \gamma_{s,min})$ was taken as equal in magnitude to the absolute value of $(\gamma_s - \gamma_{s,min})$. The expression, $(\gamma_s - \gamma_{s,min})$, can be made dimensionless with division by a constant with units of dynes/cm and then incorporated directly into the exponent in place of the functional representation. An arbitrarily chosen term such as $\gamma_{s,min}$, for example (evaluated at some reference temperature), would satisfy such a suggestion. However, because exact specification of the parameters in the expression of Eq. 1 is still in development, and an additional parameter adds no insight to the meaning of the equations, the functional representation is used. The time function, $f(t)(\mu g/(cm^2)min)$, represents a first order polynomial in t when M represents either total organic mass or mass of lipid. It is simply a constant (0 order in t) in cases where M represents mass of protein or of protein and lipid. The time dependence of deposition rate for total organic mass and mass of lipid may simply be due to detection limitations inherent in the ATR technique; an apparent lag time is therefore observed.

The scatter plot of Fig. 5 shows the fit to actual data achieved in predicting deposited mass with the integral form of Eq. 1. The points in Fig. 5 represent total organic mass adsorbed (retained). Each point is generated from six independent tests; two tests at each of the three locations in the heat exchanger (averaged, since ΔT was determined as statistically insignificant). The plot includes results of all tests performed over the entire range of surface tension, temperature and time.

The expression in Eq. 1 is physically realistic only when it describes single component adsorption. This is true since E_a represents the energy required to overcome some barrier to permanent adsorption, in this case, the transformation of a reversibly adsorbed molecule to an irreversibly adsorbed one. Therefore,
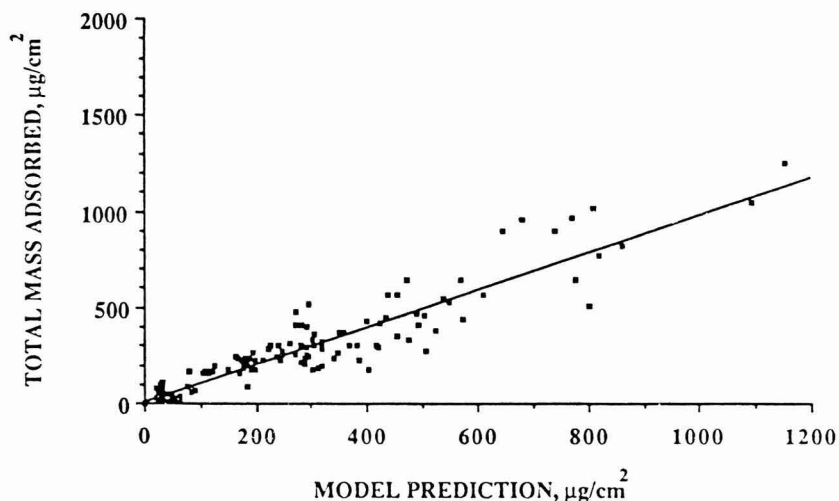
FIG. 5. RELATIONSHIP BETWEEN ACTUAL DATA AND MODEL PREDICTION
(INTEGRAL FORM OF EQ. 1) WITH RESPECT TO TOTAL MASS OF CARBOHY-
DRATE, LIPID, AND PROTEIN (r^2 = 0.839; standard error = 99.775 $\mu g/cm^2$)

the applicability of the equation to other systems and the mechanistic insight
generated by it are best discussed with reference to protein adsorption from
whole milk. Equation 1 may then be rewritten

$$\partial M_p/\partial t = C \exp(-E_a/RT_s|\gamma_s - \gamma_{s,min}|) \tag{2}$$

where M_p refers to the total proteinaceous mass adsorbed at any time and C is
a constant. The parameters of Eq. 2 are defined in Table 2. E_a is expressed as
a function of T_s and γ_s, and $\gamma_{s,min}$ is expressed as a simple function of T_s.
Actually, for all classes of molecule adsorbed, the general expressions:

$$E_a = a_1 T_s + b_1|\gamma_s - \gamma_{s,min}| + c_1 T_s|\gamma_s - \gamma_{s,min}| \tag{3}$$

and

$$\gamma_{s,min} = a_2 + b_2 T_s \tag{4}$$

where the a_i and b_i are positive and c_1 is negative, were observed to hold.

The proposal that E_a is dependent on temperature and surface tension certainly
appears speculative. In this regard it should be emphasized that Eq. 2 does not
constitute a fully developed model. Rather, it is an equation based on present
data and conveniently expressed in Arrhenius form which describes the strong

TABLE 2.
PARAMETERS OF EQ. 2

$\partial M/\partial t = C \exp(-E_a/RT_s|\gamma_s - \gamma_{s,min}|)$

$C = 20.56 \; \mu g/cm^2 min$

$E_a = 223.515T_s + 28051.711|\gamma_s - \gamma_{s,min}| - 78.087T_s|\gamma_s - \gamma_{s,min}| \; [=] \; J/mole$

$\gamma_{s,min} = 30.720 + 0.003T_s \; [=] \; dynes/cm$

where $100°C < T_s < 158°C$

influence of the magnitude of $|\gamma_s - \gamma_{s,min}|$ on protein deposition from milk. Moreover, it should be made clear that Eq. 3 is not a final representation of E_a. However, the success of such an expression, supported by the present data, suggests that E_a may indeed have separate bulk and surface components. Elucidation of the thermodynamic identity of such a surface component must await further research. Equation 4 was derived experimentally; $\gamma_{s,min}$ was observed to be a weak function of T_s.

The physical interpretation of Eq. 2 is most clearly understood with reference to Fig. 6, the typically proposed protein adsorption mechanism. The first step, denaturation, may not be a necessary prerequisite for further events leading to adsorption. The second event (mass transfer to the interface) must occur, and its importance is not well-documented in the literature. Based on the clinical work cited previously in addition to the dairy fluid fouling work cited earlier, and other tests carried out in food engineering laboratories (Ling and Lund 1978), under conditions of fully developed turbulent flow, increasing the flowrate has no significant effect on fouling rate. Therefore, since the adsorption event (step 3) is generally agreed upon as rapid (results are conflicting: the time required for adsorption has been reported as several seconds (Baier 1969, 1970, 1975, 1977, 1978; Baier et al. 1971, 1975) to many minutes (Arnebrant and Nylander 1986), the rate-determining step may indeed be the further transformation (denaturation) of an adsorbed protein to a component of an irreversibly adsorbed film. This event is described by Eq. 2, when E_a is the activation energy required for permanent adhesion of an already adsorbed species.

The protein depicted in Fig. 6, as it is adsorbed from whole milk, is not representative of a unique molecule but is representative of the several proteins initially adsorbed (especially β-lactoglobulin, and to a lesser extent α-lactalbumin, albumin, the γ-globulins and caseins). Although these proteins adsorb

BULK DENATURATION

MASS TRANSFER TO THE
INTERFACE

ADSORPTION

TRANSFORMATION TO AN
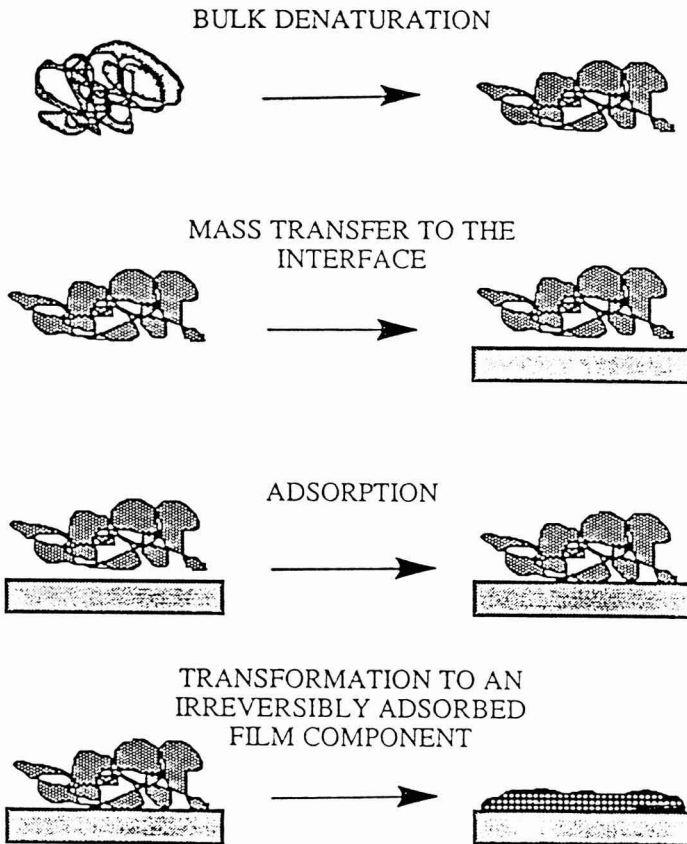IRREVERSIBLY ADSORBED
FILM COMPONENT

FIG. 6. SEQUENCE OF EVENTS IN PROTEIN ADSORPTION

by similar mechanisms, any one molecule certainly may be bound at differing numbers of sites. However, even adsorption of one protein from a single component aqueous solution may be more or less tenaciously adsorbed than another of the same species depending upon surface coverage and bare surface energetic heterogeneities. Additionally, the two most abundant whey proteins, α-lactalbumin and β-lactoglobulin, despite their great difference in size and chemistry, display remarkably similar energy requirements for denaturation (Lyster 1970; Dannenberg and Kessler 1985). In fact, the value of the activation energy required for denaturation of each molecule differs by less than 5% when the reaction takes place below 100°C. The general applicability of equation 1, however, to other groupings of biological molecules is limited due to its less clear representation of actual mechanism.
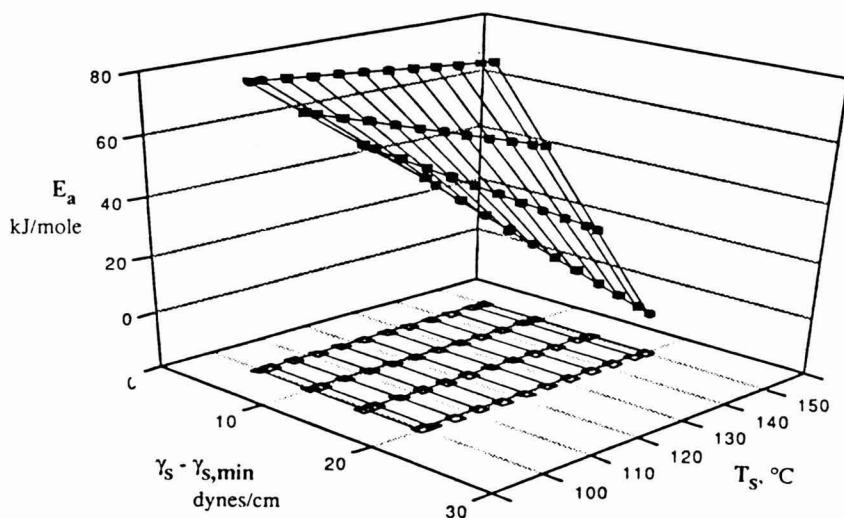
FIG. 7. INFLUENCE OF SURFACE TEMPERATURE AND SOLID SURFACE TENSION
ON ACTIVATION ENERGY.

Equation 3 suggests that the deviation in solid surface tension from some value evoking minimal adhesion affects the magnitude of the activation energy in a manner similar to that of surface temperature. This is made clear in the surface plot of Fig. 7 (a plot of Eq. 3). As the activation energy increases the number of molecules undergoing the transformation is reduced. At all temperatures in the range shown (the range at which Eq. 3 holds), E_a is increased as γ_s approaches $\gamma_{s,min}$. This increase becomes more dramatic as temperature is increased. Viewed from a different perspective, E_a decreases at constant γ_s with increasing temperature; however, as γ_s approaches $\gamma_{s,min}$, E_a barely decreases at all. These observations suggest that even at elevated temperatures, the amount of proteinaceous deposition may be reduced with a change in bare surface constitution.

The refractive index of a proteinaceous film is a useful optical property in that it provides an index of film density. Consequently, macromolecular conformational properties are indirectly observable. Table 3 lists the average film refractive index measured (ellipsometrically) on each material. Each value of n_r was averaged over the complete set of experimental variables; each value represents the average n_R evaluated at all combinations of t, γ_s and T_s. Admittedly, n_R is also a function of surface temperature, but the strong influence of surface tension is such that the following point is simply made with reference to Table 3: as γ_s

TABLE 3.
AVERAGE REFRACTIVE INDEX OF ALL ADSORBED FILMS

Material	Surface Tension, dynes/cm	Refractive Index, n_R
TFE	22.1	1.52
aluminosilicate	38.6	1.25
polished ss	46.4	1.64
rough ss	51.4	2.16

approaches $\gamma_{s,min}$, the adsorbed layer becomes increasingly diffuse, perhaps more closely approximating its native conformation, and therefore easier exchange with the bulk fluid is maintained.

Obviously, no comprehensive theory of protein adsorption will evolve from studies focused only on macromolecular adsorption from whole milk. However, it is also clear that this type of work must be done in parallel with tests using simpler, more well-defined systems. The methods used for surface characterization in this work involve no guess work as opposed to the Zisman critical surface tension approach, but are still semi-quantitative at best. The difficulties arise during determination of the polar component of the reported composite solid surface tensions. More work is needed in this area to provide information about the chemical nature of protein binding. Nevertheless, at least the qualitative aspects of the relationship between surface energy and milk component deposition presented here suggests other experiments which may enhance understanding of the subject.

ACKNOWLEDGMENT

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