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Journal of
FOOD PROCESSING AND PRESERVATION

Edited by T. P. Labuza, University of Minnesota

JOURNAL OF FOOD PROCESSING AND PRESERVATION

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CONTENTS

CHEMICAL AND CONFORMATIONAL CHANGES OF OVALBUMIN DUE TO THE MAILLARD REACTION

K. WATANABE[1] and Y. SATO[1]
Faculty of Agriculture, Nagoya University,
Chikusaku, Nagoya 464, Japan

Y. KATO
Women's College of Tokaigakuen, Tenpakuku,
Nagoya 468, Japan

Received for Publication February 27, 1979

ABSTRACT

Ovalbumin freeze-dried with or without the addition of glucose was stored at 50°C and 65% relative humidity to study the effect of the Maillard reaction on the chemical properties and conformational composition of ovalbumin. About 70% of available lysine in ovalbumin reacted with glucose before denaturation. The solubility and heat stability of the protein-glucose complex increased with the addition of the hydrophilic group and the repulsion force due to changes of such charged groups. During prolonged storage the changes of charge balance promoted the unfolding of ovalbumin at the expense of α-helix without the loss of β-structure. Insolubilization of the complex was accompanied by the high loss of lysine and arginine residues, brown color development and the formation of aggregates which were not cleaved by SDS plus 2-mercaptoethanol.

It is well known that the Maillard reaction between reducing sugars and protein results in the changes in the functional properties of foods (Coulter *et al.* 1951). For instance, the increase in solubility and heat stability of protein in the initial stage of the Maillard condensation in food under dry storage followed by the loss of the specific property during prolonged storage have been demonstrated in connection with a loss of reactivity of the ε-amino groups of lysine and a brown color development (Amaya-F. *et al.* 1976, Morales *et al.* 1976, Obanu *et al.* 1975, Tybor *et al.* 1973).

It was found in previous work that during storage of egg white solids freeze-dried with the addition of glucose that the added sugar had a protective effect against the aggregation of protein, depending on the ratio of protein to glucose in the mixture and the length of storage (Kato *et al.* 1978). However, the extent to which the protein molecules were unfolded in the course of the Maillard reaction was not investigated at that time. This study was designed to investigate the effect of the Maillard reaction on the chemical properties and secondary structure of ovalbumin.

MATERIALS AND METHODS

Preparation of Samples

Ovalbumin was prepared from fresh hen egg using the ammonium sulfate procedure (Marshall and Neuberger 1972), recrystallized four or five times at the isoelectric point by addition of ammonium sulfate, dialyzed until free of salt, and dried by lyophilization. The 2% ovalbumin solution resolubilized with the distilled water was adjusted to pH 10 with 0.1N NaOH because the browning reaction could be promoted at a higher pH level, and divided into two parts. D-glucose corresponding to 30% of the dry weight of the ovalbumin was added into one part and mixed mechanically. Both solutions were immediately frozen and then freeze-dried. The freeze-dried ovalbumin (OV) and ovalbumin-glucose (OVG) preparations were ground in a mortar, and then stored in uncapped 20 ml glass vials in sealed chambers at 50°C. The relative humidity (RH) inside the chamber at 50°C was kept constant at 65% with supersaturated potassium iodide solution.

Browning Determination

Extent of browning was measured at 420 nm with a Shimadzu spectrophotometer (type UV-200S), using 1.3% ovalbumin solution digested with pronase by the method of Clark and Tannenbaum (1970).

Determination of Available Lysine

The available lysine content was determined by the FDNB method of Carpenter (1960).

Measurement of Solubility and Heat Stability

To measure the effect of the length of storage on solubility and heat stability, the unstored and stored samples of OV and OVG were suspended at a level of 1% (w/v) in 0.1M pH 6 citrate buffer containing 2M NaCl, soaked and centrifuged at 3000 rpm for 15 min, respectively. Protein in each separated fraction was determined by the method of Lowry *et al.* (1951) and/or microKjeldahl. Solubility was expressed in percentage of soluble protein to total protein. Heat stability was expressed in terms of solubility after heating (70°C, 5 min), cooling and centrifugation of the suspension described above.

Sodium Dodecylsulfate (SDS)-Polyacrylamide Gel Electrophoresis

Samples of the unstored and stored OV and OVG were dissolved in SDS/1% 2-mercaptoethanol-Tris HCl buffer (pH 8.9). SDS-poly-acrylamide gel electrophoresis was carried out in 5% polyacrylamide gel in the presence of 0.5% SDS, and the gels were stained for proteins with amide black 10B. A Shimadzu dual-wave length scanner, Model CS–910, was used to obtain absorbance tracings.

Amino Acid Analysis

Samples were hydrolyzed for 24 h in evacuated sealed tubes at 110°C in 6N HCl. Analyses were performed with a JEOL auto amino acid analyser JLC-6AH.

Circular Dichroism (CD)

CD experiments were carried out on a JASCO J-40A spectropolari-meter at 25°C. The data were expressed in terms of mean residue ellipti-city, $[\theta]$, at a mean residue weight of 114. A protein concentration of about 0.09 mg/ml in pH 7 phosphate buffer (I=0.01) was used for measurements with 1.0 mm path length cell. When solubilization of the protein was not sufficient (e.g., with the OVG sample stored 18 days), the suspension was prepared, clarified by centrifugation and diluted to the desired concentration on the basis of nitrogen content. The conforma-tional compositions on the native and denatured ovalbumins were cal-culated using the values of Table II in the paper reported by Chen *et al.* (1974).

RESULTS AND DISCUSSION

Browning color development and loss of available lysine during storage are shown in Fig. 1 and 2, respectively. The brown color development in OVG had a rise for about 14 days followed by a slow increase, whereas no browning discoloration in OV was found during 24 days of observation. The losses of available lysine in OVG occurred abruptly as can be seen in Fig. 2 while no change in available lysine in OV was observed. It has been found that chemical reaction between protein and sugar can occur during freeze-drying (Goulden 1956). As seen, the destruction of lysine in OVG occurs prior to an increase in brown color development.
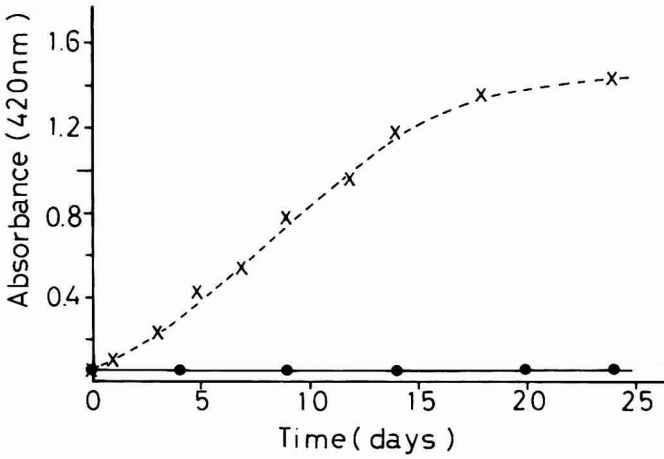
FIG. 1. BROWNING COLOR DEVELOPMENT IN FREEZE-DRIED AND STORED
OVALBUMIN AND OVALBUMIN-GLUCOSE STORED AT 50°C AND 65% RH

●———● Ovalbumin
X - - - X Ovalbumin-glucose
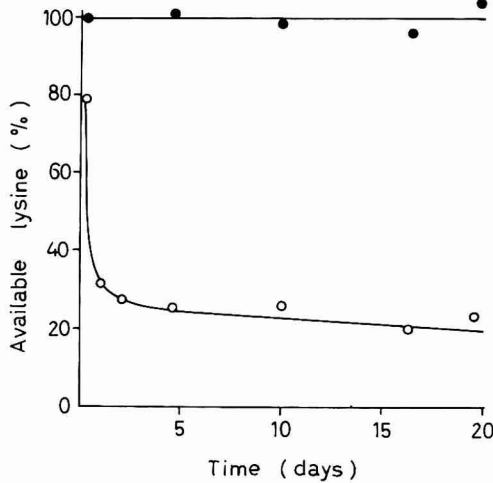
FIG. 2. LOSS OF AVAILABLE LYSINE FROM FREEZE-DRIED AND STORED
OVALBUMIN AND OVALBUMIN-GLUCOSE STORED AT 50°C AND 65% RH

●———● Ovalbumin
○ - - - ○ Ovalbumin-glucose

As can be seen from Fig. 3 in which the changes in solubility during storage are followed, the solubility of OVG slightly increases to about 100% in 9 days of storage, and then decreases to about 10% after 25 days of storage. It could be said that a small quantity of the insoluble molecule in unstored OVG produced during the treatment of the freezing and freeze-drying was converted into soluble ones in the initial stage of the Maillard reaction, and after that the soluble protein was changed in insoluble form through denaturation and aggregation with brown color development. On the other hand, the protein solubility of OV did not change in storage although there were some insoluble proteins which
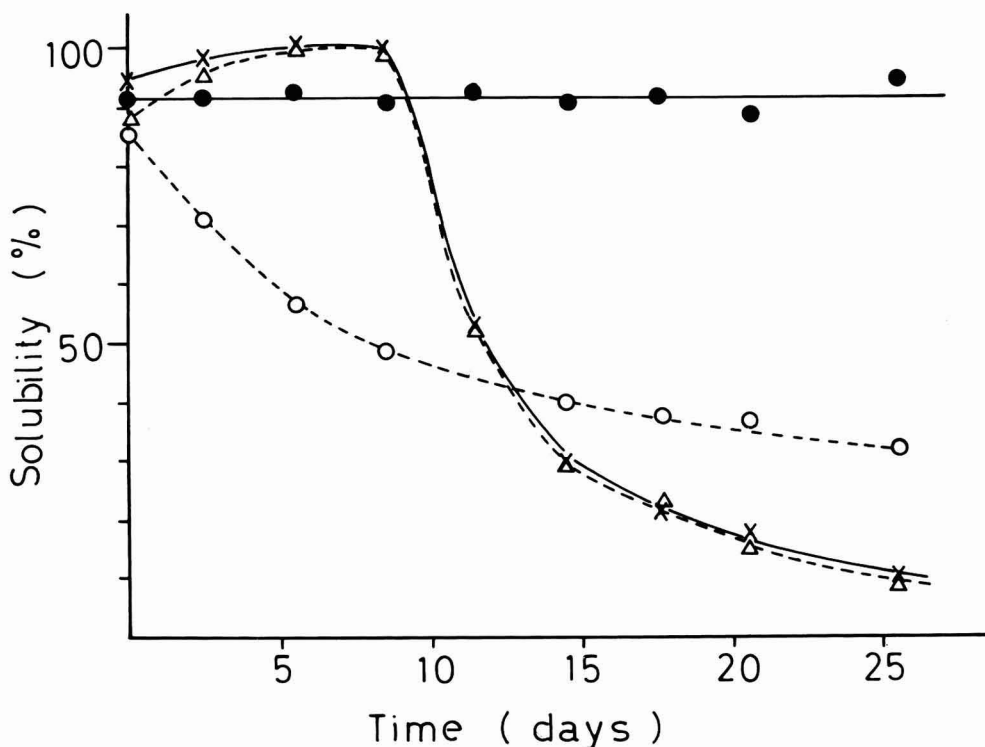
FIG. 3. SOLUBILITY AND HEAT STABILITY (70°C, 5 min) IN CITRATE BUFFER CONTAINING 2M NACL (pH 6) OF THE FREEZE-DRIED AND STORED OVALBUMIN AND OVALBUMIN-GLUCOSE

●——● Solubility of ovalbumin
○ - - - ○ Heat stability of ovalbumin
X——X Solubility of ovalbumin-glucose
Δ - - - Δ Heat stability of ovalbumin-glucose

might have been denatured during the preparation of OV. The changes during storage in the heat stability of OV and OVG are also shown in Fig. 3. The curve of heat stability of OVG showed almost the same profile as that of solubility of OVG, whereas the heat stability of OV decreased with storage to a value of about 30% of the original heat stability at 25 days of storage. From the results of the solubility and heat stability, it could be said that the proteins in the OVG system were complexed with glucose, solubilized and stabilized with the addition of the hydrophilic group and the increased repulsion force due to the changes of charged groups in the protein-glucose complex occurring in the initial stages of the Maillard reaction. The OVG system was then insolubilized through the formation of cross-links. The proteins in both systems become sensitive to the effects of heat during storage through the loss of bound water around the molecule. These are in agreement with previous findings on the protein-sugar complex (Morales *et al.* 1976, Tybor *et al.* 1973).

The SDS-polyacrylamide gel electrophoretic patterns in Fig. 4 showed a similar main band in both unstored OV and OVG. The main band with a trace one appeared after 8 and 17 days for stored OV samples. During prolonged storage it is apparent that a fraction of the protein in the sample of OVG formed aggregates having greater particle weight which could not be reduced to monomers with 2-mercaptoethanol in the presence of SDS. This suggests that stable cross-links were produced through the Maillard reaction.

The results of the amino acid analysis of unstored and stored OV showed no changes of amino acid composition, whereas those of the OVG system presented in Fig. 5 indicated the loss of lysine, arginine, serine and threonine residues during storage. The decrease of basic amino acid content is reasonable because they can react with a reducing sugar in the Maillard reaction. However, it is of considerable interest that the greatest loss occurred with arginine, that is, 68% at 8 days-storage and 83% at 19 days-storage. It has been reported that guanidyl groups might be involved in a type of cross-linking reaction requiring the presence of free amino group (F-Conrat *et al.* 1948, Mahammad *et al.* 1949).

The CD spectra of the unstored and 18 days-stored OV and OVG are shown in Fig. 6-A and -B. Both samples of unstored OV and OVG, and 18 days-stored OV showed almost the same CD spectra, in which there was a negative extreme at 222 and 209 nm. Changes of the secondary structure could be found in the CD spectrum of OVG stored for 18 days, compared with that of unstored OVG.

The variation of the secondary structure of ovalbumin calculated on the CD curves of stored and unstored OV and OVG is listed in Table 1. The contents of α-helix, β-structure and unordered form of the unstored OV and OVG were estimated to be about 33, 30 and 37%, respectively.
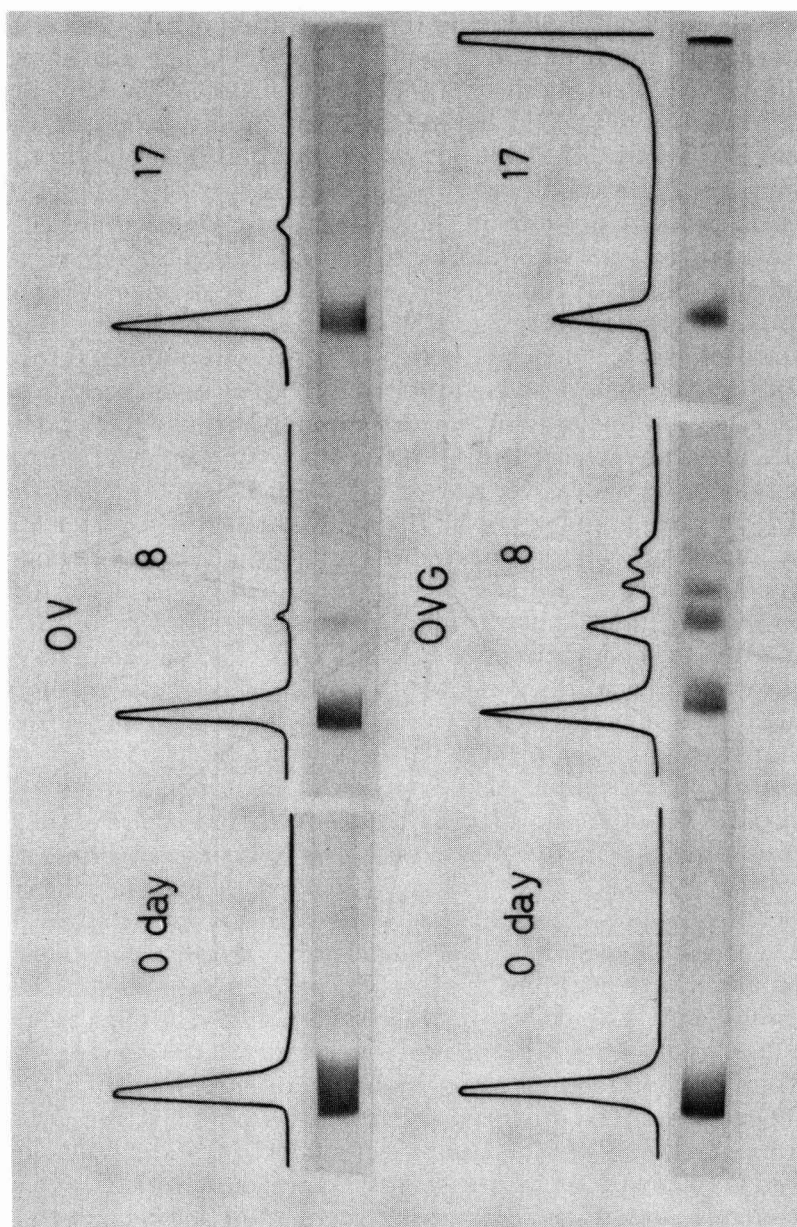
FIG. 4. SDS-POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERN AND
DENSITOMETRIC TRACING OF THE FREEZE-DRIED AND STORED OVALBUMIN
AND OVALBUMIN-GLUCOSE

OV: Ovalbumin
OVG: Ovalbumin-glucose
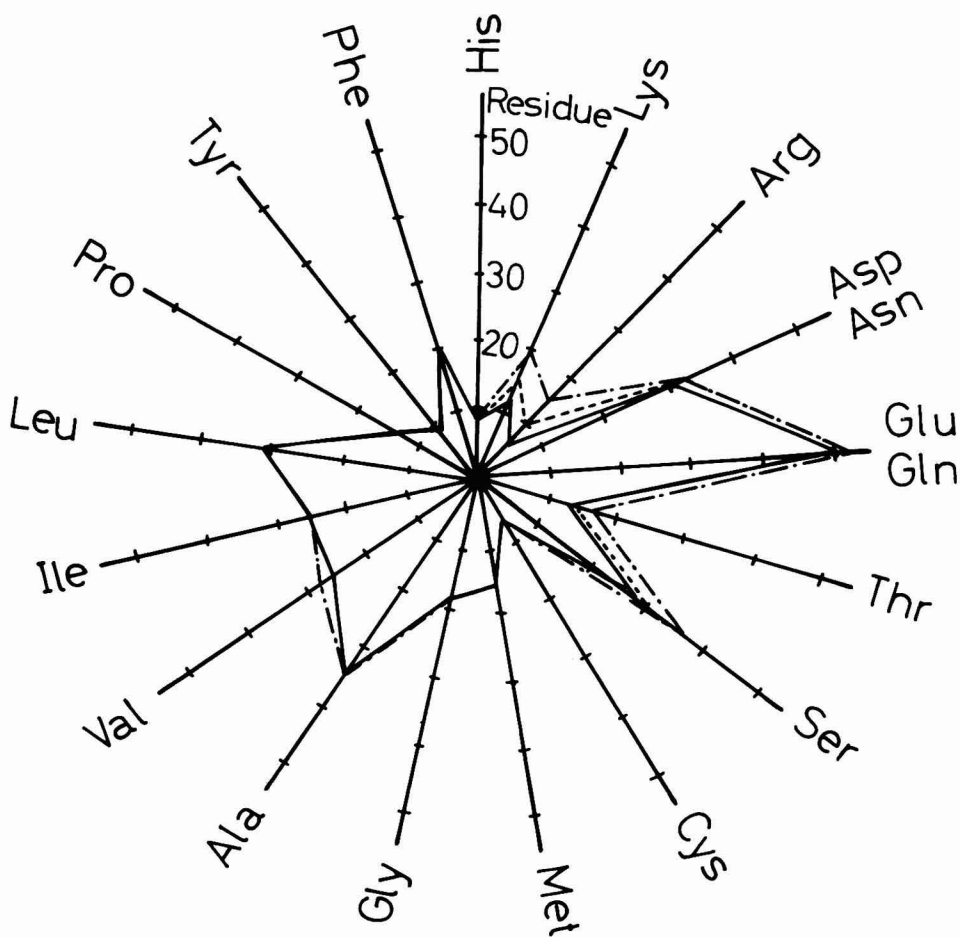
FIG. 5. AMINO ACID COMPOSITION OF THE FREEZE-DRIED AND STORED
OVALBUMIN-GLUCOSE

—·—·— Unstored ovalbumin-glucose
------ 8 days-stored ovalbumin-glucose
———— 19 days-stored ovalbumin-glucose
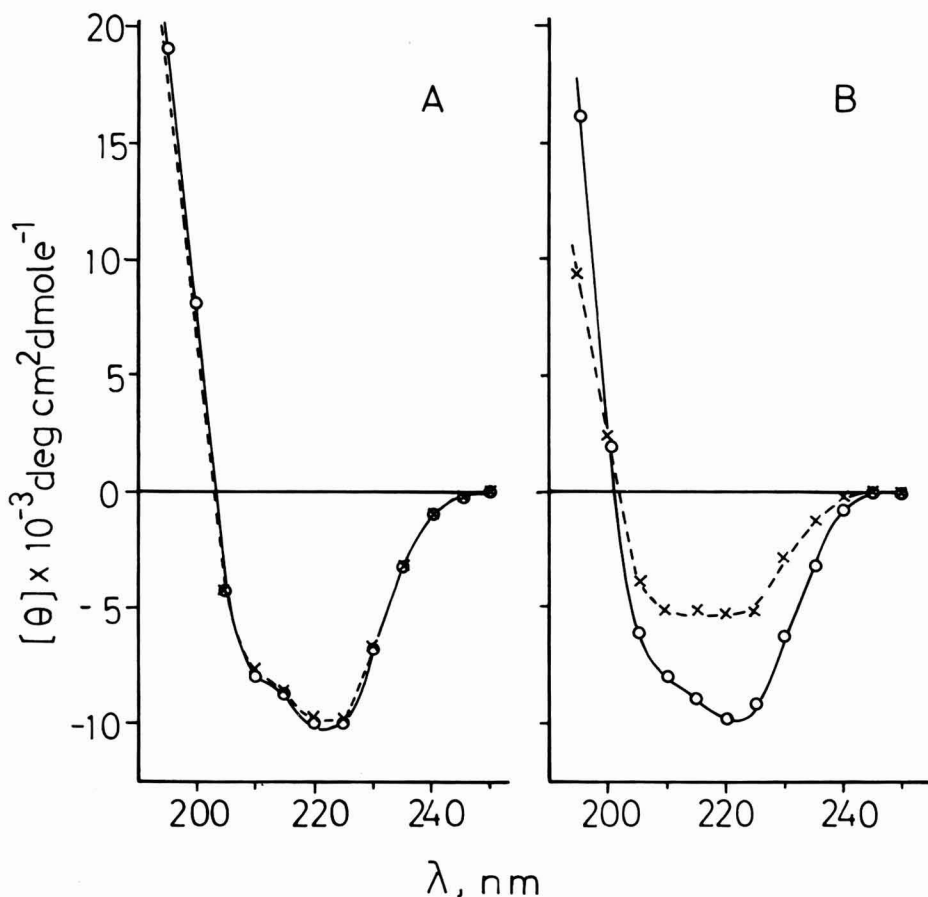
FIG. 6. CIRCULAR DICHROISM SPECTRA OF THE FREEZE-DRIED OVALBUMIN
AND OVALBUMIN-GLUCOSE

A: O——O Unstored ovalbumin
   X - - - X Unstored ovalbumin-glucose
B: O——O 18 days-stored ovalbumin
   X - - - X 18 days-stored ovalbumin-glucose

The best fit between calculated and experimental CD curves was
obtained in the regions of 250–217 nm and 205–195 nm. However, the fit
was rather poor in the region of 205–217 nm. It has been reported that the
α-helix content of ovalbumin was between 22 and 34% for several of the
optical rotatory dispersion parameters (Tomimatsu and Gaffield 1965,
Gordon 1968) and CD spectrum (Gorbunoff 1969, Timasheff and
Gorbunoff 1967). Some β-structure might also be present on the basis of

Table 1. Variation of the secondary structure of the freeze-dried ovalbumin and oval-bumin-glucose during storage

	Time in Days				
	0	3	6	11	18
	Secondary Structure Composition (%)				
OV					
α-Helix	33	32	32	32	32
β-Structure	30	30	30	30	30
Unordered form	37	38	38	38	38
OVG					
α-Helix	33	33	28	25	18
β-Structure	30	30	30	30	—
Unordered form	37	37	42	45	—

OV: Ovalbumin
OVG: Ovalbumin-glucose

optical rotatory dispersion measurement (Jirgensons 1966). Our data on the secondary structure described above clarified that ovalbumin in the unstored OV and OVG contained almost an equal amount of α-helix and β-structure. Comparison of the CD spectra of unstored OV with that of stored OV (3, 6, 11 and 18 days) indicates that no appreciable changes in the secondary structure occurred during storage of OV. The main event on the conformational change of OVG during storage seemed to be an increase in the content of unordered form at the expense of the α-helix. The content of β-structure remained essentially unaltered during storage. The percentages of β-structure and unordered form in the OVG stored for 18 days could not be obtained from the differences between the experimental and calculated values except the α-helix content. It can be seen from the relation between the loss of available lysine and the variation of secondary structure that about 70% of available lysine reacted with glucose in the period before denaturation. The comparison of the conformational changes of stored OV and OVG suggests that charge balance in a protein plays an important role in maintaining its conformation stored in a dry system, that is, the change in charge balance of the ovalbumin molecule upon the formation of protein-glucose complex promotes the unfolding of protein. This result is in agreement with the demonstration by a protease digestion method that the N$\rightleftharpoons$D transitions of lysozyme shifts toward the denatured state when net charge of lysozyme was altered upon acetylation (Imoto et al. 1976). These unfoldings of protein might be dependent on the destruction of the hydrogen bond by blocking the positive charge of amino groups in protein.

ACKNOWLEDGMENTS

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NMR-STUDY OF WATER IN SOME STARCHES AND VEGETABLES

HANS LECHERT, WALTER MAIWALD, RAINER KÖTHE and
WOLF-DIETER BASLER

*Institute of Physical Chemistry
of the University of Hamburg
Laufgraben 24, 2000 Hamburg 13, W-Germany*

ABSTRACT

The paper contains a discussion of the possibilities and limitations of pulsed and wide-line NMR-experiments for the investigation of special problems in food science and technology. In starches two kinds of bound water can be observed. For potato starch the life times and the probabilities of occupation of molecules in these regions are discussed. The self-diffusion coefficient of the more mobile kind can be determined to be about 10^{-6} cm²/s for room temperature. For some starches an anisotropic mobility of the water molecules in this region can be detected by deuteron resonance. This resonance shows further characteristic differences at temperatures above the swelling point which can be used for studies of structural influences of technical processes, like the heat and moisture treatment. For the deuterated starches possibilities of studies of swelling and retrogradation phenomena are discussed. The experiments on starches are extended to beans, peas and lentils where rehydration and cooking processes are studied. For carrot tissue a self diffusion experiment is discussed for the investigation of processes going on in the course of cooking.

INTRODUCTION

Recently, various NMR-techniques have become an important tool for investigations of special problems in food research.

NMR-methods are mostly used to look at the molecular mobility of fat components (Conway and Earle 1963; Conway and Johnson 1969; Conway 1971) or of the water belonging to the food system (Tait *et al.* 1972; Toledo *et al.* 1968; Duckworth and Kelly 1973; Blanshard and Derbyshire 1975). Another typical field is the determination of liquid solid ratios in fats (van Putte *et al.* 1974, 1975; Templeman 1975). The literature on these problems has been recently reviewed by Weisser (1977).

The most suitable techniques of NMR-spectroscopy for the indicated studies of molecular mobility are wide-line and pulsed NMR. The purpose of this paper is to show for the example of starches and some starch containing vegetables how the mentioned methods can be extended to obtain details of the mechanism of motional processes of adsorbed water and how this information can be used to study influences of processes on the structure of these materials. Because in some cases quite detailed arguments from theory are used in the explanation of the experimental results a short summary of the principal effects which can be expected for the NMR-parameters shall be given.

The mobility of a nucleus, incorporated in a moving molecule, is measured by the correlation time τ_c, giving the interval during which fixed spatial relations of the nucleus can be expected. τ_c is directly connected with the relaxation times T_1 and T_2 of the nuclear magnetization, obtainable preferably by pulsed NMR-experiments.

The dependence of T_1 and T_2 on τ_c is demonstrated in Fig. 1a. The temperature function of T_1 shows a minimum for each kind of motion. T_2 usually decreases with decreasing temperature and becomes constant if the molecules are in fixed positions and in a definite state of reorientation. At the minima of T_1, $\omega_L \cdot \tau_c \approx 0.6$ holds, from which τ_c can be obtained. ω_L is the measuring frequency of the NMR-experiment. Furthermore, at the minimum $T_1/T_2 = 1.6$ holds if the motion is isotropic and has only one single τ_c. Any deviation from this situation leads to an increase in T_1/T_2. Usually τ_c obeys an Arrhenius law with good accuracy so that the abscissa in Fig. 1a is proportional to $1/T$. The activation energy can be obtained by a measurement of the T_1-minimum for different ω_L, even in the presence of distributions of τ_c as is usually the case in sorption systems. This situation is demonstrated in Fig. 1b for different widths β of a logarithmic normal distribution of the τ_c, corresponding to a normal distribution of the activation energies of the motional process (Resing 1965, 1968). In many systems two T_2 or T_1 values can be obtained from the magnetization decays in a NMR-pulse experiment, indicating regions of different mobility and different bond strengths of the water molecules to the biopolymer. This situation is demonstrated for potato starch in Fig. 2.

Another deviation from the most simple case of a motional mechanism as in Fig. 1a is the restriction to anisotropic reorientation. This gives special decay shapes, demonstrated for potato starch with $a_w = 0.8$ in comparison with the respective wide-line spectrum in Fig. 3. From the wideline spectrum, it can be seen that the decay shape can be explained by a splitting of the resonance line. This can be shown to be due to an anisotropic motion of the water molecules (Hennig and Scholz 1976; Lechert and Hennig 1976).
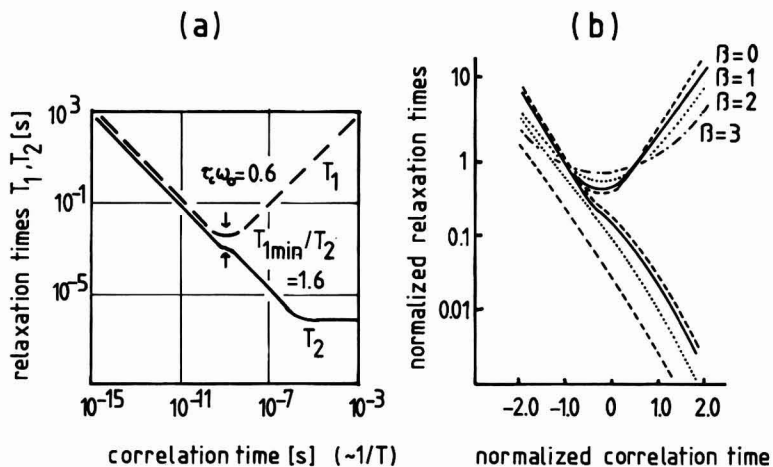
FIG. 1. a. DEPENDENCE OF THE NUCLEAR RELAXATION TIMES T_1 AND T_2 ON THE CORRELATION TIME τ_c (AND THE TEMPERATURE T) FOR A MOTIONAL PROCESS WITH ONE SINGLE CORRELATION TIME AND ISOTROPIC REORIENTATION OF THE MOLECULES

b. DEPENDENCE OF THE NORMALIZED RELAXATION TIMES

$$T_{1n}, T_{2n} = \frac{\gamma^2 M_2}{3\,\omega_L} \quad (T_1, T_2)$$

WITH DISTRIBUTIONS OF CORRELATION TIMES WITH DIFFERENT WIDTH β ON THE NORMALIZED PARAMETER

$$x = \log(\omega_L \tau_{co}) + 0.434\; \frac{E}{RT}$$

τ_{co} IS THE CORRELATION TIME FOR $\beta = 0$ AND E THE AVERAGE ACTIVATION ENERGY
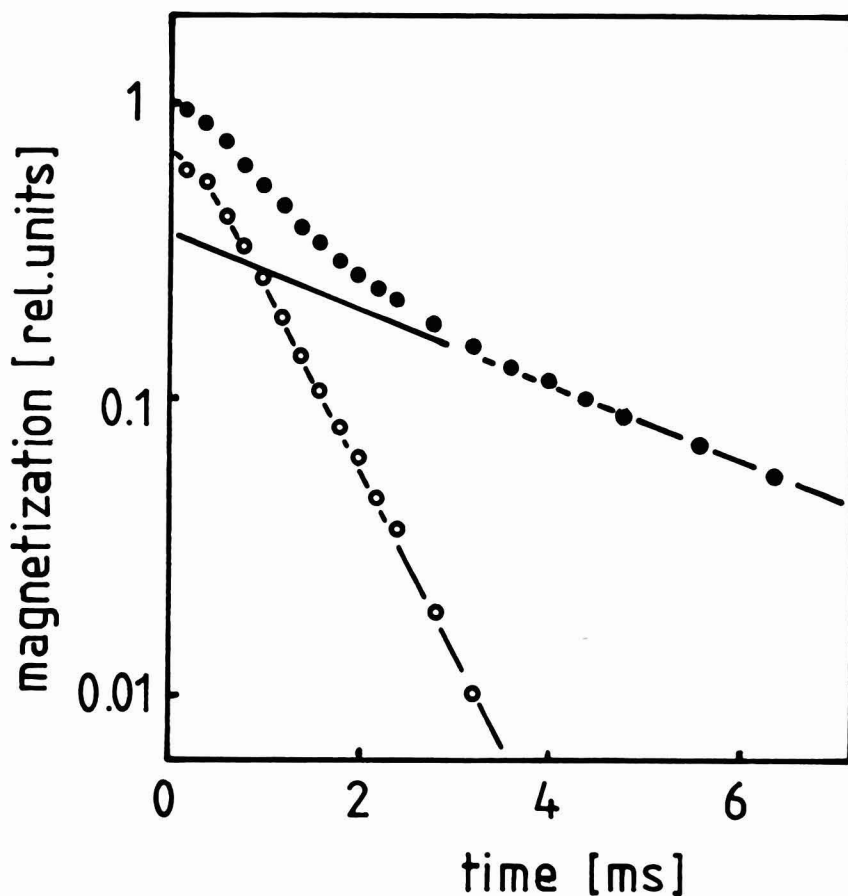
FIG. 2. LOGARITHMIC PLOT OF DECAY FUNCTION OF THE TRANSVERSE MAGNETIZATION CONTAINING TWO EXPONENTIALS WITH TWO DIFFERENT T_2, OBTAINED FROM A POTATO STARCH SAMPLE WITH 24% WATER AT 20°C

For the special case of water, proton as well as deuteron resonance can be used to study this kind of motional mechanism. For proton resonance, water must be regarded as a system of two spins coupled by the interaction of their magnetic dipoles. This causes a splitting of resonance line, the components of which have the distance

$$\Delta \omega = \frac{3 \gamma^2 \overline{h}}{2r^3} (3\cos^2 \Theta - 1) \qquad \text{Eq. 1}$$
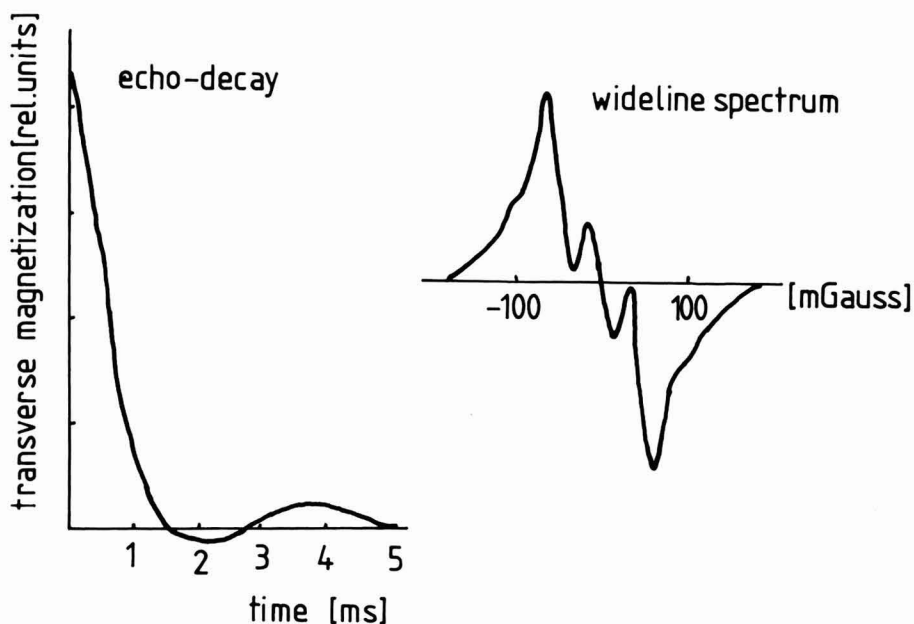
FIG. 3. COMPARISON OF A MAGNETIZATION DECAY FUNCTION, OBTAINED BY A SPIN-ECHO EXPERIMENT (ABRAGAM 1961) AND THE RESPECTIVE WIDE-LINE SPECTRUM OF THE Ca-FORM OF A POTATO STARCH WITH 20% WATER, INDICATING ANISOTROPIC MOTION OF THE WATER MOLECULES

where γ is the gryomagnetic ratio of the protons and r their distance in the water molecule. $\bar{h}$ is Planck's constant.

If a system of D_2O molecules is brought into a magnetic field, a splitting is given by the interaction of the deuteron quadrupole moment Q with the electric field gradient q in the field of the water oxygen ions. The resulting line is again a doublet with a distance similar to that of Eq.1

$$\Delta \omega = \frac{3\, e^2\, q\, Q}{4\, \bar{h}}\ (3\cos^2 \Theta - 1) \qquad\qquad \text{Eq. 2}$$

Θ in Eq. 1 is the angle between the proton-proton vector and the applied magnetic field. In Eq. 2 it is given by the direction of the OD-connection vector and the field direction.

For an anisotropic reorientating water molecule sorbed on a powdered

or fibrous material the powder pattern well known from the solid (Abragam 1961) can be observed, narrowed to an extent depending on the mobility of the molecule. The remaining splitting is a measure of the anisotropy of the motion.

The splitting of the line is quite sensitive against exchange effects of the hydrogen nuclei for the proton resonance, not, however, for the deuteron resonance, because the interaction causing this splitting is affected by the exchange in the first case far more than in the second. The anisotropic motion is, therefore, preferably studied by the deuteron resonance (Hennig and Scholz 1976; Hennig and Lechert 1977; Woessner and Snowden 1969; Berendsen 1962; Dehl and Hoeve 1969).

Deuterated samples can be used with success for investigations where the behavior of the high polymer is of interest in a system with a great excess of water, as for example in the case of the swelling mechanism and related phenomena of retrogradation and freeze-thaw-behavior (Hennig *et al.* 1976).

METHODS AND MATERIALS

The measurements of the nuclear relaxation times T_1 and T_2 have been carried out with a commercial BRUKER BKR 322 pulse spectrometer.

For the T_1 measurement, a repeated $180°$-τ-$90°$ pulse sequence has been used, where τ is the time interval between the two pulses. Readings of the signal height representing the nuclear magnetization directly after the $90°$-pulse were taken. For a time $\tau = T_1 \log 2$ this magnetization has the value 0, so that from a determination of the signal heights at different τ T_1 can be obtained.

For T_2, the spin-echo technique according to Hahn (1950) applying a $90°$-τ-$180°$ pulse sequence or the Carr-Purcell-Technique with a $90°$-τ-$180°$-2τ-$180°$-2τ-$180°$ sequence (Carr and Purcell 1954) has been used. The signals were accumulated in a signal averager and given in a logarithmic display unit, so that the logarithm of the echo-envelope was recorded, the slope of which gives T_2. The variation of temperature has been carried out with the temperature control unit belonging to the spectrometer and had an accuracy of about $\pm$ 1°C.

The temperature functions of T_1 of the starches have been measured for two frequencies 60 Mc/s and 20 Mc/s, because the extremely high value of T_1/T_2 at the T_1 minima indicated a broad distribution of the τ_c. As mentioned in the introduction, at the T_1-minimum $\tau_c \omega_L \approx 0.6$ holds. Therefore, for different ω_L the T_1-minimum appears at different temperatures, for

which the respective τ_c can be calculated. From the values obtained for τ_c and the minimum temperature an average activation energy can be estimated.

Additionally, the self-diffusion coefficient was measured according to the method of the pulsed field gradients described by Steijskal and Tanner (1965). This method is a modified spin-echo technique, where between the 90°-pulse and the 180°-pulse and between the 180°-pulse and the echo pulses of gradients of the magnetic field are applied. These gradients cause an additional damping of the echo amplitude from which the self-diffusion coefficient can be calculated. The wide-line measurements have been carried out with a Varian DP 60 spectrometer at 16 Mc/s for the proton resonances and at 10 Mc/s for the deuteron resonances.

For some starches X-ray diffraction patterns have been recorded with a ISODEBYEFLEX of the Fa. Seifert & Co. using CuKα-radiation. The amount of the C-form has been obtained by a calibration curve of mixtures of B- and C-starch supplied by the Maizena GmbH.

For our investigations of starches industrial samples have been used in most cases. As the industrial preparation is carried out always with water of some hardness, the samples are in the Ca-form in all cases where anionic groups like e.g. phosphate are present. This has been checked for all starches spectroscopically. Some of the potato starch samples have been compared with samples prepared from potatoes under careful conditions following the procedures of Richter et al. 1968.

For the experiments with vegetables, commercial dried peas, beans and lentils have been used. Carrots have been used in fresh form.

The deuteration experiments with starches have been carried out by percolating the material with D_2O until no protons could be detected in the penetrating water by NMR. This procedure leads to a complete deuteration of the adsorbed water in the OH-groups of the polysaccharide, not, however, of the CH-groups, as has been clearly demonstrated by Taylor et al. (1961) and by Hennig (1977).

For the legumes the samples were simply swollen in D_2O in most cases. For experiments with material which shows swelling already during the treatment at room temperature this material was exposed to a nitrogen stream (containing water) with $a_w = 0.8$ until the condensed water did not contain any proteins after passing the sample. This procedure takes about one week and results in incomplete proton exchange in most cases.

RESULTS AND DISCUSSION

Relaxation Measurements on Protons in Starches

First, careful studies on the relaxation times T_1 and T_2 were carried out

on different starches as a function of water content (Hennig and Lechert 1974).

These measurements showed that for starches containing up to 15% of water a single relaxation time T_2 as well as a single T_1 exists. Above this water content the magnetization decays can be separated into two exponentials following the procedure indicated in Fig. 2, corresponding to two T_2's and different states of mobility.

Among the starches studied until now, the most distinct effects have been observed for potato starch. Therefore more detailed studies were carried out especially with this starch. In the following, some examples shall be given where deeper insight into the mobility mechanism of water sorbed on the starch structure has been possible.

In Fig. 4 the temperature behavior of the two components of the relaxation time T_2 in a potato starch sample with $a_w = 0.8$ is given. From the predictions of theory, T_2 should increase with increasing temperature. The relaxation time curves in Fig. 4 can be interpreted as showing an exchange of protons or water molecules between the two regions of water. According to theoretical considerations of Woessner and Zimmermann (1963), the apparent relaxation times taken from the observed magnetization decays and plotted in Fig. 4 depend in a quite complicated way on the
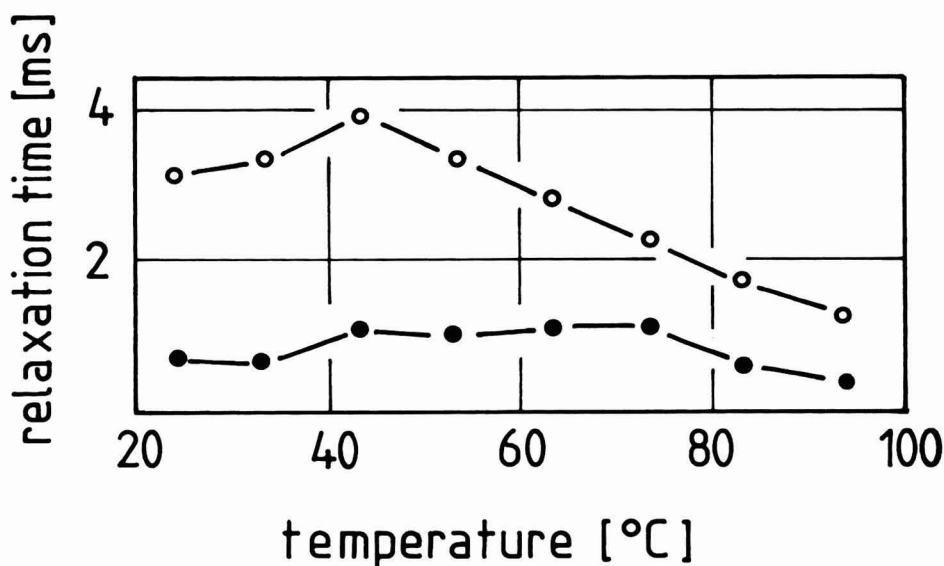

FIG. 4. TEMPERATURE BEHAVIOR OF THE RELAXATION TIME T_2 IN A POTATO STARCH SAMPLE WITH WATER OF $a_w = 0.8$

exchange rates, the real relaxation times and the probabilities of occupation of the two regions. For slow exchange the relaxation times as well as the probabilities of occupation can be taken undisturbed from the magnetization decays, because the decay is then simply the sum of the two exponentials belonging to the respective regions weighted by the relative number of water molecules in these regions.

For the sample demonstrated in Fig. 4 the probabilities of occupation are nearly equal in both regions. For this case the theory of Woessner and Zimmermann (1963) allows simplifacations which finally lead to the relation

$$\frac{1}{T'_{2a}} = \frac{1}{T_{2a}} + \frac{1}{\tau_a}$$ Eq. 3

for the values of the apparent relaxation time T'_{2a} above the maximum. T_{2a} is the real relaxation time of the protons in the region a and τ_a the life time of the molecules in this region. Sufficiently above the maximum, T_2 is quite long and the apparent relaxation rate is equal to the exchange rate $1/\tau_a$. The data of Fig. 4 have been carefully analyzed according to this theory. The results are given in Table 1. From the data of Table 1, the activation energy of the exchange is 31 kJ/mole. Estimations made with results obtained at higher water contents show that this activation energy decreases slightly with increasing water content. These values lie fairly below the values of about 50 kJ/mole usually observed for these processes in hydrogen bonded systems. For water contents above about 30% a third component with a fairly long T_2 is present in the magnetization decays (Lechert and Hennig 1976). Comparative calorimetric studies have shown that the water belonging to the third T_2 is freezable and can be regarded as "free" water, whereas the other two components have to be

Table 1. Temperature dependence of the life-times of water molecules in a region a with a higher mobility and a region b with lower molecular mobility and the ratios of probabilities of occupation for these regions for a native potato starch sample with $a_w = 0.8$

Temperature °C	τ_a ms	τ_b ms	$P_a/P_b = \tau_b/\tau_a$
43	4.5	—	—
53	3.7	11.8	3.2
63	3.0	3.7	1.3
73	2.3	2.7	1.2
83	1.6	1.9	1.2
93	1.3	—	—

regarded as "bound," following the suggestions of Kuprianoff (1958). The T_1 usually consists on only one component and decreases from about 650 to about 150 ms, going from the dry starch to about 30% water content. The temperature behavior shows for all starches a flat minimum which indicates rather broad distributions of correlation times. In Table 2, for some starches and three water contents the values of the T_1-minima are shown for two measuring frequencies. From the position of the minima the activation energies have been estimated. Because of the flatness of the minima the values of the activation energies can be obtained only with large limits of error. In the region of "bound" water at 11% and 20% water content the activation energies increase with the cross-linking of the starch. At the highest water contents, where already "free" water can be expected in the sample, this tendency is reversed.

Self-Diffusion Experiments on Potato-Starch

To get exact values of the activation energy at least in one case, self diffusion measurements with potato starch have been carried out using the method of pulsed field gradients of Steijskal and Tanner (1965). The results show values between $8 \cdot 10^{-7}$ cm²/s for 20% of water and $14 \cdot 10^{-7}$ cm²/s for 30% of water at 30°C. Measurements at different temperatures give an activation energy of 54 ± 4 kJ/mole which is more exact than the values obtained from the T_1-minima. These self diffusion coefficients belong to the more mobile region of the bound water.

Table 2. Position of the minima of the longitudinal relaxation time T_1 for measuring frequencies of 20 Mc and 60 Mc for different starch samples at different water contents and estimated values of the activation energy of the mobility of the water molecules

Sample	11% H_2O			20% H_2O			32% H_2O		
	60 Mc	20 Mc	E_a	60 Mc	20 Mc	E_a	60 Mc	20 Mc	E_a
Nat. potato starch	336	325	92	290	278	63	240	233	75
Crossl. potato starch	339	333	171	301	292	88	284	273	63
Nat. maize starch	318	304	63	270	263	92	242	235	75
Crossl. maize starch	348	339	121	291	282	84	243	235	67
Nat. waxy-maize starch	314	288	33	271	258	50	241	233	63
Crossl. waxy-maize starch	330	316	67	278	267	63	245	233	42
Wheat starch	329	319	96	273	264	75	244	233	46
Amylose	335	319	63	276	265	63	244	235	59
Amylopectin	357	348	125	312	302	88	—	—	—

The values of the activation energy E_a are given in kJ/mole

These results may be important for a number of processes taking place in a variety of food systems. In these processes water can serve as a vehicle for the transport of smaller molecules. One of the most important of these processes is the Maillard reaction. According to the results of Eichner (1978) the rate in tomato powder and a polysaccharide model system has a maximum at a water activity of about 0.8, corresponding to the activity of the sample discussed above. Other examples have been found by Simatos (1978) in a study of reactions of organic radicals with vitamin C.

Deuteron Resonance Studies on Starches

As discussed in the introduction, further insight into the mechanism of mobility of the bound water in starches can be obtained by the deuteron resonance. This yields information on deviations from isotropic reorientation in the course of the motion. These deviations cause a splitting of the deuteron resonance line which is a measure of the anisotropy of the motion. As has been found in earlier studies, the detectability of the splitting is subject to some conditions.

The most important condition is given by the requirement of ordered regions, which interact with the water molecule in a way that makes some orientations of the molecule with respect to the structure more probable than others.

The periods in which the molecule is in such an ordered state must be much larger than the periods during which an isotropic reorientation occurs. As the expected anisotropy is rather small, in most cases the mobility of the molecule must be great enough to make the linewidths of the components of the splitted line smaller than the splitting itself.

The condition that the molecule must be in the ordered situation long enough for the detection of the splitting in the deuteron resonance is only fulfilled if a long range ordering of crystallites in the starch granule is present. A change in the splitting can be taken as a sensitive indicator for a change in this structural element.

Figure 5 shows the deuteron resonance lines of a number of starches, deuterated following the procedure outlined above and equilibrated at $a_w = 0.8$. A distinct splitting can be observed for potato starch. Pea starch and wheat starch show only a weak splitting. Arrow-root and sweet-potato starch show indications of a splitting and tapioca, maize and waxy-maize starch show no splitting at all. More detailed classifications are possible by studies of the temperature behavior. In the range up to 120°C where water distills from the samples, two types of behavior can be distinguished.
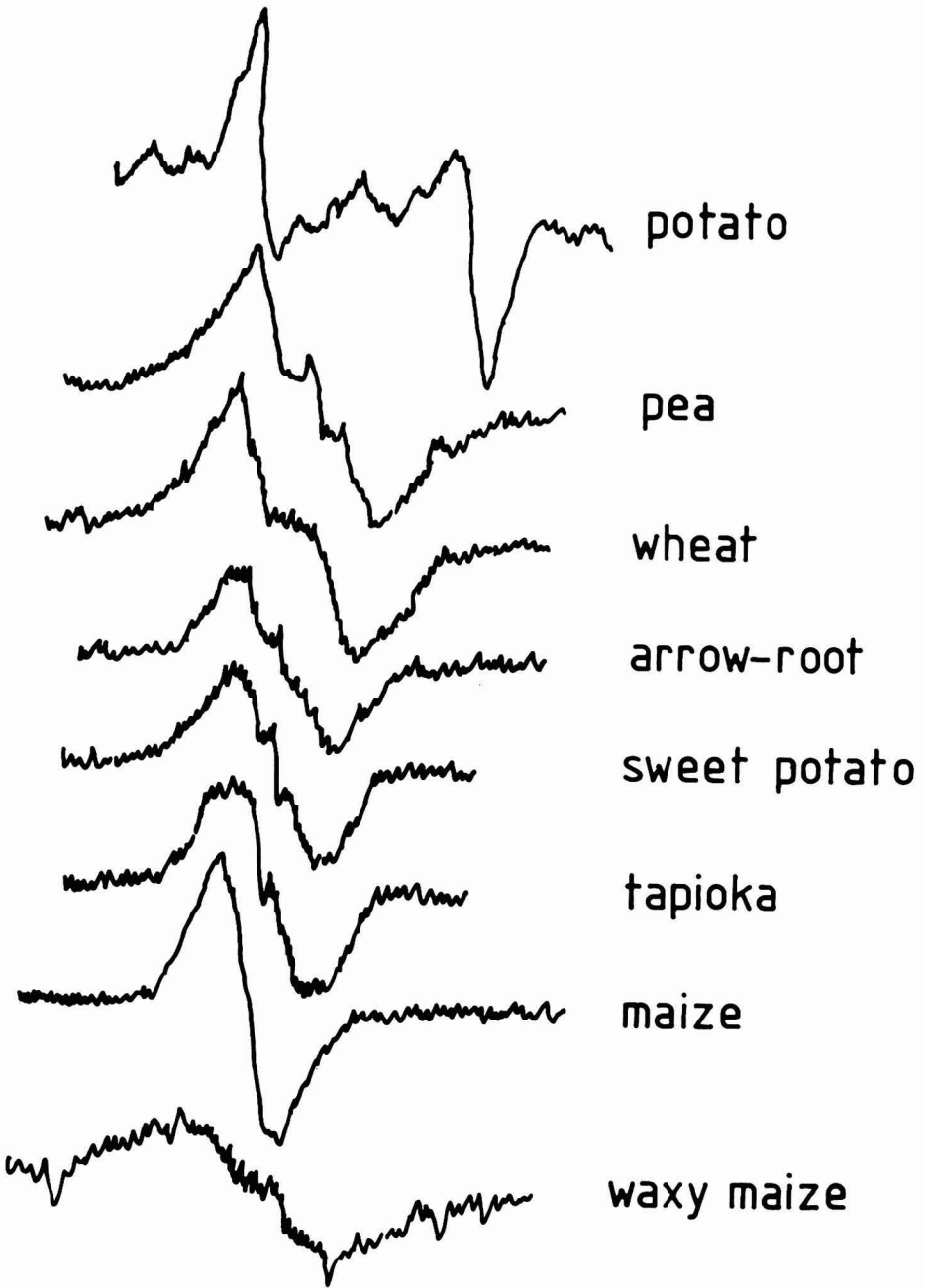
FIG. 5. DEUTERON RESONANCE SPECTRA OF DIFFERENT STARCH SAMPLES
EQUILIBRATED AT $a_w = 0.8$

Of the first type are the potato starch, the maize starch, the sweet-potato starch and the arrow-root starch (see Fig. 6). The potato starch shows at temperatures near 110°C a collapse of the splitting to a relatively broad line, indicating an isotropic reorientation of the water molecules which seems to stay, however, within the starch structure.

The maize, tapioca and arrow-root starches, that show no splitting at room temperature, develop a slight narrowing of the line in the temperature range in question. This behavior suggests that the starch structure undergoes an expanding with increasing temperature allowing for the isotropic reorientation in the potato starch and for a slightly increased mobility in the other starches. Whether the molecules occupy sites different from those they were at before the sample was exposed to the higher temperature, cannot be decided by the present experiments.

During the increase of temperature a broad line is observed with the second type of starches, as has been described for the first type. However, a region with water molecules with distinctly increased mobility exists which is indicated by a quite narrow line superimposed on the broad one. The intensity ratios vary for the different starches and show for waxy-maize and pea starch that the mobile region contains only a small amount of water. In contrast to this in the tapioca starch, the behavior of which is demonstrated on the right side of Fig. 6, all the water seems to be in the mobile region after the heat treatment. Whether this behavior belongs to a third type or whether it has to be simply regarded as another variety of the second type of water mobility is not clear. Experiments with crosslinked potato starches which show all three types of behavior, depending on the degree of the crosslinking, are in progress. If "free" water is present in the potato starch, the splitting breaks down at 80°C as has been shown by Hennig and Lechert (1977) for a potato starch sample with a D_2O content of 39%.

The reason for a splitting in some starches and a single line in others cannot be fully explained until now. The most probable explanation is that the relative residence time in the ordered state is too short, which may be caused by the starch structure itself or for instance by protein or other impurities. A support for this explanation can be seen in the fact that a splitting of the deuteron resonance can be observed in wheat starch, but not in wheat flour.

Swelling and Retrogradation Experiments

Another use of deuterated starches is made in the investigation of swelling and retrogradation and freeze-thaw-phenomena.

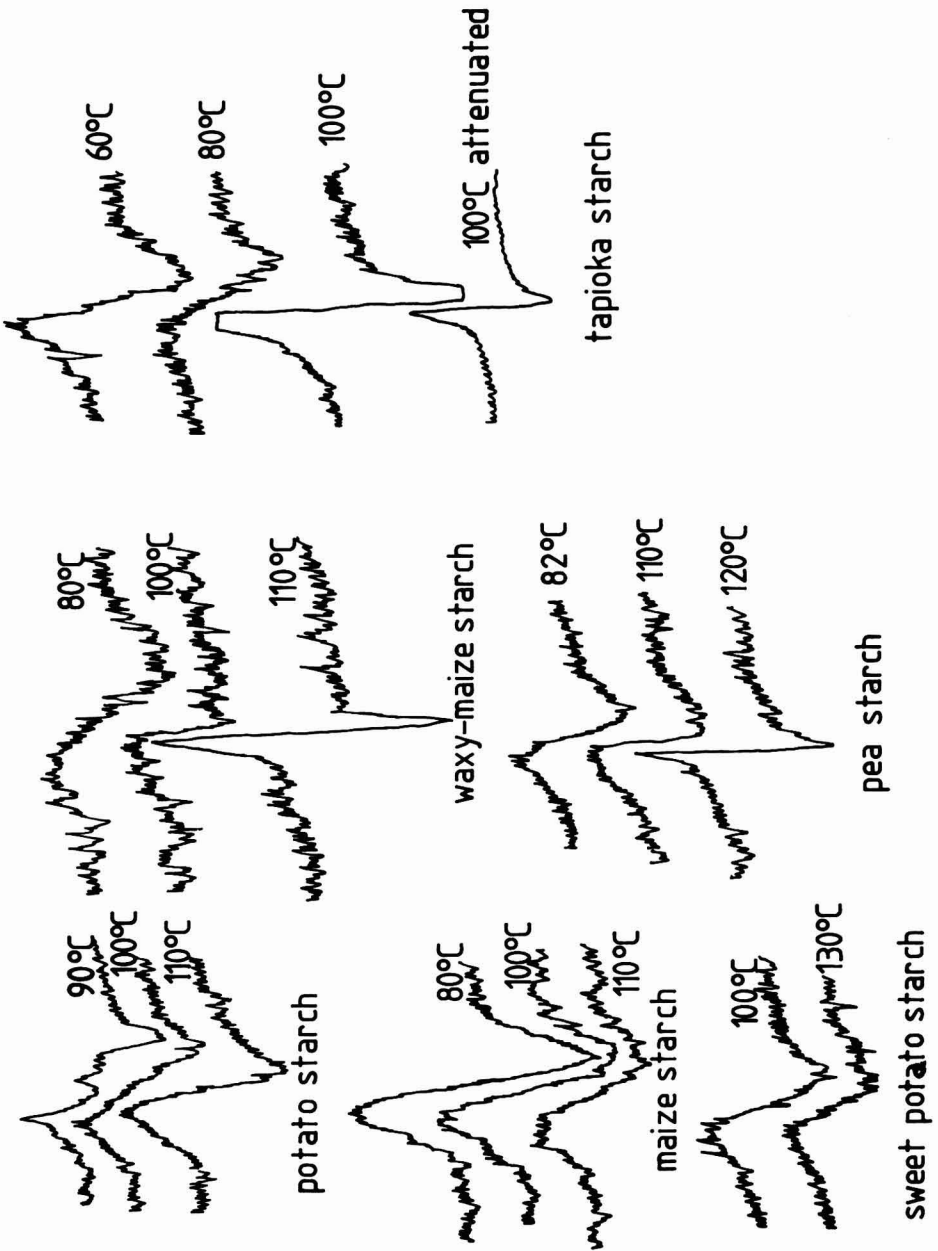Figure 7 shows a number of Carr-Purcell-experiments on the CH-

FIG. 6. BEHAVIOR OF THE DEUTERON RESONANCE OF DIFFERENT STARCHES
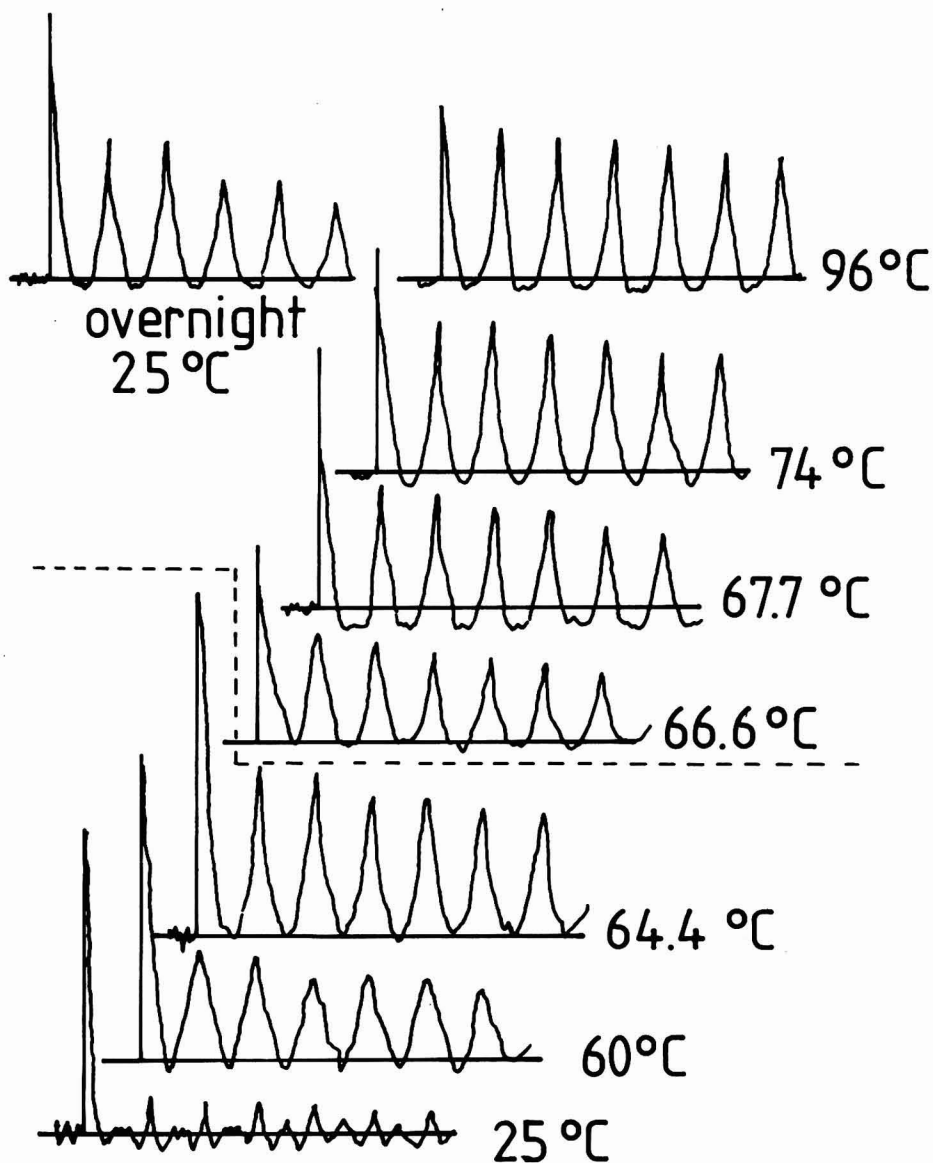WITH a_w = 0.8 AT ELEVATED TEMPERATURES

FIG. 7. MAGNETIZATION DECAYS OF THE CH-PROTONS OF A DEUTERATED
POTATO STARCH IN THE COURSE OF SWELLING IN D$_2$O
The picture in the upper left corner results after cooling the sample from 96°C to room
temperature and leaving overnight.

protons of a deuterated potato starch sample during the course of swelling. The starch content of this mixture was 3%. The fraction of mobile starch chains, given by the relative intensity of the envelope of the slowly decaying peaks, is augmented near the swelling temperature with increasing rate until at 96°C only mobile starch chains are present. The fast decay, just at the beginning of the experiment at the bottom of Fig. 7, indicating the solidlike part of the starch in the system, has disappeared. Cooling to room temperature and leaving overnight restores the signal of the solid partly, as can be seen from the diagram at the top left in Fig. 7.

Figure 8 shows the amount of the solid like component for the swelling and the retrogradation process in potato, maize and waxy-maize starch. The behavior of the mentioned signals does not differ very much for the swelling process apart from differences in the temperature at which the signal vanishes. The swelling temperature depends on the rate of heating, as has been shown in earlier investigations.

The retrogradation experiments show differences in the rate of development and the final amount of the solid-like signal which are in agreement with other experiments. It should be pointed out, however, that the described NMR-experiments give information on the molecular processes occuring in the course of the retrogradation which may differ from those obtained for instance from macroscopic quantities like the viscosity.

Deuteron Resonances of Heat-Moisture Treated Samples

In another series of experiments the influence of a heat-moisture treatment on starches has been tested.

In the first series samples of deuterated potato starch were mixed with an amount of water giving mixtures of one gram of water per gram of dry starch. Starting at 50°C these samples were heated to different temperatures each a few degrees apart. After one day of heat treatment the deuteron resonance was measured. A change of the spectrum was observed only above 70°C, resulting in decrease of the relative amount of molecules in the anisotropic state of mobility. The splitting itself remains unchanged. Above 80°C the splitting collapses, as has been mentioned before. X-ray diffraction patterns of the samples showed an increasing amount of the C-form with decreasing intensity of the splitted line. Further, a series of different starch samples has been heated to 100°C for one day. The D_2O-content of these samples was 24% corresponding to $a_w = 0.8$. A rather broad line without any structure was observed in potato starch, the width of which is about twice as large as the splitting in the untreated starch. For the pea starch the deuteron resonance is
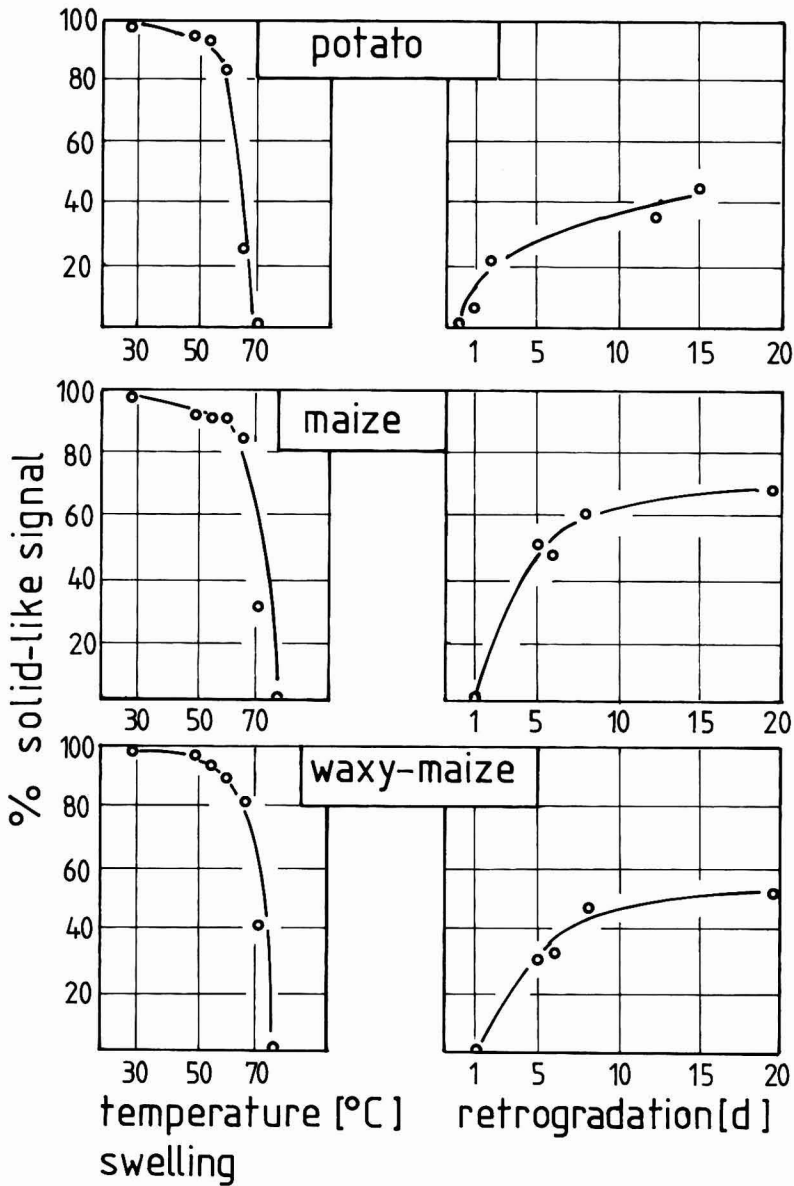
FIG. 8. DEPENDENCE OF THE AMOUNT OF SOLID-LIKE STARCH FOR THE
SWELLING AND THE RETROGRADATION OF POTATO STARCH, MAIZE STARCH
AND WAXY-MAIZE STARCH
The concentration of the gels was 10%

broadened beyond the limit of detection. Native maize and waxy-maize starch show no appreciable influence of the heat treatment.

In another experiment, potato starch was dried at 135°C and rehydrated, pouring the dry starch in an excess of D_2O and equilibrating it at $a_w = 0.8$. In both cases the resonance is restored without any change of the shape and the amount of splitting.

From these experiments it can be concluded generally that quite detailed information is obtained if the substances are of a defined kind and the questions on the system are sufficiently clearly stated. These requirements concerning the substance as well as the questions are only badly fulfilled in many cases in food research. Some examples of questions and restrictions in the informations which can be obtained from NMR results shall be given in the following examples regarding peas, beans, lentils and carrots.

NMR-Experiments With Vegetables

The question in these experiments was whether the point where samples of legumes boiled in water are done, can be observed in an NMR-experiment.

Experiments with commercial samples of dried peas, beans and lentils have been carried out first. The amount of mobile protons turned out to be about 50% in the dry sample, containing water molecules and liquid fat components of the grains. Equilibrating at $a_w = 1$ overnight did not change this value appreciably. Storing overnight in excess water reduces the amount of solid-like protons to values between 20% to 25% for the peas, to 15% for the beans and to about the same value for the lentils. Subsequent heating of the grains to 100°C yields the amounts of solid like protons demonstrated in Fig. 9a and 9b for peas and beans. The lentils showed a large variation of these values from one grain to another and have not been plotted.

It can be seen that the final state of the solid-mobile ratio of the protons is obtained already after a few minutes. No indication of change indicating when the grains are done can be observed. This point has been determined by an organoleptic test with seven different persons and occurs after about one and a half hours.

Another attempt to get information on this state of doness has been made by recording the T_2 of the mobile component during the time of cooking. As can be seen from Fig. 10a and 10b a steady increase of T_2 can be observed showing no indication of the done state.

These experiments show that the process of the rehydration can be followed easily, but no general statement can be obtained on the done
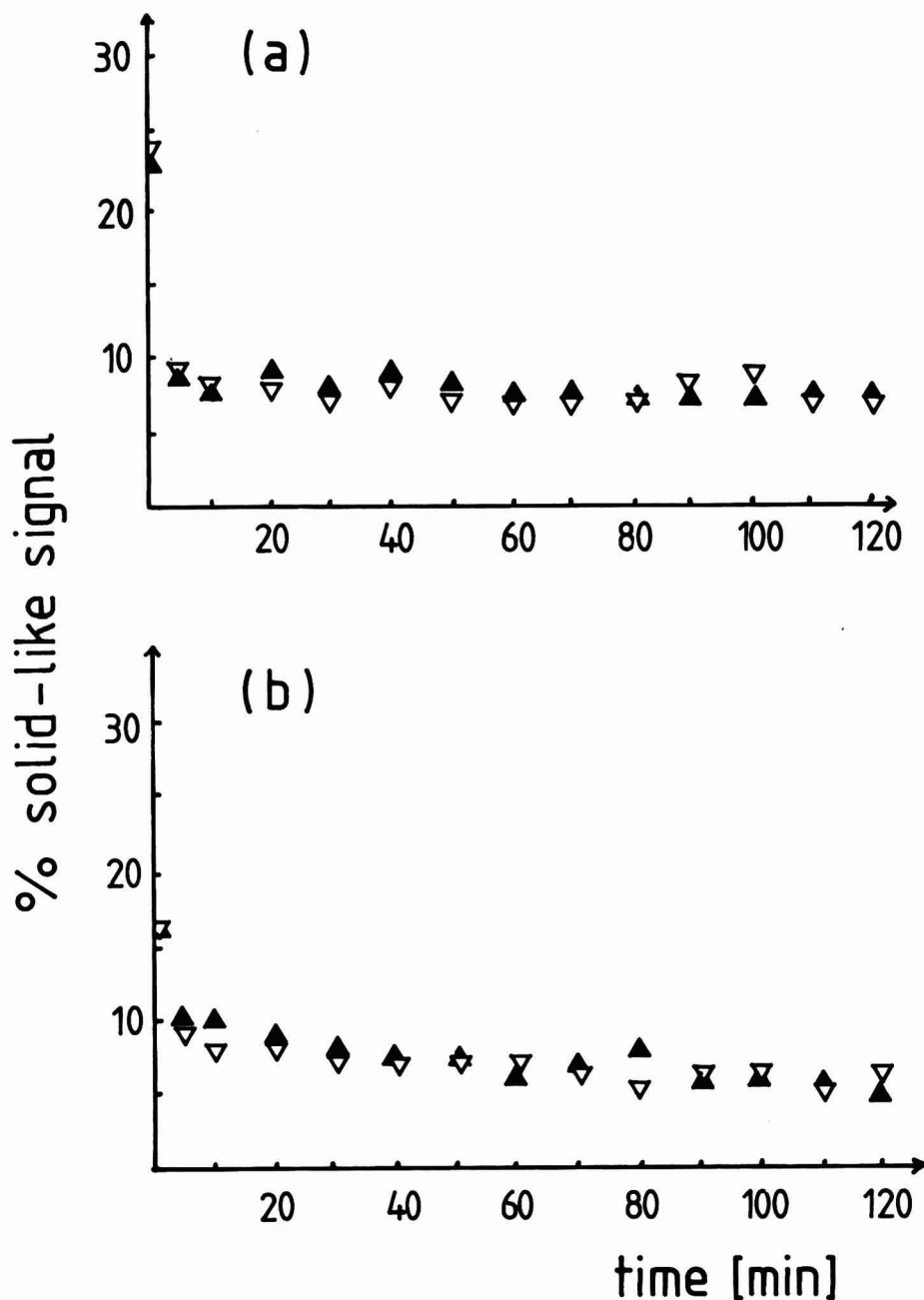
FIG. 9. DEPENDENCE OF THE AMOUNT OF SOLID-LIKE PROTONS OF a. PEAS
AND b. BEANS AFTER SWELLING IN WATER AND HEATING IN EXCESS WATER
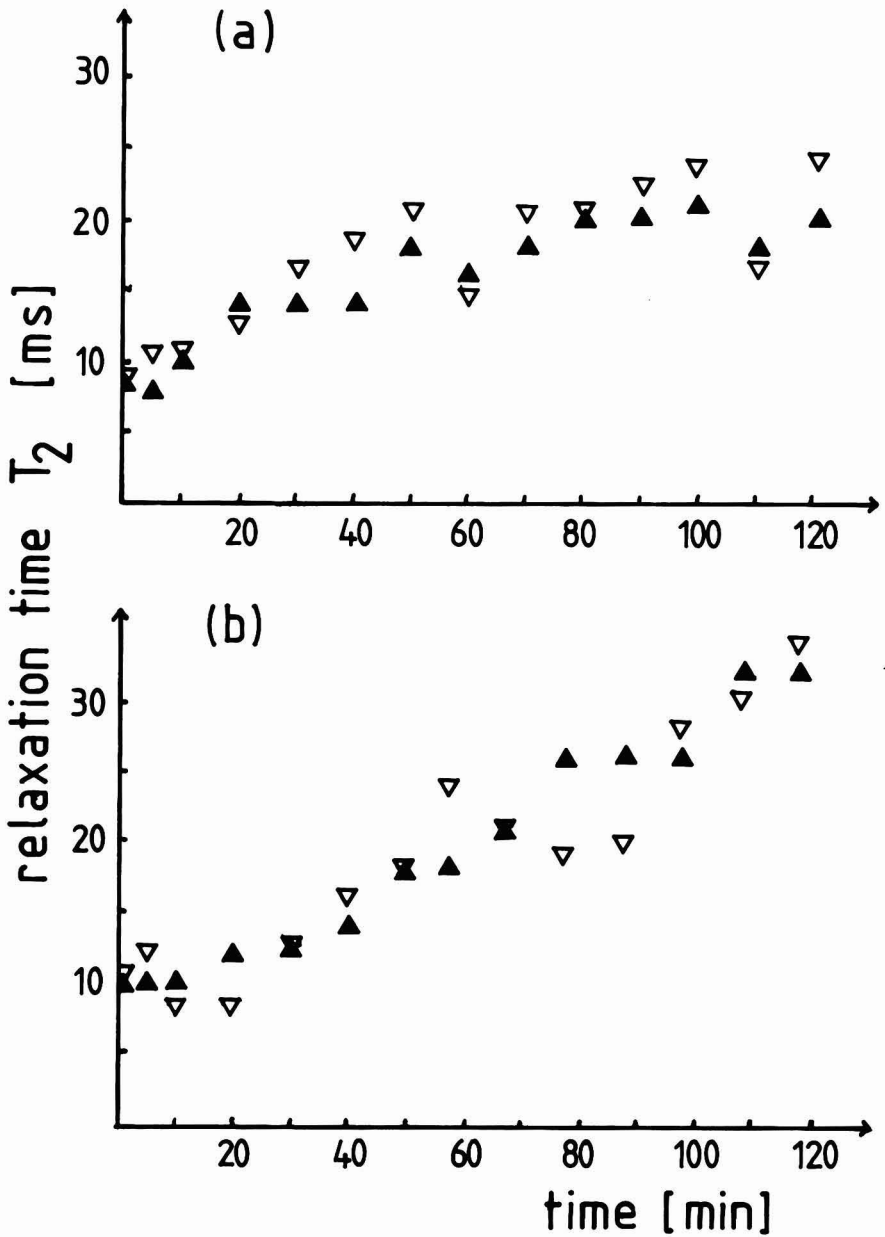TO 100°C ON THE TIME OF HEATING

FIG. 10. DEPENDENCE OF THE RELAXATION TIME T_2 OF PROTONS IN a. PEAS AND b. BEANS AFTER SWELLING IN WATER AND HEATING TO 100°C ON THE TIME OF HEATING

state of the legumes. To get information on the amount of mobile components that are not water, for instance fat, samples of beans and lentils have been evacuated and then equilibrated with D_2O vapor for some days. The result was a decrease in the corresponding T_2 of the mentioned mobile components to about 1 ms, which is about a tenth of the value obtained for the water component in the dry grains. These samples were then rehydrated in D_2O and the deuteron resonance measured. As can be seen from Fig. 11 all three samples show a weak splitting of this resonance which, bringing the samples to 90°C for 15 min, collapses.
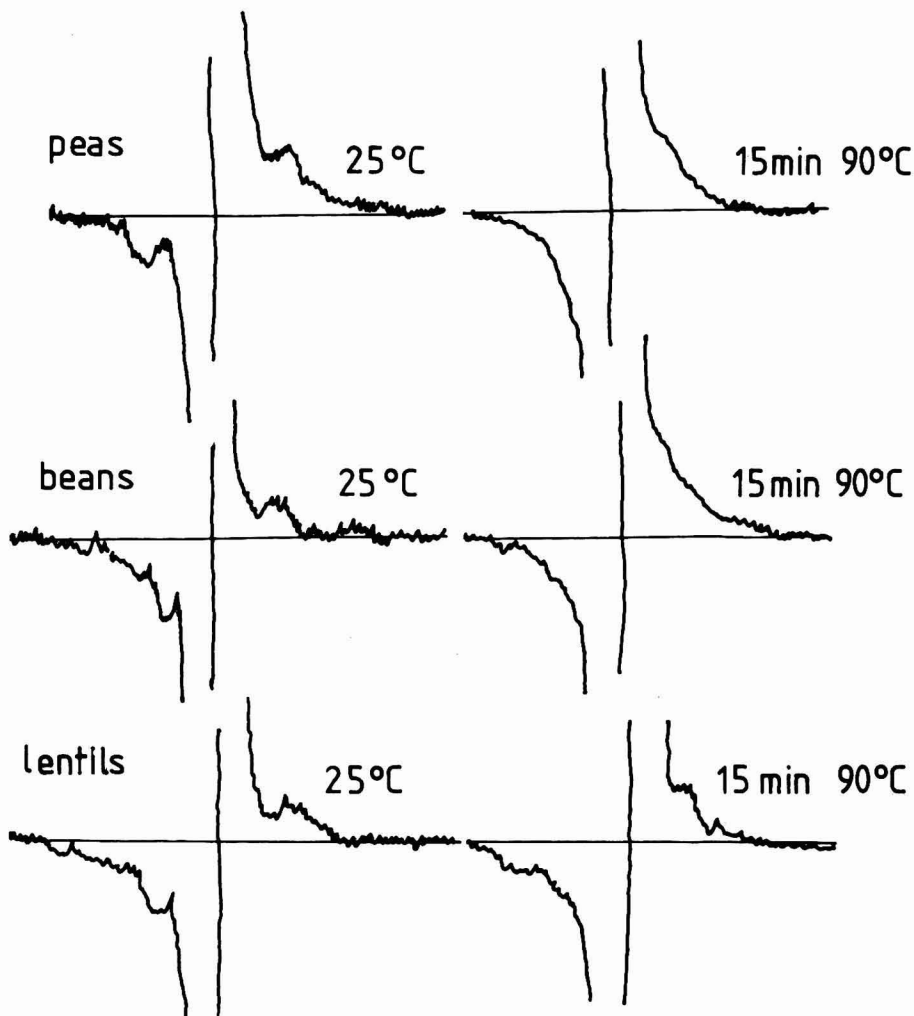
FIG. 11. DEUTERON RESONANCE SPECTRA OF PEAS AND BEANS SWOLLEN IN D_2O AND HEATED IN EXCESS D_2O TO 90°C

Finally, an example of the kind of experiment suitable for gaining information on the influence of the cooking process on cellular tissue shall be given. Figure 12 shows a self-diffusion experiment on carrot tissue according to Steijskal and Tanner (1965). In this experiment a sequence of
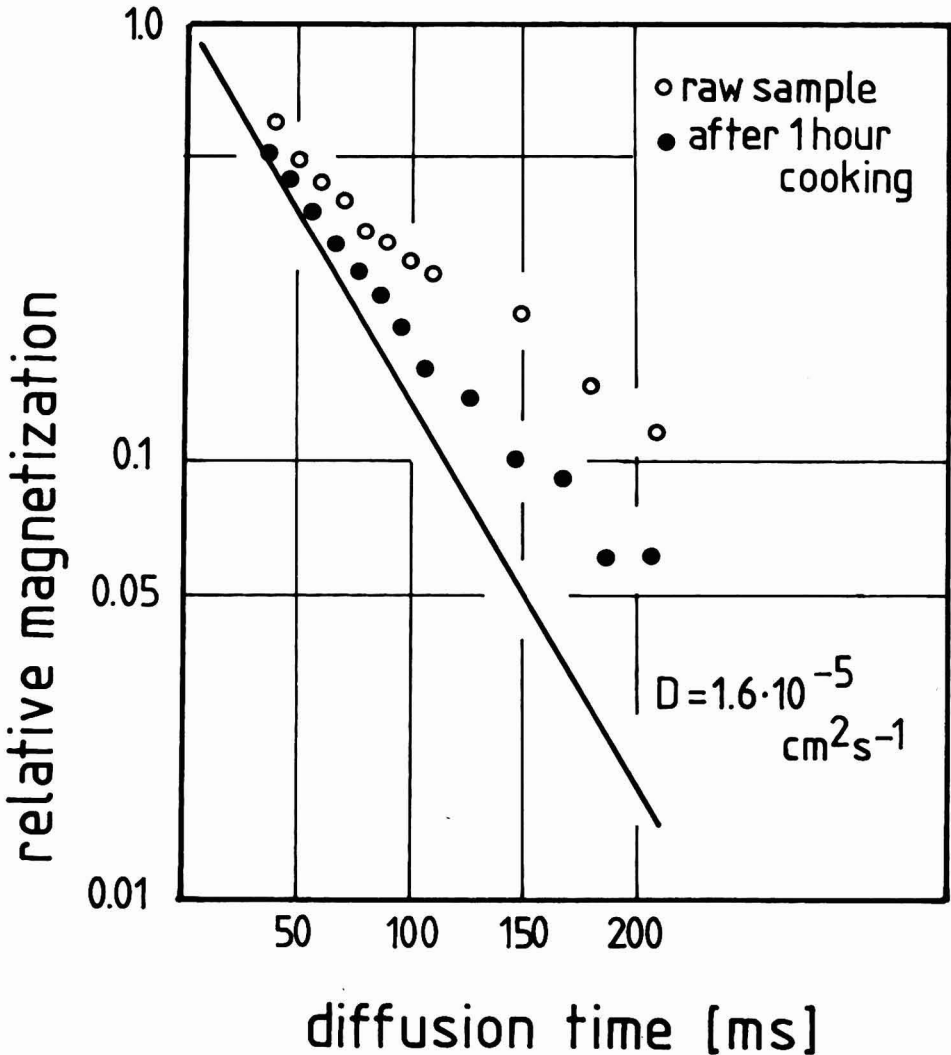

FIG. 12. DEPENDENCE OF THE MAGNETIZATION DECAY WITH THE DIFFUSION TIME IN A SELF-DIFFUSION EXPERIMENT FOR RAW CARROTS AND AFTER 1 H COOKING
The self-diffusion coefficient corresponding to the solid line has been obtained from an extrapolation of the initial slope of the measured points

pulses of high frequency and a gradient of the magnetic field is applied to the sample. These pulses are arranged so that the transverse magnetization is spread by a first field gradient pulse connecting each position in the sample with a certain phase of the processing nuclei. After that pulse, a high frequency pulse brings the fastest precessing nuclei to the end of the "fan" of spread spins and vice versa. Thus a second identical field gradient pulse should be able to restore the full magnetization. If, however, the position of the nuclei has changed meanwhile by self-diffusion, the second field gradient pulse cannot restore the full magnetization, because the nuclei are no longer in the correct position with respect to their phase at the time of the second pulse. The logarithms of the relative intensities of the magnetization with and without an applied field gradient are proportional to the time between the two field gradient pulses, allowing the self-diffusion to carry away the proper phase from its appropriate site. The slope is proportional to the self-diffusion coefficient as indicated by the solid line in Fig. 12, if the mobility is not restricted. If cell walls are present, transport of magnetization is restricted and the relative intensity remains at a larger value, as can be seen from the open circles in Fig. 12. By the cooking process the area in which self diffusion can occur is extended which may be caused by an increased diffusivity of the water molecules through the cell walls or by a damage of them.

Summarizing, it can be stated that a careful analysis of the problem to be solved by NMR-experiments and a careful examination of the questions on which different NMR-techniques can give an answer may give quite detailed information in problems of food science and technology, although the methods suffer severe restrictions in applications to complicated systems.

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WATER LOSS, THERMAL AND STRUCTURAL PROPERTIES OF DRY COOKED POTATO TUBERS

A. M. C. GALLETTI and H. T. DAVIS

Department of Chemical Engineering and Materials Science
University of Minnesota
Minneapolis, MN 55455

AND

E. A. DAVIS and J. GORDON

Department of Food Science and Nutrition
University of Minnesota
1334 Eckles Avenue
St. Paul, MN 55108

ABSTRACT

Rates of water loss and temperature profiles and concentric regional moisture contents were determined for 100 g cylindrical potato tuber samples heated at temperatures of 99°C, 143°C and 177°C. Heats of gelatinization for potato tuber were determined by differential scanning calorimetry. Structural changes were observed at various cooking times and dryness levels of heated potato tuber. It was found that the rate of moisture loss is controlled by the gelatinization of the starch which seems to occur almost simultaneously throughout the sample for this sample size. Initial water loss was also controlled by the rate at which the temperature rose to 100°C until the falling rate period began. From SEM measurement of the dry surface crust which is formed early during the heating process, it was concluded that the crust is the main thermal resistance to cooking.

INTRODUCTION

Many studies have been done on various aesthetic, nutritional, chemical and processing aspects of cooked potatoes. However, there is little literature on the dynamics of heat and mass transfer properties during dry cooking of potatoes and the intermediate or resultant structural changes that they undergo during cooking. The studies that have been

done on understanding the heat and mass transfer mechanisms operative in potato tubers have been more concerned with the complete drying of potatoes and other foodstuffs at lower temperatures (40°C to 70°C), (Gorling 1956; Saravacos and Charm 1962; Crank 1958; Van Arsdel *et al.* 1973; Chen and Johnson 1969; Fish 1956; Chiriffe 1971; Vaccarezza and Chiriffe 1972; Hollsworth 1971).

In potato, starch is the second major constituent (water being the first) and as a result plays a dominant role in the thermochemical and physical properties of cooking potatoes. Consequently significant attention has been devoted to the starch granule, its gelatinization, and its thermal and mechanical behavior either pure or in situ (French 1969; 1973; Osman 1972; Banks and Greenwood 1973, 1975; Sandstedt 1965; Leach 1965; Stevens and Elton 1971; Collison and Dickson 1971; Desai *et al.* 1973).

Potato starch granules have been examined either by light or electron microscopy in relationship to starch gelatinization of starch suspensions (Hall and Sayre 1970, 1971, 1973; Hill and Dronzek 1973; Sterling 1971). Microscopy has also been done on limited water systems such as baked products (Derby *et al.* 1975; Miller *et al.* 1973; Hoseney *et al.* 1977; Reeve 1954a,b; Sterling and Bettelheim 1955a,b,c; Barrios *et al.* 1963; Reeve 1967; Gallant and Guilbot 1971).

It was the purpose of this study to look at the interrelationship of the structural, thermal, and water loss properties of potato tuber, dry cooked in a specially constructed controlled environment oven. More specifically, water loss rates and temperature profiles were monitored continuously throughout the cooking process; moisture profiles were determined on samples cooked to various stages of doneness; differential scanning calorimetry data were collected on potato tissue and potato starch and scanning electron microscopy micrographs were obtained from raw and heated potato tuber samples.

MATERIALS AND METHODS

Potato Tuber Samples

Russet Burbank potato tubers, which are a high specific gravity variety, were used for this study.

One group of potatoes was bought from the retail market, and stored from 2 to 4 weeks at 4°C, for the part of the study dealing with water loss rates, potato temperature profiles, and localized proximate moisture profile analysis. These samples were cut into cylindrical shapes so that the length to diameter was 11.5 cm × 3.5 cm. All potato tubers were formed into cylinders along their major axis.

Another group of potatoes was bought from a farm in Anoka, Minnesota at harvest, and stored from 2 to 4 weeks at 4°C. Specific gravity was determined by weighing in air and then water by the method described by Fong and Redshaw (1973). The range of specific gravity was 1.071 to 1.087. Moisture determinations on six samples in a vacuum oven (AOAC) gave an average moisture content of 78.2% and ranged from 78.02% to 79.37%. Results agree with Treadway (1967) who found the solids contents of Western Russet Burbank potato as 22% (75% of this on a solids basis is starch). These potatoes were used for work on differential scanning calorimetry thermal analysis and SEM.

CONTROLLED ENVIRONMENT OVEN

Apparatus

Details of the design and operation of the controlled environment oven are given by Godsalve *et al.* (1977). Briefly, the purpose of the oven is to provide a uniform and controlled environment for cooking experiments. It consists of a cooking chamber, heated by electrical resistance, and a piping network that circulates air through the oven at a controlled rate and permits continuous humidity determination of the air before and after it passes through the oven chamber, thus temperature, air flow rate, and humidity are known at all times. Temperature in the oven chamber is controlled to within 2°C for oven temperatures above 100°C. At lower oven temperatures the temperature oscillates about 10°C. The potatoes baked at 99, 143 and 177°C with an air flow rate of 7.5 m³/h at ambient air.

Temperature Profiles

The rate of heat penetration in the cooking potato tubers was determined using thermocouples (Godsalve 1976) connected to a multichannel recorder. These were inserted at the centerline and ⅓ and ⅔ of the way from the surface of the sample.

Temperature profiles inside the samples were monitored during the cooking process when potato cylinders were baked at 99, 143 and 177°C with an air flow rate of 7.5 m ³/h.

Water Loss Rates

Water loss rates were measured gravimetrically. For these measure-

ments, the potato tuber cylinders, which sit on a small circular teflon disc, were placed on a cradle made of wire mesh that was attached, through an opening at the top of the heating chamber, to an aluminum frame hanging from a balance ($\pm$ 0.1 g). Readings were taken at 1 min intervals.

Determination of Radial Moisture Profile Analysis

Moisture analyses were made for separate potato cylinders heated for 45, 60 and 90 min at 177°C and air flow rate of 7.5 m³/h. At each time period, the cylinder was removed and immediately cut, perpendicularly to the axis, to form three cylindrical samples. The center sample was used for determination of the radial moisture profile, i.e., of the moisture content as a function of distance from the center of the sample.

Four concentric sections of the sample were cut each radially equidistant from one another and separately frozen in liquid nitrogen in order to avoid water loss and migration. Moisture content was determined in a vacuum oven at 30°C and 28 mm Hg until constant weight was attained.

Scanning Electron Microscopy (SEM)

Five potato cylinders (3:1 ratio) were heated in the environmental oven at a temperature of 177°C and an air flow rate of 7.5 m³/h for 15, 30, 45, 60 and 90 min, respectively. After each heating period they were cut into three sections perpendicular to the major axis (Fig. 1a). The middle of these 3 sections was used for SEM preparation and subsequent viewing. This middle section was sampled from the outer surface (crust) to the center or near the center of the section in regular intervals (Fig. 1b).

For the study on the changes that the potato cells located near the center of the wedge undergo, two methods of sample preparation for SEM were used (Davis and Gordon 1976). One was to cryofracture the sample prior to fixation and the other to mechanically excise the sample with a razor blade prior to fixation. After this initial preparation, the samples underwent glutaraldehyde-sodium cacodylate fixation, osmium tetroxide-sucrose fixation, acetone dehydration, critical point drying with acetone-carbon dioxide, mounting on aluminum stubs, and coating with gold-palladium in a vacuum evaporator. Scanning electron micrographs were taken on the Cambridge Stereoscan 600 operated at 15 Kv.

For the crust studies, SEM samples were obtained by razor sectioning the outer area of the cooked potato as shown in Fig. 1. A disk was cut from a cooked cylinder. Small slices of the outer face of the crust and of the

middle
section
used ⟶
for SEM
sampling

major
axis

(a)

crust
outer - - —
edge

(b)

Series of wedges from crust
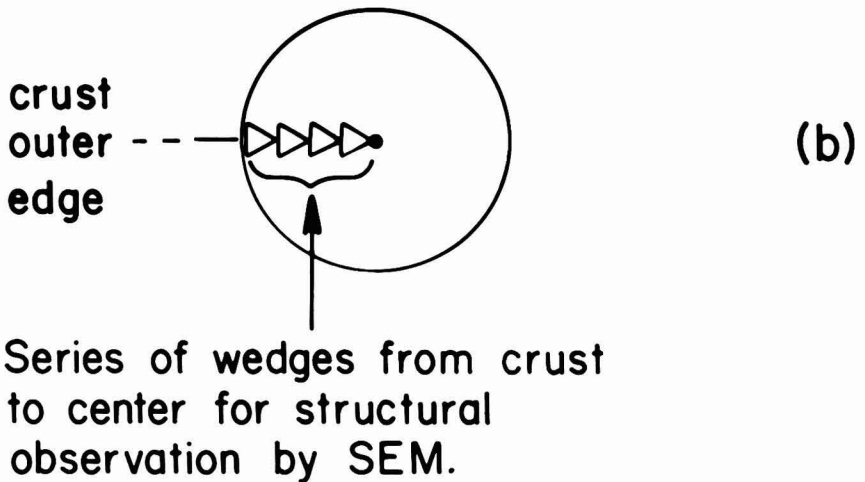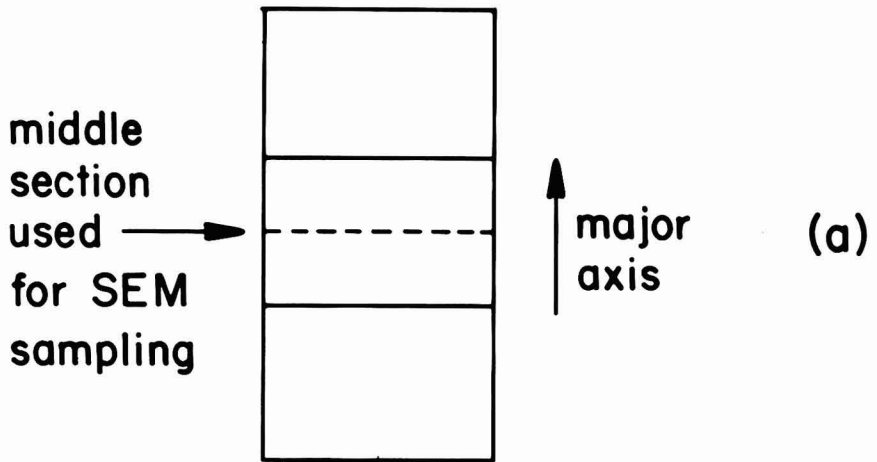to center for structural
observation by SEM.

FIG. 1. SAMPLING AREAS FOR USES IN THE SEM STUDY

cross section of the crust region were mounted on SEM stubs for observations. The cross section slices were obtained by slicing the top of a small wedge such as that shown in Fig. 1. The samples were subsequently fixed as described above prior to viewing.

Differential Scanning Calorimetry (DSC) Thermal Analysis

The thermal analysis was carried out in a Perkin-Elmer DC 2 Differential Scanning Calorimeter. The pans and lids were weighed on a microanalytical balance. The samples were cut into small pieces with a razor blade and placed in the pans, then weighed and sealed. The inert reference used consisted of aluminum sealed in a sample pan with a total weight of 36.5 mg.

The DSC cell was cooled to 3°C by means of liquid nitrogen for the baseline, which was well in advance of the endothermic peak. The chart speed was 20 mm/min and the range of the chart 5 mVolt.

The heat of gelatinization per gram of starch in the potato sample was calculated from:

$$\Delta H = \frac{KA}{W_s}$$

where A is the area under the curve, K is the cell constant determined from previous calibration using fusion of lead, tin and zinc, and W_s is the weight of starch present in the sample used. We assumed that the tubers contained 16.3% starch by weight based on Treadway's data of starch being 75% of the solids content of Russet Burbank potato tubers (1967). Sampling for DSC experiments was from the parenchyma tissue of potato tubers.

The first experiment was on samples taken from different tubers but from approximately the same parenchyma region in each tuber. The rate of heating and all other parameters of the calorimeter were held constant. From this experiment, it was hoped to obtain an average of the heat of gelatinization of the potato starch inside the cells in the tubers for this type of potato, and to correlate this value with those of 15 and 20% potato starch suspensions that were determined similarly.

In the second set of experiments, the rates of heating were varied. Samples from the parenchyma tissue from the same tuber were used. The interest was in observing how the heating rate affected the range in temperature over which the gelatinization occurred.

EXPERIMENTAL RESULTS

Water Movement and Temperature Profiles

Figure 2 presents a comparison of water loss rates for particular samples cooked at the different oven temperatures, 99°C, 143°C and 177°C. These data are representative curves taken for single samples (3.5 cm diameter × 11.5 cm length) and, consequently, have considerable oscillation due to balance fluctuations. Figure 3 shows similar results for four samples baked simultaneously at 177°C. It can be seen that the oscillations seen in Fig. 2 are smoothed out. For purposes of discussion, the representative single sample curve in Fig. 2 at 177°C was used which corresponds to the temperature profile in Fig. 4 for 177°C.

From Fig. 2 and 4 it can be seen that the water loss rate first increases to a local maximum within the first 16 min of cooking, followed by a local minimum equal to about two-thirds of the first maximum value. In the
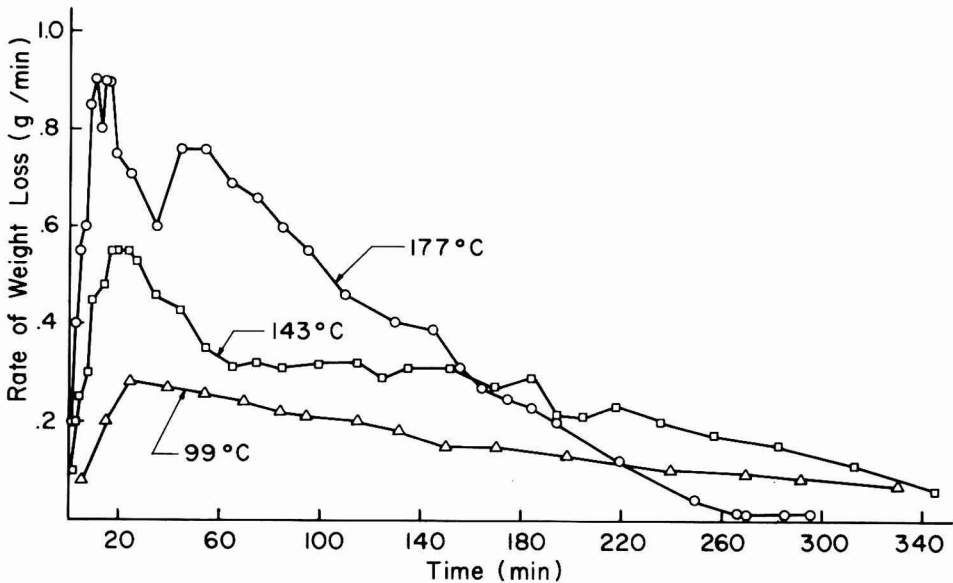
FIG. 2. WATER LOSS RATE TIME PLOTS FOR SAMPLES COOKING OVEN 177°C, 143°C AND 99°C
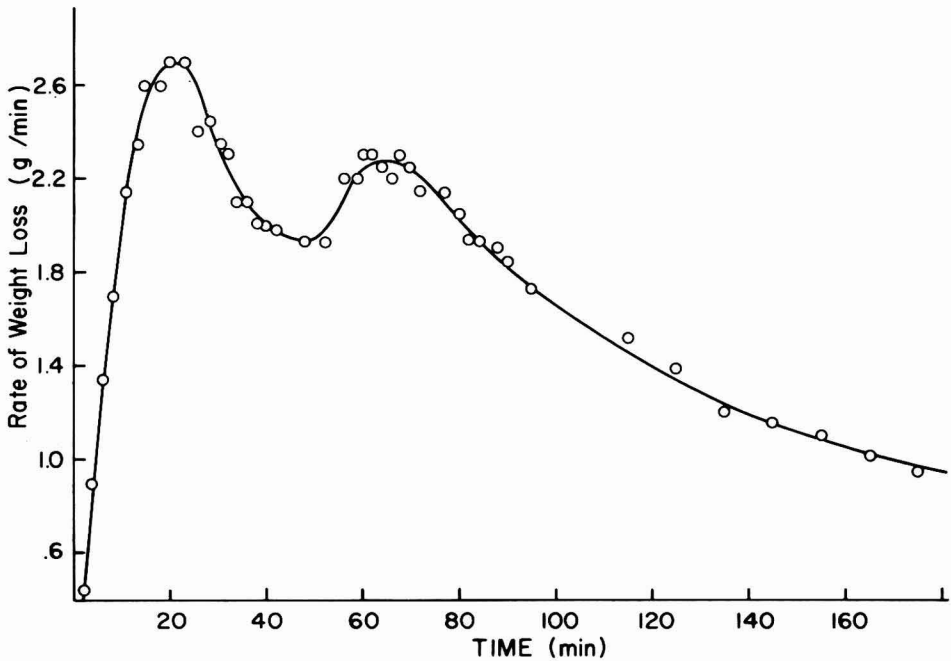
FIG. 3. WATER LOSS RATE TIME PLOT OF FOUR CYLINDRICAL POTATO TUBER SAMPLES COOKED SIMULTANEOUSLY IN A 177°C OVEN

time period between the first maximum and minimum values (16–35 min) the temperature-time curve shown in Fig. 4 passes through an inflection point. The inflection point occurs when the temperature reaches approximately 60°C, indicating the existence of a heat sink in the sample. The gelatinization reaction provides such a heat sink. DSC studies on potatoes of the type studied here demonstrate that the onset of gelatinization occurs in the temperature range of 57 to 65°C as will be presented later. Thus, the rate of moisture loss increases as the temperature of the sample increases since more energy for evaporation is provided. At the onset of gelatinization water begins to be tied up in the gelatinization reaction with a subsequent decrease in the water loss rate. When gelatinization ends, the water loss rate again begins to increase between 35 to 48 min at 177°C as seen in Fig. 2. A short constant rate period similar to that observed in dry-cooked meat (Godsalve et al. 1977) occurs as is more obvious in Fig. 3. Finally, a falling rate period follows similar to those observed in drying of solid type foods in an air tunnel dryer.

Figure 4 also shows that except for the surface temperature, all the internal thermocouples register approximately the same temperature until the interior of the sample reaches 100°C with the surface crust
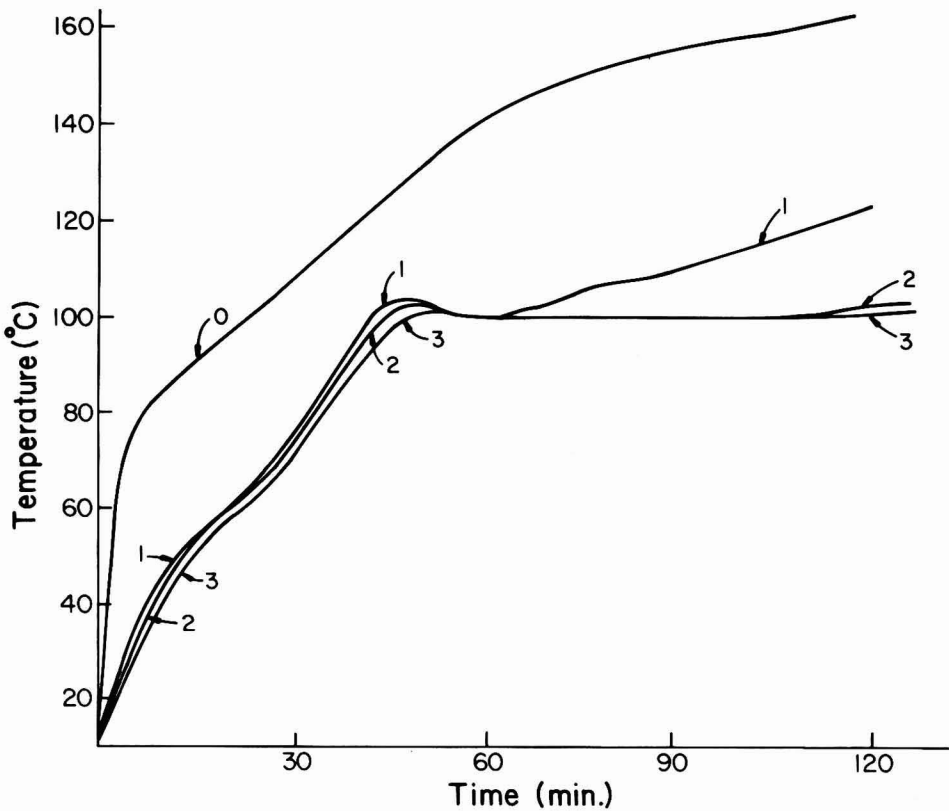
FIG. 4. TEMPERATURE PROFILE-TIME PLOTS FOR POTATO TUBER SAMPLES COOKED AT 177°C OVEN TEMPERATURE

temperature significantly higher throughout the practical cooking period. As the interior of the sample reaches 100°C a slight overheating takes place until the interior reaches 102°C causing a build-up of pressure inside the sample. The release of this pressure by blowing vapor passages through the crust is probably responsible for the upward surge in moisture loss rate seen in Fig. 2 at about 35 to 48 min with the corresponding relaxation of the interior temperatures (Fig. 4) back to 100°C.

During the falling rate period, the temperature behavior shown in Fig. 4 can be interpreted in terms of a moving evaporation front which travels radially towards the center. Interior to the moving front the temperature remains at 100°C, whereas exterior to the front the temperature is greater than 100°C. After the evaporation front begins to move through the sample, a cross-like hole begins to form as is shown in Fig. 5 for a
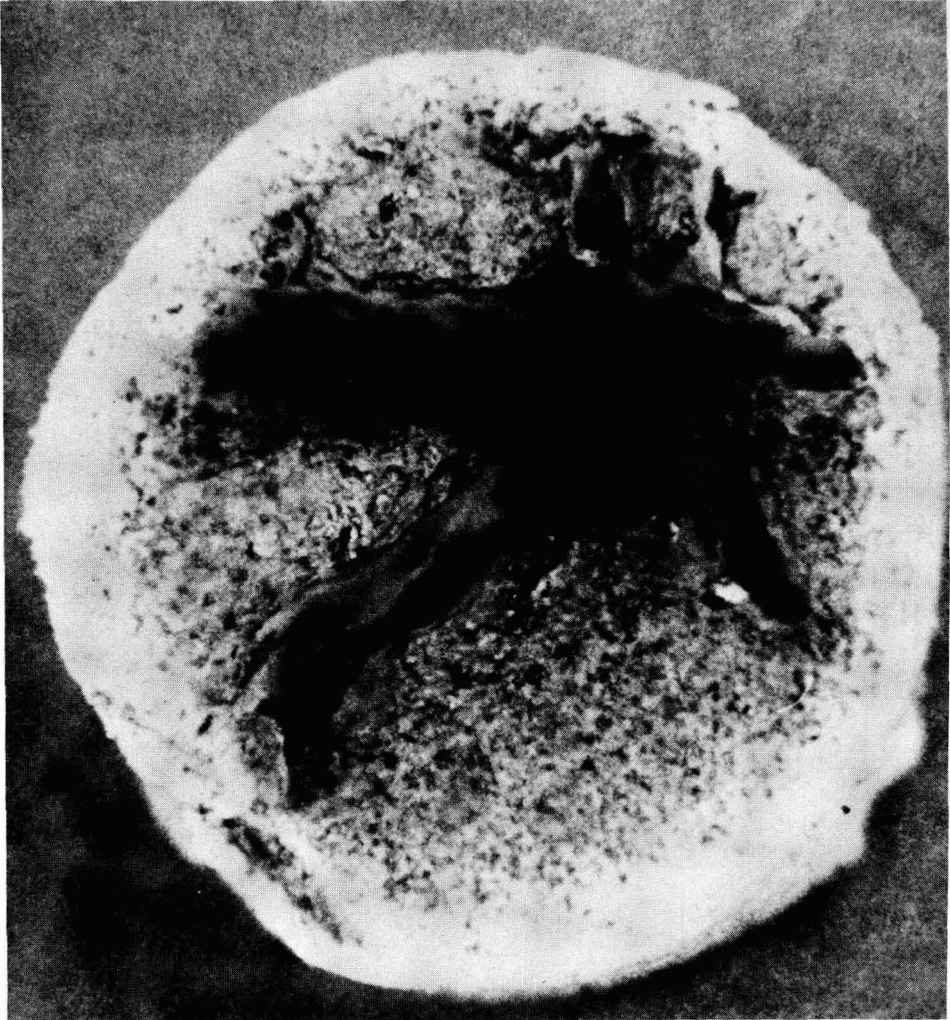
FIG. 5. CROSS SECTION OF POTATO TUBER SAMPLE AFTER 90 MIN OF COOKING
TIME

sample taken at 90 min of cooking. This hole is responsible for causing thermocouples placed in similar positions to read differently after 150 min of heating (not shown in Fig. 4).

For the sample cooked at 143°C, the moisture loss rate increases to a local maximum or short constant rate period at 20 min and then decreases to a local minimum at 65 min as the interior temperature passes through the gelatinization range. This is then followed by an almost constant rate period which lasts until the crust shows a temperature

above 100°C, at which time the falling rate period begins. In general thermocouples tended to show the same temperatures in all positions until the 100°C front began to penetrate the sample. The moving front passes the thermocouples in the order of the position of the thermocouples from the center of the sample. The center thermocouple takes about 6.5 h to reach 100°C and the sample reaches oven temperature about 7.5 h.

The curve in Fig. 2 for samples cooked at 99°C emphasizes the low rate of moisture loss even though a maximum moisture loss rate is reached at 25 to 30 min which is where a surface temperature began an inflection due to the gelatinization temperature having been reached. The basic characteristics of the moisture loss rate are similar to the other oven temperature runs except that the transitions took longer to observe since the heat transfer rate is low due to the lower oven temperature.

Figure 6 shows the rate of water loss as a function of dry basis moisture content for the three oven temperatures. In the case of the 177°C oven,
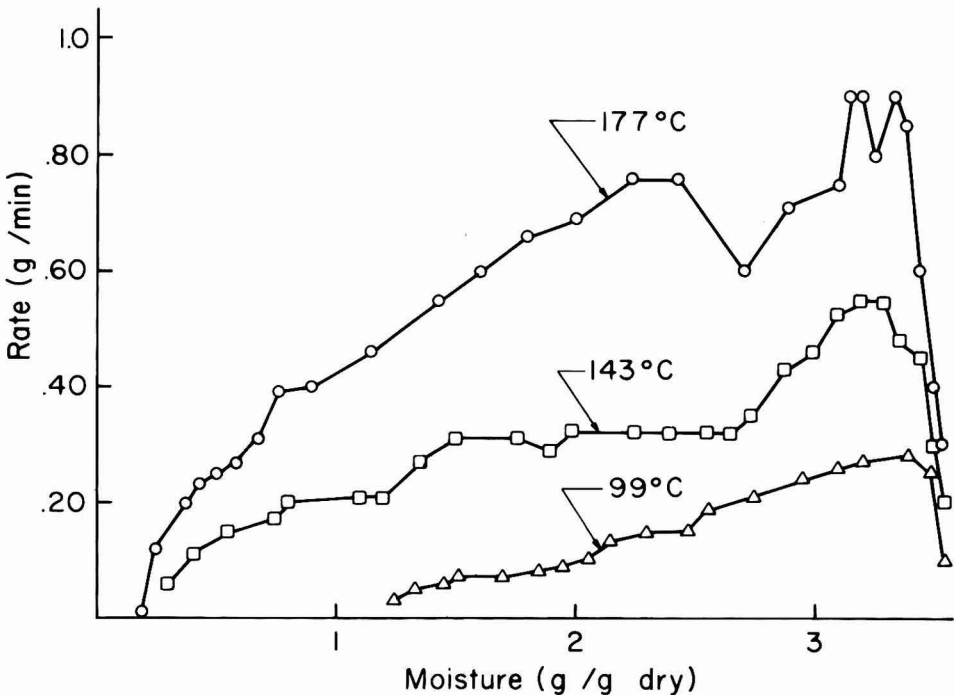
FIG. 6. RATE OF WATER LOSS AS A FUNCTION OF MOISTURE CONTENT IN THE SAMPLE PER GRAM OF DRY SOLID FOR VARIOUS OVEN TEMPERATURE USED

the drying rate part of the curve is typical of the drying rate curve for a porous hygroscopic solid (Godsalve et al. 1977). At lower oven temperatures such as 99°C where the internal temperature does not reach 100°C prior to the falling rate period, it does not exhibit this typical drying behavior. Overall, it appears from Fig. 6 that there is no single constantly falling rate period but successively different falling rate periods. These stages are probably due to the structural and moisture-distribution changes that occur inside the sample as it is being dried at these temperatures.

In order to see if moisture-distribution changes significantly from the center of the cylinder to the outer sections in concentric rings as the sample enters the falling rate period of the heating time, moisture was determined in four radial concentric sections mentioned earlier and compared to temperatures observed.

Assuming the existence of a dry crust region forming the principal thermal resistance to heat transfer from the sample surface into the interior potato sample, we can estimate the radial thickness, Δ, of the crust. At 60 min, the interior of the sample giving the data of Fig. 4 is at 100°C. Thus, all the heat transferred across the crust region is used to evaporate water. Since the temperature remains at 100°C, the pressure at the inside of the crust remains constant. This implies the vapor transport across the crust region is limited by the rate of evaporation. Thus, assuming quasi-steady state heat transfer across the crust region, we have the energy balance.

$$Ak \; \frac{T_s - T_i}{\Delta} \;=\; \Delta H_v \frac{dw}{dt},$$

where A is the area of the sample, k the thermal conductivity of the crust, T_s the temperature of the outside of the crust, T_i the temperature on the inside wall of the crust (which at 60 min is at 100°C), ΔH_v the heat of evaporation of water, and dw/dt the rate of moisture loss of the sample. At 60 min, we can find from Fig. 4 that T_s = 135°C and from Fig. 6 dw/dt = 0.75 gm/min. We have no value for k, but to get a rough estimate of Δ we can take for k the value characteristic of freeze dried apples which is 0.0062 cal/cm min°C, then, with A = 126 cm² and ΔH_v = 539 cal/gm, we estimate at 60 min the crust thickness Δ = 676 μm, a value quite close to the 400 μm region identified at the electron microscopy section as a dry, non-gelatinized crust (discussed in next section).

There is of course thermal resistance between the hot air and the sample surface. The quasi-steady state energy balance between the hot air and the sample

$$A \; h(T_o - T_s) \;=\; \Delta H_v \frac{dw}{dt}$$

where t_o is the oven temperature and h is the air-sample heat transfer coefficient. Again considering the sample corresponding to Fig. 4 at 60 min and using T_o = 177°C and the values of the other quantities already stated, we estimate h = 0.076 cal/cm² min°C. Since at 60 min we estimated k/Δ = 0.064 cal/cm² min°C, we conclude that the thermal resistances of the crust and the air-sample interface contribute about equally to the overall thermal resistance to heat transfer from the oven into the potato sample.

Moisture profiles were measured only at the oven temperature of 177°C and an air flow rate of 7.5 m³/h. The heating times for the profiles were 45, 60 and 90 min. Since the moisture of the samples were different it was not possible to relate the moisture loss of the samples and determine rates for each section, since they would depend on the initial moisture content.

Region 1 includes a substantial amount of interior material with the crust so that the dryness of the crust will not be apparent in the moisture profiles. Table 1 is a summary of the relative amount of moisture present in each of the 4 regions. Table 1 gives an estimate of how the moisture drops according to the position in the sample in relation to the moisture of the center for the three times considered. It can be that the relation between the region closest to the center (3) and the center (4) remains fairly constant, the relation between region 2 and the center appears to be constant at 45 and 60 min but decreases significantly at 90 min.

Scanning Electron Microscopy

The first study, reported in the micrographs in Fig. 7–12, was of the changes of the potato cells in the interior of the sample during cooking at 177°C. The second study, presented in the micrographs in Fig. 13–16, was of the evolution of the crust region near the sample surface during the same cooking process.

Table 1. Relative amount of moisture present in the four concentric regions in relation to Region 1

Time (min)	Relative Moisture in Region[a]			
	1	2	3	4
45	1	2.05	2.34	2.49
60	1	2.82	3.31	3.47
90	1	6.97	9.36	10.20

[a]Region 1 is the outermost region and region 4 is the innermost region.
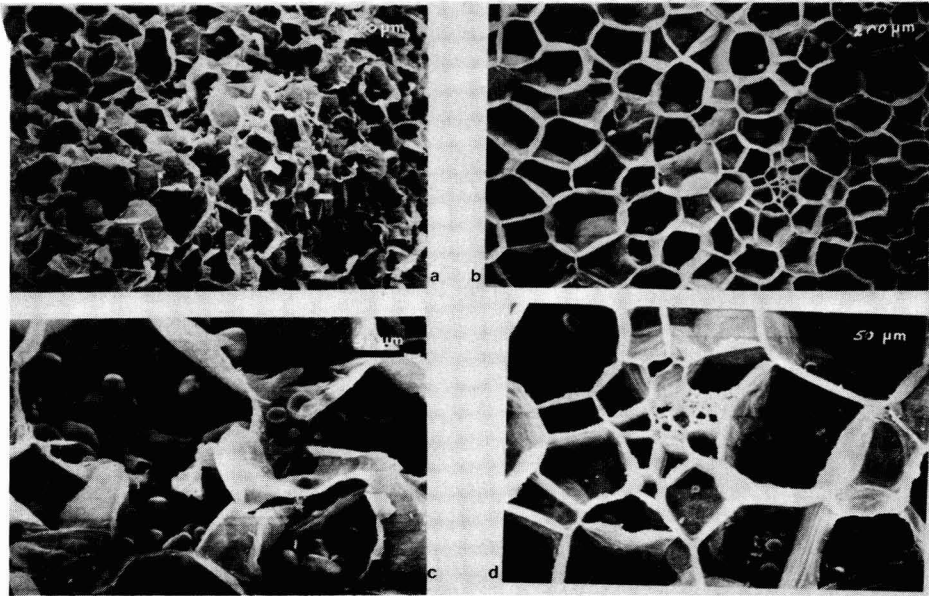
FIG. 7. SEM MICROGRAPHS: POTATO TUBER, RAW

a) Cryofractured
b) Excised
c) Detail of cryofractured area
d) Detail of starch granules

In Fig. 7 micrographs of raw potato cells are shown for samples pre-
pared by excision and cryofracture. More of the starch granules appear to
be lost from cells with interiors exposed by excision than by cryofracture.
After only 15 min of baking at 177°C, the cells and starch granules are
relatively unaffected as illustrated in Fig. 8.

By 30 min, however, as seen in Fig. 9, substantial transformation of the
starch granules has occurred which is as was noted from the drying and
temperature curves.

At 45 min, (Fig. 10), the granules can no longer be individually dis-
tinguished. A structureless mass of presumably gelatinized starch now
occupies much of the volume of a cell.

Between 45 and 60 min, the moisture loss rate increases rapidly and
the interior of the sample rises rapidly to 100°C with some overshoot to
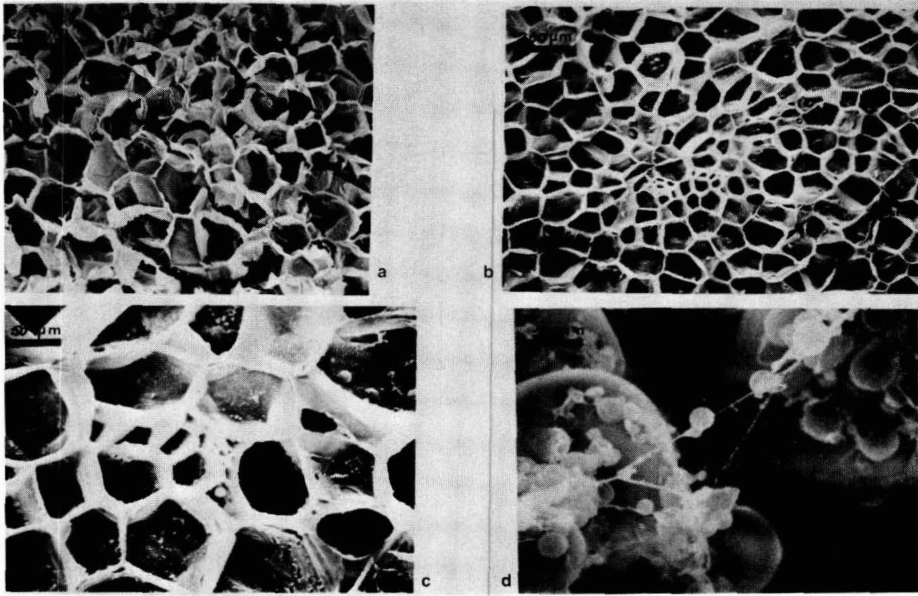
FIG. 8. SEM MICROGRAPHS; POTATO TUBER, COOKED 15 MIN

a) Cryofractured
b) Excised
c) Detail of excised cell
d) Detail of starch granules

about 102°C. At 60 min (Fig. 11b) the contents of the cells have much the same appearance as they had at 45 min. However, in the excision method of preparation (Fig. 11a) the contents of the cells are not exposed since the razor blade, instead of cutting through the cells, runs on the outside and between the cells, which now have the appearance of independent pods. The evaporation front has not at this time penetrated the interior of the sample, so the *in situ* pods must be filled with water at or slightly above 100°C. The potatoes are fully cooked at this point.

As shown in Fig. 12, at 90 min, in the falling rate period, the cells pods no longer have the fullness observed at 60 min. Excision still leaves the cells intact. When exposed by cryofracture, the cell contents appear to have become more structureless than at 45 min.

If cooked to dryness, the cell walls of the pods shown in Fig. 12b shrink down to take on the shape of the gelatinized starch masses inside the pods.
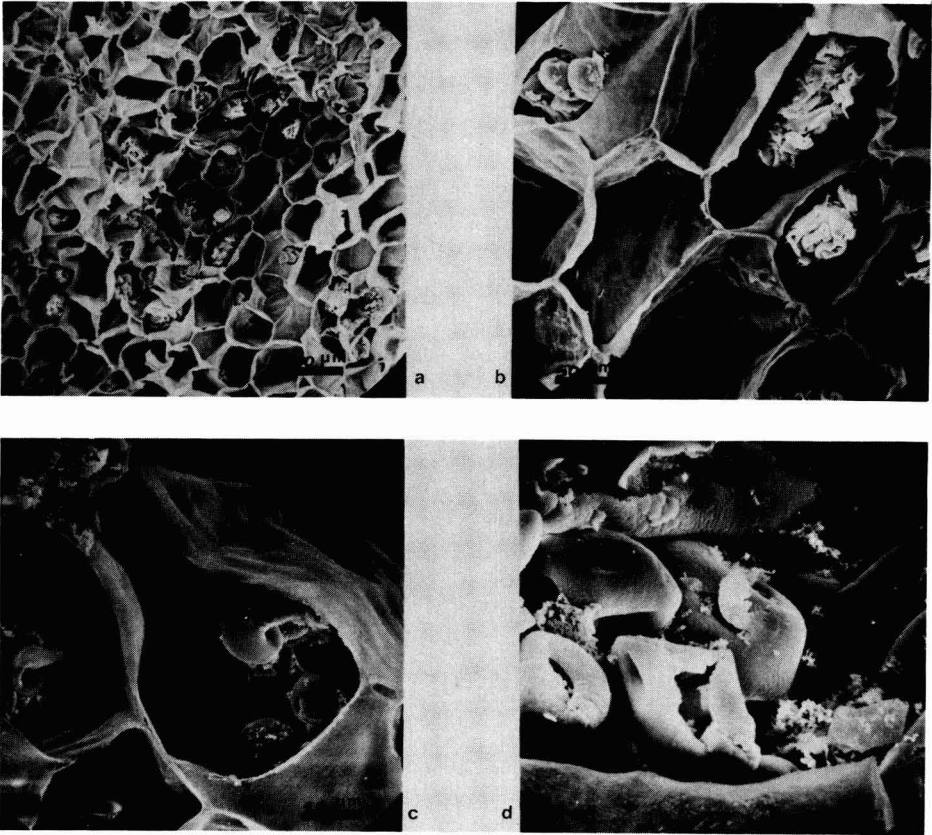
FIG. 9. SEM MICROGRAPHS; POTATO TUBER, COOKED 30 MIN

a) Cryofractured
b) Cryofractured cell detail
c) Starch in cells
d) Detail of starch granules

Micrographs of the outer face and crust cross-section are shown in Fig. 15 for a sample cooked 15 min at 177°C. There is no apparent difference between these micrographs and those of raw potato.

Figure 16 shows, however, that by 30 min of cooking substantial changes have occurred in the crust region. Starch in the outer face of the crust shows no signs of gelatinization. From the cross-section picture this
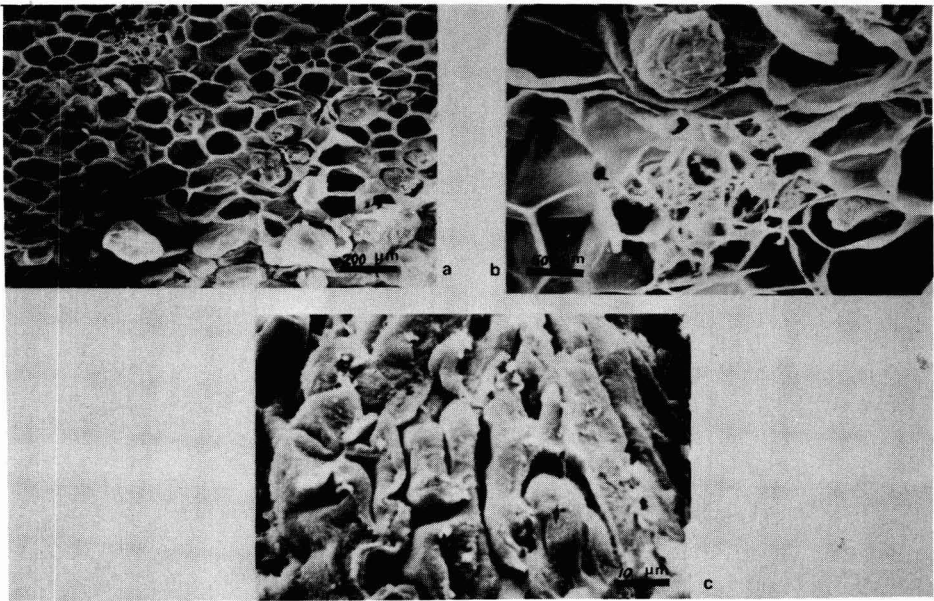
FIG. 10. SEM MICROGRAPHS; POTATO TUBER, COOKED 45 MIN

a) Cryofractured sample
b) Cell detail
c) Detail of starch granules
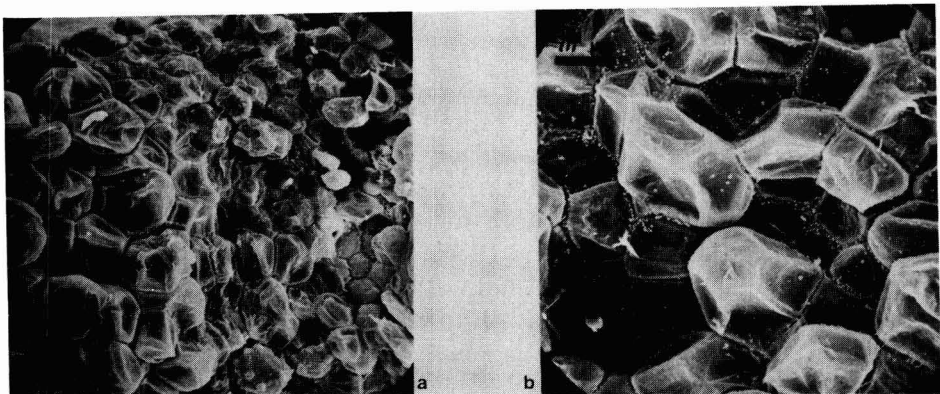
FIG. 11. SEM MICROGRAPHS; POTATO TUBER, COOKED 60 MIN

a) Excised
b) Detail of whole cells
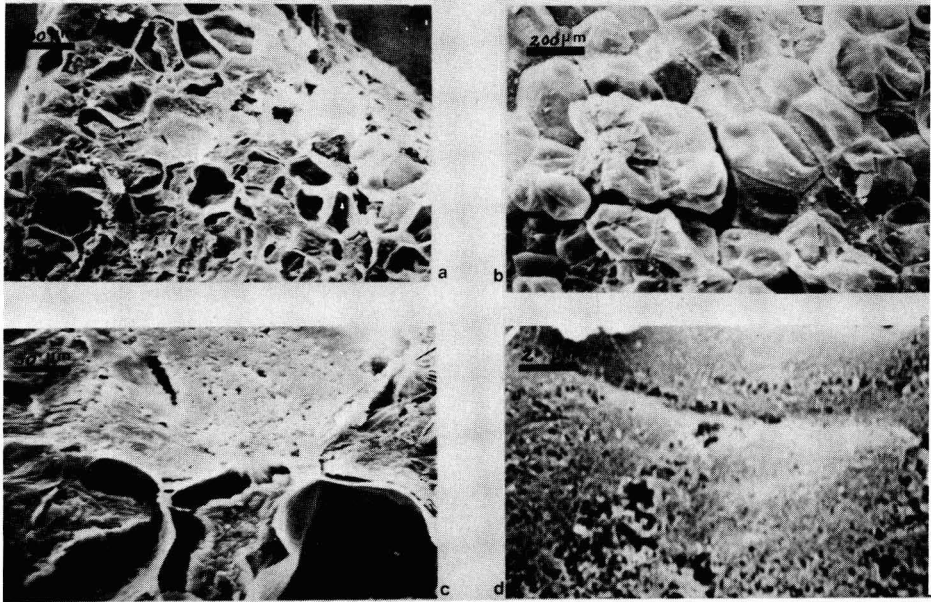
FIG. 12. SEM MICROGRAPHS; POTATO TUBER, COOKED 90 MIN

a) Cryofractured
b) Excised
c) Detail of starch in cells
d) Detail of starch

ungelatinized region which we shall call the *outer crust* appears to be about 400 μ thick. Behind this region is another region about 400 μ thick which appears fully gelatinized. We shall call this gelatinized region the *inner crust*. Behind the inner crust the structure is quite similar to that observed at 30 min in the far interior of the sample.

The micrographs in Fig. 15 and 16 show that at 60 min, the outer crust is still an ungelatinized region about 400 μ thick, the inner crust still a highly gelatinized region 300 or 400 μ thick, and the region behind the inner crust still similar to (but somewhat more dried) the far interior of the sample.

The electron micrographs provide pictorial evidence supporting the hypothesized existence of a case-hardened, dry outer layer formed during oven cooking (Van Arsdel *et al.* 1973). This layer combined with the external heat transfer resistance constitutes the principal thermal resistance in the cooking potato. From the lack of gelatinization of the starch
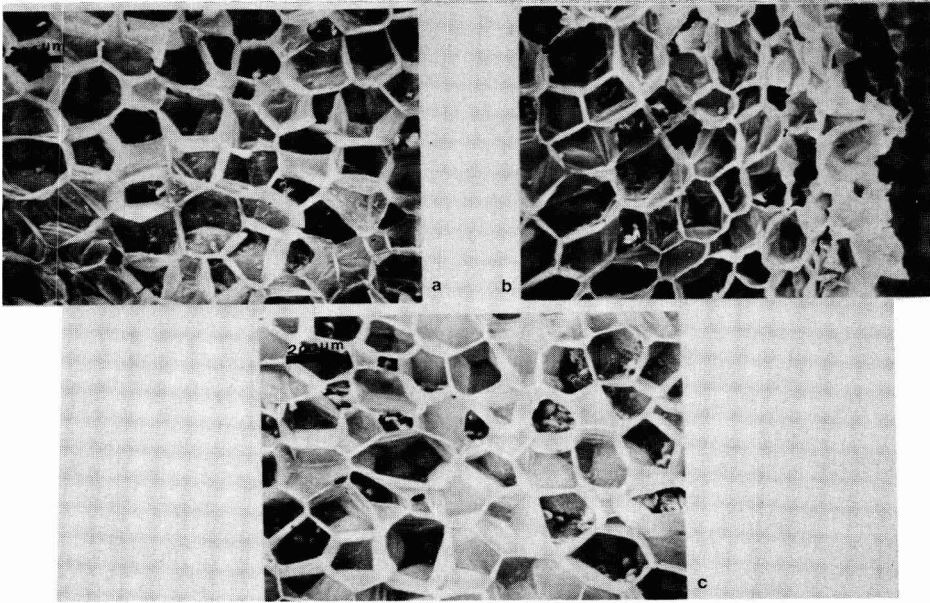
FIG. 13. SEM MICROGRAPHS; POTATO TUBER, COOKED 15 MIN

a) Outer face
b) Cross section
c) Inner face

in the outer crust, we conclude that surface evaporation dries this region before gelatinization can take place. Thus strong case hardening is indicated. As the temperature of the crust continues to increase, the compact inner crust gelatinizes and dries. Using the thermal conductivity of air dried apple, we estimated previously that the crust would have to be 676 μ thick to explain the observed temperature profiles in the sample. Given the uncertainties of the heat transfer estimate (the surface temperature may not be uniform, the measured value may be inaccurate, quasi-steady state may not be established), it agrees satisfactorily with the observed thickness of 400 μ for the outer crust. That the outer crust is somewhat thinner than that estimated from the temperature profiles is to be expected since the dry inner crust will also provide thermal resistance greater than that of the wet interior of the sample.
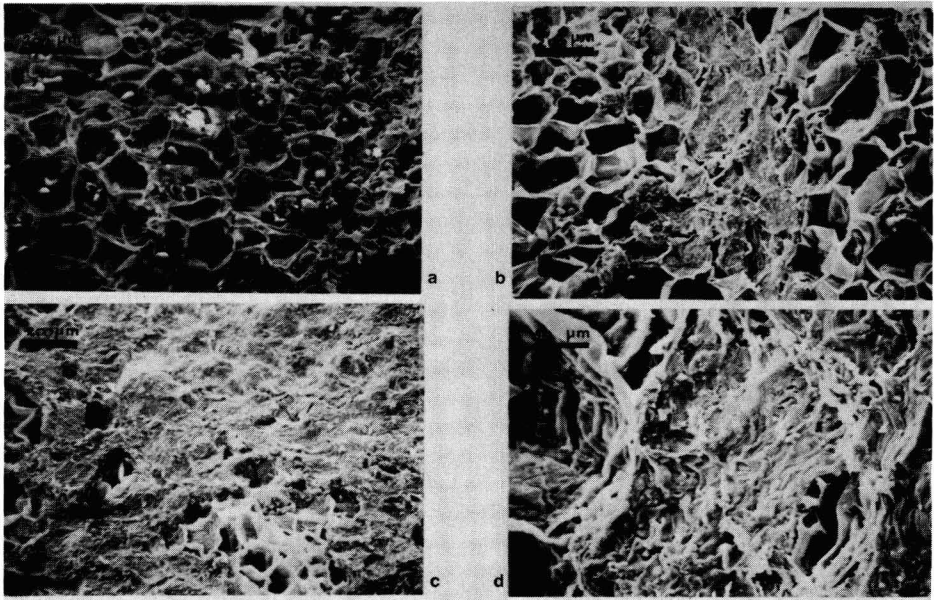
FIG. 14. SEM MICROGRAPHS; POTATO TUBER, COOKED 30 MIN

a) Outer face
b) Cross section
c) Inner face
d) Detail of compact inner crust

DSC

Table 2 contains the values of ΔH for several DSC runs at the same rate of heating each time. The total heat of gelatinization (ΔH_r), the initial temperature (T_i) at the onset of the endothermic peak, the temperature range of the endothermic peak (ΔT), and the weight of the sample (W) are all given in this table. The observed scatter of the ΔH values is probably due to sampling being from different potatoes whose age and starch contents were slightly different each time.

The average value of ΔH determined from the entries in Table 2 is 4.00 cal/g of starch. These ΔH values were compared to those for aqueous commercial potato starch suspensions of 15 and 20% concentrations which were 4.86 cal/g starch and 4.22 cal/g starch, respectively. The

Table 2. Gelatinization values for starch in the potato tuber.

ΔH (cal/g)	ΔH_T (cal $\times$ 10^{-3})	T_i (^o_C)	ΔT (^o_C)	W (mg)	Rate (deg/min)
4.20	10.05	60	10	14.7	5
3.68	6.24	66	8	10.4	5
3.66	6.48	66	6	10.8	5
4.14	8.51	65	10	12.6	5
4.28	7.04	62	10	10.1	5
4.43	12.51	62	11.5	17.3	5
3.64	6.95	61.5	12.5	11.7	5

ΔH average = 4.00

ΔH : heat of gelatinization

ΔH_T: total heat of gelatinization contributed by starch in sample

T_i : temperature onset of endothermic peak

ΔT :temperature range over which gelatinization occurs
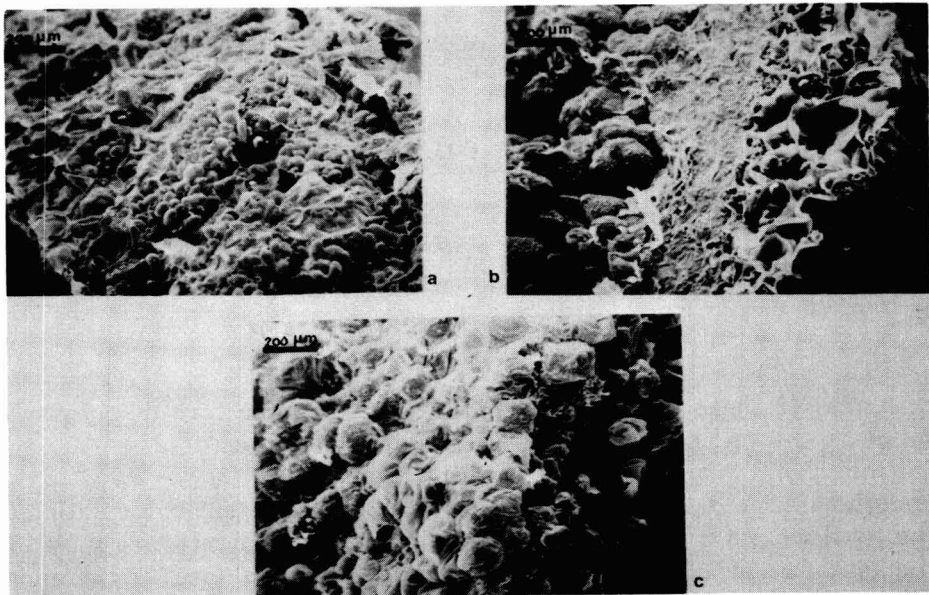
FIG. 15. SEM MICROGRAPHS; POTATO TUBER, COOKED 60MIN

a) Outer face

b) Cross section

c) Inner face
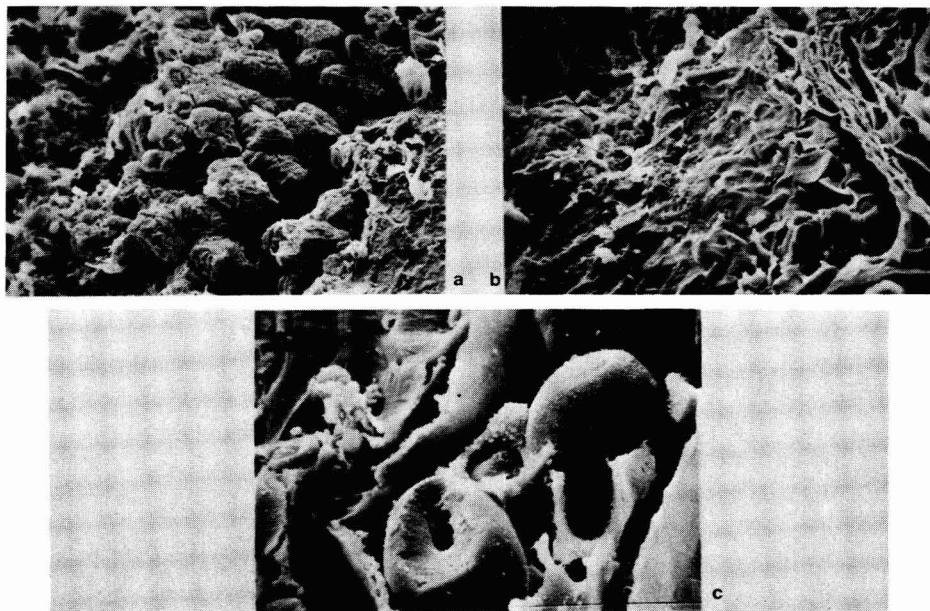
FIG. 16. SEM MICROGRAPHS; POTATO TUBER, COOKED 60 MIN

a) Detail of cells in cross section
b) Detail compact inner crust
c) Detail of starch in region between inner and outer crust

closeness of these values implies that the cell structure of nonstarch solids in the cell have little effect on starch gelatinization.

From Table 2 it can be seen that the temperature for the onset of the endothermic peak varies from 60°C to 66°C with apparently no relation to the total weight of the sample present in the experiment. Also shown is the total range, ΔT, in temperature taken from the onset of the endothermic peak to the re-establishment of the base line. There again there seems to be no effect of the total weight of the sample on the variations in the results, a fact implying that the weight influences the height of the endothermic peak but not its width. There does seem to be a correlation between ΔH and ΔT: the smaller ΔH the smaller ΔT. The start of the

endothermic peak varies from sample to sample and is higher than the constant value of 55°C observed for the suspensions of commercial potato starch.

The gelatinization reaction proved to be complete during the heating process. The samples were cooled and then reheated at the same rate, but no new peak appeared.

An experiment was also run for the same tuber but at different heating rates. In Table 3 are presented the heats of gelatinization per gram of starch (again assuming 16.3% of the total sample weight), total heat of gelatinization, temperature of onset of peak, weights of sample, and rates for this experiment.

The object of this experiment was to observe whether the rate of heating affects the range of temperature during which the gelatinization occurs. From Table 3 it is evident that the effect is considerable. The higher the rate of heating, the larger the ΔT, from a value of 26° for a rate of 20°/min, to a value of 5° for 2.5 and 1.85°/min. The heating rate has no effect on the other values of the table or the completeness of the gelatinization process since when the sample was cooled and reheated at the same rate no new peak appeared.

It is evident that there is a dependence between the rate at which the sample is heated and the ΔT during which gelatinization takes place. The ΔT becomes independent at low values of heating rate. This reflects an intrinsic range of gelatinization temperatures in the starch. This range may be due to the size or chemical distribution of the starch granules.

Table 3. Gelatinization values for different rates of heating in potato tuber[a]

ΔH	ΔH_T	T_i	ΔT	W	Rate
3.34	9.75	57	26	17.9	20
3.63	8.39	63	12	14.2	10
3.20	6.99	64	5	13.4	2.5
3.58	10.15	61	5	17.4	1.85

[a]Quantities and units same as those in Table 2.

DISCUSSION

In considering the relationship between water loss rates, temperature profiles, and thermal properties of cooking potato tubers and the use of these relationships in understanding the development of structure, water loss rates and temperature must be viewed as interacting throughout the baking period.

It was observed for a 177°C oven that the moisture loss rate showed a local maximum at about 15 min followed by a local minimum. At this point, temperatures of at least 60°C have been established internally in the potato cylinder. The decrease in the rate of moisture loss coincided with a decrease in the rate of heating, and the temperature at which this decrease took place was approximately the same as that at which the start of the endothermic peak of gelatinization occurred in the potato tuber in the DSC studies. The decrease in water loss rate in the period between the local maximum and subsequent local minimum represents a slowing due either to (a) competition between evaporation and gelatinization for the heat being transferred from the oven to the tuber and/or (b) to a decreased water mobility from gelatinization. The rate of temperature increase is reduced by the endothermic nature of the process of gelatinization (Banks and Greenwood 1975). It appears that when gelatinization occurs, it happens all through the sample at almost the same time since the temperature inside the sample is almost the same at all points. From the DSC results, we conclude that gelatinization takes place in potato tuber over a 5 to 10° temperature range regardless of experimental conditions, reflecting an intrinsic range of gelatinization temperatures for the starch. This range may be due to the size or chemical distribution of the starch granules.

Heating continues and the sample not only reaches 100°C but overheats slightly beyond that temperature. The temperature then drops back to 100°C, the drop seemingly caused by a sudden release of built up pressure in the sample. This coincides with the second local maxima in the moisture loss rate curves. The temperature of the interior of the sample then stays at 100°C until the evaporation front begins penetrating the sample. Following the period of the second local maximum, the moisture loss rate enters its falling rate period. During the falling rate period, a cross-like hole is formed along the axis (see Fig. 5) of the sample. We presume the hole results from internal shrink of the cylinder whose case-hardened crust does not allow the outside of the sample to shrink appreciably. This same type of structure was observed by Van Arsdel *et al.* (1973) when drying potato at 66°C.

SEM micrographs show ungelatinized starch granules near the sample surface. Thus, the surface seems to be porous and to dry thoroughly

before gelatinization temperatures are reached. This is confirmed by the lower moisture content observed in the outer section of the moisture profile experiments. The ungelatinized porous section once formed remains of about constant thickness during cooking. However, interior to this porous section, a more compact region is formed where starch was able to gelatinize. This compact region advances inwardly as drying takes place. From the moisture profile experiments, the moisture values in this area were found to be very low. Beyond this compact region there again is the more open structure with the usual honey combed cellular structure of the potato.

The sequence of SEM micrographs showed the physical changes that starch undergoes at different intervals throughout the heating period. The smooth starch granules of the raw samples later appeared swollen, elongated, and collapsed after which they agglomerated and finally formed what appeared to be a gelatinized mass inside the cells. All this showed the evolution of the starch in a constrained environment both due to moisture loss and cell wall limits. The cell walls, however, maintained their integrity during the heating process. There was apparently no visible change in their appearance, although it is assumed that their structure changed while cooking since their resistance to excision with a razor blade was different for the raw and 15 min of baking versus 30 min or more of baking.

From the temperature profiles observed in this study, it can be concluded that the first maximum in the moisture loss rate coincides with gelatinization of the starch, which occurs almost simultaneously throughout the sample for this sample size. The second maximum coincided with the period during which most of the interior of the sample was at 100°C. On the basis of a simple heat transfer model, we conclude that the crust, observed in our SEM studies, and air-sample interface contribute about equally and together form the main thermal resistance in the cooking sample.

ACKNOWLEDGMENTS

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THE ISOMERIC COMPOSITIONS OF MONOHYDROPEROXIDES PRODUCED BY OXIDATION OF UNSATURATED FATTY ACID ESTERS WITH SINGLET OXYGEN

JUNJI TERAO and SETSURO MATSUSHITA

Research Institute for Food Science
Kyoto University, Uji
Kyoto, 611, Japan

ABSTRACT

The isomeric compositions of monohydroperoxides produced by oxidation of unsaturated fatty acid esters with chemically produced singlet molecular oxygen were determined by mass chromatography as the trimethylsilyl derivatives of hydroxy-octadecanoate. Methyl oleate formed equal amounts of the 9- and 10-isomers. Methyl linoleate formed equal amounts of the four isomeric hydroperoxides, the 9-, 10-, 12- and 13-isomers. Six isomers, the 9-, 10-, 12-, 13-, 15- and 16-isomers, were obtained from the monohydroperoxides of methyl linolenate in which the quantities of the 9- and 16- isomers were slightly higher than those of other isomers. The isomeric compositions of monohydroperoxides obtained by chlorophyll sensitized photooxidation were very similar to those formed by chemically produced singlet oxygen except that the proportions of the 9- and 13-isomers were higher than those of the 10- and 12-isomers in methyl linoleate.

INTRODUCTION

It has been suggested that photosensitized oxidation initiates oxidative deterioration of vegetable oils (Rawls and Van Santen 1970; Clements *et al.* 1973; Carlsson *et al.* 1976). Chlorophyll like pigments present in oils seem to act as an effective sensitizer by absorbing visible light to produce hydroperoxides in unsaturated fatty acids. This reaction has been categorized into two classes, Type I and Type II, as mentioned by Foote (1976). Type I reaction involves the production of free radicals by interaction of the excited sensitizer with a substrate. In the Type II process, an excited sensitizer produces singlet oxygen by transfering excitation from the sensitizer to oxygen. This active oxygen molecule reacts with olefinic

double bonds to produce hydroperoxides by a concerted ene mechanism (Foote 1971).

There have been some reports on the structures of isomeric hydroperoxides of several unsaturated fatty acids or their esters produced by chlorophyll sensitized photooxidation (Khan *et al.* 1954; Cobern *et al.* 1966; Hall and Roberts 1966; Federi *et al.* 1971; Terao and Matsushita 1977). Chan (1977) studied the photosensitized oxidation of methyl oleate and methyl linolenate using erythrosine and riboflavin as sensitizers and demonstrated that both types of pathways could be distinguished by the analysis of hydroperoxide isomers. Terao and Matsushita (1977) also clarified that the monohydroperoxides of methyl oleate, methyl linoleate and methyl linolenate, formed by the Type II reaction of photosensitized oxidation, include positional isomers different from those formed by autoxidation. However, accurate isomeric compositions of monohydroperoxides produced by singlet oxygen oxidation are still obscure because of the difficulties in separating and quantifying these isomers. Recently, Frankel *et al.* (1977) demonstrated that the mass chromatographic approach is a powerful method to analyze the isomeric compositions of monohydroperoxides of autoxidized fatty acid esters. Mass chromatography was subsequently applied to the determination of isomeric composition of monohydroperoxides from the photosensitized oxidation of methyl linoleate by artifical colorings (Umehara *et al.* 1979).

The purpose of this report is to clarify the isomeric compositions of monohydroperoxides produced by oxidation of methyl oleate, methyl linoleate and methyl linolenate and with singlet oxygen. The mass chromatographic method was used for quantification of the monyhydroperoxide isomers in gas chromatography mass spectrometric analysis (GC-MS analysis). Oxidation reaction was carried out by two experimental systems, that is oxidation with chemically produced singlet oxygen generated by the reaction of sodium hypochlorite with hydrogen peroxide (Khan and Kasha 1963) and chlorophyll sensitized photooxidation in ethanol solution.

MATERIALS AND METHODS

Materials

Methyl oleate, methyl linoleate and methyl linolenate were obtained from Nakarai Chem. Co. Ltd., Japan 99% grade. Each fatty acid ester was purified by column chromatography using Florisil (100/200 mesh) before using for experiments. Chlorophyll was obtained from spinach chloroplast which consisted of chlorophyll *a* and *b* (Terao and Matsushita 1977).

Oxidation with Chemically Produced Singlet Oxygen

Each unsaturated ester (0.17×10^{-3} mole) was emulsified by ultrasonic treatment after addition of 0.5% Triton X (7.5 ml) and a few crystals of butyl hydroxytoluene. Aqueous sodium hypochlorite solution (10 ml; 10% w/v) was added dropwise to an intensively stirred mixture of emulsified fatty ester containing hydrogen peroxide (2.5 ml; 30 w/v). Temperature of the reaction mixture was maintained below 0°C. After completion of the reaction, the reaction mixture was extracted by ethyl ether immediately, followed by evaporation. The residue was treated with sodium borohydride in methanol and hydrogenated under a stream of hydrogen gas.

Chlorophyll Sensitized Photooxidation

The procedure of photosensitized oxidation was the same as described previously (Terao and Matsushita 1977). Light intensity was maintained at 10 mW/cm².

GC-MS Analysis

GC-MS analysis was carried out with a LKB–9000S gas chromatograph mass spectrometer. Operational conditions and preparation of trimethylsilyl derivatives were the same as described previously (Terao and Matsushita 1977). Full mass scan was recorded every 5 s. Isomeric compositions of monohydroperoxides were calculated by computer summation of the peak areas of fragment ions due to the α-cleavage of the trimethylsilyloxy group of each isomer in the mass chromatogram.

RESULTS AND DISCUSSION

Each fatty acid ester oxidized by the two systems, oxidation with chemically produced singlet oxygen and chlorophyll sensitized photooxidation, was hydrogenated and silylated. The gas chromatogram of the silylated reaction mixture gave peaks of nonoxidized esters and methyl hydroxy octadecanoate which was the derivative of monohydroperoxides. No secondary degradation products appeared in the chromatogram. Figures 1–3 show the results of mass chromatography of hydroxy octadecanoate derived from oxidation with chemically produced singlet oxygen. Contents of the monohydroperoxides produced were estimated to be 3–4% of the total esters. In the figures, peaks were obtained from the

J. TERAO AND S. MATSUSHITA
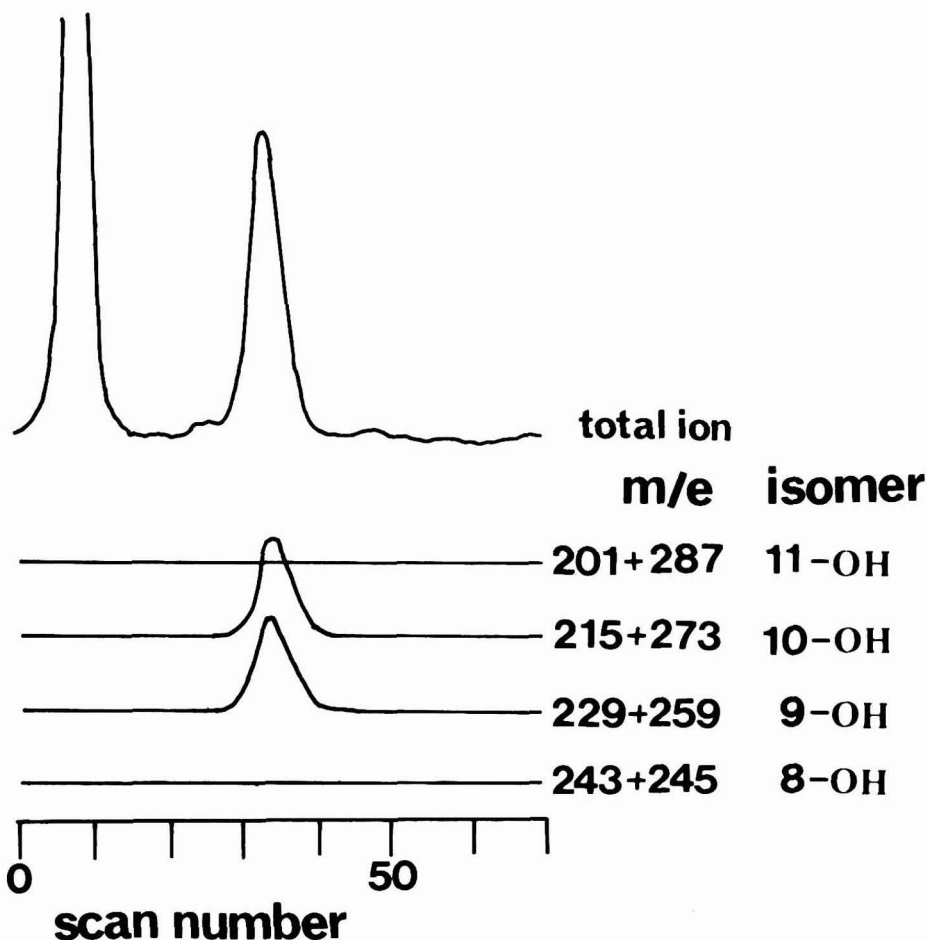
FIG. 1. MASS CHROMATOGRAPHY OF HYDROGENATED METHYL OLEATE MONOHYDROPEROXIDES PRODUCED BY OXIDATION WITH SINGLET OXYGEN

sum of the intensities of the two fragment ions produced by α-cleavage of the trimethylsilyloxy group. These fragment ions were specific to each positional isomer of the derivatives of monohydroperoxides as shown previously (Terao and Matsushita 1977). Isomeric compositions of monohydroperoxides were calculated by the ratio of the peak areas according to the report of Frankel *et al.* (1977), and are shown in Table 1. Equal amounts of the 9- and 10-isomers were present in methyl oleate monohydroperoxides. The 8- and 11-isomers, yields from autoxidation,
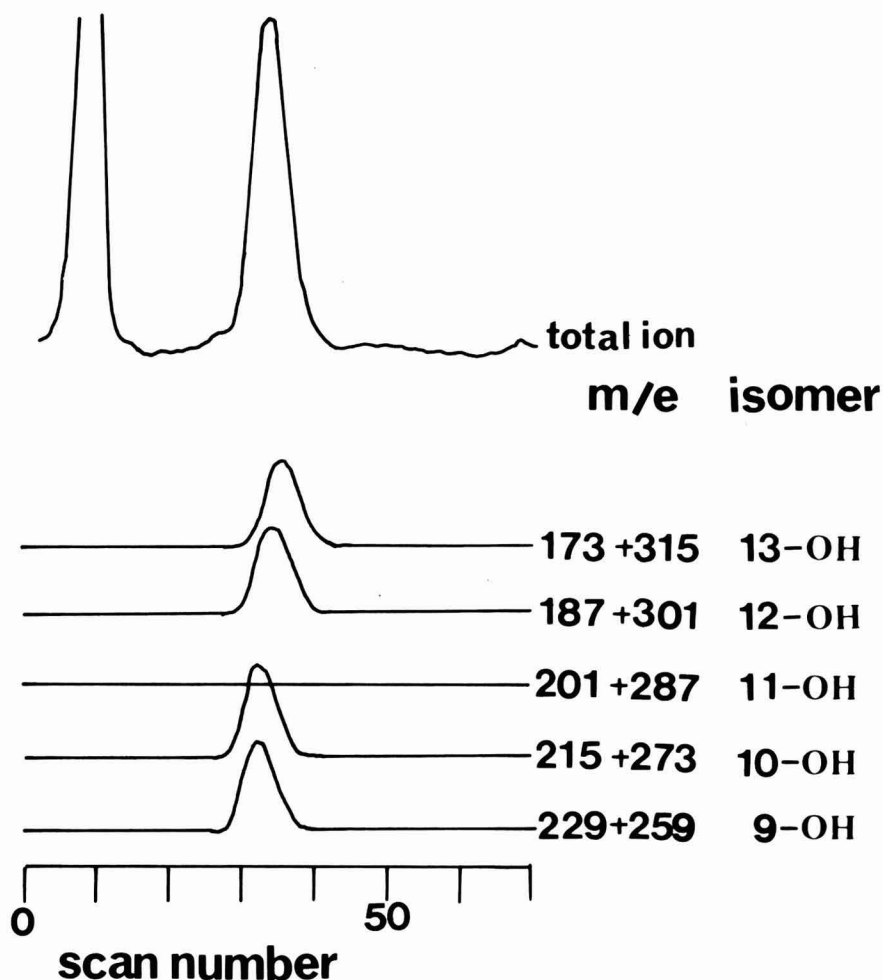
FIG. 2. MASS CHROMATOGRAPHY OF HYDROGENATED METHYL LINOLEATE
MONOHYDROPEROXIDES PRODUCED BY OXIDATION WITH SINGLET OXYGEN

were not detectable. No difference was observed between the proportion
of the four isomers in methyl linoleate. However, a slight predominance
of the 9- and 16-isomers was found in the isomeric composition of methyl
linolenate. The result of the isomeric compositions of oleate and linoleate
suggests that hydroperoxidation occurs equally at each position of carbon
forming a double bond. It is unclear why the formation of the 9- and
16-isomers occurred predominantly in methyl linolenate. One reason

Table 1. Isomeric compositions of monohydroperoxides by oxidation with chemically produced singlet oxygen

Isomer	% Composition (Peak Areas)[a]								
	8-OOH	9-OOH	10-OOH	11-OOH	12-OOH	13-OOH	14-OOH	15-OOH	16-OOH
Oleate	—	49.2±0.9	50.8±0.9	—	—	—	—	—	—
Linoleate	—	25.4±1.4	23.6±2.1	—	25.4±1.8	25.7±1.5	—	—	—
Linolenate	—	24.1±1.1	13.2±0.8	—	14.5±0.3	15.6±0.9	—	11.7±0.4	21.0±1.3

[a] Average of five experiments

Table 2. Isomeric compositions of monohydroperoxides by chlorophyll sensitized photooxidation

Isomer	% Composition (Peak Areas)[a]								
	8-OOH	9-OOH	10-OOH	11-OOH	12-OOH	13-OOH	14-OOH	15-OOH	16-OOH
Oleate	—	49.1±0.5	50.8±0.9	—	—	—	—	—	—
Linoleate	—	30.2±0.4	19.7±1.1	—	19.8±0.7	30.1±1.4	—	—	—
Linolenate	—	21.6±0.6	14.3±0.5	—	15.3±0.1	15.7±0.4	—	12.0±0.7	21.1±0.5

[a] Average of five experiments
Fatty acid ester (10.2 mM) in 3.0 ml of ethanol containing chlorophyll (13.3 μM) was incubated at 20°C for 3 h. After incubation, reaction mixture was hydrogenated and sillylated for GC-MS analysis.
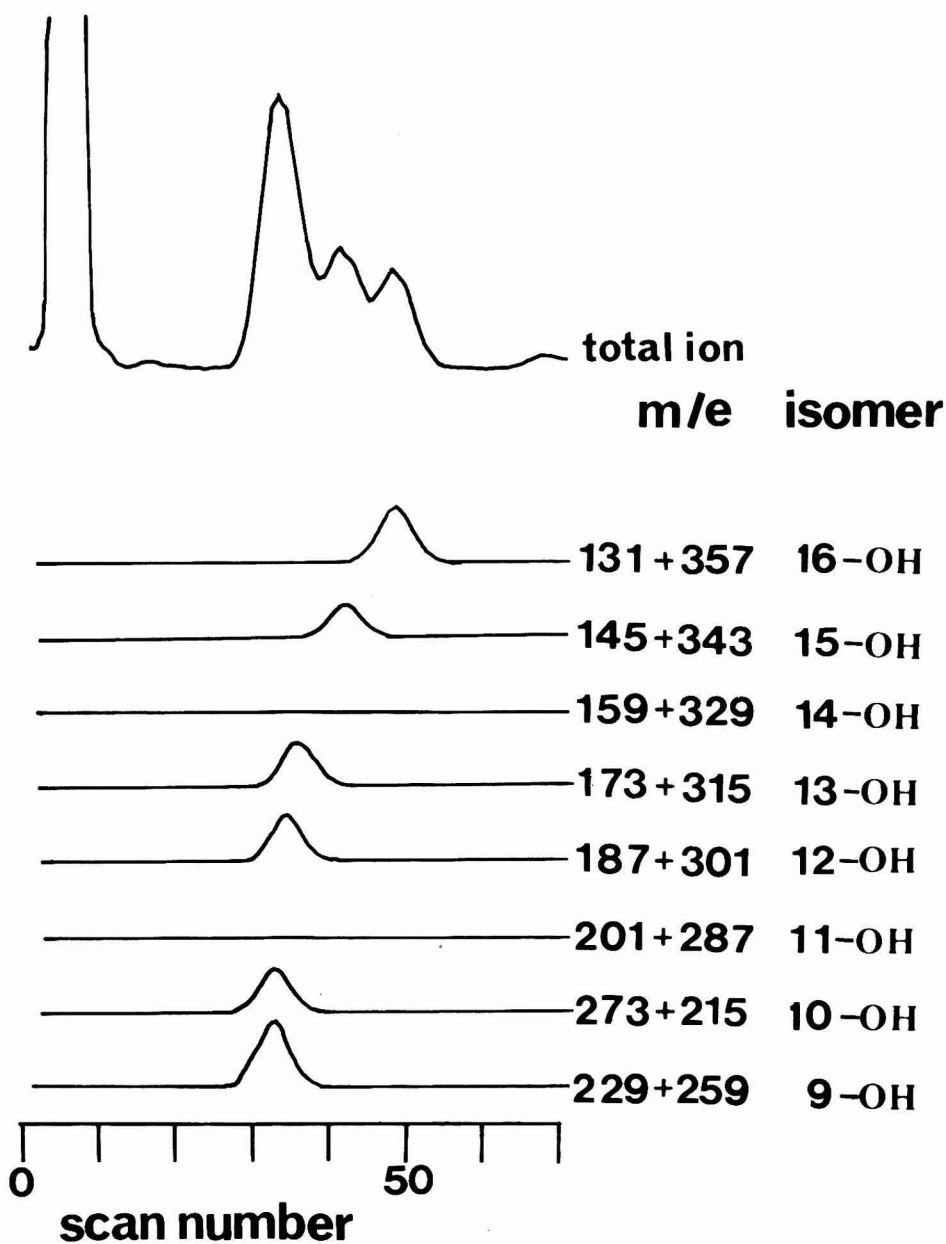
FIG. 3. MASS CHROMATOGRAPHY OF HYDROGENATED METHYL LINOLENATE
MONOHYDROPEROXIDES PRODUCED BY OXIDATION WITH SINGLET OXYGEN

may depend on the occurrence of radical reaction at the same time, because the 9- and 16-isomers are preferentially produced in autoxidation of methyl linolenate (Frankel *et al.* 1961), and methyl linolenate is more susceptible to the autoxidation than methyl oleate and methyl linoleate.

The isomeric compositions of monohydroperoxides formed by chlorophyll sensitized photooxidation was determined by the same procedure as that in the oxidation with chemically produced singlet oxygen oxidation. However, the proportions of the 9- and 13-isomers were slightly higher than those of the 10 and 12-isomers in methyl linoleate. Rawls and Van Santen (1970) supposed that chlorophyll reaction partially involved Type I mechanism. If Type I reaction occurs in the chlorophyll reaction, the 8- and 11-isomers would be produced by reaction with methyl oleate as described by Chan (1977). But, these isomers specific to Type I process did not appear as is evident in Table 2. It may be reasoned that autocatalytic oxidation reaction occurs with photooxidation of methyl linoleate in chlorophyll solution.

Application of mass chromatography to autoxidation products of unsaturated fatty acid esters has shown that oleate, linoleate and linolenate yielded the 8-, 9-, 10 and 11-isomers, the 9- and 13-isomers, and the 9-, 12-, 13-, and 16-isomers, respectively (Frankel *et al.* 1977). Therefore, isomers specific to singlet oxygen oxidation are the 10- and 12-isomers for linoleate, and the 10 and 15-isomers for linolenate. Mass chromatographic analysis can be used to distinguish between oxidation with singlet oxygen and radical oxidation including Type I process in photosensitized oxidation. In a subsequent paper, the analysis of isomeric composition of monohydroperoxides will be applied to investigating the mechanism of photooxidation of oil and the inhibitory effects of tocopherols and carotenoids.

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BOOK REVIEW

Progress in Flavour Research. Edited by D. G. Land and H. E. Nursten. Applied Science Publishers Ltd., Ripple Road, Barking, Essex, England. 1979. $46.00

This book is a compilation of thirty papers presented at the second Weurman Flavour Research Symposium. The papers deal with recent developments in the following four areas: sensory aspects-"Sensory Characterization of the Flavour of Beer," "Sensory Methods in the Work of the Flavour Chemist," "Application of Anatomical and Psychophysical Methods to Studies of Odour Interactions," "Some Factors Influencing the Perception of Flavour-contributing Substances in Food," "Determination of Personal and Group Thresholds and the Use of Magnitude Estimation in Beef Flavour Chemistry," "An Approach to Meat Flavour Research with Evaluation by the Dog and Cat"; analytical and instrumental techniques-"Review of Isolation and Concentration Techniques," "Application of Gas and Thin-layer Chromatography in Flavour Analysis," Recent Studies in Flavour Chemistry and Related Fields Undertaken in the Food Research Laboratory of C.S.I.R.O.," "Deactivation of a Metal Transfer Line between a Gas Chromatographic Column and a Flame Photometric Detector," "Developments in Mass Spectrometry," "Applications of Alternate Scan Positive Ion-Negative Ion Chemical Ionization in Gas Chromatography-Mass Spectrometry," "Optimized Coupling Techniques for Gas Chromatography-Mass Spectrometry in Flavour Research"; flavor formation-"Review of Biosynthesis of Volatiles in Fruits and Vegetables since 1975," "The Role of Micro-Organisms in Flavour Formation," "Chemical Formation of Flavour Substances," "Differences between the Volatile Compounds of Cultivated and Wild Strawberries (Fragaria vesca L.)," "Volatile Components from Thermally Desgraded Thiamin," "Factors Affecting Flavour during Growth, Storage and processing of Vegetables," "Flavour Formation in Dairy Products," "Reduction of Cooked Flavour in Heated Milk and Milk Products," "Changes in Flavour Compounds of Black Pepper during Heat Sterilization," "The Formation of Metallic Taint by Selective Lipid Oxidation: the Significance of Octa-1, cis-5-dien-3-one," "The Glucoseinolates of Two Species of Farsetia"; and consumer quality apsects-"The Evaluation of Flavour Quality in Fruits and Fruit Products," "Meat Flavour and Consumer Acceptability," "The Bitterness of Protein Hydrolysates," "Problems of Flavour Application in Food Systems" and "Why Flavour Research? How far have we come since 1975 and where now?"

Most articles contain an informative review of basic information related to the particular topic, highlighting some of the more significant

recent studies. Some chapters present an overview of recent research conducted in the author's laboratory. Because all the articles are relatively easy to read this book would make an excellent reference for undergraduate and graduate students interested in flavor, a useful addition to a flavorist's library, and a broadly informative collection of flavor-related articles for a food scientist not primarily interested in flavors.

ZATA VICKERS

AUTHOR INDEX

SUBJECT INDEX

F
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JOURNALS AND BOOKS
IN
FOOD SCIENCE AND NUTRITION

1. TITLE OF PUBLICATION	A. PUBLICATION NO.	2. DATE OF FILING
Journal of Food Processing & Preservation	4 5 6 4 7 0	October 1, 1979

3. FREQUENCY OF ISSUE	A. NO. OF ISSUES PUBLISHED ANNUALLY	B. ANNUAL SUBSCRIPTION PRICE
Quarterly	4	$50.00

4. LOCATION OF KNOWN OFFICE OF PUBLICATION *(Street, City, County, State and ZIP Code) (Not printers)*
265 Post Road West, Westport, Fairfield, CT 06880

5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS *(Not printers)*
265 Post Road West, Westport, Fairfield, CT 06880

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John J. O'Neil, 265 Post Road West, Westport, CT 06880

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

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

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

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

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

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EDITORIAL OFFICE: Prof. T. P. Labuza, Editor, Journal of Food Processing and Preservation, University of Minnesota, Department of Food Science and Nutrition, Saint Paul, Minnesota 55108 USA

CONTENTS