JOURNAL OF FOOD PROCESSING AND PRESERVATION

D.B. LUNI
EDITOR

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GUIDE FOR AUTHORS

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

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

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

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JOURNAL OF FOOD PROCESSING AND PRESERVATION

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EDITORIAL

As we embark on Volume 10 of the Journal of Food Processing and Preservation it occurred to me that this might be an opportunity to use this Journal to share technical information which frequently does not or cannot be published. There are two ideas which we would like to initiate starting with Volume 10. For some time I have thought that there was a need for a place to publish data that are collected on food products and ingredients. For example, when studies are done on textural characteristics as a function of process variables or formulation, these are often buried in theses or in industrial laboratories because there is not a convenient place to publish those data. Starting with Volume 10, the Journal of Food Processing and Preservation will devote a section to publishing short (1-2 pages) data sets on properties of food material. These "Data Bank" papers would consist of a brief description of methods and the table, figure or nomograph with the data. The "data bank" paper will be sent out for review. The turn around time on these papers will be extremely short and the papers must necessarily be very brief.

Starting with Volume 10 we would also like to devote a second section to "Computer Codes and Applications." The author would give a brief description of the code, indicate its availability (in hard disc from the author or a listing of the code) and an application of the code. In order to avoid advertising problems, we would not accept papers describing codes which are copyrighted or must be purchased. These descriptions of computer codes will not be sent out for review since it would be difficult to judge the quality of the code simply by reading about it. I would appreciate receiving your papers for both of these sections.

The goal of the Journal of Food Processing and Preservation is to serve you the reader with the best technical papers possible. We hope these two sections will add to the worth of the Journal to you. Should you have any suggestions for improving the Journal, please do not hesitate to write me. I want to take this opportunity to also thank the authors of papers who have made contributions to this Journal. I invite you to submit papers of original research for consideration in the Journal of Food Processing and Preservation.

The Journal of Food Processing and Preservation has been fortunate to have an excellent editorial board. Many of the members have served since the initiation of the Journal nine years ago. In order to make serving on the board a little more appealing, I have initiated three year terms for board members. I would like to thank the following people for their excellent work on the board and for helping make the Journal of Food Processing and Preservation a viable publication: Dr. Larry Beuchat, Dr. Daniel Farkas, Dr. James Flink, Dr. Norman Heidelbaugh, Dr. James

Kirk and Dr. Lowell Satterlee. Serving three year terms will be Dr. William Breene, continuing member of the editorial board, and Dr. Jerry Cash, Dr. Berry Swanson and Dr. Ricardo Villota, all new members to the editorial board. Serving two year terms are the following continuing board members: Dr. Frank Busta, Dr. Owen Fennema, Dr. Ted Labuza and Dr. Kenneth Swartzel. Finally, serving one year terms are continuing board members: Dr. Marcus Karel, Dr. Gary Reineccius, Dr. Romeo Toledo and Dr. Ron Wrolstad. For me personally and on behalf of the readership of the Journal, I want to thank these individuals for serving a necessary role in insuring the quality of the Journal of Food Processing and Preservation. I also want to thank the countless other reviewers who have served the Journal over these years. The following have served as reviewers for papers considered for Volume 9 of the Journal:

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RATE OF PIGMENT MODIFICATIONS IN PACKAGED REFRIGERATED BEEF USING REFLECTANCE SPECTROPHOTOMETRY

A. E. BEVILACQUA and N. E. ZARITZKY

Centro de Investigacion y Desarrollo en Criotecnologia de ALimentos.
CIDCA. (CONICET-CIC-UNLP). Facultad de Ciencias Exactas.
Universidad Nacional de La Plata
Calle 47 y 116 La Plata (1900) Pcia. Bs.As. ARGENTINA

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ABSTRACT

Equations derived from Kubelka-Munk theory were applied to determine relative concentrations of muscle pigments in packaged refrigerated beef using reflectance spectrophotometry. Effects of storage temperatures and gaseous film permeabilities on myoglobin oxygenation and oxidation rates were analyzed in unsterile beef tissue. Kinetic constants for metmyoglobin accumulation during aerobic storage were measured assuming a first order reaction with respect to unoxidized myoglobin; values were in the range of autoxidation rate constants reported for solutions. Myoglobin oxygenation was described as a first order reversible reaction. The influence of vacuum aging time on kinetic constants was determined considering microbial growth and losses of enzymatic activity during previous storage.

INTRODUCTION

Measurement of meat color is determined by the relative proportion of oxy (MbO_2), myo (Mb) and metmyoglobin (MetMb) (Francis and Clydesdale 1975). Broumand *et al.* (1958), developed spectrophotometric methods for determining the states of myoglobin contained in extracts of meat surfaces. Since extraction procedure is a source of considerable error, the reflectance spectrophotometry method of analysis was preferred. Dean and Ball (1960) developed a quantitative method for measuring meat pigments based upon the absorbancy ratio of Broumand *et al.* (1958), but using reflectance measurement of meat surfaces; Stewart *at al.* (1965) used

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reflectance spectrophotometry to determine total pigment and percent metmyoglobin; Snyder (1965) adjusted the spectral curves to a common reflectance at 525 nm to overcome the problem of the meat matrix influence; Franke and Solberg (1971) measured relative concentration of MetMb adjusting the spectra to a constant value at 750 nm and measuring absorbance at 632 nm; Van den Oord and Wesdorp (1971a,b) used differences in absorbances at 580 nm and 630 nm to determine MbO_2 and MetMb content in samples with negligible Mb content; Strange *et al.* (1974) related reflectance measurements to tristimulus parameters and trained panel observations; Eagerman *et al.* (1978) proposed a rapid procedure for following oxidative or reductive changes in meat MetMb based on differences in reflectances at 632 and 614 nm; Attrey and Sharma (1979) compared different methods and concluded that K/S ratio at 572 and 525 nm gave more consistent results than other wavelengths for MetMb evaluation; Krzywicki (1979) proposed a method for determining relative concentration of the three pigments using reflectance spectrophotometric measurements but adopting the absorption coefficients obtained by Broumand *et al.* (1958) in meat extracts.

Many of the proposed techniques are only based on measuring certain spectrophotometric reflectance ratios or differences, as indicators of beef muscles visual color (Harrison *et al.* 1980); some are limited to the presence of only two pigment derivatives and others have not been sustained on theoretical basis since linear relationships were used between absorbance and pigment concentration. Most of the reactions which interconvert ferrous and ferric forms of myoglobin set up a dynamic color cycle in meats (Fox 1966). MetMb accumulation in beef and factors affecting discoloration have been analyzed mostly in sterile muscles (Ledward 1970; Van den Oord and Wesdorp 1971a; Hood 1971, 1980; Hood and Riordan 1973; Ledward *et al.* 1977).

Rate constants of oxymyoglobin autoxidation in solutions were determined by George and Stratmann (1952a,b); Brown and Mebine (1969); Bembers and Satterlee (1975). Little information is available in the literature on kinetics constants for myoglobin oxygenation and oxidation reactions in beef muscles (Satterlee and Hansmeyer 1974; Ledward *et al.* 1977).

The objectives of this study were: (1) to obtain equations derived from Kubelka-Munk theory for evaluating relative concentrations of the three myoglobin derivatives in beef muscle by reflectance spectrophotometry; (2) to apply this methodology for determining kinetic constants of meat myoglobin oxidation and oxygenation rates in unsterile muscle and (3) to analyze the effects of temperature and gaseous packaging permeabilities on pigment modifications.

MATERIALS AND METHODS

Beef samples were obtained from gluteus medium bovine muscles (eye of rump) removed from steers (carcass weight 240 kg) with 24 h postmortem at 4 °C and packaged into two types of plastic films with different oxygen permeabilities: low density polyethylene, 60 μm thick (oxygen transmission rate OTR = 6500 cm^3/m^2 atm day at 23 °C) and a composite EVA/SARAN/EVA coextruded film for vacuum packaging, 60 μm thick (trade name Super Cryovac, OTR = 37.5 cm^3/m^2 atm day at 25 °C and RH = 75%). Oxygen transmission rate of composite film decreases at low temperatures following an Arrhenius relationship; OTR for this type of film is generally 10% of the value at 25 °C. Beef cuts were packaged under vacuum immediately after being sectioned from muscle to avoid oxygenation using a Minidual Equipment with a single chamber at 4.5 mm Hg and heat sealing; average pressure inside packages was 45 mm Hg. The pH of all tested samples was betwen 5.7-5.9 determined by a meat electrode (INGOLD LOT 405-M4).

During storage at 0 °C and 4 °C color measurements and bacteriological analysis were performed on packaged samples at different intervals of time. Total aerobic counts were made on Plate Count agar (Oxoid) with incubation at 30 °C for 48 h. Storage periods were terminated when total microbial counts reached 10^7 CFU/cm^2, starting from initial contamination levels of 10^4 CFU/cm^2.

Relative pigment concentration was measured in a Varian Super Scan 3 spectrophotometer equipped with an integrating sphere reflectance attachment. Reflectance spectra were recorded between 400 and 800 nm using a barium sulphate coating as the reference standard. Meat samples of 3.2 × 2.3 cm in cross-section and 2 cm thickness were placed in special sample ports with muscle fibers parallel to the surfaces to be analyzed and were covered with a thin optical glass removing trapped air; the same glass was used to cover the reference standard. Absorbance readings of the glass slice were considered in further calculations. Thickness of the samples was selected to satisfy requirements for R_∞ measurements (R_∞ = reflectance of a layer so thick that an increase in thickness does not change this value). To establish standard spectra, meat pigments were converted to the three forms of myoglobin. Reduced Mb samples were obtained from fresh cuts in deep zones of muscles and/or by spreading a thin layer of sodium hydrosulfite over the meat surface. For conversion to MetMb, potassium ferricyanide (1%) in aqueous solution was sprayed three times at intervals of one hour over meat slices at 4 °C to a level of 0.1 ml per 100 cm^2 of beef surface. Slices with myoglobin completely converted to MbO_2 were obtained by blooming in an oxygen atmosphere at 4 °C during two hours.

Scattering coefficients were determined from reflectance measurements of beef slices on black and white backgrounds; thickness of samples varied between 1.7 and 20 mm because most accurate determinations were obtained when the difference between the reflectance of the thin slice and of the infinitely thick slice was large (McDougall 1971).

RESULTS AND DISCUSSION

Equations for Determining Relative Pigment Concentration

Diffuse reflectance refers to reflected radiant energy that has been partially absorbed and partially scattered by a surface with no defined angle of reflection. The most generally accepted theory concerning diffuse reflectance has been developed by Kubelka and Munk (Wendlant and Hecht 1966; Kortum 1969) and it is valid for weakly absorbing substances in diffuse reflecting systems only. Most other theories are special cases or adaptations of Kubelka-Munk theory. For the purpose of Kubelka and Munk analysis, meat can be considered to be a light scattering matrix of cellular material, myofibrillar proteins, connective tissue and light absorbing pigments (McDougall 1970). The intensity of reflected light and therefore its color and appearance is governed by the interrelationship of the light scattering components in the system, and the concentration and spectral absorption properties of the pigments. The red pigments are absorbers of light and the uncolored structure and myofibrillar proteins both scatter and absorb.

The Kubelka-Munk function, $F(R_\infty)$ in the case of meat tissue, can be expressed as:

$$F(R\infty) = \frac{(1 - R_\infty)^2}{2\,R\infty} = \frac{\Sigma Ki_\lambda\, Ci}{\Sigma Si_\lambda\, Ci} =$$

$$\frac{K_\lambda^{oxy} C^{oxy} + K_\lambda^{myo} C^{myo} + K_\lambda^{met} C^{met} + K_\lambda^{m} C^{m}}{S_\lambda^{oxy} C^{oxy} + S_\lambda^{myo} C^{myo} + S_\lambda^{met} C^{met} + S_\lambda^{m} C^{m}} \qquad (1)$$

where: Ki = absorption coefficients of pigments and matrix; Si = scattering coefficients; Ci = concentration of the components in meat tissue. Considering that scattering is attributed to the matrix of fibers ($S_\lambda^m = S_\lambda$), the following was obtained:

$$F(R_\infty) - Fo(R_\infty)\,\Big|_\lambda = \frac{K_\lambda^{oxy} C^{oxy} + K_\lambda^{myo} C^{myo} + K_\lambda^{met} C^{met}}{S_\lambda C^{m}} \qquad (2)$$

where $Fo(R_\infty) = \frac{K^m}{S}$.

Reflectance spectra of beef shows a minimum at approximately 730 nm (Krzywicki 1979) where it is not dependent on pigment concentration and can be considered as the $\frac{K}{S}$ value by free pigment meat.

Analysis of reflectance spectra of meat is concerned with changes in reflectance at specific wavelengths: 525 nm is isobiestic for all three derivatives, 572 nm is isobiestic for MbO_2 and reduced Mb, 473 is isobiestic for MbO_2 and Met Mb.

Writing Eq. (2) for $\lambda = 525$ nm and $\lambda = 572$ nm and defining $\Delta F_\lambda = F(R_\infty)_\lambda - F(R_\infty)_{730}$, the following expressions were obtained:

$$\Delta F_{525} = \frac{K_{525}^{oxy=myo=met} (C^{oxy} + C^{myo} + C^{met})}{S_{525} C^m} \tag{3}$$

$$\Delta F_{572} = \frac{K_{572}^{myo-oxy} (C^{myo} + C^{oxy}) + K_{572}^{met} C^{met}}{S_{572} C^m} \tag{4}$$

Equation (3) shows that $(\Delta F)_{525}$ is an indicator of the total myoglobin content and the overall color intensity of the meat.

Defining the fractional concentration of each pigment as $x^i = \frac{C^i}{C^T}$, with $C^T = C^{oxy} + C^{myo} + C^{met}$, from Eqs. (3) and (4) the value of x^{met} was obtained as follows:

$$x^{met} = \frac{\frac{\Delta F_{572}}{\Delta F_{525}} \left(\frac{K}{S}\right)_{525}^{oxy=myo=met} - \left(\frac{K}{S}\right)_{572}^{myo=oxy}}{\left(\frac{K}{S}\right)_{572}^{met} - \left(\frac{K}{S}\right)_{572}^{myo=oxy}} \tag{5}$$

In a similar way writing Eq. (2) for $\lambda = 473$ and considering Eq. (3), the value x^{myo} was expressed as follows:

$$x^{myo} = \frac{\frac{\Delta F_{473}}{\Delta F_{525}} \left(\frac{K}{S}\right)_{525}^{oxy=myo=met} - \left(\frac{K}{S}\right)_{473}^{oxy=met}}{\left(\frac{K}{S}\right)_{473}^{myo} - \left(\frac{K}{S}\right)_{473}^{oxy=met}} \tag{6}$$

The relative concentration of oxymyoglobin can be obtained directly from the values of x^{met} and x^{myo} ($\Sigma x^i = 1$). To check the validity of these equations an independent expression was derived, selecting $\lambda = 580$ nm, one of the characteristic wavelengths of oxymyoglobin absorption spectra, then:

$$x^{oxy} = \frac{\frac{\Delta F_{580}}{\Delta F_{525}}\left(\frac{K}{S}\right)_{525}^{oxy=myo-met} + x^{met}\left\{\left(\frac{K}{S}\right)_{580}^{myo} - \left(\frac{K}{S}\right)_{580}^{met}\right\} - \left(\frac{K}{S}\right)_{580}^{myo}}{\left(\frac{K}{S}\right)_{580}^{oxy} - \left(\frac{K}{S}\right)_{580}^{myo}} \quad (7)$$

Table 1. Comparison of average K/S ratio for meat pigments.

Wave length (nm) ratio λ_1/λ_2	Ratio of K/S values at two wavelengths			Source of data
	OxyMb	Mb	MetMb	
572/525	1.40 (0.16)[1]	1.40 (0.16)	0.56 (0.04)	Stewart et al (1965)
572/525	1.33 (0.07)	1.33 (0.08)	0.61 (0.04)	Snyder and Armstrong (1967)
473/525	0.96 (0.04)	0.54 (0.04)	0.96 (0.02)	
572/525	1.37 (0.05)	1.34 (0.05)	0.65 (0.02)	Pierson et al (1970)
473/525	0.92 (0.03)	0.58 (0.04)	0.90 (0.02)	
572/525	1.49 (0.04)		0.55 (0.03)	Van den Oord and Wesdorp (1971a)
473/525	0.91 (0.01)		1.06 (0.02)	
572/525	1.50 (0.03)	1.50 (0.02)	0.55 (0.02)	Present Study[2]
473/525	0.94 (0.02)	0.52 (0.03)	0.94 (0.04)	
580/525	1.97 (0.03)	1.43 (0.03)	0.52 (0.03)	

[1]Standard deviation between parenthesis.
[2]Mean of 10 samples.

Standard spectra were used to determine K/S values for each form of myoglobin derivatives at different wavelengths. The observed values as well as those reported in literature are shown in Table 1.

The inclusion of these results in Eqs. 5, 6 and 7 led to the following equations for evaluating relative pigment concentration:

$$x^{met} = 1.58 - 1.05 \frac{\Delta F_{572}}{\Delta F_{525}} \tag{8}$$

$$x^{myo} = 2.24 - 2.38 \frac{\Delta F_{473}}{\Delta F_{525}} \tag{9}$$

$$x^{oxy} = 1.685\ x^{met} + 1.85 \frac{\Delta F_{580}}{\Delta F_{525}} - 2.65 \tag{10}$$

Scattering Coefficient

Absorption coefficients in reflectance spectrophotometry are similar to molar absorptivity coefficients used in transmission spectroscopy. The ratio of (K/S) values at two given wavelengths (Table 1) differs from the ratio of absorbances at the same wavelengths obtained by Broumand *et al.* (1958) in extract solutions (Table 2). This difference can be attributed to the dependence of scattering coefficient on wavelength, calculated from the following expression:

$$\left.\frac{S_{\lambda 2}}{S_{\lambda 1}}\right|_{predicted} = \frac{(K/S)_{\lambda 1}}{(K/S)_{\lambda 2}} \cdot \frac{A_{\lambda 2}}{A_{\lambda 1}} \tag{11}$$

Predicted values (Table 2) show a slight decrease of S when λ increases. These results were confirmed by experimental determinations of scattering coefficient on beef slices of different thickness. For such experiments the following equation was applied:

$$S = \frac{1}{2bx} \ln \left| \frac{(Rg - R_\infty)(1 - R R_\infty)}{(R - R_\infty)(1 - Rg R_\infty)} \right| \tag{12}$$

where R = reflectance of the sample of x thickness over a background of reflectance Rg; $b = (a^2 - 1)^{1/2}$; $a = \frac{1}{2}\left(\frac{1}{R_\infty} + R_\infty\right)$.

Table 2. Variation of scattering coefficient with wavelength

	Ratio of absorbance coefficients. Broumand et al (1958)		Predicted ratio of scattering coefficients (Eq. 11)	
	$\frac{A_{473}}{A_{525}}$	$\frac{A_{572}}{A_{525}}$	$\frac{S_{525}}{S_{473}}$	$\frac{S_{525}}{S_{572}}$
MbO_2	0.99	1.38	1.09	0.95
Mb	0.57	1.38	1.09	0.90
Met Mb	0.99	0.40	1.37	0.95

Scattering coefficients were obtained under such conditions that Rg of the background differed by more than 20% from R_∞. (Cairns *et al.* 1976).

Kinetics of Metmyoglobin Accumulation in Aerobic Packages

Samples packaged in polyethylene (aerobic conditions) and stored at 0 °C and 4 °C were analyzed for relative pigment concentration using reflectance spectrophotometry measurements and applying Eq. 8, 9 and 10. Storage times of the meat cuts were established by maximum total bacterial counts of 10^7 CFU/cm² starting from initial average values of 10^4 CFU/cm². These conditions led to the following periods: 14 days at 0 °C and 7 days at 4 °C.

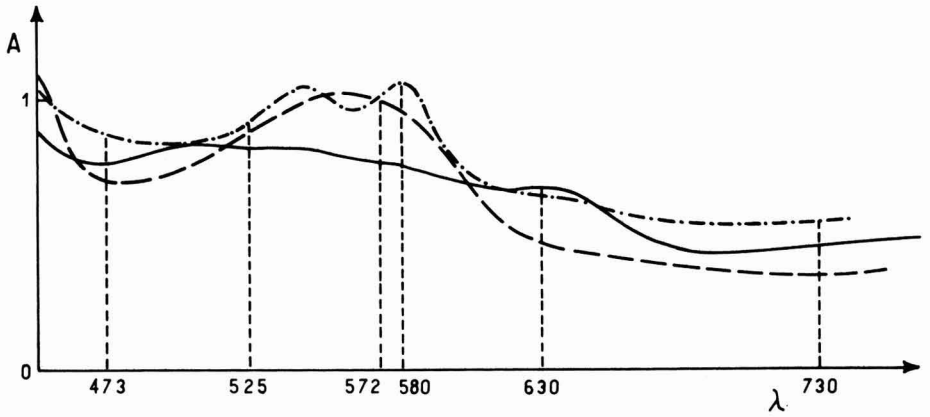


FIG. 1. REFLECTANCE SPECTRA OF BEEF DURING AEROBIC STORAGE AT 0 °C
—— initial; —·— 1 day; —— 14 days.

The evolution of reflectance spectra for aerobic packaging conditions (Fig. 1) and the changes in relative proportions of myoglobin derivatives

during storage time at 0 °C (Fig. 2) show that myoglobin was rapidly converted into the oxygenated form, followed by a gradual autoxidation of the pigment to metmyoglobin. Pigment modifications can be interpreted on the basis of the color cycle in fresh meat (Fox 1966). Reduced myoglobin, which lacks a ligand in the sixth position, upon exposure to air, reacts with oxygen to yield the bright red oxygenated pigment (MbO_2). The red complex once formed is stabilized by the formation of a highly resonant structure. Oxygen is continually associating and dissociating from the heme complex.

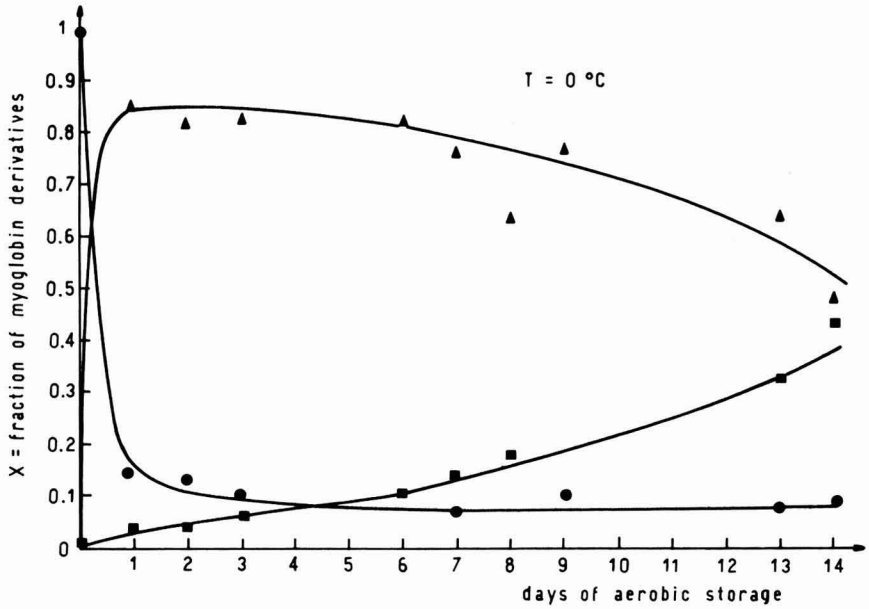


FIG. 2. CHANGES IN RELATIVE CONCENTRATION OF MYOGLOBIN DERIVATIVES DURING AEROBIC STORAGE AT 0 °C (Packaging film: low density polyethylene; 60 μm thick)
▲ Oxy Mb; ● Mb; ■ Met Mb.

In the presence of oxygen the ferrous ion in the heme group tends to oxidize slowly to the ferric state, producing the undesirable brown metmyoglobin. In fresh meat, this autoxidation of myoglobin is pseudo reversible; the enzymatic reducing system present in the tissue is capable of converting metmyoglobin back to one of the reduced forms (Ledward 1970). Deoxygenation of myoglobin was proposed as a likely intermediate step in the autoxidation because this reaction proceeds with oxygen as the sole reducing species, and one electron transfer from the iron center to a high-field ligand is a thermodynamically unfavorable reaction (Livingston and Brown 1981).

Obtained data of metmyoglobin production at 0 and 4 °C were interpreted considering that myoglobin autoxidation proceeds as a first order reaction with respect to unoxidized myoglobin.

The kinetic equation was expressed as follows:

$$d\, x_{MetMb}/dt = k\,(X_{Mb} + X_{MbO_2}) \tag{13}$$

resulting in:

$$\ln\left(\frac{1 - x_{MetMb}}{1 - x^{\circ}_{MetMb}}\right) = k\,t \tag{14}$$

where x°_{MetMb} is the initial relative concentration of metmyoglobin present in beef sample.

Plotting experimental data according to Eq. (14) kinetic constants of metmyoglobin formation in M. gluteus were: $k = 0.00104\ h^{-1}$ at 0 °C (Fig. 3) and $k = 0.00203\ h^{-1}$ at 4 °C.

Information available in literature on autoxidation rate constants correspond, mainly, to data obtained in solutions at different temperatures. George and Stratmann (1952a) reported a first order rate constant (k) of $0.325\ h^{-1}$ at 30 °C in solutions of pure equine myoglobin. Bembers and Satterlee (1975) working on muscle extracts from normal porcine muscles

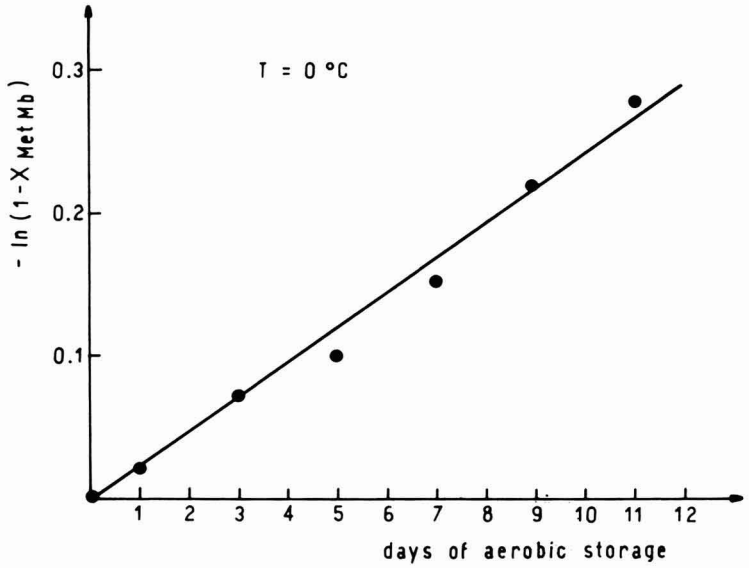


FIG. 3. FIRST ORDER KINETIC PLOT FOR MYOGLOBIN AUTOXIDATION AT 0 °C

(pH between 5.8 and 6.0) obtained values of $k = 0.003\ h^{-1}$ at 5 °C, $k = 0.025\ h^{-1}$ at 30 °C; in purified Mb solutions, the values were higher: $k = 0.027\ h^{-1}$ at 5 °C and $0.119\ h^{-1}$ at 30 °C. Satterlee and Hansmayer (1974) obtained $k = 0.00325\ h^{-1}$ at 5 °C for semitendinosus muscle, a value similar to that reported for muscle extracts.

As it can be seen, autoxidation is strongly temperature dependent. Brown and Mebine (1969) determined activation energies for this reaction reporting values between 24 to 27.8 Kcal/mol ($Q_{10} = 5$ for temperatures between 0 °C and 22 °C).

Differences in kinetic constants can be attributed to the intermuscular variability with reference to the aerobic reducing activity of the muscles. M. psoas major is the least stable to discoloration followed by M. gluteus medius, M. semimbranosus and finally M. longissimus dorsi, the most stable muscle with respect to color (Hood 1980).

Extremely high values of autoxidation constants were obtained by Ledward *et al.* (1977) in sterile muscles; the authors attributed these results to the presence of myoglobin oxidation catalysts in beef. Actually, the difference arose from an erroneous application of first order kinetics (data were plotted as log % MetMb versus time instead of applying Eq. (14)). When their experimental data were plotted in the correct way, the kinetic constants were similar to the values reported in the present study (e.g., $k = 0.003\ h^{-1}$ for samples 10 mm thick at 1 °C).

Different criteria are shown in literature with reference to the levels of metmyoglobin that represent the end of meat shelf life. Van den Oord and Wesdorp (1971a) affirmed that at approximately 50% conversion to MetMb the meat is unacceptable to most consumers and unsuitable for retail sale. Hood and Riordan (1973) found that the sales ratio of discolored beef to bright red beef is approximately 1:2 when 20% metmyoglobin is present. It is important in practical terms to know how long it takes to reach this level of discoloration.

According to experimental data, MetMb reached a relative concentration of 0.2 in approximately 216 h at 0 °C (Fig. 2) and in 96 h at 4 °C. These values agree with those reported by Hood (1980) in samples where bacteriological causes of contamination were eliminated, showing the negligible effect of normal microbial growth on color stability, specially at short storage times.

Effects of Vacuum Aging Time on Myoglobin Oxygenation and Oxidation Rates

Vacuum packaging permits refrigerated storage of fresh meat for longer periods; meat is normally aged to improve tenderness and eating quality.

Effects of vacuum aging time on myoglobin oxygenation and oxidation

rates were analyzed. Vacuum packaged beef sections were stored at 0° and 4 °C during 38 and 14 days, respectively; storage time was established by total bacterial counts of 10^7 CFU/cm^2 (Zamora and Zarirzky 1985). In these samples reduced myoglobin levels of approximately 100% persisted throughout the entire storage period. Samples stored during 4, 7 and 23 days at 4 °C and 0 °C in low permeability films were removed from the packages and allowed to oxygenate in air. Reflectance spectra were recorded continuously during a period of one hour (Fig. 4). Application of Eq. (8, 9, 10) showed an increase of oxymyoglobin relative concentration as a function of time.

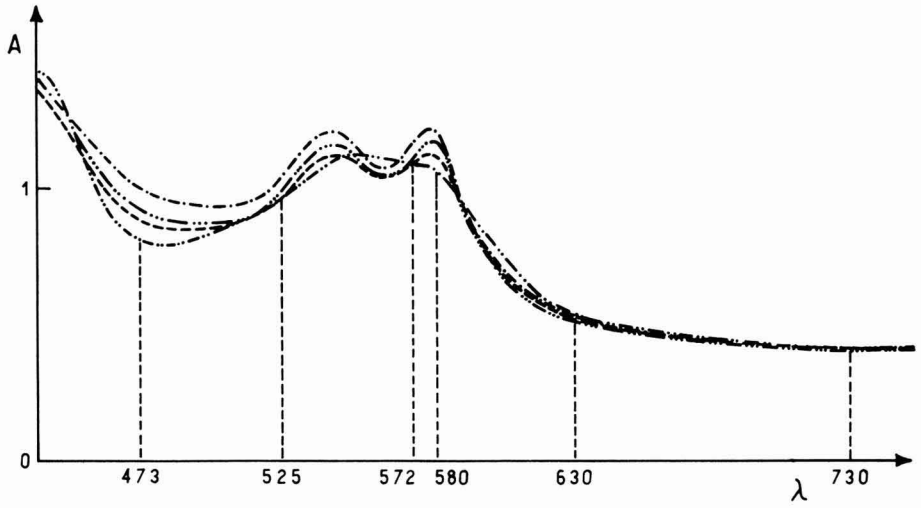


FIG. 4. REFLECTANCE SPECTRA FOR BEEF DURING OXYGENATION
—··— initial, --- 5 min, —···— 12 min, —·— 20 min.

Aging time led to lower levels of oxymyoglobin in meat tissues (Fig. 5). Oxygenation was also affected by storage temperatures; samples aged at 0 °C during 7 days showed higher levels of oxymyoglobin than those stored at 4 °C during the same period of time. Formation of oxymyoglobin from myoglobin in solutions is a rapid reaction; the half time of myoglobin oxygenation in solution was found to be 4×10^{-4} s (Krzywicki 1982) and at oxygen partial pressures higher than 60 mm Hg only oxymyoglobin is present (George and Stratmann 1952b), but in meat tissue this reaction is controlled by the rate of oxygen penetration and conversion depends on meat characteristics (type of muscle, aging conditions). Oxygenation was interpreted as a first order reversible reaction with myoglobin equilibrium concentration, x^e_{Mb}, depending on temperature and duration of conditioning storage.

When a meat sample was exposed to air an equilibrium constant for the oxygenation process can be established:

$$Mb \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} MbO_2; \qquad K\ eq = \frac{k_1}{k_1'}$$

where k_1 and k_1' are forward and reverse constants for the elementary process. The integrated form of the rate expression was used with concentration-time data to evaluate kinetic constants:

$$-\ln\left(\frac{x_{Mb} - x^e_{Mb}}{x^o_{Mb} - x^e_{Mb}}\right) = \left(\frac{K+1}{K}\right) k_1\, t = k_R\, t \qquad (15)$$

where x^o_{Mb} is the initial relative concentration of myoglobin in the meat.

Table 3 shows the effect of previous vacuum storage at 4 °C on oxygenation rates; k_R was practically constant but K values decreased with vacuum aging time. This loss of oxygenation capacity proceeded with a rapid accumulation of metmyoglobin in muscle.

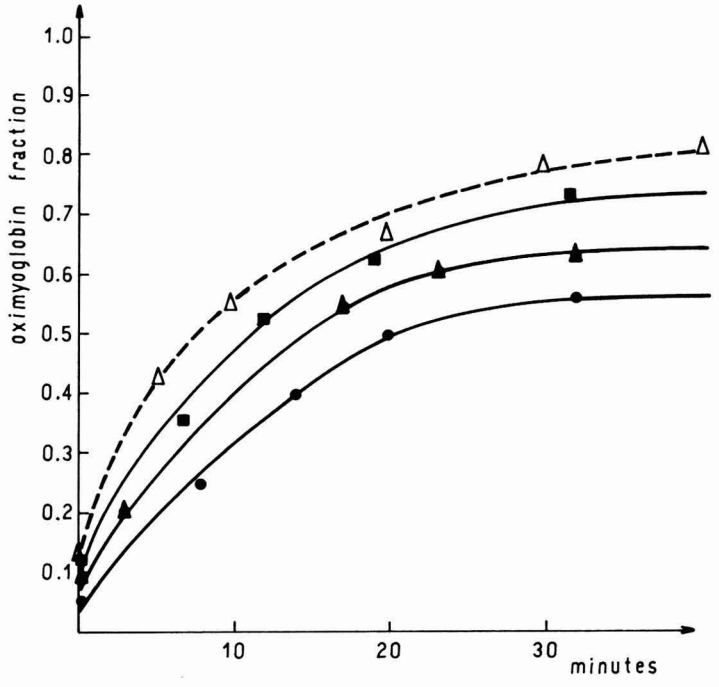


FIG. 5. AGING TIME EFFECT ON OXYGENATION RATE
Previous vacuum storage at 4 °C: ■ 4 days; ▲ 7 days; ● 23 days. At 0 °C: △ 7 days.

Table 3. Effects of vacuum aging time at 4 °C, on myoglobin oxygenation rates (M. gluteus medium)

Aging time (days)	X^{e}_{Mb}	k_R (min^{-1})	k_1 (min^{-1})	$K = k_1/k'_1$
1	0.13	0.14	0.12	6.53
3	0.15	0.12	0.10	5.66
7	0.25	0.13	0.09	2.87
11	0.44	0.13	0.07	1.27

Experiments storing beef samples in vacuum during 7 and 14 days at 0 °C and then, wrapped with an oxygen permeable film (polyethylene) and maintained during a subsequent period of 14 days at 0 °C showed the influence of aging time on metmyoglobin accumulation. Figures 6a and 6b show that conditioning of meat diminished the stability of the oxy-deoxy myoglobin mixture to oxidation.

Initial microbial counts of the samples were 2×10^4 CFU/cm^2. These values remained practically constant (lag phase) during the vacuum aging period of 7 days at 0 °C; a slight increase was observed for the aging period of 14 days (3.1×10^4 CFU/cm^2). During subsequent aerobic storage, microbial counts reached 1.3×10^6 CFU/cm^2 and 2.1×10^6 CFU/cm^2 respectively, at the sixth day of storage.

During postmortem time, respiratory activity of enzymes decreases leading to higher oxygen penetration values when meat is exposed to air. A deeper zone of oxymyoglobin masks the inner metmyoglobin layer which is formed at low oxygen partial pressures. This beneficial effect of conditioned meat color is opposed by the increase of metmyoglobin accumulation rate.

Differences between metmyoglobin relative concentrations in conditioned meat samples (Fig 6a and b) and in fresh meat (Fig 3) during aerobic packaging are observed from the beginning of the storage period. These results cannot be attributed to microbial growth because total counts remained lower than 10^7 CFU/cm^2 during the first six days of storage.

Considering the oxygen permeability of polyethylene (6500 cm^3O_2/m^2 day atm at 23 °C), the bacterial demand of oxygen (Pseudomonas sp. consume 0.970×10^{-7} μl O_2/h cell at 30 °C, Greig and Hoogerheide 1941) and respiration activity of postmortem tissue (Bendall and Taylor 1972), levels of contamination in the order of 10^6 CFU/cm^2 cannot accelerate metmyoglobin formation by reducing oxygen partial pressures to values lower than 30 mm Hg. In summary, meat color deterioration should be

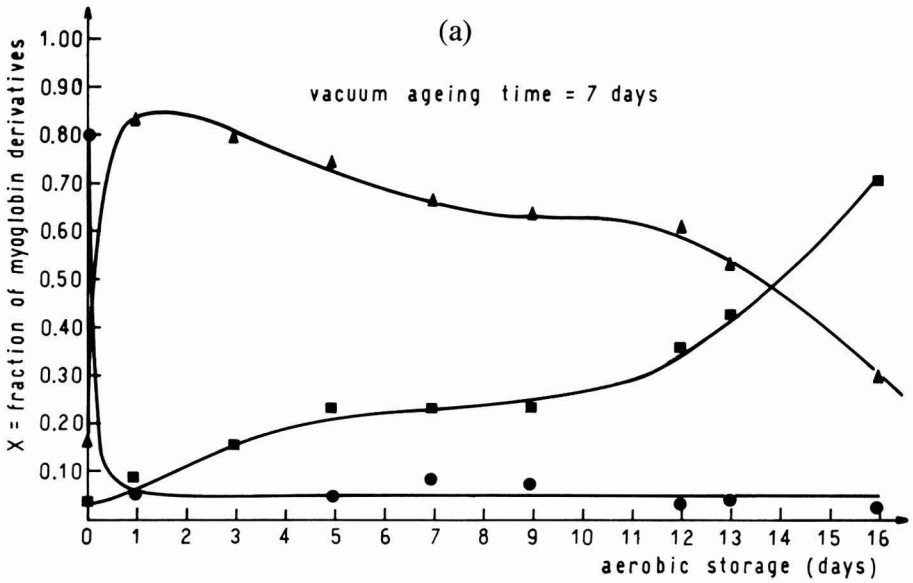
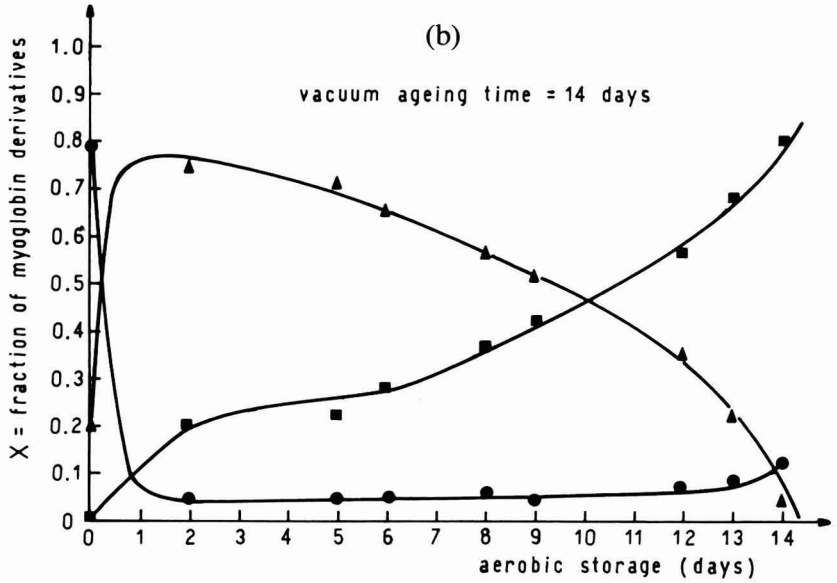


FIG. 6. CHANGES IN RELATIVE CONCENTRATION OF MYOGLOBIN DERIVATIVES DURING AEROBIC STORAGE OF AGED MEAT Vacuum aging time: a) 7 days; b) 14 days.
▲ Oxy Mb; ● Mb; ■ Met Mb.



attributed to the loss of metmyoglobin reducing activity in the tissue (Stewart *et al.* 1965; Fox 1966; Ledward 1970; Hood 1980) and the incipient denaturation of the globine fraction of myoglobin during storage time.

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PRODUCTION OF ENRICHED β-LACTOGLOBULIN AND α-LACTALBUMIN WHEY PROTEIN FRACTIONS

A. W. SLACK[1], C. H. AMUNDSON[2], and C. G. HILL,[3] JR.

University of Wisconsin-Madison
Madison, WI 53706

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ABSTRACT

This is a process based on (1) 90% volume reduction of whey by ultrafiltration, (2) partial demineralization of the UF retentate by electrodialysis or diafiltration, (3) pH adjustment of the UF retentate before and after demineralization to pH 4.65, and (4) centrifugation of the retentate produced β-lactoglobulin-enriched fractions containing 33% of the original acid whey protein and 17% of the original sweet whey proteins. Lactose and ash impurities in the β-lactoglobulin-enriched fraction were reduced by 18% by resuspending the pellet in distilled water at pH 4.65 and recentrifuging. Diafiltration of the α-lactalbumin-enriched fraction with 2, 4 and 6 volumes of distilled water reduced the lactose and ash content of this fraction by 53%, 62% and 81%, respectively. Gel electrophoresis patterns of the two protein enriched fractions confirmed selective enrichment of β-lactoglobulin or α-lactalbumin in each fraction.

INTRODUCTION

Amundson *et al.* (1982) have previously reported a method for fractionating whey proteins into β-lactoglobulin and α-lactalbumin-enriched fractions This fractionation procedure (see Fig. 1) utilizes the techniques of ultrafiltration and electrodialysis to produce two individual whey pro-

[1]Department of Food Science.
[2]Departments of Food Science and Agricultural Engineering.
[3]Department of Chemical Engineering.

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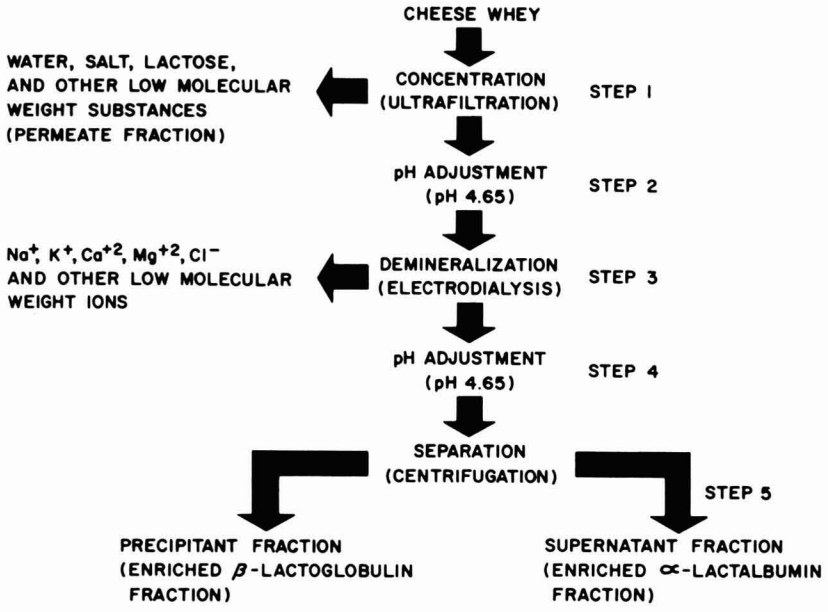

FIG. 1. FLOW DIAGRAM FOR FRACTIONATION OF β-LACTOGLOBULIN AND α-LACTALBUMIN

tein enriched fractions which have improved functional properties relative to currently available whey protein concentrates. In order to better define the conditions needed to optimize this fractionation procedure a series of experiments were caried out using pilot-plant scale production equipment to prepare β-lactoglobulin and α-lactalbumin enriched products. Specifically, the purposes of the experiments reported here are: (1) to establish the degree of separation and yield of protein obtained in the two enriched fractions when the starting whey material is reduced to 10% of its original volume by ultrafiltration prior to pH adjustment; (2) to assess the relative merits of electrodialysis and diafiltration as alternative methods of demineralization; and (3) to investigate possibilities for further purification of the two enriched fractions.

MATERIALS AND METHODS

A flow diagram (Fig. 1) depicts how the β-lactoglobulin and α-lactalbumin enriched fractions were isolated from the whey. The fractionation procedure was performed in the University of Wisconsin food engineer-

ing pilot plant. Fresh sweet whey produced during cheddar cheese manufacture was obtained from Associated Milk Producers, Inc., Madison, Wisconsin and fresh acid whey produced during cottage cheese manufacture was obtained from the Bancroft Dairy, Madison, Wisconsin. Initially fresh whey was pasteurized (161F - 17 s) and clarified in the University of Wisconsin dairy plant.

A modified model HF25SS UF unit (Romicon, Inc., Woburn, Ma.) was used for all ultrafiltration procedures. The modifications involved the use of a larger pump (7.5 hp vs. 2.5 hp) and the installation of sanitary disc valves at both ends of each UF cartridge (Fig. 2). Romicon hollow fiber UF cartridges (type HF 26.5-43-PM50, 2.5m² surface area, and type HF 13.5-43-PM50 0.71m² surface area, polysulfone composition) were used to reduce the volume of the whey protein by at least 90% volume reduction.

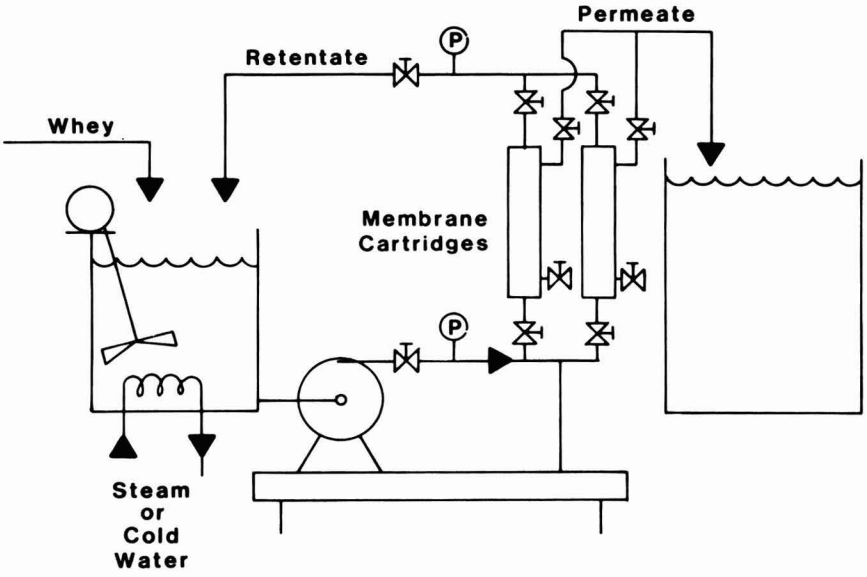

FIG. 2. SCHEMATIC DIAGRAM OF THE ROMICON ULTRAFILTRATION UNIT

Prior to ultrafiltration of the whey, the UF equipment was cleaned and sanitized until a pure water permeate flux of 1.222×10^4 liter/m²/day was obtained at 52°C, 136 kPa average transmembrane pressure. Ultrafiltration of the whey involved continuous recirculation of a measured amount of preheated whey (49°C) for 15 min at an average transmembrane pressure of 136kPa to achieve steady state operating conditions. After this initial period, recycle of the permeate was discontinued and additional

measured amounts of whey were added to the feed tank. The retentate was continuously recycled to the feed tank until the desired quantity of whey had been ultrafiltered to remove the specified amount of permeate. To produce a whey retentate corresponding to the desired 90% volume reduction, 950 liters of whey were ultrafiltered to produce a volume of 95 liters. Upon completion of the UF experiment the liquid whey protein retentate was drained from the system and collected. The UF system was then cleaned according to the manufacturers instructions until the original water fluxes were restored.

Demineralization of the retentate by diafiltration was accomplished with this same UF equipment. The diafiltration procedure utilized start up, shutdown and cleaning procedures identical to those of the UF experiment. Diafiltration of the 95 liters of preheated (49 °C) retentate consisted of recirculating this retentate through the UF equipment at 136kPa average transmembrane pressure while adding 285 liters of warm (49 °C) water to the feed tank at the same rate that permeate was being removed. Diafiltration was continued in this manner until the desired extent of demineralization was achieved.

Demineralization of the retentate by electrodialysis was conducted using a pilot plant electrodialysis unit from Ionics, Inc., Watertown, Ma. (Fig. 3). The membrane stack assembly consisted of alternating 23 cm × 25 cm type 111 EZ L-219 anion selective membranes, polyethylene spacer sheets, type 61CZL-183 cation selective membranes and polyethylene spacer sheets. The membrane stack was initially equilibrated by circulating pretreated whey (pH adjusted to 4.65), UF permeate (pH 4.65) and electrolyte solution (0.15N sodium sulfate, (Na_2SO_4), pH 2.54) through the concentrate, dilutate and electrolyte flow loops, respectively, for 30 min at 18 °C while a current of 0.2 amps was passed through the stack. Once the ED membrane stack was equilibrated, the ED unit was turned off and the solutions were drained from the unit. The concentrate tank was then filled with 32 liters of the retentate (pH adjusted to 4.65) to be demineralized and the diluate and electrolyte tanks were refilled with fresh permeate and 0.15N Na_2SO_4 solutions. Once the temperature of these recirculating streams reached 18 °C, a constant voltage of 110 volts was applied across the membrane stack. As demineralization of the retentate occurred the current dropped from 4.5 amps to 0.5 amps. Once the current dropped to 0.5 amps, electrodialysis of the retentate solution was continued for 30 min longer and then stopped. Throughout the electrodialysis solution temperatures and the applied voltage were maintained at 18 °C and 110 volts, respectively. The demineralized solution was then drained and collected from the ED unit and the ED unit was then cleaned following the manufacturer's instructions.

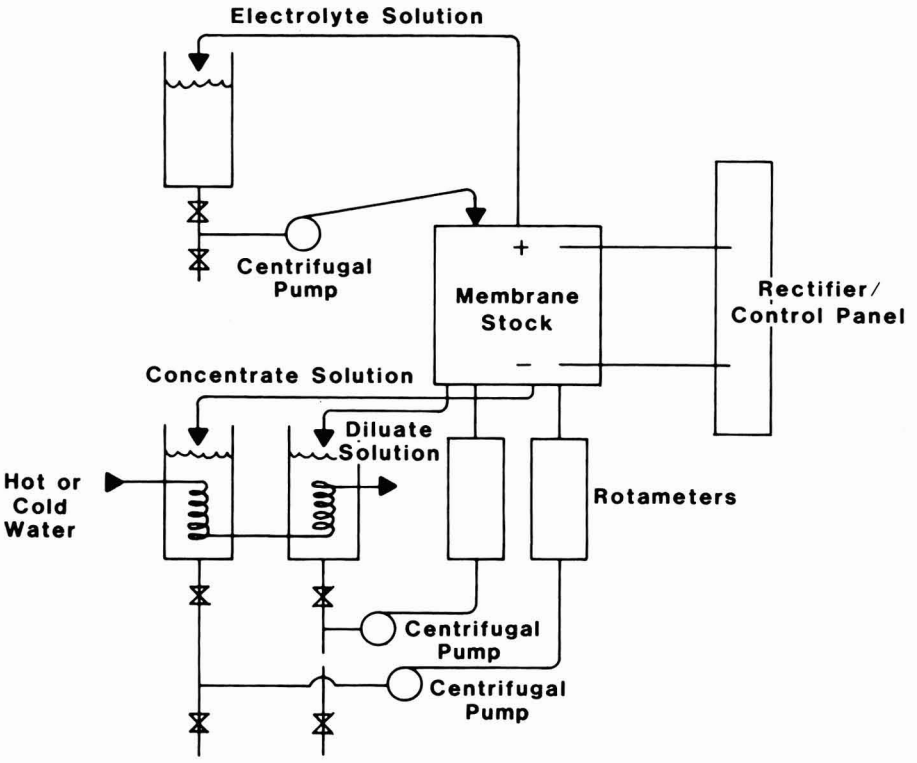

FIG. 3. SCHEMATIC DIAGRAM OF THE IONICS ELECTRODIALYSIS UNIT

The demineralized whey retentate was then adjusted to pH 4.65 by the addition of NaOH or HCL solutions with agitation. A Radiometer 26 pH unit with a glass combination electrode was used to measure pH.

The pH adjusted, demineralized whey retentate samples were then centrifuged in a Sorvall RC-5 super speed refrigerated centrifuge. The centrifugation conditions were chosen to expose the samples to a force of 9000 × g for 30 min at a temperature of 20 °C.

The centrifuged samples were then decanted and the supernatant liquid and the solid residue were analyzed by the following methods: total solids and fats by the Mojonnier method (Mojonnier and Tray 1965); total noncombustible ash by AOAC gravimetric method (1975); total nitrogen by the macrokjeldahl method (Bradsheet 1965); nonprotein nitrogen as that nitrogen soluble in 12% trichloroacetic acid. The factor 6.38 was used to convert nitrogen to protein. Analyses were performed in duplicate using reagent grade chemicals and distilled water.

Polyacrylamide gel electrophoresis (PAGE) was used to evaluate protein purity and quality. The procedure utilized was that of Darling and Butcher (1975).

RESULTS AND DISCUSSIONS

Fresh acid and sweet whey's were ultrafiltered to 90% volume reduction and demineralized by electrodialysis at pH 4.65 to an ash content of 0.07% (w/w) and 0.72% (w/w), respectively. The resultant solutions were centrifuged to yield semi-hard white precipitates and clear yellow supernatant liquids. Table 1 indicates that 33.51% (w/w) of the acid whey protein and 16.90% (w/w) of the sweet whey proteins were recovered in the pellet and 11.62% of the acid whey proteins and 38.13% of the sweet whey proteins remained in the supernatant liquids. Material balances indicate that 54.87% (w/w) of the acid whey protein was not recovered in the overall fractionation process; 32.50% (w/w) of the acid whey protein is lost during ultrafiltration; 18.32% is lost during electrodialysis and 3.55% is lost during centrifugation. When sweet whey is the feedstock a total of 44.97% (w/w) of the original protein is lost during processing; 24.36% (w/w) is lost during ultrafiltration, 19.03% (W/w) during electrodialysis and 1.58% during centrifugation.

These data imply that more of the acid whey protein can be recovered in the precipitate from this fractionation process than is possible in the corresponding sweet whey process. Moreover the data indicates that the particular electrodialyses procedure employed was more efficient in demineralizing the acid whey retentate than the sweet whey retentate. Previous work on the fractionation procedure (Amundson *et al.* 1982) has shown that the lower the ash content of the whey retentate, the higher the percentage of protein recovered. Therefore if the ash content of the sweet whey retentate could have been reduced to 0.07%, more protein would probably have been recovered from the sweet whey.

Much of the protein loss during ultrafiltration and electrodialysis is due to protein fouling of the membranes, incomplete removal of the retentate from the two systems and losses encountered during transfer from one sys-tem to another. Whey protein is also lost during filling and draining of the centrifuge tubes and as a consequence of incomplete removal of the precipitate from the centrifuge tubes. A large fraction of these protein losses could be recovered with more efficient drainage systems for the UF and ED units, reprocessing the rinse water used to clean these systems, and more efficient transfer of the fluids from the UF system to the ED system.

Table 1. Comparison of electrodialysis and diafiltration in the fractionation procedure[a]

	Method of Demineralization			
	Electrodialysis		Diafiltration	
	Acid Whey	Sweet Whey	Acid Whey	Sweet Whey
% Ash content of ultrafiltered whey	0.72	0.77	0.72	0.77
% Ash content of ultrafiltered, demineralized whey	0.07	0.07	0.27	0.38
% Protein content of ultrafiltered whey	4.44	6.03	4.44	6.03
% Protein content of ultrafiltered, demineralized whey	3.70	5.74	4.80	6.13
% Total solids content of ultrafiltered demineralized whey	8.82	13.13	7.26	9.88
Protein precipitated from ultrafiltered, demineralized whey, g/100g of feedstock	1.24	0.97	1.63	0.52
Protein yield in precipitate g/100g protein in ultrafiltered, demineralized whey	33.51	16.90	33.96	8.48

[a] Percentages given are on a weight basis

Figure 4 and 5 depict PAGE patterns of the acid and sweet whey retentates and the precipitate and supernatant liquids obtained from the fractionation of the demineralized retentates. The PAGE patterns indicate that the retentates contain both β-lactoglobulin and α-lactalbumin whey proteins (samples 7 and 8 of Fig. 4). Samples 9 and 11 of Fig. 4 are PAGE patterns of the pellets obtained from the fractionated acid and sweet whey electrodialyzed retentates. Qualitatively these PAGE patterns indicate a fractionation of β-lactoglobulin protein into the pellet fraction. PAGE patterns of the supernatant obtained from the fractionation of the acid and sweet whey retentate are seen on Fig. 5 (samples 13 and 15, respectively). These patterns indicate that some β-lactoglobulin is still present in the supernatant liquid. The PAGE patterns show that a β-lactoglobulin enriched fraction is produced but the total recovery of the β-lactoglobulin whey proteins into the precipitate is not achieved. Visual observations of the PAGE patterns of the retentates and the enriched fractions produced from the retentates indicate that the fractionation procedures do not harm the whey protein.

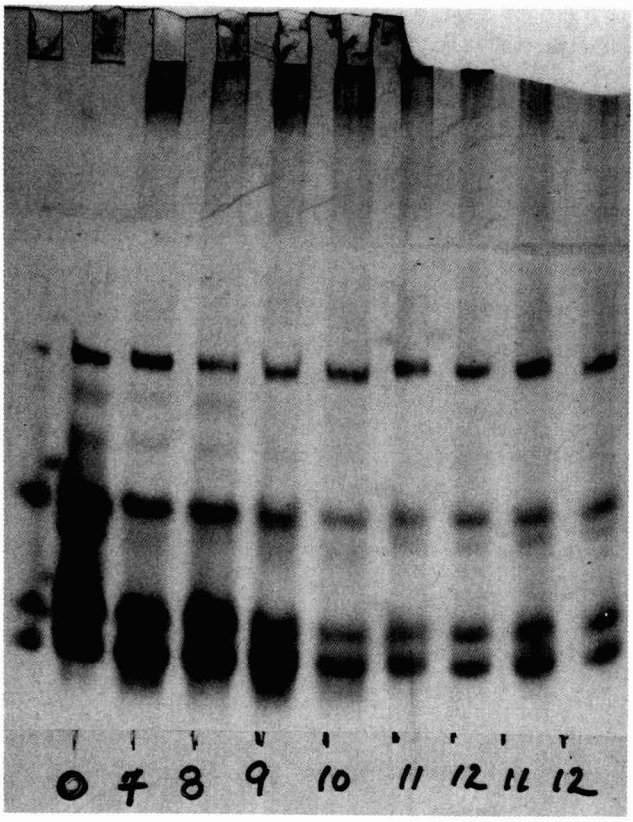

FIG. 4. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS

0) Mixture of β-lactoglobulin and α-lactalbumin from Sigma Chem Corp.
7) Freeze dried sweet whey protein concentrate
8) Freeze dried acid whey protein concentrate
9) β-lactoglobulin fractionated from electrodialyzed sample #7
10) β-lactoglobulin fractionated from diafiltered sample #7
11) β-lactoglobulin fractionated from electrodialyzed sample #8
12) β-lactoglobulin fractionated from diafiltered sample #8

With a view towards avoiding the high energy consumption and capital investment costs associated with the use of electrodialysis we investigated diafiltration of the retentate as an alternative to electrodialysis as the method of demineralization in the fractionation process. The acid whey retentate demineralized by diafiltration resulted in a solution with nearly 4 times the ash content and 1.3 times the protein content of the electrodialyzed acid whey retentate (see Table 1). Upon centrifugation the diafiltered retentate spun down into a semi-hard, white pellet and a clear yellow supernatant products similar to those obtained in the separation scheme utilizing

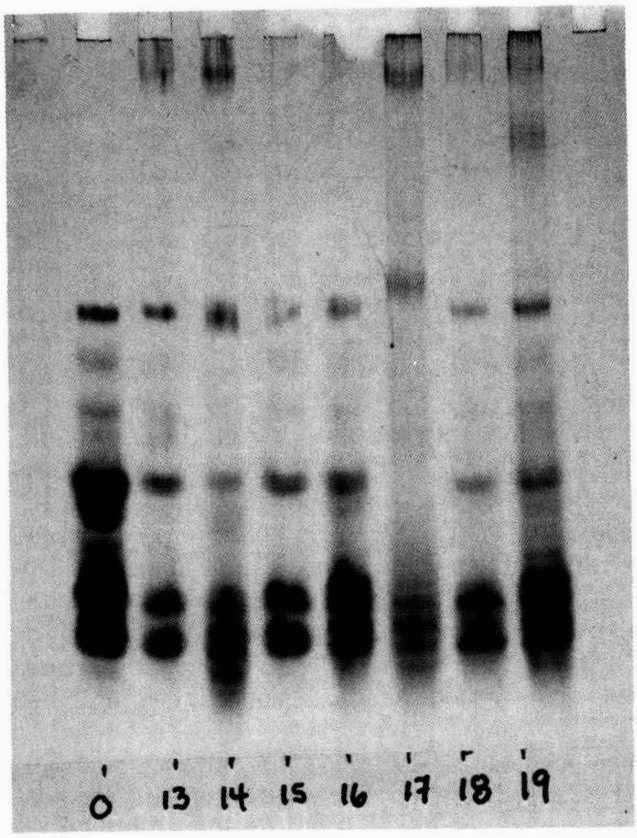

FIG. 5. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS

0) Mixture of β-lactoglobulin and α-lactalbumin from Sigma Chem Corp.
13) α-lactalbumin fractionated from electrodialyzed sample #7
14) α-lactalbumin fractionated from diafiltered sample #7
15) α-lactalbumin fractionated from electrodialyzed sample #8
16) α-lactalbumin fractionated from diafiltered sample #8
17) α-lactalbumin fractionated from electrodialyzed sample #1
18) α-lactalbumin fractionated from electrodialyzed sample #2

electrodialysis of the acid whey retentate. Approximately the same amount of protein was recovered in the pellet fraction; 33.96% (w/w) of the original acid whey protein was recovered in the precipitate after diafiltration while 33.5% (w/w) of the original acid whey protein was recovered in the precipitate after electrodialysis. In processing the acid whey retentate demineralized by diafiltration only 31.76% (w/w) of the original protein was lost during the fractionation procedure compared to the 54.87% (w/w) of the original protein which was lost when electrodialysis was employed.

The diafiltered sweet whey retentate solution was 5.5 times higher in ash and 1.07 times higher in protein than the electrodialyzed sweet whey retentate solution. Upon centrifugation the diafiltered retentate separated into a soft, white precipitate and a cloudy yellow supernatant. The sizes of the precipitate derived from the diafiltered sweet whey retentate were noticeably smaller than those derived from the electrodialyzed sweet whey retentate. In this case only 8.46% (w/w) of the original sweet whey protein was recovered in the precipitate compared to 16.90% of the original sweet whey protein recovered in the precipitate derived from the electrodialyzed retentate. However, in processing the sweet whey retentate via diafiltration only 25.35% (w/w) of the original whey proteins were lost compared to the 44.95% (w/w) of the original whey proteins which were lost in the process employing electrodialysis.

These results indicate that higher recoveries of protein can be obtained in the precipitate from acid whey than is the case with sweet whey. Material balances on the various steps in the fractionation procedure indicate that elimination of the electrodialysis step reduces the losses of whey protein that are incurred during transfer from the UF unit to the ED unit, in fouling of the ED membranes, and in the holdup volume of the ED unit. Furthermore, diafiltration appears to "wash off" or resolubilize some of the whey proteins deposited on the UF membranes. PAGE patterns of the retentates and separated fractions (Fig. 4 and 5) indicate substantial enrichment of β-lactoglobulin in the precipitate.

In an attempt to further purify the β-lactoglobulin-enriched fractions, centrifuged pellets were resuspended in distilled water, the pH of the solution was adjusted to 4.65, and the solution was centrifuged at 9000 $\times$ g for 30 min. The results of these experiments indicate that the protein contents of the β-lactoglobulin-enriched precipitates were increased from 67% to 80% (w/w) for acid whey retentates and from 59% to 69% (w/w) for sweet whey retentates. However, some protein was lost in the supernatant wash waters; during the first and second washings, 9.34% and 5.39% (w/w), respectively, of the protein were lost from the β-lactoglobulin-enriched precipitate derived from acid whey. During the first and second washings 20.24% and 7.56% (w/w) were lost from the β-lactoglobulin-enriched precipitate derived from sweet whey. It is not known if α-lactalbumin protein was washed from the relatively insoluble β-lactoglobulin-enriched pellets, but this hypothesis would provide a reasonable explanation for the above protein losses.

Diafiltration was also used to eliminate more of the lactose and ash from the α-lactalbumin enriched fractions. Original α-lactalbumin enriched fractions were diafiltered to their original volumes after dilutions with 2, 4, and 6 times their original volumes of distilled water. On a dry basis the lactose and ash contents of these diafiltered retentates were 72.31% (w/w)

for the original α-lactalbumin supernatant, 34.21% (w/w) for the diafiltered (2X distilled water dilution) solution, 26.88% (w/w) for diafiltered (4X distilled water dilution) solution, and 13.55% (w/w) for diafiltered (6X distilled water dilution) solution. These results indicate that diafiltration further purifies the α-lactalbumin-enriched fractions.

Gel filtration was also used to "desalt" the α-lactalbumin-enriched fraction. The α-lactalbumin-enriched fraction was applied to a vertical glass column filled with Sephadex G25 medium gel and the α-lactalbumin was eluted from the column with distilled water. The protein was eluted after the void volume and the ash and lactose were eluted after a volume equal to three times the void volume.

Purification by gel filtration reduced the lactose and ash contents (dry basis) of the α-lactalbumin-enriched fraction from 72.31% to 32.48% (w/w). Hence gel filtration yielded an α-lactalbumin-enriched fraction similar in dry weight composition to that obtained by 2X dilution with distilled water and diafiltration. One disadvantage of this method is that the eluted sample was four times more dilute than the original sample. In commercial applications this would add extra processing costs for water removal.

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NITROGEN SOLUBILITIES OF β-LACTOGLOBULIN AND α-LACTALBUMIN ENRICHED FRACTIONS DERIVED FROM ULTRAFILTERED CHEESE WHEY RETENTATES

A. W. SLACK[1], C. H. AMUNDSON[2] and C. G. HILL, JR.[3]

University of Wisconsin-Madison
Madison, Wisconsin 53706

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ABSTRACT

The nitrogen solubilities of β-lactoglobulin and α-lactalbumin-enriched fractions derived from acid and sweet wheys were measured over a range of concentrations as a function of pH. The β-lactoglobulin-enriched fractions had relatively low nitrogen solubilities at concentrations above 5% (W/V), especially at pH 4.65. This fraction was soluble at pH 7.0 and at concentrations below 5% (W/V). The α-lactalbumin-enriched fraction had a high nitrogen solubility at concentrations of ⩽ 20% (W/V) within the pH range of 3.0 to 9.0.

INTRODUCTION

A protein with good solubility in different ionic environments is important for optimum functionality in beverages, foams, emulsions, gels and similar applications. Solubility measurements also provide indications of the degree of protein denaturation and thereby serve as useful guides to selection of optimum conditions for protein isolation and manufacture. Solubility can be affected by any condition to which the protein has been exposed, e.g., its original protein source, its processing history, and the conditions of the solubility determination.

Several researchers have studied the solubilities of whey protein concentrates (WPC) prepared by different methods (Beck 1981; Burgess and Kelly 1979; Hildalgo and Gamper 1977; Modler and Emmons 1977; Morr

[1]Department of Food Science.
[2]Departments of Food Science and Agricultural Engineering.
[3]Department of Chemical Engineering.

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et al. 1973; Richert *et al.* 1974; Sternberg *et al.* 1976). These studies found WPCs to be soluble over the pH range of 3 to 8 usually with a sudden decrease in solubility near pH 4.65. The decrease is attributed to the insolubility of β-lactoglobulin at this pH. WPC manufacturing procedures which involve excessive heat, pH extremes, and exposure to $CaCl_2$, iron, carboxymethylcellulose or metaphosphates result in WPCs with reduced solubility which may be due to protein denaturation.

In this paper the nitrogen solubilities of WPC and WPC fractions enriched in β-lactoglobulin and α-lactalbumin were measured over a wide range of WPC concentrations, pH, and salt concentrations.

MATERIALS AND METHODS

Protein solubilities were measured at several pH values, ionic strengths, protein concentrations and temperatures. The nitrogen solubility index (NSI), an official method of the American Oil Chemists Society, was employed. Solutions of WPC and β-lactoglobulin enriched fractions were made up at sample concentrations of 0.5, 1.0, 2.0, 3.0 and 5.0% (W/V) in distilled water. Solutions of the α-lactalbumin-enriched fraction were made up in distilled water at sample concentrations of 1.0, 5.0, 10.0, 15.0 and 20.0% (W/V) in distilled water. Once the sample was dissolved, the pH of the mixture was adjusted to 7.0 with HCl or NaOH. Each of the solutions was stirred with a magnetic stirring bar for 30 min and then centrifuged at 100Xg for 10 min. The amounts of nitrogen in both the sediment and supernatant were determined by the semi-micro Kjeldahl method (Bradsheet 1965). The nitrogen solubilities of the various samples were expressed as the percentage of the nitrogen in the soluble fraction relative to the total nitrogen in the mixture before centrifugation.

The effect of pH on the nitrogen solubilities of the sample was determined by making 2.0% (W /V) solutions of the whey and β-lactoglobulin-enriched fraction and 10.0% (W/V) solutions of the α-lactalbumin-enriched fraction in distilled water. The pH values of aliquots of the various solutions were then adjusted with HCl or NaOH to produce samples with pH values of 3.0, 4.65, 5.0, 7.0 and 9.0. The percent solubility at each pH was determined as noted above.

To determine the effect of salt concentration on the nitrogen solubilities, 2.0% (W/V) solutions of the whey and β-lactoglobulin-enriched fraction and 10.0% (W/V) solutions of the α-lactalbumin-enriched fraction were prepared with 0.1 or 0.3M NaCl solutions. One set of samples, comprised of mixtures made from the 0.1 or 0.3 M NaCl solutions, was adjusted to pH 7.0; the other set was adjusted to pH 4.65. The percent solubility was determined as before.

All nitrogen solubility tests were performed in duplicate. Error analysis estimates based on the nitrogen solubility calculations indicate an error limit of ±3% (determined as a standard error of the method).

The whey protein samples tested were derived from fresh cheddar cheese or cottage cheese wheys which had been ultrafiltered to 90% volume reduction. Portions of these ultrafiltered cheese wheys were then fractionated into β-lactoglobulin and α-lactalbumin enriched fractions. This fractionation procedure consisted of electrodialysis and pH adjustment of the WPC to 4.65 followed by centrifugation. Centrifugation resulted in a β-lactoglobulin enriched solid and an α-lactalbumin enriched supernatant. The β-lactoglobulin enriched solid was then further purified by resuspending or washing the solid with an aqueous buffer solution (pH 4.65). The suspension was again subjected to centrifugation to remove some of the lactose and ash. Lactose and ash were removed from the α-lactalbumin enriched supernatant via diafiltration with distilled water. The α-lactalbumin samples were then spray dried or freeze dried. The compositions of the samples tested are summarized in Table 1.

RESULTS AND DISCUSSION

Effect of Sample Concentration on Nitrogen Solubility

At pH 7.0 the effect of sample concentration on the nitrogen solubilities of the rehydrated WPC was very small, except for the spray dried acid whey protein concentrate (AWPC) (#1) and the freeze dried concentrated AWPC (#14) (see Fig. 1). These two WPCs exhibited marked decreases in nitrogen solubility as their concentrations were increased. In contrast, the nitrogen solubility of the freeze dried sweet whey protein concentrate (SWPC) (#9) ranged from 99% to 105% and the nitrogen solubility of the spray dried SWPC (#2) ranged from 91% to 93% over the concentration range of 0.5 to 5.0% (W/V). The SWPC samples contained more protein than the AWPC samples (37% vs 31%). This disparity may be partially responsible for the differences in the nitrogen solubilities of the acid and sweet WPCs which were prepared in similar fashion.

The effect of the procedure used in the initial drying step (spray versus freeze drying) on the nitrogen solubilities of the WPC products is ambiguous. At concentration levels of 3% and below, the nitrogen solubility of the AWPC prepared by spray drying was greater than that prepared by freeze drying. However, at the 5% (W/V) concentration level, the nitrogen solubility of the freeze dried AWPC was higher than that of the spray dried AWPC. At concentration levels of 1-5% (W/V), the freeze dried concentrated AWPC had a higher nitrogen solubility than either the

Table 1. Sample Composition (% dry basis)

No.	Sample	Protein	Fat	Ash	Residual*
1	Spray Dried Acid Whey Protein Concentrate (Sp. D. AWPC)	27.92	1.95	7.94	62.19
2	Spray Dried Sweet Whey Protein Concentrate (Sp. D. SWPC)	34.63	6.04	6.03	53.30
3	ß-Lactoglobulin Source: Electrodialyzed Sp. D. AWPC	66.82	3.00	1.48	28.70
4	Washed ß-Lactoglobulin Source: Electrodialyzed Sp. D. AWPC	80.30	3.73	1.68	14.29
5	ß-Lactoglobulin Source: Electrodialyzed Sp. D. SWPC	58.71	8.16	0.74	32.39
6	Washed ß-Lactoglobulin Source: Electrodialyzed Sp. D. SWPC	69.13	9.64	0.78	20.40
7	Freeze Dried Egg Yolks	31.70	63.80	3.58	0.92
8	Freeze Dried Acid Whey Protein Concentrate (Fr. D. AWPC)	33.94	2.99	5.93	57.14
9	Freeze Dried Sweet Whey Protein Concentrate (Fr. D. SWPC)	38.91	9.16	5.22	46.71
10	ß-Lactoglobulin Source: Electrodialyzed Fr. D. AWPC	67.34	4.45	1.38	26.83
11	ß-Lactoglobulin Source: Diafiltered Fr. D. AWPC	75.31	11.44	2.05	11.02
12	ß-Lactoglobulin Source: Electrodialyzed Fr. D. SWPC	37.98	35.93	0.95	25.14
13	ß-Lactoglobulin Source: Diafiltered Fr. D. SWPC	51.96	26.28	2.41	19.35
14	Concentrated Acid Whey Protein Concentrate (Freeze Dried)	71.75	3.18	1.86	23.21
15	α-Lactalbumin Source: Electrodialyzed Sp. D. AWPC	25.02	0.92	0.44	77.62
16	Diafiltered α-Lactalbumin Source: Electrodialyzed Sp. D. AWPC	49.50	0.70	0.70	49.10
17	α-Lactalbumin Source: Electrodialyzed Sp. D. SWPC	55.60	0.70	8.0	35.70
18	α-Lactalbumin Source: Electrodialyzed Fr. D. AWPC	27.79	1.38	0.51	70.32
19	α-Lactalbumin Source: Diafiltered Fr. D. AWPC	52.36	2.05	2.68	42.91
20	α-Lactalbumin Source: Electrodialyzed Fr. D. SWPC	39.00	0.57	1.42	59.01
21	α-Lactalbumin Source: Diafiltered Fr. D. SWPC	64.49	2.41	1.34	31.76
22	Freeze Dried Egg Whites	68.80	1.90	5.04	24.26

Sp. D = spray dried
AWPC = acid whey protein concentrate
SWPC = sweet whey protein concentrate
Fr. D. = freeze dried
* = primarily lactose (by difference)

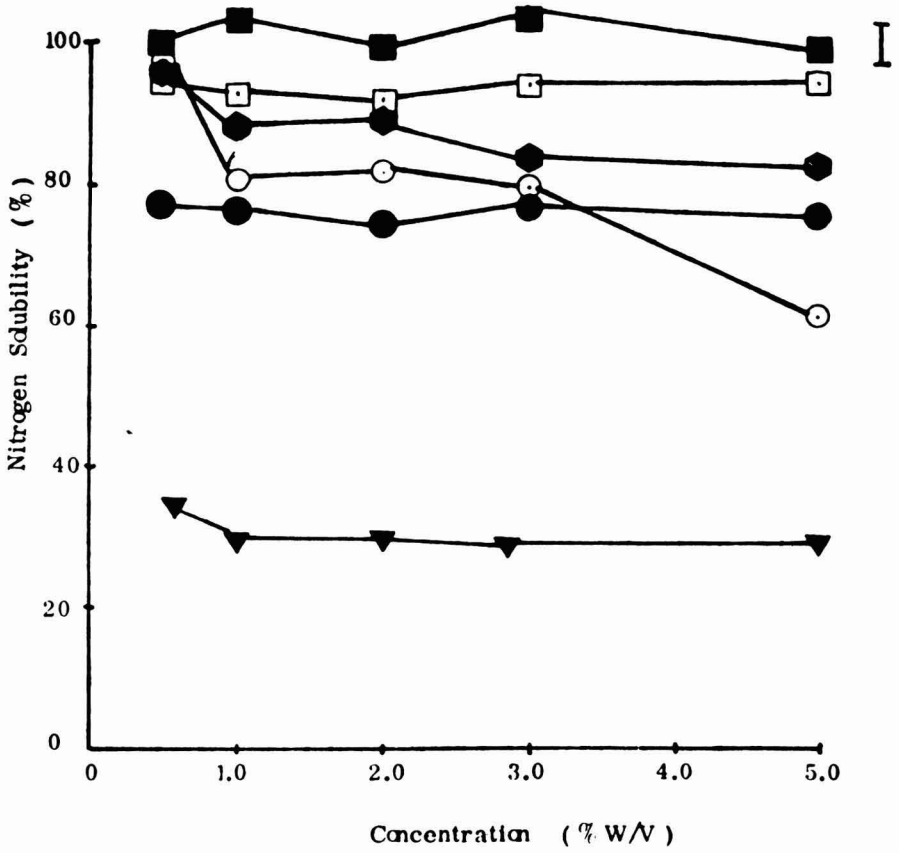

FIG. 1. NITROGEN SOLUBILITY PROFILES OF WHEY PROTEIN CONCENTRATES.

○ #1 Spray dried acid whey protein concentrate
□ #2 Spray dried sweet whey protein concentrate
● #8 Freeze dried acid whey protein concentrate
■ #9 Freeze dried sweet whey protein concentrate
⬢ #14 Freeze dried concentrated acid whey protein concentrate
▼ #7 Freeze dried egg yolks
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spray or freeze dried AWPC samples. In the case of the SWPC, the freeze dried products were essentially completely soluble at all concentration levels tested, whereas the nitrogen of the spray dried SWPC solubilities were only 91 to 93%.

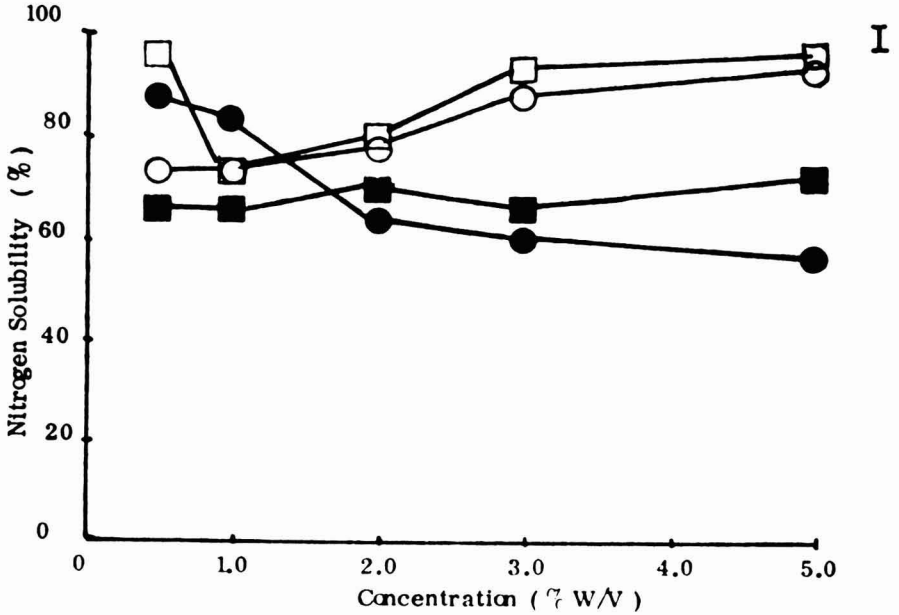

FIG. 2. NITROGEN SOLUBILITY PROFILES AT pH 7.0 FOR β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM ACID WHEY.

○ #3 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried AWPC
□ #4 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried AWPC
● #10 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried AWPC
■ #11 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried AWPC
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The overall effect of concentration on the nitrogen solubilities of the β-lactoglobulin enriched fraction is also ambiguous (Fig. 2 and 3). Figure 2 depicts the nitrogen solubility profiles of the β-lactoglobulin enriched samples from acid whey. This figure indicates that the nitrogen solubility of the β-lactoglobulin-enriched fraction fractionated from electrodialyzed spray dried AWPC (#3) increased with concentration; that the washed β-lactoglobulin enriched sample (#4) exhibited a minimum in nitrogen solubility as its concentration was increased; that the nitrogen solubility of the sample fractionated from electrodialyzed freeze dried AWPC (#10) decreased with increasing concentration; and the nitrogen solubility of the sample fractionated from diafiltered freeze dried AWPC (#11) showed very little change in nitrogen solubility as its concentration was increased. At the low concentration level of 0.5% (W/V), the washed sample (#4) was

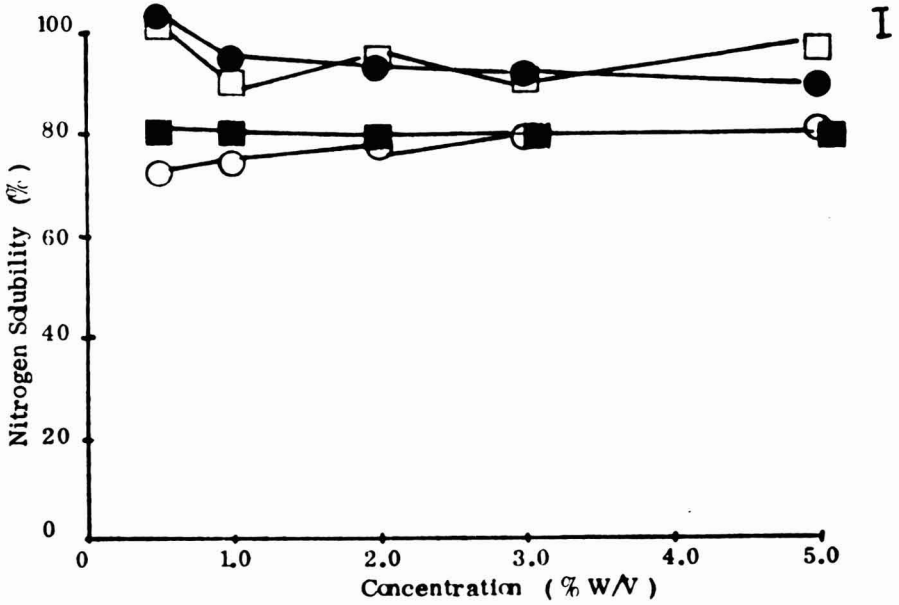

FIG. 3. NITROGEN SOLUBILITY PROFILES AT pH 7.0 FOR β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM SWEET WHEY.

○ #5 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
□ #6 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
● #12 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried SWPC
■ #13 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried SWPC
I error bracket

the most soluble, followed by the sample fractionated from the electrodialyzed freeze dried AWPC (#10), the sample fractionated from the electrodialyzed spray dried AWPC (#3) and finally the sample fractionated from the diafiltered freeze dried AWPC (#11). However, at the highest concentration level tested, the two samples fractionated from the electrodialyzed spray dried AWPC (#3 and #4) were the most soluble. These β-lactoglobulin-enriched fractions (#3 and #4) had significantly higher nitrogen solubilities than those fractionated from the diafiltered freeze dried AWPC and the electrodialyzed freeze dried AWPC.

Figure 3 depicts the nitrogen solubility profiles of the β-lactoglobulin enriched fractions derived from sweet whey. As the concentrations of the fractions derived from electrodialyzed freeze dried SWPC (#12) and the washed electrodialyzed spray dried SWPC (#6) were increased, slight

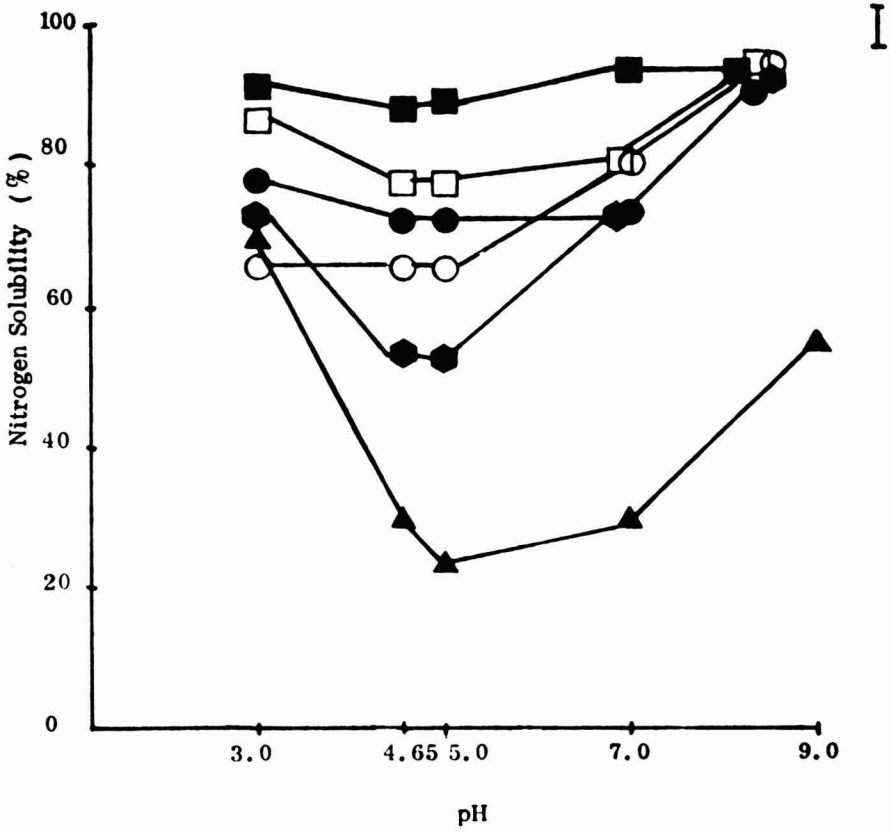

FIG. 4. THE EFFECT OF pH ON THE NITROGEN SOLUBILITIES OF WHEY PROTEIN CONCENTRATES (CONCENTRATION = 2% (W/V))

○ #1 Spray dried acid whey protein concentrate
□ #2 Spray dried sweet whey protein concentrate
● #8 Freeze dried acid whey protein concentrate
■ #9 Freeze dried sweet whey protein concentrate
⬢ #14 Freeze dried concentrated acid whey protein concentrate
▲ #7 Freeze dried egg yolks
I error bracket

decreases in nitrogen solubility to constant values of 89% and 96%, respectively, were observed. The other two samples (#5 and #13) exhibited little change in their nitrogen solubilities as their concentrations were increased, both being characterized by nitrogen solubility levels of approximately 80%. No conclusions can be drawn from these data concerning

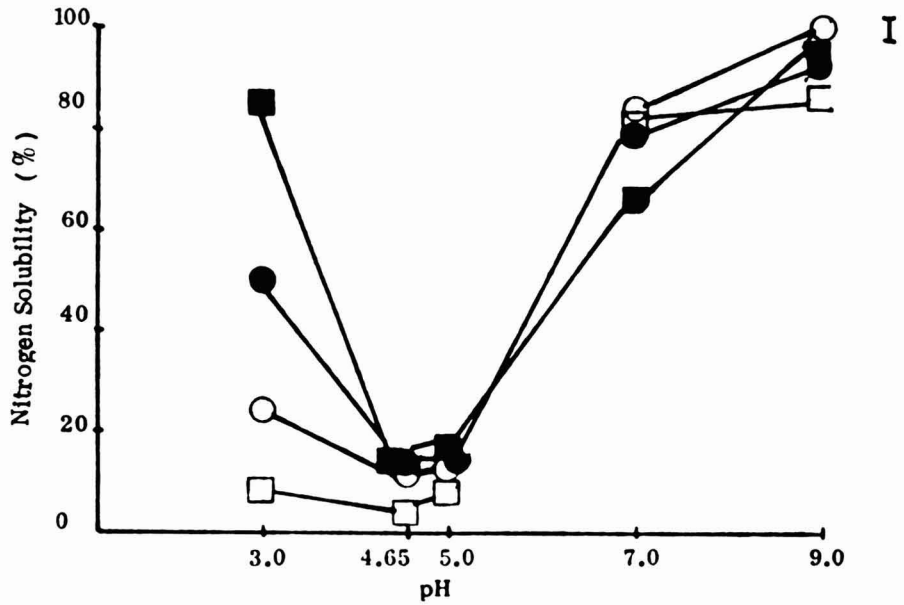

FIG. 5. THE EFFECT OF pH ON THE NITROGEN SOLUBILITIES OF β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM ACID WHEY (CONCENTRATION = 2% (W/V))

○ #3 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried AWPC

□ #4 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried AWPC

● #10 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried AWPC

■ #11 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried AWPC

I error bracket

the effects of whey source, processing history or method of drying on the nitrogen solubilities of the β-lactoglobulin-enriched fractions and their dependence on product concentration.

From a nitrogen solubility standpoint, the various α-lactalbumin enriched fractions were almost completely soluble at concentrations of $\leqslant$ 20% (W/V), regardless of protein or ash concentration or the method of drying. The nitrogen solubility of the rehydrated egg whites was also approximately 100% over the concentration range tested.

Effect of pH on Nitrogen Solubility

The solubilities of WPC and the β-lactoglobulin enriched fraction were also influenced by pH (Fig. 4 through 6). Over the pH range of 3.0 to

9.0, the WPC and β-lactoglobulin-enriched fractions exhibited minimum solubilities near pH 4.65. As the pH was increased from 5.0 to 9.0, the nitrogen solubilities of these materials increased markedly. Throughout the pH range studied, the nitrogen solubilities of the spray dried WPCs and the β-lactoglobulin-enriched fractions derived from sweet whey were lower than those of the corresponding freeze dried samples. For pH values below 5.0, the spray dried AWPCs were also less soluble than the corresponding freeze dried AWPC samples.

The type of whey (i.e. acid vs sweet) also appeared to influence the nitrogen solubilities of the WPCs. At each pH condition the spray dried SWPCs were either more soluble or comparable in solubility to the spray dried AWPCs and the freeze dried SWPCs were more soluble than the freeze dried AWPCs.

The β-lactoglobulin-enriched fractions lacked solubility near pH 4.65 but were very soluble at pH 7.0. The β-lactoglobulin enriched fractions derived from AWPC were less soluble at pH 4.65 than those derived from SWPC (see Fig. 5 and 6). The presence of salts and lactose by and large improved the solubilities of the β-lactoglobulin-enriched fraction derived from either AWPC or SWPC. (Compare the nitrogen solubility of samples 3 and 4 and the solubilities of samples 5 and 6 at pH 4.65. Also see Fig. 8 and 9.) The method used in the initial drying step (spray drying or freeze drying) did not have a major influence on the nitrogen solubilities of the β-lactoglobulin enriched samples. However, for the large majority of the conditions investigated in this aspect of the study, the nitrogen solubilities of whey protein products processed by spray drying were slightly greater than those of the comparable products processed by freeze drying (see Fig. 5 and 6). The rehydrated freeze dried egg yolks were least soluble in the pH range of 4.65 to 7.0 and the most soluble at pH 3.0.

The nitrogen solubilities of the α-lactalbumin-enriched fractions and the freeze dried egg whites were again essentially 100% and were relatively insensitive to pH in the range of 3.0 to 9.0. The large effect of pH on the nitrogen solubilities of the β-lactoglobulin enriched products relative to the pH insensitivity of the α-lactalbumin-enriched products was expected. The lack of solubility of the former protein at pH 4.65 was the basis of the fractionation process.

Effect of Ionic Strength on Nitrogen Solubility (at pH 4.65 and 7.0)

The ionic strength of a solution is known to affect the nitrogen solubilities of some proteins. Figures 7 through 9 summarize the data collected on the nitrogen solubilities of several WPCs and β-lactoglobulin enriched fractions at pH values of 4.65 and 7.0 in salt solutions at two different ionic strengths. The addition of salt did not have a major effect on the nitrogen solubilities of the WPC (Fig. 7).

Increasing the concentration of salt added to the solutions of the β-lactoglobulin enriched samples increased their nitrogen solubilities at pH 4.65 especially those derived from sweet whey (Fig. 8 and 9). However, when the same concentration of salt was added to these solutions at pH 7.0, different results were obtained in that the nitrogen solubilities of three of the β-lactoglobulin-enriched fractions derived from acid whey (#4, 10 and 11) decreased. At each salt level, increases in the nitrogen solubilities of the β-lactoglobulin enriched fraction were observed as the pH was increased from 4.65 to 7.0 (Fig. 8 and 9). The nitrogen solubilities of the β-lactoglobulin enriched fractions derived from acid whey in solutions at

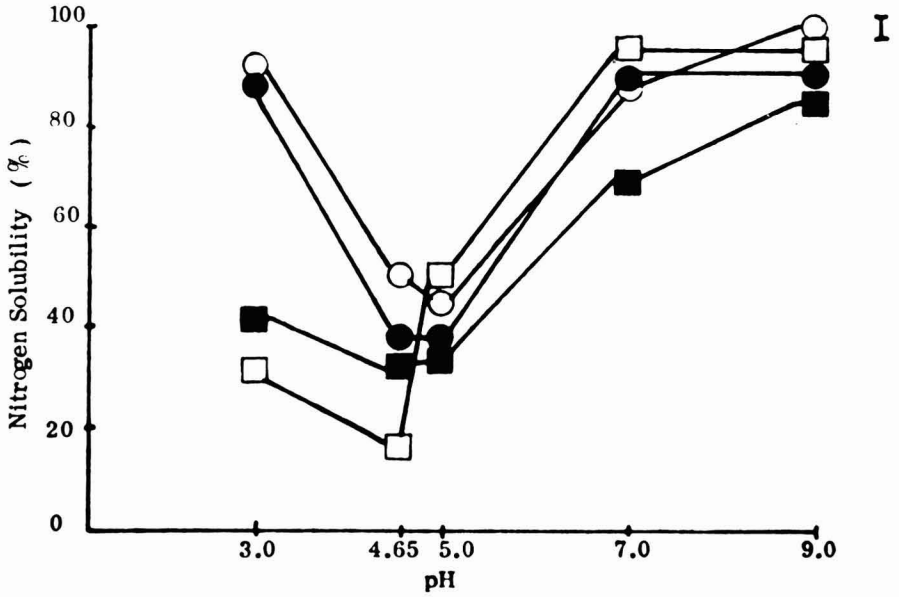

FIG. 6. THE EFFECT OF pH ON THE NITROGEN SOLUBILITIES OF β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM SWEET WHEY (CONCENTRATION = 2% (W/V))

○ #5 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
□ #6 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
● #12 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried SWPC
■ #13 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried SWPC
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1M concentrations increased by factors of 2.0 (sample #11) to 7.2 (sample #3) as the pH was increased from 4.65 to 7.0; in 3M salt solution the

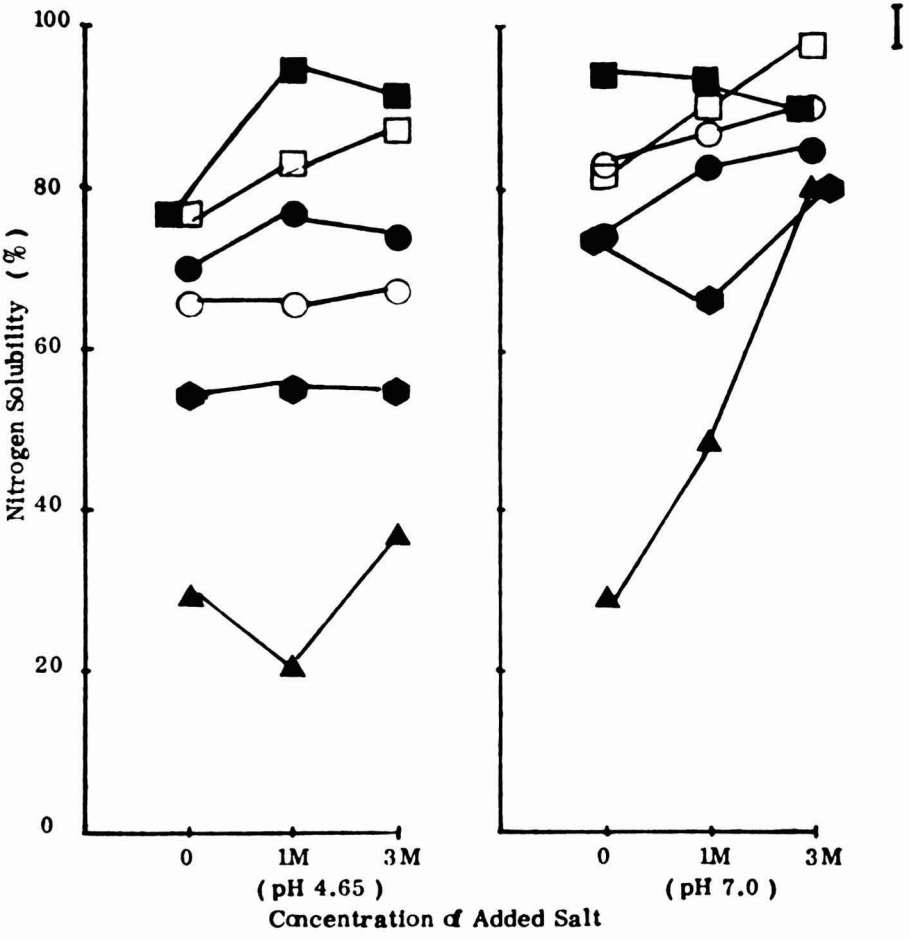

FIG. 7. THE EFFECTS OF pH AND IONIC STRENGTH ON THE NITROGEN SOLUBILITIES OF WHEY PROTEIN CONCENTRATES (CONCENTRATION = 2% (W/V))

○ #1 Spray dried acid whey protein concentrate
□ #2 Spray dried sweet whey protein concentrate
● #8 Freeze dried acid whey protein concentrate
■ #9 Freeze dried sweet whey protein concentrate
⬢ #14 Freeze dried concentrated acid whey protein concentrate
▲ #7 Freeze dried egg yolks
I error bracket

nitrogen solubility of sample #11 increased by a factor of 1.4 while the nitrogen solubility of sample #3 increased by a factor of 5.1. The nitrogen

solubilities of the β-lactoglobulin-enriched fraction derived from electrodialyzed SWPC increased markedly with pH at both salt concentrations. These data concerning the effect of pH on the nitrogen solubilities of the β-lactoglobulin enriched samples support the data in Fig. 5 and 6 discussed

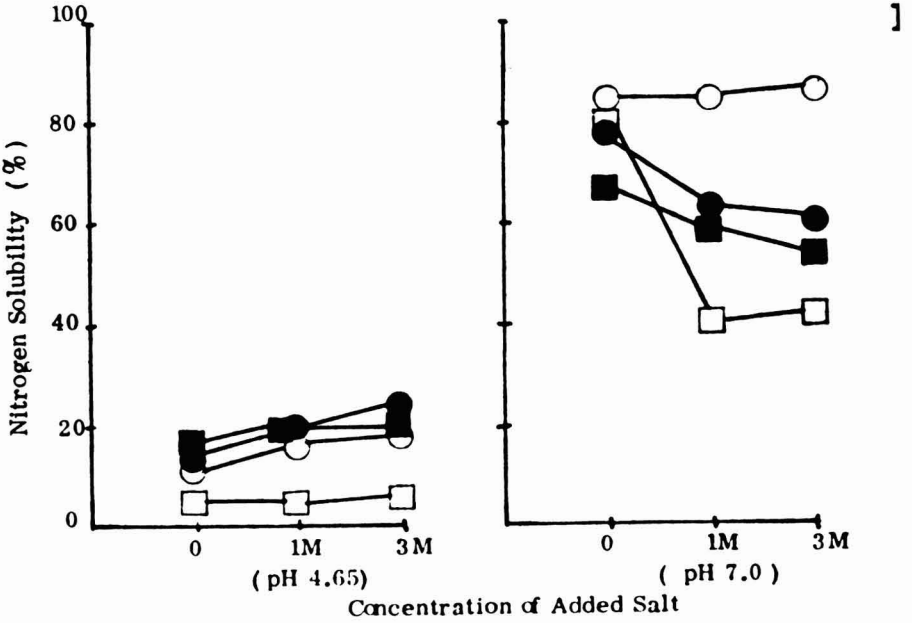

FIG. 8. THE EFFECTS OF pH AND IONIC STRENGTH ON THE NITROGEN SOLUBILITIES OF β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM ACID WHEY (CONCENTRATION = 2% (W/V))

○ #3 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried AWPC
□ #4 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried AWPC
● #10 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried AWPC
■ #11 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried AWPC
I error bracket

earlier. The addition of salt was expected to increase the solubilities of the β-lactoglobulin enriched fractions since demineralization is required in the fractionation process to cause the β-lactoglobulin to precipitate.

Neither the addition of salt nor the change in pH had a significant effect on the nitrogen solubilities of the α-lactalbumin enriched fractions. In all cases essentially complete solubilization was observed.

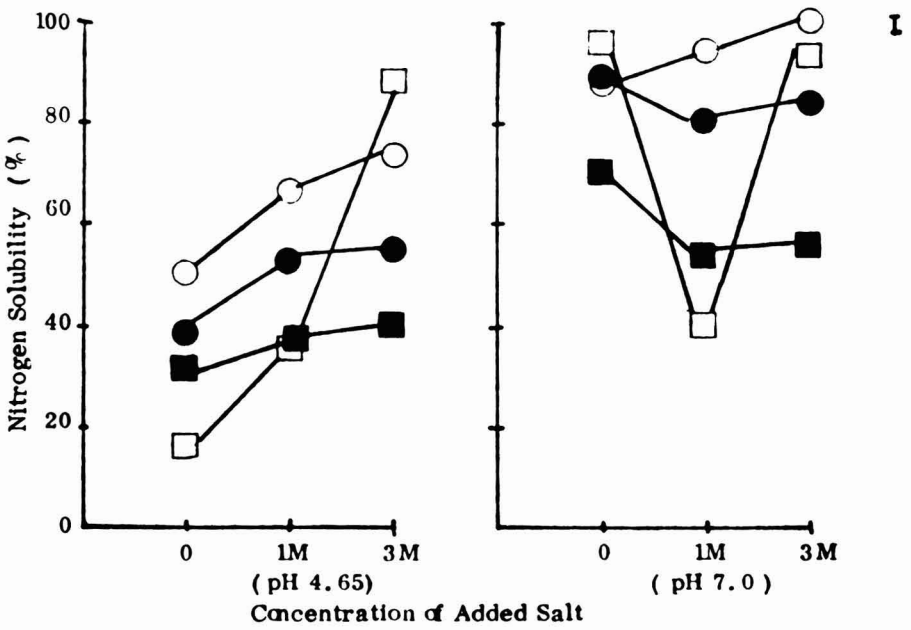

FIG. 9. THE EFFECTS OF pH AND IONIC STRENGTH ON THE NITROGEN SOLUBILITIES OF β-LACTOGLOBULIN ENRICHED SAMPLES DERIVED FROM SWEET WHEY (CONCENTRATION = 2% (W/V))

○ #5 Freeze dried β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
□ #6 Freeze dried washed β-lactoglobulin fractionated from electrodialyzed spray dried SWPC
● #12 Freeze dried β-lactoglobulin fractionated from electrodialyzed freeze dried SWPC
■ #13 Freeze dried β-lactoglobulin fractionated from diafiltered freeze dried SWPC
I error bracket

CONCLUSIONS

The results of this study show that the α-lactalbumin enriched fraction derived from demineralized WPC has improved solubility relative to both unfractionated WPC and the β-lactoglobulin enriched fraction. The dry α-lactalbumin enriched fractions were 100% soluble up to concentrations of 20% (W/V) at pH 7.0, and were 100% soluble at concentrations of 10% (W/V) over a pH range of 3.0 to 9.0 and in 0.3 M NaCl solutions. This type of solubility is comparable to that of dried egg whites. In contrast, WPCs and the β-lactoglobulin enriched fractions showed limited solubility at concentrations of 5% (W/V) and at pH 4.65.

The high solubility of the α-lactalbumin enriched samples over wide ranges of concentration, pH, and ionic strength indicates that this fraction could be used as a nutritional supplement in fruit juice or soft drinks. These materials may also be useful in coffee whiteners because of their high solubilities in acidic solutions. The sensitivity of the solubilities of the β-lactoglobulin enriched samples to changes in concentration and pH implies that one may have problems in using this fraction as a constituent in liquid foods due to the likelihood that these proteins will precipitate during processing or storage if changes occur in the protein concentration and/or in pH.

ACKNOWLEDGMENTS

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SOME PROPERTIES OF SOURED AND DE-ACIDIFIED BUFFALO'S MILK — MINERAL COMPOSITION

O. N. MATHUR and B. SUJATHA

Dairy Chemistry Division
National Dairy Research Institute
Karnal-132001 India

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ABSTRACT

The influence of passing electric current in the acidic buffalo milk with a view to explore the possibility of retaining and utilizing such milk for the manufacture of dairy products was studied. Twelve trials were conducted on de-acidification of milk and the quality of milk was judged by the Heat Coagulation time and the concentration of major minerals, namely, calcium, magnesium, phosphate and citrate in normal buffalo milk, its rennet whey, acidic milk whey and the de-acidified milk whey. The average HCT for normal milk, acidic milk and de-acidic milk was 42.66±1.40, 2.74±0.03, 44.41±1.78 min, respectively. The average pH of these milks were 6.72±0.19, 6.21±0.02 and 6.77±0.03, respectively.

The soluble calcium, in different types of whey obtained from whole milk, acidic milk and de-acidified milk were 24.03%, 33.11% and 21.37%, respectively. Similarly, magnesium contents were 56.78%, 66.58% and 56.61% and the phosphate contents were 43.87%, 60.74% and 42.24%; and citrate 67.93%, 81.48% and 62.16%.

INTRODUCTION

In India, souring of a large quantity of milk particularly during summer is not uncommon, primarily due to unhygenic conditions, adverse climatic conditions and lack of refrigerated transport. A significant portion of milk turns acidic or curdles when received by the dairies, especially during summer months. The need for utilizing sour milk, particularly for human consumption has been discussed by Vijai Kumar and Mulay (1979). Gupta *et al.* (1980) discussed the use of curdled milk for the manufacture of ghee.

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Under the rules of PFA (1950) addition of neutralizers to milk has been prohibited. An investigation was conducted to ascertain if such milk can be admitted under PFA and suitable protocols can be developed which reduce the acidity to a considerable extent by passing electric current.

Benson (1949) used aluminum electrodes and passed a low tension current for de-acidification of milk. Casalis (1949) used 2-8 volts current for de-acidification of milk and afterwards it was pasteurized. The present study was conducted with the objectives that whether the electricity de-acidified milk retain most of its properties namely (1) heat stability (2) the status of minerals etc. as that of normal milk.

MATERIALS AND METHODS

Composite milk samples from not less than twenty buffaloes of Murrah breed of the Institute herd were collected in 2 kg lots using plastic containers. For all operations involved in the analysis of major minerals corning glasswares were used after thorough cleaning. All reagents used for the study were of "Analar" or "G.R." grade except otherwise mentioned.

De-acidification of milk. De-acidification of acidic milk was made by passing an electric current using the method described by Yadav (1981). The apparatus consists of a glass vessel of 1 litre capacity with built in tap at the bottom, 3 electrodes of 7.5 × 6 cm each, a voltmeter calibrated from 0.95 volts, an ameter (0-3) amps, a magnetic stirrer with teflon coated magnetic rod and a pH meter.

The acidity of milk was developed by keeping it at room temperature till the pH reached between 6.0-6.2. The acidic milk was transferred to a specially fabricated beaker of 1 litre capacity. Aluminum electrodes were used at 12 volts DC. The samples of milk were drawn at regular intervals of 5 min and checked for its pH value. It continued till the pH reached the level of normal milk.

Heat Stability of Milk. The heat stability of milk was determined by heat coagulation time of milk heated to 130 ± 1 °C. The HCT of samples of normal milk, acidic milk and de-acidic milk were measured with an equipment using the procedure based on White and Davies (1958). Milk samples in duplicate of 5 ml volume were pipetted into two clean, dry and thick walled corning glass tubes (or 10 ml capacity, and dimensions of 15 mm diameter and 125 mm height) provided with tightly stoppered screw caps fitted with tigon washers. The tubes were placed in the sockets of a specially given a uniform to-and-fro rocking motion. The stand along

with the tubes was dipped in a temperature controlled oil bath maintained at 130±1 °C. The time required for clotting was measured with a stop watch having a sensitivity of at least 0.1 s.

Mechanism Used for the Partitioning of the Minerals

Rennet Coagulation. Measured quantity of milk was taken in a beaker and was brought to 30±1 °C in a water bath and Hansen's rennet in the solution form (3% w/v) was added at the rate of 0.1 ml to 100 ml of milk. The curd was allowed to set for 30 min and was cut with a stainless steel spatula. The whey was drained and was collected after filtering through Whatman No. 30 filter paper. The same procedure was repeated with acidic and de-acidic milk, respectively. The milk samples and the whey contents were analysed for major minerals.

pH. The pH of the milk samples were determined using a combination of glass electrode and a reference calomel electrode.

Analytical Procedure. The details of various methods followed for the estimation of calcium, magnesium, phosphorus and citrate are described as follows.

Calcium and Magnesium

Calcium and magnesium were estimated simultaneously by the complexiometric method of Davies and White (1962) using E.D.T.A. with little modifications. About 20 g of weighed samples was diluted with T.C.A. solution (24% w/v) to 50 ml in volumetric flask. The flask was shaken and kept for 30 min and filtered through Whatman No. 40 filter paper and the filtrate was collected. Ammonia-Ammonium chloride buffer of pH 10 was used instead of ethanolamine buffer of the same pH employed in the original method. The rest of the procedure was unaltered.

Phosphate

Phosphate was estimated in the form of phosphorus, colorimetrically, essentially by the method of Fiske and Subba Rao (1925), after digesting the samples according to Verma and Sommer (1957). Weighed quantities of samples were diluted (1:25) before digestion. For digestion 2.0 ml of diluted sample was used along with 2.5 ml of 5N H_2SO_4 and 0.5 ml of 60% perchloric acid. Phosphorus concentration was calculated from the colorimeter reading and a standard curve prepared with standard solutions of potassium dihydrogen orthophosphate.

Citrate

Citrate was estimated colorimetrically according to the method of Marrier and Boulet (1958), as modified by White and Davies (1963). About 5.0 g of sample was accurately weighed in a 100 ml volumetric flask and a protein free filtrate was prepared by using T.C.A. as in the case of calcium determination as given earlier. The color development was carried out with the filtrate, and citrate concentration was estimated with a standard curve prepared with standard solutions of trisodium citrate.

RESULTS AND DISCUSSION

The results collected during the present investigations are described through Tables 1-3.

Heat Coagulation Time (HCT) and pH of Buffalo Milk

The heat coagulation time (HCT) and pH of normal buffalo milk of 12 samples, acidic milk due to developed acidity by keeping the milk and de-acidified milk by passing electric current are described in Table 1. The results are averages with standard error for the 17 samples studied. The average HCT for normal buffalo's whole milk was 42.66±1.40 min (pH ranging from 6.65-6.80). These values are slightly lower than 61.6 min (for 17 samples) at 130 °C as reported by Singh and Roy (1978). Puri *et al.* (1963) on the other hand observed a higher average of 106.2 min in the range of 100-110 min for the HCT at 130 °C for buffalo milk.

Table 1. Heat coagulation time (HCT) at 130±1 °C and pH of buffalo milk

(Mean ± standard error)[a]

Type of milk	HCT (min)	pH
Normal milk	42.66 ± 1.40	6.72 ± 0.19
Acidic milk	2.74 ± 0.05	6.21 ± 0.02
De-acidic milk	44.41 ± 1.78	6.77 ± 0.03

[a]Mean of 12 determinations in duplicate.

When the pH of milk was lowered with an average of 6.21±0.02 in the range of 6.10-6.35 on keeping the milk, the HCT decreased appreciably and averaged to 2.74±0.03 min at an average pH 6.21±0.02 in the range of 6.10-6.35. This is also supported by the work of Rose (1961a). He observed that the heat stability of individual milks could be greatly influenced by slight adjustment of their pH by small additions of acid or alkali.

As the milk was de-acidified by passing electric current and the pH brought to the level of fresh buffalo milk the HCT again increased and averaged to 44.41±1.78 min in the range of 38-55 min at the average pH 6.77±0.03. The HCT ranged between 33 min to 55 min (pH ranging from 6.60-6.90). From the results, it is obvious that when milk is de-acidified by passing electric current, it regains its heat stability and it takes longer time to coagulate than when it is acidic.

Mineral Content and pH of Milk

This section deals with the concentration of major minerals, namely calcium, magnesium, phosphorus and citrate content in milk and rennet whey obtained from normal milk, acidic milk and de-acidified milk through rennet coagulation. The average concentrations for the polyvalent minerals, namely calcium, magnesium, phosphate as phosphorus and citrate as citric acid in 12 samples were 177.24±4.52, 16.03±0.54, 114.09±4.43 and 189.42±8.28 mg/100 g, respectively in the buffalo milk samples having average pH 6.72±0.19.

Calcium. The average calcium concentration in milk was 177.24±4.52 mg/100 g and the amount of soluble calcium was 24.03%. These results are in conformity with those of Sindhu and Roy (1976, 1978); Verma and Ananthakrishnan (1946). Further, it has been noted that the soluble calcium obtained from the rennet whey from acidic milk (pH 6.2±0.02) averaged to 33.11%. The concentration of soluble calcium increased on acidifying the milk. Zittle *et al.* (1958) and Davies and White (1960) also noted the increase soluble calcium content in acidic milk.

The percentage of soluble calcium in de-acidified milk was found to be 21.37. The de-acidification of acidified milk by passing electric current resulted in the decrease of soluble calcium content. The differences in the soluble calcium of normal buffalo milk and de-acidified milk were not statistically significant (Table 3) though whey from de-acidified milk showed slightly lower calcium content than in normal milk whey.

Magnesium. The results pertaining to magnesium content are tabulated in Table 2. These are based on analysis of 12 samples of milk. The average magnesium content in milk was found to be 16.03±0.54 mg/100 g. About

Table 2. Distribution of calcium, magnesium, phosphorus and citrate in buffaloe's milk and milk fractions

	No.of samples	Whole milk mg/100 g	Rennet whey mg/100 g	%	Rennet whey from acidic milk mg/100 g	%	Rennet whey from de-acidic milk mg/100 g	%
Calcium	12	177.24 ± 4.52	42.59 ± 1.64	24.03	58.69 ± 3.61	33.11	37.88 ± 1.67	21.37
Magnesium	12	16.03 ± 0.54	9.10 ± 0.28	56.78	10.68 ± 0.28	66.58	9.08 ± 0.27	56.61
Phosphate	11	114.09 ± 4.34	45.89 ± 2.34	43.87	63.52 ± 5.94	60.74	44.18 ± 6.78	42.24
Citrate	12	189.42 ± 6.28	128.67 ± 7.70	67.93	154.53 ± 9.36	81.48	117.75 ± 9.92	62.16

Table 3. Analysis of variance for influence of different treatments of buffalo milk on the concentration of calcium, magnesium, phosphorus and citrate in rennet whey.

Source of variation	Calcium		Magnesium		Phosphate		Citrate	
	df	Mean sum of squares	df	Mean sum of squares	df	Mean sum of squares	df	Mean sum of squares
Between samples	11	60.6038	11	2.5219**	10	810.022**	11	2394.674**
Between treatments	2	1428.7888**	2	10.0590**	2	1261.3655**	2	4252.5835**
Error	22		22		20		22	

**Significant ($P < 0.01$)

56.78% of magnesium was found in soluble form from milk having pH 6.72±0.19. These results agree with the result of Ismail *et al.* (1971a) who reported 56.90% of soluble calcium in buffalo milk. Ghosh *et al.* (1972) recorded 48.87% soluble magnesium in whey. It has been recorded that 66.58% of magnesium was in acidic milk whey and in acidified milk there was a considerable increase in the soluble magnesium. In de-acidified milk, 56.61% magnesium was present in soluble form. The de-acidification resulted in the decrease of soluble magnesium.

Analysis of variance of results (Table 3) showed that the effect of acidification and de-acidification of milk on the partitioning of magnesium into milk fractions were statistically significant ($P < 0.01$). In this case, the difference between the soluble magnesium content in normal milk and acidic milk was statistically significant and the content in acidic milk was significantly higher than that of normal milk ($P < 0.01$).

Phosphate. Average phosphate content in milk was found to be 114.09±4.34 mg/100 g. Approximately 43.8% phosphate was present in soluble form. The present results are to a great extent in conformity with the findings of Yadav and Singh (1970).

When the milk was acidified by keeping it at room temperature the concentration of soluble phosphate increased to about 60%. These results agree with the reports of Zittle *et al.* (1958) and Davies and White (1960). In de-acidified milk about 42.24% phosphate was found in soluble form.

Analysis of variance of results from different samples indicated that the effect of different treatments of milk on the partitioning of phosphate into milk fractions were statistically significant ($P < 0.01$) (Table 3). The soluble phosphate concentrations of normal milk and acidic milk differed significantly and acidic milk differed significantly from the de-acidified milk. The difference between normal milk and de-acidified milk was not significant and could be compared with each other.

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THERMAL DIFFUSIVITY OF NONHOMOGENEOUS FOOD

M. OLIVARES, J. A. GUZMAN and I. SOLAR

Depto. de Ingenieria Quimica
Universidad Catolica de Chile,
Casilla 6177. Santiago, Chile

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ABSTRACT

Thermal diffusivity of nonhomogeneous canned food were evaluated through heat penetration experiments. The values obtained are in the range between 1.5 and 2.0 × 10−7 m²/s, which agreed reasonably with previous values reported in the literature. The reliability of the applied theoretical model, the heat conduction equation for a finite cylinder, was fairly good. Previous correlations between thermal diffusivity and humidity is attributed to the existence of other variables that affect the thermal diffusivity, namely the particle size of the disperse phase, its distribution, and the viscosity and water content of the continuous phase.

INTRODUCTION

Thermal diffusivity is a very important thermal property of materials. It determines the heat flux in nonsteady processes according to the general heat transfer conduction equation. It is defined as the ratio between the thermal conductivity and the product of density by specific heat. In the case of food, thermal diffusivity is important because it regulates the calculation and design of thermal processes concerning the sterilization and/or preservation of foods.

Several papers have been published concerning the value of thermal diffusivity for different kinds of foods. A good summary of the state of the art is given by Singh (1982), where the thermal diffusivity is presented for a large number of foods. Some attempts have been made to correlate thermal diffusivity with the water content of foods. These correlations were obtained specifically for different foods such as cherries (Parker and Stout 1967), sweet potatoes (Wadsworth and Spadaro 1969), potatoes (Mathew and Hall 1968), peanuts (Suter 1975) and rice (Morita and Singh 1979).

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Some work has also been published concerning the prediction of the thermal diffusivity for homogeneous foods in terms of the humidity of the food (Riedel 1969; Martens 1980). However, for the case of nonhomogeneous foods, a relatively common case in canned food, there are not many data available (Lenz and Lund 1977; Rao *et al.* 1975); furthermore, none of the correlations published appear applicable since there are other variables apart from water content that affect thermal diffusivity.

The purpose of this work is to present the values of thermal diffusivity for several nonhomogeneous foods that have not been published and to determine whether these foods can be compared with homogeneous food regarding the correlation of thermal diffusivity with water content.

For this purpose, measurements of the heat transfer process in the nonsteady state period in a cylindrical can surrounded by a constant temperature source were made for different nonhomogeneous foods. The experimental data were fitted with solution to the heat transfer conduction equation of a finite cylinder and the thermal diffusivity was calculated by an iterative least squares procedure. It is important to note that the thermal diffusivity obtained by this method corresponds to an equivalent thermal diffusivity since the heat transfer process in most cases is not occurring only through pure conduction, but through a combination of conduction and convection. Therefore, the prediction for the case of a highly convective heating food is not expected to be very accurate.

Mathematical Considerations

Considering a cylindrical can at an initial temperature, T_0, which is placed at time zero ($t = 0$) in a constant temperature heat source at temperature T_1. Figure 1 shows a schematic diagram of the described situation for two different times.

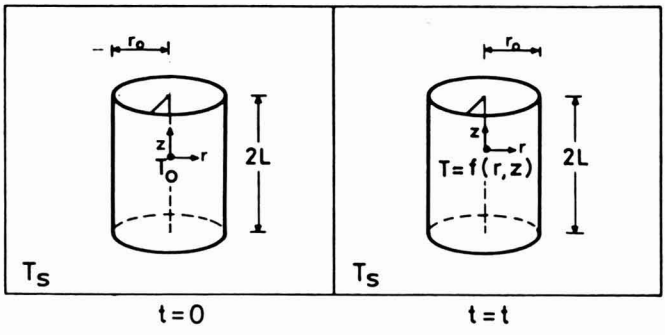

FIG. 1. SCHEMATIC DIAGRAM OF THE TEMPERATURE VARIATION WITH TIME FOR A FINITE CYLINDER

The solution of the heat transfer conduction equation of the cylindrical can may be obtained through the combination of the partial solutions of an infinite cylinder and two infinite plates according to the Newman's rule (Eckert and Drake 1972).

The following general solution for the finite cylinder is obtained (Singh 1982):

$$\theta = \sum_{m=1}^{\infty} \sum_{m=1}^{\infty} A_m B_n J_o \left(\delta_m \frac{r}{r_o}\right) \cos\left(\lambda_n \frac{z}{L}\right) e^{-\frac{\alpha t}{r_o^2}} \left[\delta_m^2 + \lambda_n^2 (r_o/L)^2\right] \quad (1)$$

where:

θ is the excess temperature $\dfrac{T_s - T}{T_s - T_o}$
r is the radial coordinate
z is the rectangular coordinate, normal to radius
α is thermal diffusivity
t is time

and: $A_m = \dfrac{2}{\delta_m J_1(\delta_m)}$

$$B_n = (-1)^{n-1} \frac{4}{(2n-1)\Pi}$$

$$\lambda_n = \frac{2n-1}{2} \Pi$$

$$\delta_m = \lambda_m r_o$$

The boundary conditions for this case are:

1) $\theta\ (r, z, o) = 1$ for $t = o$
$\theta\ (r_o, z, t) = 0$ for $r = r_o$
$\theta\ (r, L, t) = \theta\ (r, -L, t) = o$ for $z = L, Z = -L$

The application of Eq. (1) to the case of a cylindrical can filled with a nonhomogeneous food immersed in a steam autoclave at constant temperature involves the following main assumptions: (1) The can is a perfect cylinder, (2) Heat resistance of the can is negligible, (3) An initial uniform temperature of the food, (4) Heat is transferred through the food by conduction with an equivalent thermal diffusivity, (5) Physical properties of the food are invariable with time and temperature, and (6) The

temperature of the autoclave remains constant and equal to the temperature of the external can surface at any time.

The series Eq. (1), is rapidly convergent for a Fourier number greater than 0.2 (Singh 1982), and it is possible to consider only the first two terms with an accurate result (Yanez 1983) after some time has elapsed. The resultant expression is:

$$\theta = \sum_{m=1}^{2} \sum_{n=1}^{2} A_m B_n J_o \left(\delta_m \frac{r}{r_o}\right) \cos\left(\lambda_n \frac{z}{L}\right) e^{-\alpha t/r_o^2} \left[\delta_m^2 + \lambda_n^2 \left(\frac{r_o}{L}\right)^2\right] \quad (2)$$

In this equation the value of θ can be obtained experimentally by placing thermocouples inside and outside the cylindrical can. Therefore it is possible to obtain for a certain time, at which temperatures are measured, the value of thermal diffusivity of the food inside the can.

In order to obtain an average thermal diffusivity, the experimental values and the predicted ones for θ are compared for some arbitrarily selected value of thermal diffusivity. Using an iterative technique, the value of thermal diffusivity is changed until the difference is minimized.

MATERIALS AND METHODS

Thermal diffusivity of 16 different institutional canned foods were determined. This was done during industrial sterilization process of infant foods to be used in national programs of infant feeding.

The kinds of foods, their characteristics and can sizes are shown in Table 1. Two of the foods practically consisted of a pure component with very small particle size: meat sauce and cooked bovine blood. The rest were homogeneous mixtures of different ingredients. Most of the ingredients (except chickpeas, beans, lentils, corn, peas and rice) were diced approximately 1 cm cubed. Meat croquettes were approximately 4 cm in diameter and 1 cm in height, and were packed in an homogeneous thick sauce. The foods were all precooked before filling the cans. During this stage, ingredients like onions, squash and tomatoes were nearly crushed. The rest of the ingredients approximately maintained their structure during processing.

Regarding heat transfer, the foods were mostly conductive in nature. Before heat penetration tests, the slowest heating points were determined by placing thermocouples along the vertical axis of cans during sterilization. These experiments showed that the slowest heating points were located between ½ and ⅓ of can height measured from the bottom (Table 1).

Table 1. Experimental characteristics of the different food tested

	Water content (weight %)	Drained weight (%)	Temperature range inside can during sterilization (°C)	Processing time, including CUT (min)	Transient step (min)	Can size diameter x height (mm)	Thermocouple position (slowest heating point) (mm from the bottom)
1. Meat Sauce	77,3	68	60-112	75	72	84 x 96	40
2. Meat croquette	74,0	70	59-115	85	85	84 x 96	40
3. Cooked seaweed (Durvillea Antartica)	75,9	89	56-110	150	146	157 x 109	36
4. Cooked chick-peas with pork sausages	81,4	62	71-114	75	75	84 x 96	40
5. Frankfurters	73,4	93	58-109	85	74	103 x 119	56
6. Cooked lentils and rice	77,8	89	64-114	75	70	84 x 96	48
7. Cooked abalones	68,5	91	56-108	85	80	103 x 119	48
8. Cooked clams	75,1	70	51-108	85	80	103 x 119	50
9. Cooked clams and rice	80,1	73	60-114	75	75	84 x 96	40
10. Chicken and rice	75,1	74	65-113	75	72	84 x 96	48
11. Chicken and peas, diced potatoes and carrots	67,6	92	63-109	95	95	103 x 119	56
12. Chicken and diced potatoes and carrots	73,7	83	72-109	85	85	103 x 119	56
13. Cooked beans, corn & diced squash	68,3	99	56-113	130	120	157 x 109	48
14. Cooked bovine blood	74,0	99	58-104	80	72	103 x 119	56
15. Chopped meat & tomatoes & diced potatoes	66,4	97	65-106	85	78	103 x 119	56
16. Chopped meat & diced potatoes & carrots	82,1	74	58-113	75	70	84 x 96	40

The sizes of cans (84 × 96 mm, 103 × 119 mm and 157 × 109 mm) were determined by the production program; this was the only reason for using different sizes.

Heat Penetration Tests

During normal processing, cans containing different foods were immersed in a vertical stationary steam autoclave at approximately constant temperature. The number of cans in each experiment was at least 7, which assured a fairly good average value for the temperature variation. Each can had inside, in a known position, a thermocouple of copper constantan (O. F. Ecklund Inc.). A thermocouple was also placed inside the autoclave to measure the heat source temperature. The slowest heating point was chosen as the thermocouple location. Temperatures were recorded every 2 min through a digital thermometer (Thermoelect, 1/10 °C) using a manual selecting device of ten positions (O. F. Ecklund Inc.). The autoclave used was 600 L capacity and operated with steam at approximately 116 °C. The moisture content was previously determined for each food by drying to a constant weight at 70 °C.

RESULTS

Table 1 shows the different characteristics of each experimental run. The temperatures obtained experimentally, as well as time and other parameters involved, were introduced in Eq. (2).

Thermal Diffusivity

The calculus of equivalent thermal diffusivity, α, was done by adjusting the heat penetration data to the model expressed in Eq. 2. The iterative technique minimized the function:

$$\phi(\alpha) = \sum_{i=1}^{N} (\theta_i^E - \theta_i^C)^2$$

where:

θ_i^E = experimental value of θ for time i

θ_i^C = calculated value of θ for time i and for a determined value of α

Through a computational program that adjusts the data to the model Eq. (2), an average thermal diffusivity was obtained.

The correlation of experimental results with the theoretical values calculated with the average thermal diffusivity were highly satisfactory. The lowest correlation factor obtained was 0.996. Figure 2 shows some representative experimental results with the corresponding theoretically calculated average values.

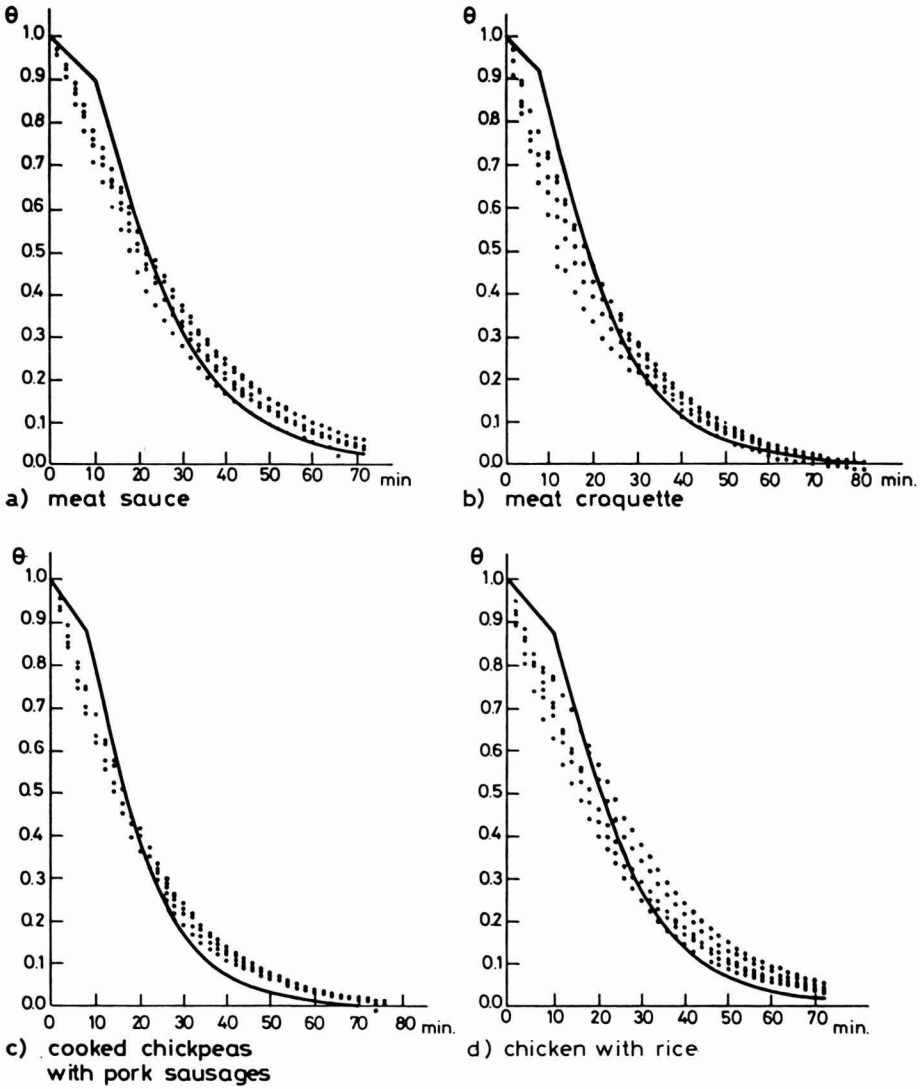

FIG. 2. EXPERIMENTAL VALUES AND CALCULATED CURVE (AVERAGE THERMAL DIFFUSIVITY) OF THE EXCESS TEMPERATURE (θ) VARIATION WITH TIME FOR DIFFERENT FOODS

Table 2 shows the calculated average thermal diffusivity and its standard deviation for each one of the different foods treated, together with its water content and the estimated valid temperature range.

Table 2. Experimental values of average thermal diffusivity of several foods

Food	Water content (weight %)	Temperature range (°C)	Average thermal diffusivity 10^{-7}(m2/s)	Standard deviation 10^{-7}(m^2/s)	Number of cans
1. Meat sauce	77,3	60-112	1,46	0,05	8
2. Meat croquette	74,0	59-115	1,98	0,22	8
3. Cooked seaweed (Durvillea Antartica)	75,9	56-110	1,90	0,03	8
4. Cooked chickpeas with pork sausages	81,4	71-114	2,24	0,13	7
5. Frankfurters	73,4	58-109	2,36	0,34	9
6. Cooked lentils with rice	77,8	64-114	1,64	0,15	8
7. Cooked abalones	68,5	56-108	1,85	0,004	8
8. Cooked clams	75,1	51-108	1,96	0,08	8
9. Cookcd clams with rice	80,1	60-114	1,98	0,08	8
10. Chicken with rice	75,1	65-113	1,93	0,21	8
11. Chicken with peas & diced potatoes & carrots	67,6	63-109	1,65	0,06	8
12. Chicken with diced potatoes & carrots	73,7	72-109	1,70	0,03	8
13. Cooked beans with corn and diced squash	68,3	56-113	1,83	0,07	8
14. Cooked bovin blood	74,0	58-104	1,62	0,04	8
15. Chopped meat with tomatoes & diced potatoes	66,4	65-106	1,57	0,02	8
16. Chopped meat with diced potatoes and carrots	82,1	58-113	1,77	0,15	8

Figure 3 shows the relation found between the thermal diffusivity and the humidity of the different tested foods as well as the theoretical estimated values predicted by Riedel (1969) and Martens (1980).

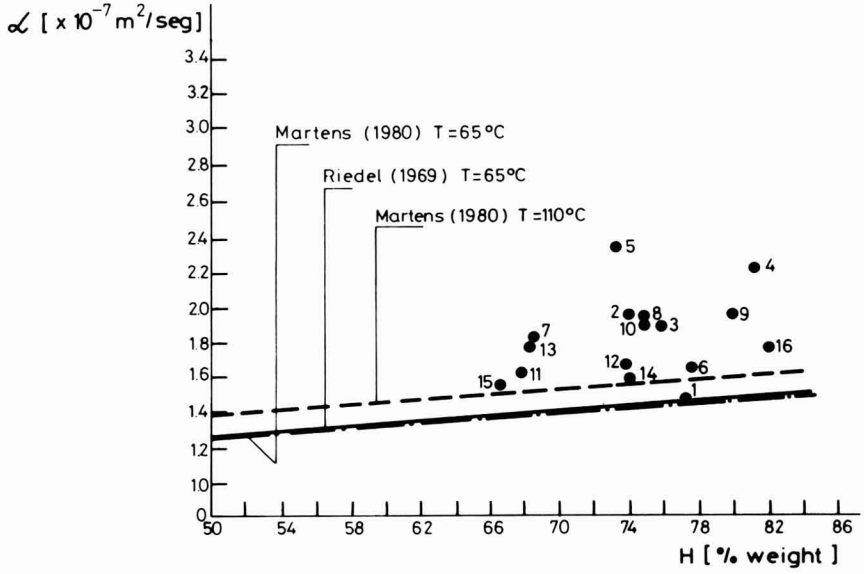

FIG. 3. LITERATURE AND EXPERIMENTAL RELATION BETWEEN THERMAL DIFFUSIVITY AND WATER CONTENT (numbers correspond to Table 2 foods).

CONCLUSIONS

The results obtained for thermal diffusivity of the different nonhomogeneous food are in the range between 1.5 and 2.0 $\times$ 10^{-7} m^2/s, which agree reasonably well with previous reported values in the literature for similar materials. The reported value for beans (Lenz and Lund 1977) is 1.68 X 10^{-7} m^2/s and for potatoes (Rao *et al.* 1975) is 1.7 $\times$ 10^{-7} m^2/s.

It is important to comment the possible error sources which may affect the accuracy of the obtained thermal diffusivities. Larkin and Steffe (1982) have largely reviewed error sources, most of which have been selected for comments:

(1) Time and temperature were measured as accurate as possible using well calibrated instruments.

(2) Difference between retort temperature and initial temperature inside the can was, in all cases, over 40°C (44° to 65°C).

(3) The sizes of cans ensures that the error due to probe location was not significant.

(4) The length/radius ratio of cans was in some cases, the recommended optimal value, 0.7-0.8. In others, it was close to it (1.14-1.15), and did not introduce an important error in α estimation.

(5) In all cases the excess temperature, θ, used for α estimation, was in the optimal range (0.15-0.85).

With reference to the reliability of the applied theoretical model, agreement between experimental values and the predicted ones is reasonably good. Better adjustment is obtained in more homogeneous foods: meat sauce and cooked bovine blood (No. 1 and 14); cooked abalones and cooked clams (Nos. 7, 8 and 9), where the food was chopped in small particle size; and in foods containing chicken as pulp, chopped meat, or chopped seaweed (Nos. 3, 11, 12 and 15). In the case of less homogeneous foods, as meat croquettes, frankfurters and cooked chickpeas with sausages (Nos. 2, 5, and 4), less adjustment is obtained. Standard deviations obtained for thermal diffusivities of the different foods are small in general, 0.04-0.08 $\times$ 10^{-7} m^2/s. It becomes greater, 0.15-0.22 $\times$ 10^{-7} m^2/s in less homogeneous foods (Table 2).

Determination of α using f_h factor, (Singh 1982), was made in order to correlate the calculated values. Just a fair agreement was obtained, probably due to the fact that α estimation in our case was made using the first 2 terms of series Eq. (1).

Finally and probably the most important result concerns with the correlation established for the thermal diffusivity and the humidity of the food. From the observation of Fig. 3 it is obvious that the reported prediction models (Riedel 1969; Martens 1980) are not applicable for the case of nonhomogeneous food. This discrepancy is, in view of the authors, due to the existence of other variables that affect thermal diffusivity. These are the particle size of the different phases, its distribution and the water content and viscosity of each phase. It will be necessary to study the influence of these variables and probably others, in order to establish a more reliable model for predicting the value of thermal diffusivity in nonhomogeneous food.

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EFFECT OF DIFFERENT TYPES OF BLANCHING ON THE COLOR AND THE ASCORBIC ACID AND CHLOROPHYLL CONTENTS OF GREEN BEANS

NEZIH MUFTUGIL[1]

Research Fellow
TUBITAK, Marmara Research Institute
Nutrition and Food Technology Department
Gebze, Kocaeli, Turkey

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ABSTRACT

Green beans were blanched by four methods: water blanching, steam blanching, microwave blanching and convection oven blanching. Minimum adequate blanch time for complete peroxidase enzyme inactivation was less in the microwave oven. Weight loss was significant in the samples blanched in the convection oven due to moisture vaporization. Ascorbic acid was found to be significantly ($P < 0.05$) higher in the microwave blanched sample. The ascorbic acid contents of water blanched green beans were higher than those of steam blanched ones. Convection oven blanched green beans were found to be lowest in ascorbic acid and chlorophyll contents, and microwave treated samples were found to be highest. For equivalent peroxidase inactivation, more losses of chlorophyll were detected in steam blanched samples than in water blanched samples. The results of color measurements with a Hunterlab colormeter indicated that blanching with water and steam resulted in a more intense greenness of the samples.

[1]Correspondence address: Dr. Nezih Muftugil, Dr. Faruk Ayanoglu cad., Dr. Faruk Ayanoglu Apt., No. 7, D.23, Feneryolu, Istanbul, Turkey.

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INTRODUCTION

Most vegetables must be blanched before the freezing or drying process to prevent the development of bad flavor in subsequent storage (Feinberg 1973; Baardseth 1978; Bottcher 1975). The blanching process involves a short heat treatment usually by either steam or hot water. The object of the blanching process is to produce the following effects in the material being processed; (1) to inactivate the enzymes present, (2) to shrink the material, (3) to expel air trapped intracellularly, (4) to reduce any initial infection, (5) to remove any undesirable flavors and aromas (Voirol 1980; Lee 1958). As a result of heat and leaching of soluble constituents, the blanching of vegetables leads in many instances to a significant loss of nutrients, decrease in colors and product yield, and also the generation of highly polluting effluent (Feaster 1973; Odland and Eheart 1975; Carroad *et al.* 1980). When blanching vegetables before freezing, inactivation of the enzymes is dependent upon both time and temperature of the heat treatment (Adams 1978). For this reason peroxidase activity is widely used as an index of blanching. The enzyme is used because it is easy to determine its activity and it is the most heat stable in the majority of vegetables; if it cannot be detected then other less stable enzymes are taken to be inactive (Burnette 1977; Dietrich *et al.* 1955). Many studies have been conducted to determine whether steam or water blanching was the more efficient when one considers the loss of nutrients as a result of blanching (Lee 1958; Feaster 1973; Voirol 1980). Steam blanching has usually been found to conserve more soluble nutrients than blanching in water, but there is some indication that color of green vegetables is adversely affected by steam (Odland and Eheart 1975). Microwaves can be used industrially for blanching vegetables, but it is claimed that this is a costly method (Philippon 1981). Drake *et al.* (1981) reported that microwave blanched asparagus had higher shear values and less green color than either water or steam blanched but no significant difference was found for green beans.

The purpose of this study was to investigate the effects of four different blanching methods on the ascorbic acid and chlorophyll contents and the color of the green beans.

MATERIALS AND METHODS

In this study green beans (*Phaseolus vulgaris*) were used as the raw materials. The green beans were washed, snipped and cut into 30 mm pieces. Samples were size graded to range from 12 mm to 18 mm in width. Blanching was carried out by four different methods. The blanching times

used in all four methods was sufficient to inactivate the peroxidase enzyme. For this reason blanching times for each method had been predetermined by qualitative peroxidase test. For each blanching method two replicates were done. In each replicate two lots of 300 g each were blanched. Therefore, the total of 1200 g of green beans were used for each method.

Water Blanching

300 g of samples were placed in a wire mesh basket which was then placed into 2400 ml of water at 100 °C in a blanching kettle. The green beans were blanched for 120 s and the blanching time was measured from when the water began to reboil.

Steam Blanching

300 g of samples were placed in a wire mesh basket which was then placed at a distance of 30 cm above the steam pipe of a screwdrive blancher. Samples were treated with steam at atmospheric pressure for 90 s.

Convection Oven Blanching

About 300 g of green beans were spread in a single layer on two glass trays. The uncovered trays were placed in an oven. The temperature in the oven was set at 200 °C. The treatment was carried out for 8 min.

Microwave Blanching

About 300 g of green beans were spread in a single layer on two glass trays. The trays were placed in a microwave oven, Model 2005, 650 W and an operating frequency of 2450 MHz. The trays were rotated automatically in the microwave oven to distribute the microwaves for uniform blanching. Samples were kept in the microwave oven for 60 s.

After each blanching treatment, samples were immediately weighed and then cooled in 1 liter of tap water at 15 °C for 3 min and then drained on a stainless steel wire mesh tray for 4 min and reweighed again.

Fresh and blanched homogenized samples were dried in a vacuum oven at 70 °C for 3 h for the determination of moisture content. Qualitative peroxidase activity was determined according to the method reported by Twigg and Kramer (1968). Ascorbic acid was assayed using the 2.6 dichlorophenolindophenol spectrophotometric method (Pearson 1979). The quantitative determination of chlorophyll was based on the method of Bruinsma (1963). Color of samples was measured on the Hunter color difference meter Model D25 D2P. Measurements were taken for the cut, raw and blanched samples. Reflected light readings (10 mm light path) for values

L, a and b were taken. Data were statistically analyzed by variance and Tukey's test was used to determine the significance of the difference between treatment means at $P < 0.05$.

RESULTS AND DISCUSSION

The changes in weight which occurred during the blanching treatments and then cooling, and the total solid contents of the green beans after blanching are shown in Table 1. Weight losses were not significant in the samples blanched in water, steam and in the microwave oven. In water blanching, the loss of solids and minerals to the blanching media was thought to be the probable cause of the weight loss. In microwave and convection ovens, the weight loss was mainly due to the drying action of heat which caused moisture vaporization. High-temperature and long-time blanching in a convection oven resulted in significant weight loss. Weight losses which occured in the four different blanching methods were regained by cooling in water. Weight increases after water cooling may be attributed to water adhering to the vegetables or to an increased water holding capacity of vegetable colloids. Moisture uptake was less in microwave blanched samples than those blanched in water, steam and in the convection oven.

Table 1. Dry matter contents of raw and blanched green beans and changes in weight occurred during blanching[1]

Method of Blanching	Average weight changes (%)		Dry matter (%)
	After blanching	After cooling	
Raw	-	-	12.81
Water	-1.41	+1.78	11.60a
Steam	-1.28	+2.12	11.46a
Microwave oven	-2.48	-1.67	13.35b
Convection oven	-15.21	-12.15	18.23c

[1]Numbers with letters a, b, c, in common within a column are not statistically different (5% level)

Minimum adequate blanch time for complete peroxidase enzyme inactivation was less in the microwave oven. Qualitative peroxidase data indicated that the steam blanched samples had absorbed more heat than the

water blanched samples. Slow penetration of heat to the centers of the vegetable pieces could explain slow enzyme inactivation by water. The effects of various methods of blanching on the ascorbic acid and chlorophyll contents and color of green beans are reported in Table 2. The ascorbic acid and chlorophyll values were reported after making corrections for the weight changes which occured during blanching.

Table 2. Influence of various blanching methods upon the ascorbic acid and chlorophyll contents and the color of green beans[1]

Method of blanching	Ascorbic acid (mg/100g fresh weight)	Chlorophyll (mg/100g fresh weight)	L	a	b
Raw	15.65	167.91	57.9	-18.7	22.6
Water	13.44bc	147.34b	51.2a	-27.7b	22.6a
Steam	12.92ab	139.55b	51.6a	-29.4b	21.3a
Microwave oven	14.20c	163.12b	55.2b	-22.7a	22.2a
Convection oven	11.87a	103.10a	52.6a	-23.6a	21.2a

[1]Numbers with letters of a, b, c, in common within a column are not statistically different (5% level)

Ascorbic acid was found to be significantly higher ($P < 0.05$) in microwave blanched samples. The effect of microwave blanching on ascorbic acid was considerably less marked than that of the other methods. The average losses of ascorbic acid after blanching were 14.2%, 17.5%, 9.3% and 24.2% in water, steam and in the microwave and convection ovens, respectively. High ascorbic acid loss occurring in convection ovens indicated that the duration and the temperature of the heat treatment adversely affected the ascorbic acid content of green beans. Probably, the samples blanched in the microwave and convection ovens lost a further amount of their ascorbic acid during cooling in water. Total ascorbic acid was higher in water blanched samples than in steam blanched. A number of earlier studies, reviewed by Feaster (1960), have reported generally opposite results although in many cases blanching times in steam and water had not been based on enzyme inactivation. Philippon (1985) reported that advantage of steam blanching for retention of soluble compounds is partly lost when steam blanching is followed by cooling in water. Steam causes disruption of cells during blanching and more soluble compounds are leached out during cooling in water. It seemed that the extraction in the blanching and cooling water and the temperature of the blanch had direct

influence on the ascorbic acid losses. Morrison (1975) noted that the loss of ascorbic acid in water blanching was due to the leaching action of hot water and to enzymatic destruction. Voirol (1980) reported that the rate at which the ascorbic acid is destroyed depends on the duration and the temperature of the blanching process.

Convection oven blanched green beans were significantly lower ($P < 0.05$) in total chlorophyll and the microwave blanched were highest. The average chlorophyll losses after blanching were 12.3%, 17.0%, 3.2% and 38.6% in water, steam and in the microwave and convection ovens, respectively. Walker (1964) found that chlorophyll is converted to pheophytin when beans are blanched, and the conversion increaases with increasing blanch time. The other type of chlorophyll degradations are hydrolysis and oxidation, and the rate of degradation is largely dependent on the temperature. A rise in temperature accelerates the degradation (Walker 1964). Chlorophyll destruction was higher in convection oven than in steam, water and microwave oven due to long time treatment at a higher temperature. Short-time blanch in microwave ovens gave better retention of chlorophyll. For equivalent peroxidase inactivation, more chlorophyll losses were detected in steam than in water blanched samples, but the difference was not statistically significant. Dietrich and Neumann (1965) and Feaster (1973) stated that steam blanching caused more chlorophyll conversion than water blanching in most instances, where time and temperature were similar. Chlorophyll is insoluble in water, but during blanching in water, it diffuses throughout the cell to the blanching medium and is leached out into the blanching media through damaged cell walls (Lee 1958). Mirza and Morton (1977) reported that in blanched carrots chromoplasts were disintegrated and this breakdown resulted in liberation of carotene molecules into the blanching media.

Table 2 shows that the Hunter(a) values of water and steam blanched samples became more negative, indicating that those samples were more green. Hunter(a) values were significantly lower ($P < 0.05$) for water and steam blanched samples than for microwave and convection oven blanched green beans. The increase in greenness was not accompanied by a decrease in yellowness as reflected by the Hunter(b) values. Samples blanched in water, steam and in convection oven decreased significantly ($P < 0.05$) in lightness of color (L values). Microwave blanched green beans were found to be lighter than that which had been blanched by the other three methods. The initial brightening of the green color of green vegetables after water and steam blanching resulted from the removal of air from the vegetable surface and from the intercellular spaces (Adams 1981). Lee (1958) noted that when green vegetables are heated in water, the chloroplasts become swollen and distorted or may even burst, and the green color becomes more or less diffused throughout the cell. This causes

the more intense green color on the surface of the vegetable. Odland and Eheart (1975) reported better retention of color in broccoli cooked in water than the broccoli steamed at atmospheric pressure.

CONCLUSIONS

Samples blanched in water and steam had more intense green color than those blanched in the microwave and convection ovens. Blanching in microwave and in convection ovens resulted in weight loss in samples due to the drying action of the heat. Retention of ascorbic acid and chlorophyll was higher in microwave blanched samples. While the use of microwave has interesting possibilities, much more work of an economic as well as scientific nature should be done. It seems that destruction of vegetable cells during blanching causes leaching of soluble compounds into the cooling water. Application of different cooling methods (i.e. air cooling, water spraying, etc.) should be considered.

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BOOK REVIEWS

Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk. F. L. Davies and B. A. Law, (eds.). Elsevier Applied Science Publishers, London. pp. 268. 26 illustrations, $52.00.

This book contains 9 chapters written by 9 authors (some chapters are written by a single author and some by several authors) all associated with the National Institute for Research in Dairying in Reading, England. Each chapter deals with a topic of current interest to persons working with cheese or fermented milks.

The opening chapter discusses coagulation of milk and development of texture in cheese. Mentioned are enzymes from several sources that are used to cause the clotting of milk; mechanisms of milk coagulation, curd assembly and syneresis of whey; development of cheese structure and texture; and the suitability of milk for cheesemaking.

The second chapter deals with classification of bacteria important in the making of cheese and fermented milks. This is a particularly useful chapter to microbiologists who need to identify bacterial isolates obtained from fermented dairy products. The chapter describes newer and also more traditional tests done to characterize lactic streptococci, lactobacilli, leuconostocs, pediococci, propionibacteria and brevibacteria. For many, this chapter alone will be worth the cost of the book.

The third chapter gives current views on physiology and growth of the lactic acid bacteria. Described are processes involved in use of carbohydrates and proteins. The proteolytic "equipment" of lactic acid bacteria is of considerable current interest because of studies on its involvement in cheese ripening. Nutrient uptake and growth of lactic acid bacteria also are discussed.

Genetics of lactic acid bacteria is discussed in the fourth chapter. Major topics include arrangement of genetic material, gene transfer processes, molecular analysis of plasmids and gene transfer and applications for genetic manipulation. Not surprising, more than one-third of the references at the end of this chapter are reports of work done in the laboratory of Dr. Larry McKay at the University of Minnesota.

Bacteriophages of lactic acid bacteria are considered in chapter five. Topics covered include differentiation of phages, lysogeny, resistance of lactics to phage and control of bacteriophages in factories.

The sixth chapter deals with development of flavor in fermented milks. Metabolic pathways by which flavor compounds are produced are described. Products discussed include cultured buttermilk, Scandinavian buttermilks, ymer, villi, Bulgarian buttermilk, yakult, acidophilus milk, yogurt, dahi, kefir, laban and kumiss.

Development of flavor in cheeses is discussed in the seventh chapter. It lists major flavor components of common types of cheese and then describes production of these substances from carbohydrates, lipids and proteins. The author of the seventh chapter also prepared the eighth chapter which deals with accelerating the ripening of cheese. Methods to speed-up the ripening process include storage of cheese at an elevated temperature, addition of enzymes, use of adjunct starter cultures, and use of a cheese slurry in which flavor develops rapidly.

The final chapter deals with nonsensory methods for assessment of cheese flavor. Included for discussion are compositional analysis, volatile compounds, neutral volatile compounds and redox potential. Brief comments are included on sensory assessment of flavor and on statistical methods associated with assessment of flavor.

Overall, this is an excellent book. It deals with timely topics. It is up-to-date. There is an extensive list of references at the end of each chapter. Generally, the book is well written, although variation in style, as would be expected in a book by a group of authors, is evident. The typeface selected for the book is clean and makes the text easy to read. The book appears to be well made. It is recommended for all persons interested in current research developments related to cheese, dairy starter cultures and cultured milks.

ELMER MARTH

Immunoassays in Food Analysis. B. A. Morris and M. N. Clifford, Editors. Elsevier Applied Science Publishing Co., Inc., New York. pp. 248. $37.50.

Immunoassays have assumed increasing importance in the analysis of food-borne substances over the past 15 years. Immunoassays have found use in the analysis of proteins, bacterial toxins, mycotoxins, antibiotic residues, naturally-occurring chemicals, food additives, and residues of food contaminants. These assays are often more sensitive and simple than alternative methods, and the development of enzyme-linked immunoassays (ELISAs) has removed many of the problems inherent in the earlier radioimmunoassays or RIAs (expensive equipment, need for radioisotopes, need for personnel trained in the use of radioisotopes).

This excellent book describes the current state-of-the-art in the application of immunoassays in food analysis. It also provides a brief, but interesting historical account of the development of food immunoassays and tries to predict some of the future developments in this field. This volume is a compilation of the presentations made at a 1983 symposium at the University of Surrey, England, on this topic. Most of the contributions are from British authors, although other European and Australian authors are also represented.

This text will be especially valuable to scientists who have limited knowledge of immunoanalysis. The book begins with an excellent glossary which defines the unique terms used in this field of analysis. The first section containing four chapters provides an excellent background for the novice and some interesting reading for the expert. It includes chapters on the history of immunoassays in food analysis, the principles of immunoassays in general and enzyme immunoassays in particular, and a chapter on alternative labelling approaches. These chapters are especially well written, and easy to read and understand. The second and third sections of the book contain seven and four chapters, respectively, on application of immunoassays to macromolecules (section II) and small molecules (section III). Since the symposium was held in 1983, these chapters may be slightly

out-dated. However, these chapters are included to demonstrate the potential of immunoanalysis in the assay of a wide range of foodborne substances. For that purpose, these sections are very helpful especially to the novice. Two of these chapters are very short (2 pages) and not terribly helpful but the others are well written and referenced.

I would heartily recommend this book for food analysts who are novices in the development and application of immunoassays. This technology is bound to spread rapidly in the near future, and this book will help immensely in establishing a good foundation of knowledge in this area. Most food analysts probably fall into this novice category since application of immunoassays in food science is still in its infancy. I would also recommend this book for laboratory managers, administrators, and supervisors. Immunoassays often have considerable economic advantages, and it will be necessary for such individuals to develop a reasonable understanding of their potential. This easily read book should fill that role. The book will probably be less useful to food scientists expert in the use of immunoassays. However, the breadth of coverage of specific analytical methods and the inclusion of the historical material may be of interest to scientists in this category. This book will also be a valuable teaching aid in universities and a good addition to academic and industrial libraries.

STEVE L. TAYLOR

Microbiology of Frozen Foods. R. K. Robinson, Editor. Elsevier Applied Science Publishers. pp. 289.

This is certainly a book you might select to look up the technology of freezing or the fate of microorganisms exposed to freezing, storage and thawing. You won't find out a lot about any given microorganism, but then again there isn't a lot to find out about. For any given commodity, the microorganisms associated with the frozen product are very much the same as those associated with the fresh product. Hence, the microbiology which is unique to frozen foods is very limited. As one of the book authors observed, "Once a product is frozen, there is very little change in its overall microbial population." As a generalization, however, Gram-negative bacteria are less likely to survive the freeze-storage-thaw process than Gram-positive bacteria. Exceptions to this generalization are pointed out in the book.

The eight chapters are: The Technology of Freezing by M. F. G. Boast; Effects of Freezing/Thawing on Foods by L. Boegh-Soerensen and M. Jul; Response of Micro-organisms to Freeze-Thaw Stress by R. Davies and A. Obafemi; Microbiology of Frozen Meat and Meat Products by P. D. Lowry and C. O. Gill; Microbiology of Frozen Fish and Related Products by C. K. Simmonds and E. C. Lamprecht; Microbiology of Frozen Dairy Products by J. Rothwell; Freezing for the Catering Industry by R. K. Robinson; and Laboratory Examination of Frozen Foods by C. A. White and L. P. Hall. The chapter titles reflect the scope of the book. Most of the commodities considered are those which are very sensitive to microbial spoilage (meat, fish, and dairy products) while less perishable

items (doughs, fruits, vegetables, and juices) are not included. Each chapter has an extensive reference list. One reference list, however, would indicate that no literature has been produced since the seventies.

All of the chapters were easy to read, understandable and informative. From a microbiological viewpoint, this volume would be a useful reference to have in the library. One involved in the technology of freezing might consider a possible personal copy.

LLOYD D. WITTER

Role of Fats in Food and Nutrition. By M. I. Gurr. Elsevier Applied Science Publishers, hardbound. pp 170. $36.00.

This book provides an important perspective on dietary fats which is absent from many other works on the subject. In addition to a comprehensive coverage of the chemistry and occurrence of food lipids, author Gurr examines the role of fats in health and disease. Individual fats which may have toxic effects are discussed in well researched sections, as are diseases which may have a dietary fat component. The book is easy to use as a resource, as the early chapters on basic concepts support the later diet-disease discussions, which are well cross referenced.

Gurr omits the references which are traditionally cited throughout a technical work. This practice works well with his fluent writing style and makes this text remarkably easy to read. Although it becomes less easy to connect specific information to a source, Gurr provides a description of content for each reference listed at the end of each chapter. Most consumption data cited are specific to the United Kingdom, but may still be applicable to the U.S. and other developed countries.

Gurr states that he walked the tight-rope between comprehensiveness and readability for the non-specialist. He has succeeded in this respect as readers with backgrounds ranging from a course in college chemistry to advanced degrees in lipid chemistry or nutrition will find this work quite useful. The information is up-to-date and unbiased, the writing is lucid, and the perspective of disease prevention or causation by lipids is novel. This book has the distinction of being both a useful reference as well as being interesting reading for anyone interested in the role of fats in nutrition or disease.

KEN LEE

F N P

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