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EFFECTS OF COOKING METHODS ON PEELABILITY AND APPEARANCE OF EGGS[1]

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ABSTRACT

Ease of peeling and appearance of eggs cooked by cold water, boiling water and steam pressure methods were compared using oiled and unoiled fresh and stored eggs. Several time-pressure combinations for steam cooking of hard cooked eggs were investigated.

Best results for both appearance and peeling time were from eggs cooked at 7.5 psi and 12.5 min. Ten psi for 10 min and 15 psi for 7.5 min also gave excellent results. Fresh eggs and eggs stored up to 16 days, oiled and unoiled, peeled quickest and had the best appearance when cooked by the steam pressure method. Rapid release of pressure at the end of cooking caused many shells to crack which added to ease of peeling.

INTRODUCTION

Problems of peeling eggs cooked in the shell continue to plague both consumers and commercial processors. Because ease of peeling has been associated with the rise in pH, which occurs when eggs are stored, a frequent recommendation is to use older eggs. This requires the user to segregate eggs into higher quality fresh eggs used for most cooking purposes and lower quality old eggs used for peeling. Additional disadvantages are that aging of eggs contributes to a larger air cell, weight loss and off centered yolks. Modern production and processing methods bring fresher and higher quality eggs to the user. Because eggs are gathered several times each day, sometimes oiled, and placed under refrigeration within hours after being laid, difficult peeling often

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persists even with extended aging before cooking. Demand for wholesale production of hard cooked peeled eggs is increasing and the average product loss in commercial kitchens is 11% due to breakage and albumen adhering to shells (Anon. 1975a).

There are disagreements in the preferred method for cooking eggs in the shell. Some directions suggest placing eggs in cold water and bringing to boiling or simmering temperatures, while others suggest placing eggs in simmering or boiling water. The January 1975 revision of the USDA Home and Garden Bulletin No. 103 states for hard cooked eggs: lower eggs into gently boiling water, reduce heat and simmer just below boiling for 20 min (Anon 1975b). Britton and Hale (1972) used 98°C water, Nath *et al.* (1973) used 93°C water, and Grunden *et al.* (1975) used 90°C water. Irmiter *et al.* (1970) suggested that many of the cookbook recommendations appear to be based on methods recommended years ago, which may not be applicable to the type and quality of eggs marketed today. They observed better peelability from a boiling water method where eggs were lowered into boiling water, heat was reduced and eggs were held at 85°C for 18 min than from a cold water method where eggs were placed into 20°C water, heated in a covered pan to boiling, immediately removing the heat and left in the water for 25 min. Another method of cooking eggs is the use of steam. The instruction plate on a Steam-chef[3] stack steamer recommends 10 min at 6 psi. Recent correspondence with the manufacturer of this cooker recommended eggs be placed in a preheated steamer and steamed for 8 to 10 min at 5 psi, but the origin of the research which led to this recommendation was not revealed. The wide variation in recommended cooking procedures indicates that the relationship between cooking methods and ease of peeling has not been elucidated.

The studies presented here were conducted to investigate methods of preparing hard cooked peeled eggs. The main variables include the effect of cooking eggs by cold water, boiling water and steam pressure methods on ease of peeling and appearance. Several time-pressure combinations for steam cooking of hard cooked eggs were investigated.

MATERIALS AND METHODS

Eggs were obtained from the University of Connecticut Poultry Farm on the day they were laid. All eggs were stored at 13°C and only sound shell, grade A and AA quality eggs were used. Except where specified,

[3] The Cleveland Range Co., 971 East 63rd St., Cleveland, Ohio 44103

eggs were not oiled. When eggs were oiled, Sta-good Egg Spray[4], a liquid paraffin with silicone was applied to the large end on 1-day old eggs.

Optimum Time-Pressure Combination for Steam Cooking

Five large 1-day old eggs were cooked in a portable autoclave that was preheated to 100°C. Eggs were processed at the time-pressure combinations indicated in Table 1. After cooking, pressure was released in approximately 30 sec and eggs were cooled in 13°C running tap water for 5 to 10 min. The procedure was repeated two or three times per test method.

Table 1. Effect of time-pressure cooking combinations on appearance of peeled hard cooked eggs

	Cooking Time, Min											
Pressure	7.5			10.0			12.5			15.0		
psi	S_1	S_2	C[1]	S_1	S_2	C	S_1	S_2	C	S_1	S_2	C
0		—[2]			—			—		4	5	1
5.0		—			—		5	3	2	2	8	0
7.5		—		7	8	0	15	0	0	8	7	0
10.0	9	1	0	12	2	1	7	2	1		—	
12.5	7	3	0	9	1	0		—			—	
15.0	9	1	0		—			—			—	

[1] S_1 is the number of eggs that had an appearance score of 1 or 2,
S_2 is the number of eggs that had an appearance score of 3 or 4,
C is the number of eggs that cracked

[2] Not tested because it would result in either undercooking or overcooking

Comparison of Cold Water, Boiling Water and Steam Pressure Cooking Methods

The three cooking methods were tested on oiled and unoiled medium eggs that were stored for eight time intervals from 1 to 16 days. All eggs were washed after one day of storage and one-half of the eggs were oiled immediately after washing. Six oiled and six unoiled eggs were cooked simultaneously by each method. In the cold water method, eggs were placed into tap water at 13°C, brought to a boil and held at a slow boil for 10 min. In the boiling water method, eggs were placed gently

[4] Mattox & Moore Inc., 1503 East Riverside Drive, Indianapolis, Indiana 46207

into boiling water and held at a slow boil for 15 min. In the steam cooking method, eggs were placed into the preheated autoclave and processed for 12.5 min at 7.5 psi and then the pressure was released in approximately 30 sec. Eggs cooked by the three methods were cooled in 13°C running tap water for 5 to 10 min. Each method was replicated twice using a total of 576 eggs.

Peeling Method and Evaluation

All eggs in the above studies were peeled by the same individual. The cooled eggs were cracked by tapping and rolling against a table top and were peeled as rapidly as possible using the thumb and forefinger. The time required to peel each egg was recorded. After all eggs were peeled, appearances were rated by a subjective score. A score of 1 was given when none of the albumen adhered to the shell; 2, when a small amount of the albumen adhered to the shell although the egg still had a desirable appearance; 3, when enough albumen adhered to the shell to detract from the egg's appearance; and 4, when a major portion of the albumen adhered to the shell. Texture and color of the albumen and dryness and mealiness of the yolk were subjectively evaluated to determine the optimum time-pressure combination for steam cooking.

RESULTS AND DISCUSSION

Optimum Time-Pressure Combination for Steam Cooking

The results of steam pressure cooking on ease of peeling of 1-day old eggs are shown in Tables 1 and 2. Peeling time and appearance scores were not determined on eggs that cracked during cooking. Eggs cooked in boiling water, i.e. 0 psi, required more time for peeling and had the poorest appearance scores. Eggs cooked at 7.5 psi for 12.5 min had the best appearance scores and peeled rapidly. The 15 eggs, i.e. 3 replicates of 5 eggs, peeled easily without albumen adhering to the shell. Cooking time was an important variable because 7.5 psi for either 10 or 15 min did not give as good results as did 12.5 min. The eggs cooked for 10 min had a softer albumen indicating slight undercooking and those cooked for 15 min had tougher albumen indicating overcooking. Cooking at 10 psi for 10 min and 15 psi for 7.5 min also gave excellent results. The other time-pressure combinations that had good appearance scores or peeling times were either slightly undercooked or overcooked. Preliminary studies indicated that cooking time was dependent upon size of egg and desired doneness. Time-pressure combinations greater

Table 2. Effect of time-pressure combinations on mean peeling time

Pressure psi	Cooking Time, Min			
	7.5	10.0	12.5	15.0
	Peeling Time, Sec			
0	—[1]	—	—	27 ± 6[2]
5.0	—	—	26 ± 18	29 ± 12
7.5	—	34 ± 12	18 ± 7	21 ± 9
10.0	18 ± 7	18 ± 5	20 ± 10	—
12.5	22 ± 5	13 ± 6	—	—
15.0	17 ± 4	—	—	—

[1] Not tested because it would result in either undercooking or overcooking
[2] Standard deviation and means do not include eggs that cracked during cooking

than those shown caused albumen to have a yellowish-brown color and rubbery texture. Baker and Darfler (1969) observed a similar discoloration and reported the higher the holding temperature following cooking, the greater the discoloration. They concluded that the discoloration was due to the browning reaction of amines and glucose in the albumen.

Cooking under steam pressure rather than elevated temperature appeared to be responsible for ease of peeling. Eggs cooked at atmospheric pressure in 112, 115.5 and 121°C oil which corresponds to the temperature of steam at 7.5, 10 and 15 psi, did not peel easily.

Comparison of Cold Water, Boiling Water and Steam Pressure Cooking Methods

The mean peeling times and mean appearance scores for the three cooking methods as effected by days of egg storage are shown in Figures 1 and 2, respectively. Results from the two replications were similar and were combined in preparing the figures. Values for 1-day stored eggs showed a rather random distribution for oiled and unoiled eggs. This was expected because eggs were oiled at one day, and storage time after oiling was only minutes. At two days of storage, unoiled eggs peeled quicker and had better appearance scores than did oiled eggs for each cooking method. By three days of storage, eggs cooked by the steam method peeled consistently quicker and had the best appearance scores; eggs cooked by the boiling water method were intermediate; and

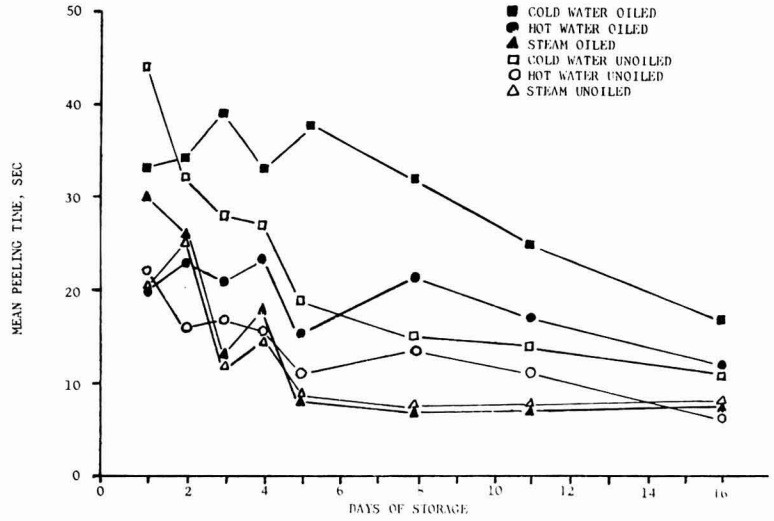

FIG. 1. EFFECT OF STORAGE ON PEELING TIME AS A FUNCTION OF COOKING METHODS

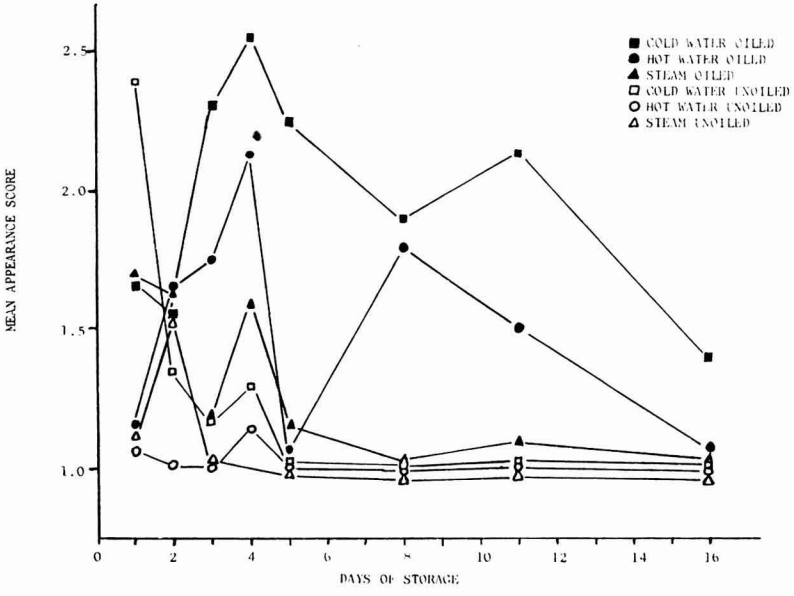

FIG. 2. EFFECT OF STORAGE ON APPEARANCE SCORE AS A FUNCTION OF COOKING METHODS

eggs cooked by the cold water method required the greatest time for peeling and had the poorest appearance scores. By three days of storage, two days after oiling, even the oiled eggs cooked by the steam method peeled quickly and had excellent appearance. There were no differences in the incidence of cracked eggs. Out of 192 cooked by each method the number of eggs that cracked during cooking were 7, 6 and 8 for the cold water, boiling water and steam methods, respectively.

Variables associated with egg production such as age of laying hens, ambient temperature and management practices affect egg quality, the rate of quality deterioration and consequently the minimum storage time needed for satisfactory peeling. Swanson (1959) reported that two days storage of fresh eggs was necessary before satisfactory peeling was possible. Spencer and Tryhnew (1973) did not consider eggs stored for two days at 1.1°C before cooking to peel easy. Hard *et al.* (1963) reported that even after eight weeks of storage at 0°C, some difficulty in peeling was experienced when egg quality was preserved by oil, silicone grease or 95% carbon dioxide atmosphere. It is well known that oiling retards the rise in egg pH during storage and increases the storage time needed for satisfactory peeling. However, oiled eggs held for seven days at 4°C peeled readily when rapidly cooled in ice water followed by 10 sec reheating (Hale and Britton 1974). Storage of oiled eggs at 25°C for seven days was not recommended due to quality loss related to off-centered yolks.

Greater ease of peeling eggs cooked in hot water as opposed to cold water has been reported by Irmiter *et al.* (1970). Eggs cooked by starting in boiling water gave more predictable results than starting in cold water, because cooking time in cold water was dependent upon the quantity of water used and the rate of heating.

Figures 1 and 2 were based on slow release of steam. It was later discovered that rapid release of steam following cooking, i.e. 2 to 3 sec, gave even greater ease of peeling. Some shells could be pulled off in halves and others would fall off in the process of picking up an egg. Because the eggs were fully cooked before the shells cracked, there were no marks on the albumen or adverse effects on appearance.

Steam pressure cooking of eggs could be adapted for both commercial and consumer use. The 7.5 psi for 12.5 min appears best suited for commercial processing. The 10 psi for 10 min or 15 psi for 7.5 min could be readily adapted by consumers who have pressure cookers which are usually calibrated at 10 or 15 psi. Organizations which periodically cook large quantities of eggs could use pressure canners.

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MICROBIAL ACTIVITY IN HEATED AND UNHEATED TOMATO SERUM CONCENTRATES

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ABSTRACT

Tomato serum (4.25% total solids), which contained all the water-soluble constituents of the tomato but little of the water insoluble fibrous material, was concentrated to 37.17, 42.53, 47.64 and 53.70% levels of total solids. Half of each concentrate was given a heat treatment designed to destroy vegetative cells and to activate spore germination and outgrowth. Microbial activity in both heated and unheated concentrates was monitored.

The unheated serum concentrates spoiled rapidly at the lower concentration levels. Spoilage seemed to be caused by bacteria at the lower concentration levels and by yeasts at the higher solids levels. At 53.70% solids the microbiological stability of the unheated concentrates was questionable following 28 days of incubation.

Data on serum concentrates heated 13 min at 80°C indicated microbiological stability at all four concentration levels. Malt agar plate counts indicated the absence of both viable yeasts and molds in the heated serum concentrates.

INTRODUCTION

The growth of spoilage-causing organisms in high solids tomato concentrates depends on the interaction of many factors. The most important factors were identified as water activity (a_w) (Mossel and Westerdyk 1949), natural antimicrobial constituents (Ingram 1955),

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acidity, and incubation temperature (Mossel 1971).

All living organisms need water to survive. The interaction of water and solids affects microbial survival and growth which depend on the microorganisms' ability to balance osmotic pressure (Christian and Hall 1972; Mossel 1971). Most bacteria of concern in tomato concentrates are inhibited below 0.91 a_w, but various yeasts and fungi may be capable of growing at as low as 0.60 a_w (Mossel 1971). Since tomato concentrates are classified as acid food, they are not subject to spoilage by organisms of public health significance, such as *Clostridium botulinum*, because these do not proliferate at pH values below 4.5 (Gould 1974). Members of the lactic acid bacteria and the yeast and fungi groups grow quite well within the pH range of tomato products whereas the activities of spore-forming organisms and the germination and growth of spores are limited. Townsend (1958) showed that spores of butyric acid anaerobes failed to germinate below pH 4.5 in tomato juice. Pederson and Becker (1949) found that while vegetative cells of *Bacillus coagulans* could grow in tomato juice at pH 4.15–4.25, the spores of the organisms could not germinate and grow below pH 4.32.

The main natural antimicrobial constituent in tomato concentrates is the product of a Maillard-type browning reaction, i.e. hydroxymethylfurfural (HMF) (Ingram *et al.* 1955). The amount of HMF depends on enzyme inactivation and on storage time and temperature of the tomato concentrates (Luh *et al.* 1958) which also influence whether spoilage will occur.

The aim of this investigation was to evaluate whether concentration with or without pasteurization would control microbial activity in a model system using tomato serum.

MATERIALS AND METHODS

Tomato Serum Concentrates

Ripe tomatoes of the variety GS-12 were hand harvested into 22.7 kg lug boxes from a field located 10 miles southwest of the Food Science Department at the University of California, Davis. The tomatoes were transported to the department and held in storage at 13°C until processed the following day, approximately 20 hr later.

Tomatoes were washed in chlorinated water in three 2.75 × 0.33 × 0.305 m troughs which allowed 2 min residence time in each trough. The wash water was chlorinated to a minimum of 5 ppm residual in the third wash. Chlorine levels were monitored. Tomatoes were conveyed

onto a mechanical sorting table where cracked and moldy tomatoes were removed.

The washed sorted tomatoes were given a steam injection hot break in which the whole tomatoes were crushed and brought from 20°C to 104°C in less than two seconds. The crushed tomatoes were held at this temperature for 45 seconds in a holding tube and then the skins and seeds (two and one-half to three and one-half percent by weight) were removed in a Brown Citrus Finisher (Model 3600) fitted with an 0.84 mm screen. The condensation of steam increased the weight of juice extracted by 11%. The hot juice was cooled to 54°C in a Cherry-Burrell heat exchanger (Model #324) and collected into a sanitized, covered stainless steel tank.

Tomato juice was separated in two passes through a Westfalia continuous centrifuge (Model #SAQOH205) operated at 10,000 rpm. The sludge fraction was discarded and the serum was concentrated in a wiped film evaporator (Pfaudler Co., Rochester, N.Y.). The unit operated with 23.5 mm Hg pressure and a steam jacket temperature of 121°C. Product temperature was approximately 38°C. Four concentration levels ranging from 38.0 to 54.5°Brix were prepared. The concentrates and samples of the serum were filled into sterile 404 × 502 enameled tomato cans and sealed. Cans were closed at 303 mm Hg pressure, and stored at 0°C until used. Storage at 0°C ranged from 1 to 3 months. It is possible that this low temperature may have changed the flora distribution and number however because of the size of the study no other alternative was available.

One can from each of the four concentration levels was removed from cold storage and agitated for 20 min to assure homogeneity. The can top was flamed with alcohol and opened. The serum was divided into three portions.

The first portion was filled into 15 sterile 16 × 150 mm glass test tubes each containing 10 ml serum. The tubes were capped with vaspar (equal volumes of petroleum jelly and paraffin) and incubated at 37°C.

The second portion was filled into a sterile 2,000 ml round bottom flask. The flask was attached by means of a long steam duct adaptor to a variable speed rotating motor. Serum was given a rotary agitated thermal process of 80°C for 13 min to destroy all vegetative microorganisms and induce spore germination and outgrowth. Serum temperature was monitored with a mercury-in-glass thermometer immersed in the serum. The heat-treated serum was removed into sterile 16 × 150 mm glass test tubes, sealed in the same manner as the first portion, and incubated at 37°C.

The third portion was used for initial microbial counts and for chemical analyses.

Microbiological Analysis. Microbial growth in the serum concentrates was monitored on SMA (Standard Methods Agar, DIFCO, Detroit, MI), OSA (Orange Serum Agar, DIFCO), FTA (Fluid Thioglycollate Agar, DIFCO) and MA (Malt extract 50 g/l, + Bacto-Agar, DIFCO, 30 g/l) after 0, 3, 7, 14 and 28 days of incubation.

The SMA (pH 7.0) was used as a nonselective aerobic and facultative aerobic growth medium. The OSA (pH 5.5) was used for its selectivity for spoilage organisms in acid fruit and vegetable products, i.e. lactic acid bacteria and yeast. Samples of SMA and OSA were pour plated and incubated at 37°C for 48 hours. The FTA (pH 7.1) was filled into anaerobic flat tubes, inoculated, and sealed with vaspar. The FTA tubes were used to determine strict and facultative anaerobic counts after 48 hr of incubation at 37°C. Samples were spread plated on MA (pH 3.5) and incubated at 25°C for 96 hr to determine yeast and mold counts. After incubation all colonies were counted and visual descriptions of colony differences were noted. Morphological variations were observed with a phase contrast microscope (Carl Zeiss, Germany) at 100× magnification. The samples for observation were concentrated by filtration when necessary.

Gas production coupled with plate counts were used to monitor spoilage in the serum concentrates. A serum concentrate sample was considered spoiled when the vaspar cover rose 6.8 mm from the concentrate's surface.

Growth rates on the various media were calculated as follows:

$$\mu = \frac{\log A_2 - \log A_1}{T_2 - T_1}$$

where A_1 = microbial count when logarithmic rate of growth was initiated
A_2 = microbial count when spoilage occurred
T_1 = day when logarithmic rate of growth was initiated
T_2 = day when spoilage occurred.

Chemical Analyses. Total solids, serum solids, and titratable acidity were determined according to the methods of AOAC (1975). Percent total solids was determined according to Section 32.004 of AOAC (1975) and was calculated as:

$$\% \text{ Total Solids} = \frac{(W_3 - W_1)100}{W_2 - W_1}$$

where: W_1 = Tare weight of dish and DE (diatomaceous earth)
W_2 = Weight of the dish, wet sample and DE
W_3 = Weight of the dish, dry sample and DE

Soluble solids by vacuum drying was also determined according to Section 32.004 of AOAC (1975). Four replications were made and the results were averaged. The sample was prepared for analysis by centrifuging for 30 min in a Sorvall Model SS-4 centrifuge equipped with a GSA rotor (13200 × g). True soluble solids in the original uncentrifuged material was calculated according to the following equation

$$SS = T - \frac{100(T - S_0)}{100 - S_0}$$

where: T = % total solids in uncentrifuged sample
S_0 = % total solids in centrifuged supernatant
SS = % soluble solids in uncentrifuged sample

Titratable acidity, determined according to Section 22.061 of AOAC (1975), was calculated as milliequivalent H^+ per 100 g sample:

$$\text{T.A. (Milliequivalents/100 g sample)} = \frac{\text{ml NaOH} \times \underline{N} \times 100}{\text{sample weight (g)}}$$

Three determinations were made for each concentration level, and the results were averaged.

(1) pH. The pH of each concentration level was measured in terms of H^+ activity using a Beckman Zeromatic (Model #SS-3) glass-calomel electrode system (NCA 1968).

(2) Serum Color. Tomato serum color was determined on a 4°Brix (natural juice strength) dilution of the concentrated serums. The diluted serum was filled into a 26 × 100 mm plastic centrifuge tube, capped and centrifuged in a Sorvall (Model #SS4) at 18,000 rpm for 30 min. The resulting serum was drawn into a syringe and filtered through 2.54 cm diameter Gelman glass fiber (GF-E) and 0.2 μ Gelman membrane filters. The clear serum was filled into a cleaned 1 cm Pyrex cell. Serum color was read on a Bausch-Lomb Spectronic 70 at 420 nm against a water blank. Absorption measurements were recorded.

(3) Brix. Sugar scale (Brix), i.e., NTSS (natural tomato soluble solids) values were determined by use of a Zeiss Opton Abbe refractometer at 20°C. Samples of tomato serum were prepared for measurement by centrifuging in an ultracentrifuge equipped with a special rotor for

tomato products (Clay-Adams Division of Becton, Dickinson & Company, Parsippany, New Jersey 07054).

A few drops of serum were applied to the refractometer prism and the prism was closed immediately. The ocular lens was adjusted to provide a neutral colored field (neither red nor blue). The main control knob was turned to raise the dark field to the cross hairs, with the field always moving in an upward direction, stopping when the dark field just blocked out the light projecting through the lower inverted V of the cross hairs.

(4) Water Activity. Water activity measurements were made using a Beckman Hygroline Meter (Model #EZFBA-4-02-05-16) equipped with a Sina equi-Hygro-Scope Humidity Sensor (Model #ezFBA/ePP). Samples were filled into 40 × 12 mm plastic dishes, then placed into the sensor unit. Each sample was allowed to equilibrate for 24 hr before water activity measurements were taken. Sensor reliability was monitored by alternating standard saturated salt solutions with the samples. The meter remained within manufacturer's specifications of ±1% accuracy.

RESULTS AND DISCUSSION

The factors which influence spoilage in a food system are strongly interrelated. Variation in concentration levels and thermal processing affected both the rate and type of spoilage in this model system.

Unheated samples from the first concentration level (38°Brix) spoiled within three days. Large amounts of gas production were observed in every tube. Microscopic examination showed predominantly nonmotile cocci in chains of 2–4, which is characteristic of lactic acid bacteria.

Similar results were obtained at the next higher concentration level (43.5°Brix) where spoilage occurred within seven days. Gas production was heavy in all samples and microscopic observation showed nonmotile cocci in chains to be the predominant organism.

At the third concentration level (48.9°Brix), spoilage appeared between the eighth and fourteenth days. At this level a single tube spoiled on days 8, 9, 13 and 14 with 4 tubes spoiling on days 10 and 12. Gas production was less than in the lower concentrates. Microscopic observation showed a shift from nonmotile cocci to a predominance of large, oval-shaped cells, characteristic of yeasts.

The fourth concentration level (54.5°Brix) did not spoil within the definition of spoilage established for the experiment. Gas production

was minimal, and microscopic examination showed large, oval yeast cells to be the only significant organisms present.

Microbial growth in the four serum concentrates, as determined on SMA, OSA, FTA, and MA is shown respectively in Fig. 1–4. The enumerations on SMA, FTA and OSA, when viewed as a whole, indicate that most of the microorganisms in the natural flora were facultative i.e. capable of growing under either aerobic or anaerobic conditions, and were acid tolerant, i.e. potential spoilage organisms in the serum concentrates (Fig. 1–3). The data obtained on MA depict the number of yeasts only (Fig. 4).

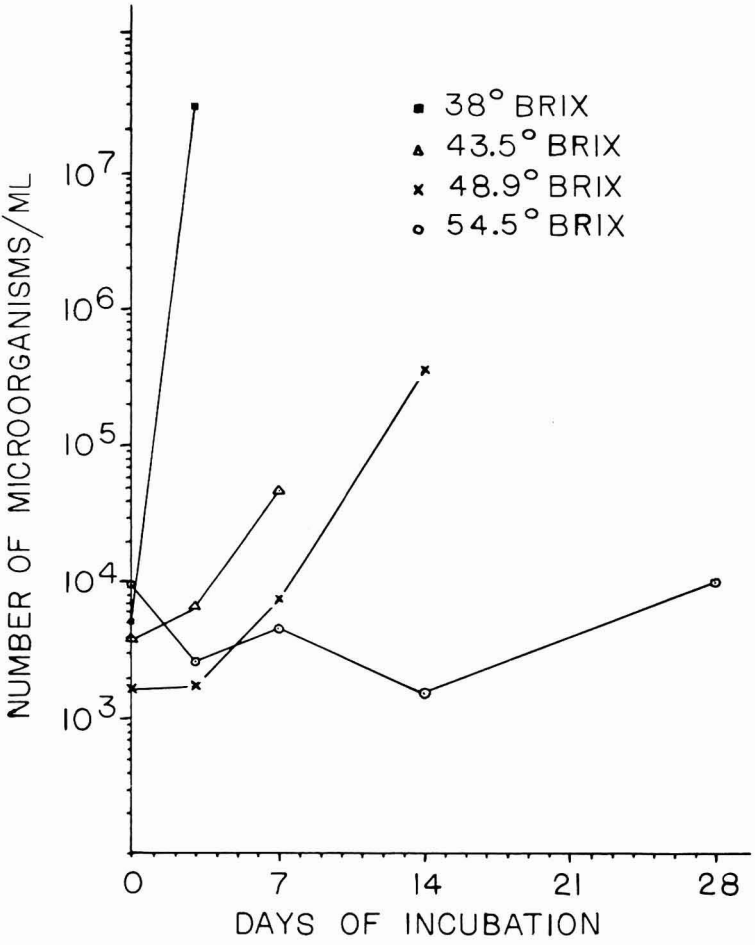

FIG. 1. GROWTH OF MICROORGANISMS IN THE RESPECTIVE UNHEATED TOMATO SERUM CONCENTRATES THROUGH THE TIME OF SPOILAGE, AFTER 0, 3, 7, 14 AND 28 DAYS OF INCUBATION, ENUMERATED IN STANDARD METHODS AGAR (SMA)

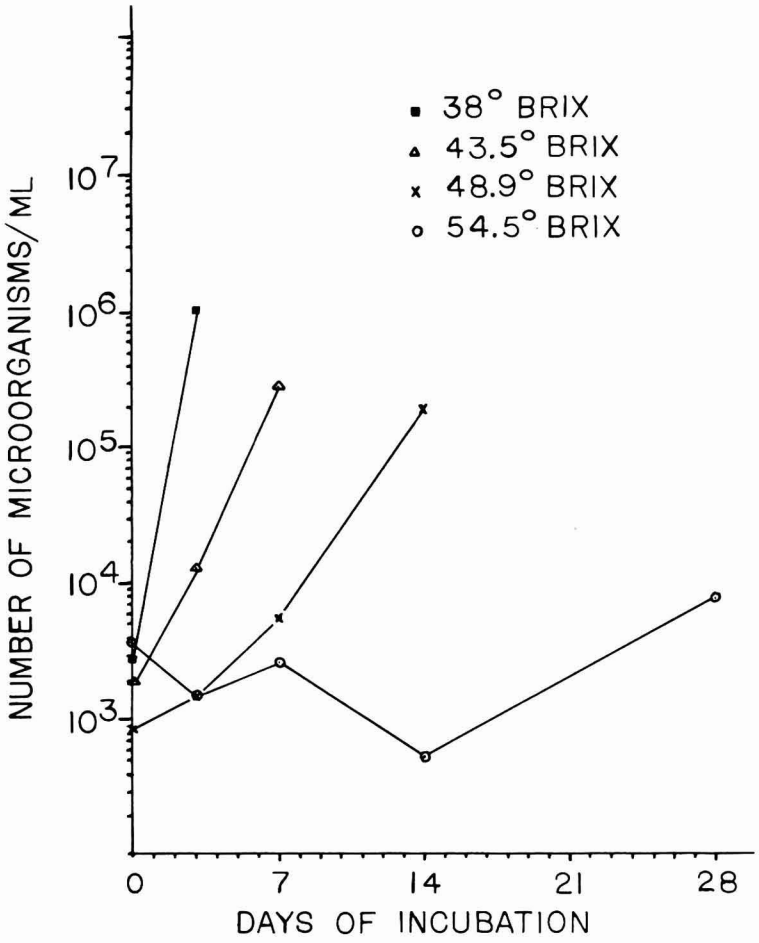

FIG. 2. GROWTH OF MICROORGANISMS IN THE RESPECTIVE UNHEATED TOMATO SERUM CONCENTRATES THROUGH THE TIME OF SPOILAGE, AFTER 0, 3, 7, 14 AND 28 DAYS OF INCUBATION, ENUMERATED IN ORANGE SERUM AGAR (OSA)

The data on the 38 and 43.5°B concentration levels strongly indicate that spoilage was probably caused by species of the genus *Leuconostoc*. The compositional data on these two concentrates, listed in Table 1, were within the tolerance limits for growth of these organisms. Species of this genus of spoilage organisms are capable of growing at pH of 4.21 and 4.20 and are not inhibited by water activities of 0.951 and 0.935 (Buchanan and Gibbons 1974, and Mossel 1971). When conditions are

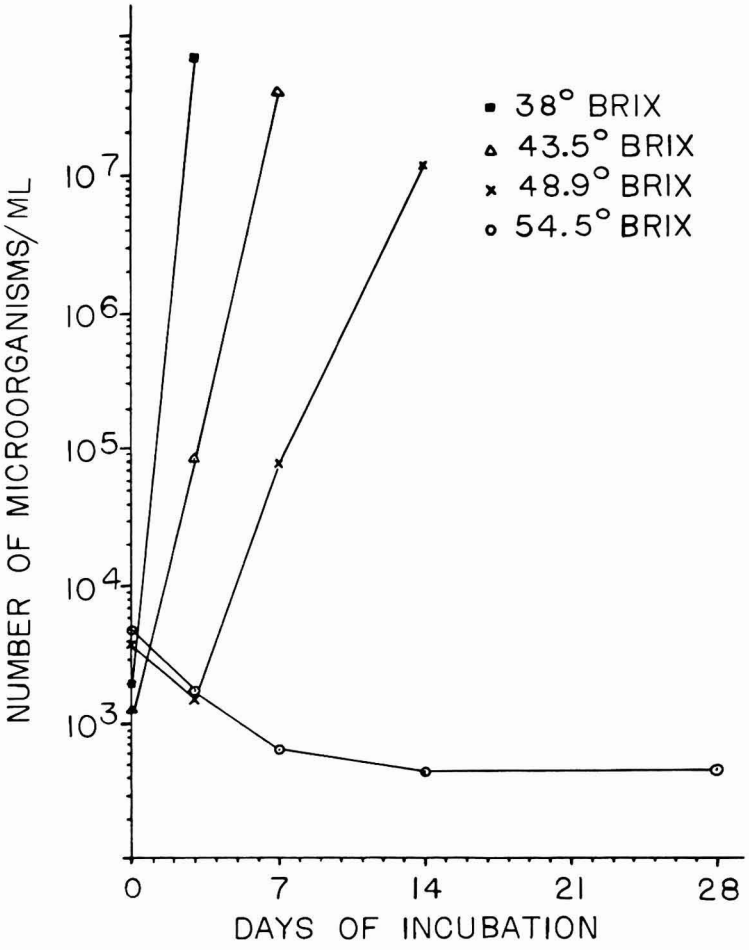

FIG. 3. GROWTH OF MICROORGANISMS IN THE RESPECTIVE UNHEATED TOMATO SERUM CONCENTRATES THROUGH THE TIME OF SPOILAGE, AFTER 0, 3, 7, 14 AND 28 DAYS OF INCUBATION, ENUMERATED IN FLUID THIOGLYCOLLATE AGAR (FTA)

favorable *Leuconostoc* can grow rapidly and produce large amounts of gas in the presence of fermentable sugars. At concentration levels of 38 and 43.5°Brix there was a rapid increase in the number of organisms (Fig. 1–4). According to Buchanan and Gibbons (1974), the presence of the nonmotile cocci observed through the microscopic examination of the serum samples could confirm the assumption that species of the genus *Leuconostoc* caused the spoilage at the lower concentration

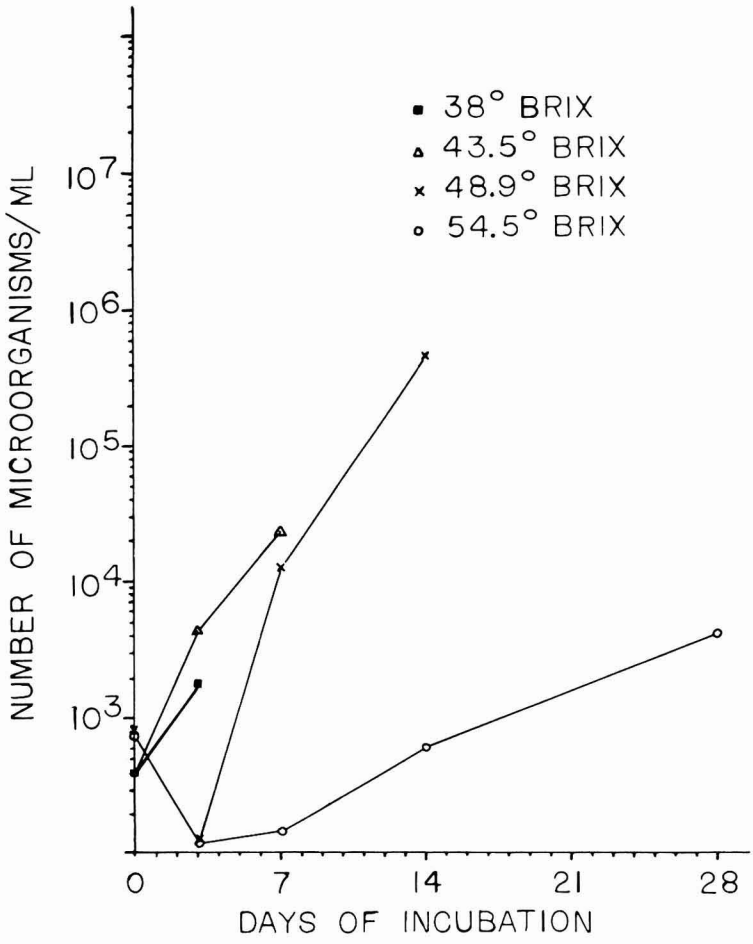

FIG. 4. GROWTH OF MICROORGANISMS IN THE RESPECTIVE UNHEATED TOMATO SERUM CONCENTRATES THROUGH THE TIME OF SPOILAGE, AFTER 0, 3, 7, 14 AND 28 DAYS OF INCUBATION, ENUMERATED ON MALT AGAR (MA)

levels, although further isolation and identification would be necessary for absolute confirmation.

A shift in spoilage organisms occurred in the 48.9°B concentrate and continued into the 54.5°B concentrate. In these concentrates, spoilage appeared to be caused by yeasts. The rate of spoilage decreased, and tubes began to spoil sporadically. This varying spoilage rate is characteristic of the slower growing yeasts. The yeasts grew approximately 9 to

Table 1. Analytical data on the whole tomato juice, the serum from which the concentrates were prepared, and the resulting concentrates

Sample	Total Solids	Soluble Solids	Brix °B	A_W at 20°C	Serum Color (4°B)	Total Acidity	Experimental pH
Whole juice	4.54	3.86	4.1	0.999	0.190	4.86	4.36
Serum, single strength	4.25	3.86	4.0	0.999	0.192	4.71	4.36
1st concentration level	37.17	—	38.0	0.951	0.194	43.17	4.21
2nd concentration level	42.53	—	43.5	0.935	0.195	47.64	4.20
3rd concentration level	47.64	—	48.9	0.916	0.202	53.49	4.18
4th concentration level	53.70	—	54.5	0.888	0.251	60.45	4.13

10 times slower than the bacteria and therefore did not produce as much gas per unit time as bacteria. Yeasts, because of their slower rates of growth, are poor competitors with bacteria and cannot begin to dominate the spoilage picture until the bacteria are inhibited (Walker 1977). The analytical data on the 48.9 and 54.5°B concentrates are also presented in Table 1. Yeasts are quite capable of growing at the a_W and pH range of the 48.9°B concentrate, whereas most bacteria start to be inhibited as a_W nears 0.91 (Mossel 1971). Although the 54.5°B concentrates did not meet the criterion set for spoilage within the incubation period, counts on MA between days 14 and 28 showed a 100× increase (Fig. 4). The large oval-shaped cells observed under the microscope indicated yeasts were the dominating organism present in this concentrate. Since certain strains of less osmotolerant yeasts are repressed near 0.88 a_W, they could require more than 28 days of incubation to spoil the product (Duckworth 1975), and the microbiological stability of the 54.5°B concentrate remains questionable.

The increase of lag periods with increasing concentration levels (Fig. 1–4) indicated that the environment was becoming increasingly inhibitory to the microorganisms. As the lag period for growth increased, the growth rates decreased on SMA, OSA, and FTA, as seen in Fig. 5. The growth rate on MA, unlike on the other media, increased at the 48.9°B level. The increase in growth rate on MA, a selective medium for yeasts and fungi, coupled with the microscopic observation, confirmed that yeasts were the causative agents of spoilage in the 48.9°Brix concentrate (Buchanan and Gibbons 1974).

Results from the heat-shocked concentrates indicated no significant changes in microbial population over the 28 day incubation period. Figure 6 shows the growth of microorganisms in the concentrates, determined on the various media. No growth on MA was observed. Chemical composition of the heat-shocked concentrates was the same as the unheated concentrates (Table 1). Microscopic examination of the various concentrations showed refractive spores and some nonmotile rods to be the only observable microorganisms.

Data on the heat-shocked concentrates is limited, and the inhibitory influence of additional factors other than heating and concentration must be considered. Heating destroyed the inherent vegetative cell population in these concentrates and also destroyed the yeasts and molds as determined by the MA plate counts. The surviving population of viable spores did not germinate and grow sufficiently to cause spoilage during 28 days of testing.

Several researchers have shown that pH is inhibitory to spore germination for members of the *Bacillaceae* family (Pederson 1929;

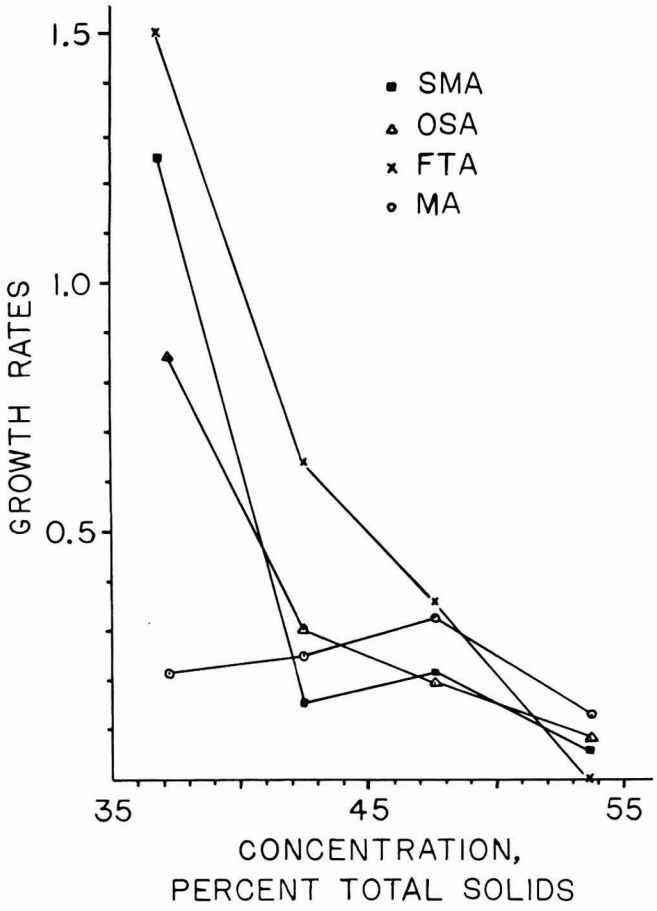

FIG. 5. GROWTH OF MICROORGANISMS AT THE RESPECTIVE UNHEATED CONCENTRATION LEVELS, AS DETERMINED ON SMA, OSA, FTA, AND MA

Rice and Pederson 1954; Townsend 1958, and York *et al.* 1975). The pH level in all concentrates measured below 4.22, and germination of spores of most members of the family *Bacillaceae* is inhibited at pH values below 4.3.

The presence of natural antimicrobial constituents, such as hydroxymethylfurfural, which form in tomato products did not seem to be an important factor in this study. Luh *et al.* (1958) showed that hot break pulp would be very low in hydroxymethylfurfural, and through the conditions and duration of this study the formation of this compound would not reach levels that Ingram *et al.* (1955) found inhibitory to yeasts.

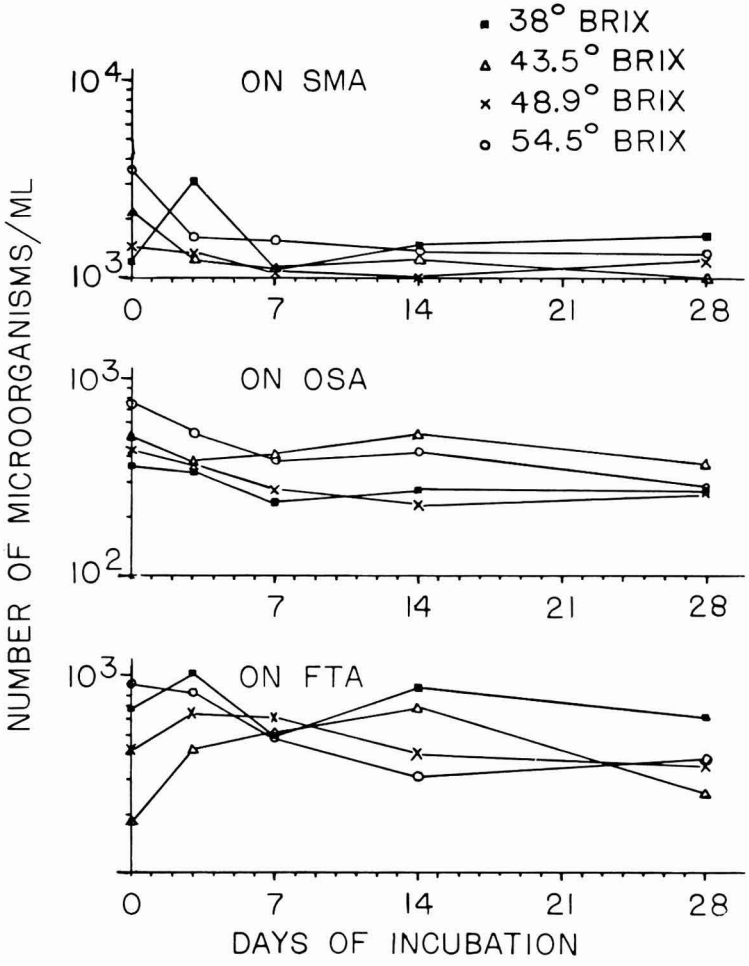

FIG. 6. GROWTH OF MICROORGANISMS IN THE RESPECTIVE HEAT TREATED SERUM CONCENTRATES AFTER 0, 3, 7, 14 AND 28 DAYS OF INCUBATION AS DETERMINED ON SMA, OSA AND FTA

There was no growth on MA when plated with the heat treated serum concentrates.

The use of tomato serum instead of tomato paste to study the effects of concentration on microbial growth was made necessary by the limitations of the experimental concentration equipment. Whole tomato pastes which exceeded 46°Brix became difficult to evaporate due to the increased viscosity and reduced heat transfer coefficient.

Serum which lacked the fibrous insoluble solids was less affected by this problem, and higher concentrations could be obtained. With the removal of the fibrous insoluble solids, the water-binding capacity of the serum was reduced (Mossel 1971), thus whole tomato pastes at the same °Brix values could be expected to have lower a_w, and greater microbial stability than the unheated serum concentrates. The latter possibility needs further investigation.

CONCLUSION

The use of concentration without thermal processing to achieve biological stability in tomato serum was not accomplished in this study. Although serum at 54.5°Brix did not spoil, the yeast counts leave questions about the stability of this concentrate over a longer period of incubation. The ability of yeasts to grow in unheated high acid foods at low a_w, and the mechanical feasibility of producing low a_w whole tomato concentrates need further investigation. This is especially true as this study was based on one year's production. This may vary from year to year.

Serum concentrates which received a thermal treatment of 13 min at 80°C showed stability at all concentration levels. This stability can be credited to a combination of several factors. The first was the processing of the raw fruit and serum under Good Manufacturing Practices. The next factor was the destruction of the vegetative microorganisms including yeasts and mold. The third controlling factor was the chemical composition of the concentrates. While the microbiological stability of the unheated concentrates in this study is not considered successful, the heat treatment and modification of conventional process for tomato concentrate shows some promise. By reducing the pH of comparable concentrates to below 4.3, the processing temperature may be reduced from 99°C to 80°C. The addition of food grade hydrochloric acid to control pH would permit the use of the milder heat treatment, making possible a stable product with less thermal degradation and some conservation of energy.

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PREDICTION OF DIELECTRIC PROPERTIES IN SOLID FOODS OF HIGH MOISTURE CONTENT AT ULTRAHIGH AND MICROWAVE FREQUENCIES

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ABSTRACT

Recent studies have shown that dielectric properties of raw potato can be predicted over the range of 300–3000 *Mhz and* 5–65°*C by a noninteractive Distributive model derived from lumped circuit analysis by a two-phase approximation which treats the potato as a binary system consisting of an inert solid phase and an active liquid phase. Dielectric behavior was seen to result primarily from water and ion activities of aqueous regions but subject to appreciable modification by a mechanism of volume exclusion due to effects of colloidal solids. Cell-free and whole potato extract measurements showed cation binding and complexing effects, resulting in considerably lower effective salts concentrations than implied by ash content. In addition, intracellular cation and biochemical constituent levels were significantly higher than extracellular levels. However, dielectric behavior of aqueous regions of the potato appeared to be based on bulk average fluid properties subject to displacement by colloidal solids. Low-frequency measurements of raw potato showed other regions of relaxation and conductivity effects rather than free water and bulk conductivity at low frequencies. But these appeared not to contribute to high-frequency dielectric response of the potato since observed relaxations were of small magnitude or occurred at frequencies well below the ultrahigh and microwave regions, suggesting that surface properties of solid foods may not be of much significance at high frequencies. Preliminary analysis of solid food measurements by other workers suggests the feasibility of modelling solid food behavior by two-phase approximations of Distributive, Maxwell or Rayleigh model behavior based on physical-chemical properties. For example, raw beef measurements were*

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predicted closely by a two-phase model in Rayleigh form, suggesting modelling characteristics similar to the potato but with specific model behavior due to differences in biological structure of beef and potato.

A general physical-chemical model is proposed for high-frequency dielectric behavior of solid foods based on observed mechanisms of interaction between water and the biochemical constituents of foods.

INTRODUCTION

Previous studies by Mudgett *et al.* (1971, 1974a, 1974b) have shown that the dielectric properties of certain aqueous mixtures are generally predicted at high frequencies by electrophysical models based on observed mechanisms of interaction between water and the biochemical constituents of foods. In studies of nonfat dried milk solutions (1971, 1974a), dielectric constants and loss factors were predicted at frequencies from 300–3000 Mhz and temperatures from 5–65°C by the Hasted-Debye (Hasted *et al.* 1948) models for aqueous ionic solutions based on modified proximate analysis. These studies showed a substantial fraction of milk salts to be associated, thus the effective salts concentrations were considerably lower than suggested by total ash content. This was seen to result from binding effects related to colloidal milk salts equilibria and from nonbinding effects related to the formation of hydration complexes of dissolved ions by hydrogen bonding. Further studies (1974b) showed that the dielectric properties for oil-water emulsions of varying composition at 3000 MHz, 25°C were predicted by the Fricke (1955) model for the complex conductivity of colloidal suspensions, expressed in terms of complex permittivity. The Fricke model reduces to the Maxwell (1881) model for spherical inclusions and the Rayleigh (1892) model for long cylindrical inclusions perpendicular to an applied field for appropriate values of a geometric form factor. Intermediate values of the form factor are assumed by Fricke to represent oblate spheroidal inclusions of random orientation to the applied field. The major effect of suspended oil droplets of low relative permittivity in a continuous water phase of high relative permittivity, and vice versa, was seen to result in volumetric exclusion of one material by the other subject to effects of droplet size, shape and orientation. The Fricke model predictions for noninteractive constituent mixtures are based on the pure component properties of a continuous and a suspended phase. Also in these studies, an effect observed by Buck (1965) and Roebuck *et al.* (1972) in chemically interactive alcohol-water mixtures which results in synergistic loss, i.e.

the loss of a mixture is higher than the loss of either pure component, was predicted by an empirical combination of the Maxwell model for colloidal suspensions and the Debye models for polar liquids, based on pure component properties. The resulting empirical equations were designated as the Maxwell-Debye model for interactive mixtures. In view of these results, a physical-chemical basis for high-frequency dielectric behavior in liquid systems was suggested which related the biochemical constituents of foods, as broadly classified in proximate analysis, and their physical state, i.e. dissolved vs colloidal, to observed modifications of water behavior by the various classes of food constituents.

Based on these studies, it was conceived that dielectric behavior in solid foods is broadly related to chemical composition, activity, and structure, subject to complex variations with frequency and temperature, and may be generally predicted by considering physical-chemical properties of foods in terms of the interactions of their chemical constituents with water. The development of a predictive modelling basis then requires analysis of modifications to the dielectric properties of free water by individual constituents of foods. As a first approximation, high-frequency dielectric behavior in solid foods was seen as a function of two major variables, water and ionic activity, subject to other considerations of chemical composition and biological structure.

Chemical composition of foods is generally classified in proximate analysis by moisture, ash, lipid, protein and carbohydrate contents. These are given for a wide variety of raw, processed, and prepared foods by the U.S. Department of Agriculture (1963). General concepts of the relative dielectric activities of these constituents are of interest. That is, water and salts levels, as represented by moisture and ash contents, are seen to be dielectrically active at high frequencies in terms of free water relaxation and ionic conductivity. Lipids are generally inert and, except for the phospholipids, hydrophobic. Thus the lipid content of foods is expected to depress overall dielectric activity by the mechanism of volume exclusion seen for colloids. Proteins appear to have two levels of dielectric activity. High activity may result from surface charge effects due to ionization of carboxyls, sulfhydryls, and amines at reactive surface sites. These are subject to water binding effects by hydrogen bonding and binding interactions with salts. For example, Waugh and Noble (1965) have observed the formation of milk micelles of calcium-phosphate-protein complexes. Water and ion binding levels of proteins are generally determined by acid-base equilibria, as represented by Henderson-Hasselbalch equation given by Lehninger (1970). Dissolved proteins may possess net positive or negative charge, depending

on pH of their suspending medium with respect to a characteristic isoelectric point. Such behavior is shown by Lehninger to determine steric conformation and solubility of proteins and the number and quality of reactive sites available for binding interactions. The active nature of soluble proteins is of possible importance in analyzing anomalous relaxations and conductive effects in foods. But, proteins also exist in a dielectrically inert state due to conformational exclusion of water from substantial portions of their total mass by interactions between charged surface groups which give molecular folding. Hydrophobic regions of the folded molecule may thus exclude aqueous phases from considerable molecular volumes. High-frequency response due to protein content in high-moisture foods may then be largely determined by its colloidal nature with modification of aqueous phases in contact with charged surfaces through water and binding interactions. Carbohydrates may also exist in dielectrically active or inert states. Dissolved sugars and polysaccharides may be active, as seen in carbohydrate-water mixtures. However, particularly in vegetables, polysaccharide materials such as starch and cellulose may be highly colloidal and therefore may exclude large aqueous volumes.

The effects of biological structure impose a second order of variability on the prediction of dielectric behavior in solid foods. Foods are highly-differentiated multicellular tissues with solid and liquid phases of great structural diversity. Some aspects are considered based on information given by Clowes and Juniper (1968), Lehninger (1970) and Briskey *et al.* (1965). Food tissues of animals and plants are eukaryotic at the cellular level and may contain subcellular organelles which are membrane-bounded. Mammalian cells are characterized by a semipermeable membrane, while plant cells also possess a rigid polysaccharide cell wall and are highly interconnected by cytoplasmic bridges. Three structural regions are identified which may contribute to dielectric activity at essentially different levels. First is a bulk aqueous intracellular matrix, the cytoplasm, containing colloidal or dissolved salts, metabolites, enzymes, sugars, nucleic acids, and organelles. The cytoplasm may contain colloidal inclusions of storage materials, for example as starch in vegetables or as glycogen in muscle tissue. A second region of interest is the cell membranes and walls. The cell membrane is believed to be continuous with the endoplasmic reticulum, a center of biochemical activity in the cell, which appears to result from surface invagination to increase surface-to-volume ratios for transport of nutrients and waste products. A unit membrane hypothesis conceives a uniform structure for biological membranes in general composed of lipid-protein units and subject to biochemical control of permeability.

Membranes are characterized by three transport mechanisms controlling the flow of nutrients and waste products to and from the cytoplasm. Normal diffusion results from natural porosity due to concentration gradients across the membrane which is freely permeable to water. Facilitated diffusion is associated with membrane surface charge effects which provide coulombic forces to attract charged particles from bulk to surface regions. Active transport is seen as an enzyme-mediated process providing biochemical energy for modification of membrane permeability in the transport of larger molecules and is generally subject to genetic control. The membrane is seen to be highly charged by surface ionization and surrounded by a diffuse layer of counterions which give rise to surface potentials expressed electrokinetically as zeta-potentials. The cell wall of plant tissues is a porous open mesh of polysaccharide-protein subunits and may bind water by means of hydroxyl interactions or be ionized by acid-base reactions. Cell wall and membrane regions are seen to be the locus of unusual mechanisms of dielectric behavior in terms of molecular relaxation and conductive processes. A third region of interest is an extra-cellular bulk aqueous phase containing salts, nutrients and waste products and, in mammalian tissues, the complex fluid, blood. Blood contains high levels of dissolved proteins, including clotting factors and antibodies, and a variety of specialized cells, including erythrocytes and leukocytes, which provide gas-transport carriers and resistance to disease and toxins.

These factors suggest possible modifications of dielectric behavior in solid foods based on a variety of structural patterns observed in meats, fish, fruits and vegetables. Preliminary physical-chemical models involving the concept of a two-phase heterogeneous mixture of colloidal solids and aqueous ionic fluids were considered as a basis for such variations. One of these was obtained on a lumped-parameter basis:

$$K^* = K^*_c V_c + K^*_f V_f \qquad (1)$$

where K^*, K^*_c and K^*_f are the relative complex permittivities of the solid food, its colloidal solids and its aqueous ionic fluids, respectively, V_c is the volume fraction of colloidal solids and V_f is the volume fraction of fluids. This equation, designated in further discussion as the Distributive model, implies a structural configuration of uniformly distributed colloidal solids and aqueous ions and is seen to involve the possibility of fluid compartments at substantially different levels of relative dielectric activity, i.e. extracellular vs intracellular. Additional models based on two-phase heterogeneous mixtures of colloidal solids

and aqueous ionic fluids were obtained from the Fricke model in Maxwell and Rayleigh forms which suggest spherical and cylindrical colloidal inclusions, respectively. The Maxwell model may be expressed as:

$$K^* = K^*_c \left[\frac{K^*_f(1 + 2V_f) + 2K^*_c(1 - V_f)}{K^*_c(2 + V_f) + K^*_f(1 - V_f)} \right] \quad (2)$$

The Rayleigh model may be expressed similarly:

$$K^* = K^*_c \left[\frac{K^*_f(1 + V_f) + K^*_c(1 - V_f)}{K^*_c(1 + V_f) + K^*_f(1 - V_f)} \right] \quad (3)$$

Thus, three models for the prediction of dielectric behavior in solid foods were obtained for comparison with experimental measurements.

Raw potato was chosen as a model system for detailed studies to be reported in this work. The selection of a vegetable rather than a meat tissue as a model system for solid foods was based on several factors. First, potato cells are much smaller, more homogeneous and structurally less complex than meats. Physiologically, as suggested previously, vegetables have a more uniform structure, with cell membrane surfaces and cell walls interspersed with pectins which hold the cells together. Individual cells are cuboidal in shape, vary somewhat in size and include starch storage granules up to 30% of cell volume. These may be enzymatically degraded to give high free sugar levels under various conditions of aging and storage. Cells are interconnected by cytoplasmic bridges with interstitial free space permeated by extracellular fluids. The extracellular matrix is also more homogeneous and structurally simpler than meats. Second, vegetables are considerably more stable to deteriorative processes at high moistures due to low lipid content and a more limited microbial host range. Third, the data of de Loor and Meijboom (1966) and Pace *et al.* (1968) show higher loss levels in potatoes than most other solid foods measured including those of Bengtsson and Risman (1971). On the other hand, measurements of Van Dyke *et al.* (1969) and Bengtsson and Risman (1971) show generally lower loss levels in ground beef and other meats and fish. These data, in conjunction with carbohydrate-water measurements of Roebuck *et al.* (1972) showing synergistic loss effects suggest that high carbohydrate foods such as the potato would be the best systems in which to look for loss synergism as a significant effect in solid foods. Finally, a variety of meats and fish show anisotropic variation in

thermal conductivity, a closely related property to electrical conductivity, depending on muscle fiber orientation in the temperature field. Such behavior has been observed by Harper and Chichester (1960) and others. Anisotropic variation of dielectric behavior in meats and fish has also been demonstrated by Bengtsson *et al.* (1963).

Distributive, Maxwell and Rayleigh model predictions for two-phase heterogeneous mixtures based on physical-chemical properties were made for raw potato at frequencies and temperatures of interest for comparison with standing wave measurements. Similar predictions were also made based on proximate analysis data for comparison with raw beef measurements of dielectric constant and loss reported by To *et al.* (1974) by the standing wave method and with meat, fish and vegetable measurements of dielectric constant reported by Bengtsson and Risman (1971) by a cavity perturbation method. Additional measurements of complex conductivities at low frequencies were also made for raw potato to investigate the possible significance of relaxation and conductivity effects on high-frequency dielectric behavior. While de Loor (1968) has previously suggested that relaxation of free water and ionic conductivity may be the major determinants of dielectric behavior in solid foods at high frequencies, he also indicated the possibility of surface conductivity and bound water relaxation effects. Regions of dispersion indicated by Schwan and Cole (1960) for packed red blood cells are of interest with respect to this possibility. Measurements of intracellular and extracellular constituent levels in raw potato were also made to determine relative levels of biochemical constituents and their physical state.

EXPERIMENTAL METHODS

High-Frequency Dielectric Properties

Standing wave measurements at frequencies from 300 Mhz to 3 Ghz and temperatures from 5–65°C were made in water-sealed sample holders with temperature control by immersion of sample holders in a water bath heated or cooled to various temperatures and with internal flushing of center conductors by a circulating pump. At 5°C, the waveguide was flushed with dry nitrogen to prevent condensation of ambient moisture. Duplicate measurements were obtained for all samples. The method of Roberts and Von Hippel (1946) and the equipment used were described by Pace *et al.* (1968b). Calibration studies of sample holders were made to determine moisture loss levels for

measurements from 5–65°C. These showed that tight-fitting Teflon covers were required to provide a moisture seal between the cover and the center conductor. Similar studies of sample temperatures by a thermistor probe imbedded in a Teflon cover showed that sample temperatures during thermal measurements were within 1°C of water bath control temperatures from 5–65°C.

Low-Frequency Dielectric Properties

Lumped-circuit measurements of real and imaginary conductivities were obtained for solid samples by a capacitance bridge assembly for frequencies from 100 Hz to 18 MHz designed by Charles *et al.* (1966).

Total Cation Concentrations

Total calcium and magnesium concentrations were measured by a Perkin-Elmer (Norwalk, Conn.) model 303 atomic absorption spectrophotometer calibrated by standard concentrations of chloride salts with supression of interfering cations by lanthanum trichloride and hydrochloric acid at final concentrations specified by the manufacturer. Total sodium and potassium concentrations were measured by an Instrumentation Laboratory (Lexington, Mass.) model 143 flame photometer calibrated by standard concentrations of chloride salts with suppression of interfering cations by lithium diluent supplied by the manufacturer.

Free Cation Concentrations

Free cation concentrations were measured at temperatures from 25–55°C by ion-selective electrodes for sodium (model NAS 11-18), potassium (model 476235), calcium (model 476041) and divalent (model 1476235) cations and a single-junction silver/silver chloride (model 476029) reference electrode (Corning Glass Company, Medfield, Mass.). Temperature control was obtained in a constructed water-jacketed sample holder by a circulating water bath. Additional measurements at 25°C were made for cell-free potato extracts by an ion-selective electrode for potassium (model 92-19-00) and a double-junction reference electrode (model 90-02-00) filled with lithium trichloroacetate (Orion Research Associates, Cambridge, Mass.). Cation concentrations were calibrated by standard concentrations of chloride salts. Electrode potentials were measured on an expanded millivolt scale by a model PHM26C pH meter (Radiometer Company, Copenhagen, Denmark).

pH Levels

pH levels were measured on an expanded pH scale by the radiometer model PHM26C meter, with a glass electrode and a calomel reference electrode.

Moisture

Moisture content of whole potato samples were obtained in triplicate by the AOAC 22.003 (1965) vacuum oven method.

Ash Content

Ash content of whole potato samples were obtained in triplicate by ashing moisture samples by the AOAC 29.012 (1965) muffle oven method.

Protein Content

Total nitrogen content of whole potato samples were obtained in triplicate by the micro-Kjeldahl method of AOAC 38.010 (1970). Total nitrogen contents obtained were multiplied by 6.25 to obtain crude protein contents, as indicated in AOAC 7.016 (1970).

Soluble Protein Levels

Soluble protein levels of cell-free potato extracts were obtained in duplicate by the method of Lowry *et al.* (1951) for standard bovine serum albumin concentrations of 0, 25, 50, 75 and 100 μg/ml.

OD280/260 Levels

Soluble protein and nucleic acid levels in the potato extract were estimated from duplicate optical density measurements at 280 and 260 mμ. The method was calibrated by measuring extinction coefficients at both wavelengths for 100 μg/ml bovine serum albumin and yeast RNA solutions with calculation of soluble protein and nucleic acid levels by simultaneous solution of Beer's Law equations in two unknowns:

$$OD_{280} = \epsilon_{BSA}^{280} C_p + \epsilon_{NA}^{280} C_n \quad (4)$$

$$OD_{260} = \epsilon_{BSA}^{260} C_p + \epsilon_{NA}^{260} C_n \quad (5)$$

where C_p and C_n are the unknown concentrations of protein and nucleic acid, respectively. Measured extinction coefficients of the bovine serum albumin standard used compare closely with those of Warburg and Christian as given by Herbert *et al.* (1971), i.e., 4.32 v. 5.31 OD/λ at 260 mμ and 6.20 v. 8.95 OD/λ at 280 mμ. Measured extinction coefficients for the yeast RNA standard used were considerably higher than Warburg and Christian values, i.e., 34.0 v. 22.1 OD/λ at 260 mμ and 17.5 v. 10.8 OD/λ at 280 mμ. But, the measured extinction coefficient for yeast RNA at 260 mμ compared favorably with that observed by Ceriotti (1955) for an RNA obtained from animal tissue extracts, i.e., 30.0 OD/λ.

Reducing Sugar Levels

Soluble reducing sugar levels of cell-free and whole potato extracts were obtained in duplicate by the modified Somogyi alkaline copper reagent method of Nelson (1944) for standard concentrations of D-glucose of 0, 25, 50, 75 and 100 μg/ml. Whole potato extracts were obtained by the method of AOAC 7.058 (1970).

Total Carbohydrate Levels

Soluble total carbohydrate levels of cell-free potato extracts were obtained in duplicate by the anthrone reagent method of Herbert *et al.* (1971).

Starch Content

Starch contents of whole potato extracts were obtained in triplicate by the method of Nelson (1944). Whole potato extracts were obtained by the method of AOAC 8.017 (1970).

Cell-Free Potato Extracts

Cell-free extracts of raw potato were obtained as follows:

a. 690.8 g wet weight of whole raw potato (peeled) was fed to an electric grinder with holes approximately 6 mm in diameter. The ground potato was then filtered under vacuum and the supernatant centrifuged for 45 min at 25,000 g. Final extract volume was measured in a graduated cylinder as 152 ml.

b. 20.0 g wet weight of whole raw potato (peeled) was ground in a mortar and pestle for 15 min. The ground potato was then filtered under vacuum and the supernatant centrifuged for 45 min at 25,000 g. The final extract volume was measured in a graduated cylinder as 10 ml

and then diluted to 100 ml with deionized water to obtain enough material for biochemical and conductivity measurements.

Extract (a) was designated as the coarse grind extract and extract (b) was designated as the wet grind extract. A number of extraction procedures were evaluated in preliminary measurements including the Waring blendor, the Manton-Gaulin homogenizer, sonication, grinding with powdered alumina and dry grinding of freeze-dried potato samples. The wet grind method was chosen because released protein levels were highest without serious disadvantages observed in some of the other methods.

Estimation of Extracellular and Intracellular Levels in Cell-Free Potato Extracts

Extracellular and intracellular levels of dissolved proteins, reducing sugars, total carbohydrates, total and free cation concentrations, pH and conductivities were estimated by calculating extracellular and intracellular volumes by means of nucleic acid measurements in coarse and wet grind extracts. Soluble nucleic acid levels in these extracts were assumed to be associated entirely with the intracellular fluid contained by each extract. Computations based on this assumption led to the approximations that the coarse grind extract contained 94% extracellular and 6% intracellular fluids and that the wet grind extract contained 42% extracellular and 58% intracellular fluids. Extracellular and intracellular concentrations of the constituent levels were then estimated by simultaneous solution of the equations:

$$0.94C_e + 0.06C_i = C_c \tag{6}$$

$$0.42C_e + 0.58C_i = C_w \tag{7}$$

where C_e and C_i represent the extracellular and intracellular levels, respectively, of a particular constituent and C_c and C_w represent measured levels of the constituent in the coarse and wet grind extracts. On this basis, the volume fraction of total moisture for the potatoes extracted was estimated as 0.26 in extracellular and 0.74 in intracellular fluid, i.e., most of the moisture appeared to be contained in the intracellular regions of the potato. Total constituent levels were then projected to the whole potato level by weighting extracellular and intracellular concentrations by these volume fractions to obtain estimates of selected constituent levels for comparison with whole potato extract measurements.

Whole Potato Extracts

A dry ash extract was prepared by ashing 5.0 g (wet weight) of raw potato, adding a small amount of water and heating in concentrated hydrochloric acid at a low boil for 10 min. The extract was then filtered and diluted to 100 ml for assay of total cation levels (Hawke *et al.* 1963). A wet ash extract was prepared by extracting 5 g of raw potato in a 3:1:1 mixture of nitric, sulfuric and perchloric acids, incubating at 65°C for 45 min and diluting to 50 ml for final assay (Gorsuch 1959).

Raw Potato Samples

Uniform raw potato slices for dielectric measurements in solid sample holders were obtained in a micrometer-drive slicing fixture whose advance was controlled to within 0.01 mm by a Starrett Company (Athol, Mass.) model 263 M micrometer head. Slices of 0.140 cm nominal thickness were then measured in a test fixture by means of an accurately milled surface plate and a Starrett Company (Athol, Mass.) model 440 M depth gauge accurate to within 0.01 mm. Moisture and ash content samples for each potato slice measured were taken in triplicate from the same potato core as the measured slice. Potato cores for the cylindrical space of the slicing fixture were obtained by an accurately machined stainless-steel core cutter which was larger than required for the solid sample holders. Final samples of toroidal shape with a hole for the center conductor and of measured thickness were then obtained by an accurately machined stainless-steel "donut cutter" to give samples of the required shape and dimensions for solid sample holders.

Frequency and Temperature Variation in Raw Potato

Standing wave measurements at 300 and 915 Mhz and 3 Ghz and 5, 25, 45 and 55°C were made for Long Island potato slices to determine the effects of variation in frequency and temperature in raw potato. Similar measurements were made for a California variety at 3 Ghz and 5, 25, 45 and 65°C to compare species differences.

Raw Potatoes

Commercial Long Island and California potatoes were initially obtained. The moisture and ash content for these varieties as measured in triplicate samples are shown in Table 1. The Long Island variety was chosen for detailed studies because of its high moisture and ash contents.

Table 1. Moisture and ash content of commercial raw potatoes

Variety	% (Wet Weight)	
	Moisture	Ash
California	85.0	0.6
Long Island	85.0	1.0

RESULTS AND DISCUSSION

The noninteractive models described for prediction of dielectric behavior in raw potato were obtained by considering the potato as a two-phase system with a dielectrically inert solid phase and a dielectrically active liquid phase. The solid phase was assumed to consist of potato solids, as represented by carbohydrate, protein and lipid contents. The liquid phase was assumed to consist of aqueous ions at an effective salts concentration determined by conductivity measurements of cell free potato extracts obtained by coarse and wet grinding of raw potatoes. These indicated a bulk effective salts concentration of 0.12 M sodium chloride equivalents. Dielectric properties of the solid phase were obtained from measurements of finely ground freeze-dried (48 hr 100μ) potato as shown in Table 2. These values are essentially assumed to be independent of temperature and frequency. Dielectric properties of the aqueous phase were calculated by the Hasted-Debye models for 0.12 M sodium chloride. These values were then substituted in the Maxwell, Rayleigh and Distributive models to obtain predictions for comparison with raw potato measurements.

Table 2. Measured dielectric constants and losses of freeze-dried potato at 3 GHz, 5–65°C

Temperature (°C)	K′	K″
5	4.1	0.1
25	4.3	0.1
45	4.4	0.2
65	4.6	0.2

Constituent levels in cell-free extracts were measured for coarse and wet grind preparations. Extracellular and intracellular constituent levels were then estimated by the nucleic acid method previously described and projected to obtain volumetric average properties of the bulk liquid phase for comparison with selected whole potato extract measurements. Estimated extracellular and intracellular constituent levels are shown in Tables 3 and 4 and will be discussed in related groups to consider the effects of dissolved sugar and effective salts concentrations as a basis of loss modelling in the potato.

Table 3. Approximate constituent levels in potato extracts (mg/100 g wet wt)

Constituent	Cell-Free Extracts			Whole Potato Extracts
	Extracellular	Intracellular	Whole Potato	
Moisture	21000	59000	80000	80000
Ash				1000
Protein				
Folin	39	822	610	
OD280/260	2	990	730	3100
Carbohydrate				
Red. sugar	107	303	250	150
Total	247	536	459	16000
Nucleic acid	0	268	199	—

Table 4. Approximate cation levels in potato extracts (mg/100 g wet wt)

Constituent	Cell-Free Extracts			Whole Potato Extracts
	Extracellular	Intracellular	Whole Potato	
Total —				
K^+	69	390	306	339
Na^+	0	27	20	17
Mg^{++}	0	20	15	17
Ca^{++}	1	6	5	6
Free —				
K^+	57	310	243	
Na^+	0	27	20	
Divalent	0	28	21	
Weight fraction bound	0.18	0.18	0.18	

Extracellular-Intracellular Liquid Volume

As shown by moisture recovery, the wet grind extract contained approximately 63% and the coarse grind extract 28% of the total moisture in whole potato samples, determined in proximate analysis as 80.3% wet weight. Analysis of these results by the nucleic acid approximation showed about 26% of the total moisture to be associated with extracellular fluid and 74% of the total moisture to be associated with intracellular fluid. On this basis, differences between intracellular and extracellular constituent levels are weighted heavily by the intracellular fluid in determining bulk average constituent levels. The figure of 26% total moisture associated with the extracellular fluid was found to be consistent with a free space measurement of 29% as determined by mannitol uptake by the method of Briggs and Robertson (1957). Free space is defined as the volume contained by cell walls and any portion of extracellular space that is liquid or air-logged and thus freely accessible by diffusion to solutes in a suspending medium. Since mannitol is generally not taken up by active transport in vegetables due to lack of a permease, it is particularly suitable for free space measurements since bulk levels of high concentration may be assayed by the method of Graham (1963) for prolonged heating with 0.15% anthrone in concentrated sulfuric acid. Solutes taken up by active transport may also be used in free space measurements, however equilibration time for such solutes, e.g., sucrose, fructose and glucose, is critical and must be shorter than the transport enzyme induction period. From the data of Table 3, it is seen that critical concentrations for all constituents appear to be the intracellular levels. This is particularly important if dielectric behavior in composite mixtures with significantly different levels of dielectric activity is primarily a volumetric function, as suggested by noninteractive models.

Protein Levels

Extracellular protein levels are seen in Table 3 to be very low, as determined by Folin and OD280/260 measurements. Results of these assays are generally consistent, although OD280/260 measurements gave somewhat higher intracellular and lower extracellular levels. Total nitrogen measurements by the micro-Kjeldahl method indicate a total protein level of 3100 mg% as compared with 670 mg% for soluble protein in the whole potato. This suggests that much of the total protein in potato is in colloidal form. However, micro-Kjeldahl measurements were not corrected for nonprotein nitrogen which, as seen in nucleic acid measurements, is greater than 200 mg%.

Carbohydrate Levels

Extracellular reducing sugar and total carbohydrate levels are seen in Table 3 to be significant, i.e., 107 and 247 mg%, respectively. Intracellular levels are seen to be about twice these levels. Projected whole potato levels of soluble reducing sugars and total carbohydrates are consistent with normal potato levels reported by Talburt and Smith (1967). That is, total sugar levels in potato typically range from trace amounts to as much as 10% of dry weight. On this basis, maximum dissolved sugar content is calculated as 2.3% and dissolved reducing sugars as 1.3%, suggesting that the potatoes measured in this work were acceptable for processing since reducing sugars were less than 2%. Whole potato extracts showed a reducing sugar level of 150 mg%, which is appreciably less than projected for cell-free extracts. This may be due to the breakdown of intracellular starch granules in extraction, particularly for the wet grind extract. Talburt and Smith also point out that sucrose tends to accumulate in storage of potatoes at low temperatures so that the 1:1:1 ratio of sucrose:fructose:glucose generally observed in freshly harvested tubers may vary in storage to give higher nonreducing-to-reducing sugar levels. The 1:1 nonreducing:reducing ratio observed in projection of reducing sugar and total carbohydrate levels to the whole potato does not seem unreasonable for potatoes which may have been previously stored at low temperatures. Finally, the starch content of whole potato extracts is seen to be 14.4% wet weight, i.e., 90% of total carbohydrate measured as glucose equivalents in whole potato extracts. This is within the range of values given by Talburt and Smith and is consistent with a value obtained by the method of Von Scheele (1937) based on whole potato density. That is, an estimated starch content of 12.4% wet weight is obtained by the Von Scheele correlation for a measured potato density of 1.07 g/cm^3. As observed by Talburt and Smith, this correlation was derived for European potatoes and should be revised for American varieties.

It is concluded from these measurements that the possibility of significant carbohydrate-water interactions is very unlikely since maximum concentrations of dissolved sugars estimated for intracellular fluid are 0.3% wet weight for reducing sugars and 0.6% wet weight for total carbohydrates. These levels are not high enough to significantly affect loss behavior of the liquid phases, as seen in the data of Roebuck *et al.* (1972). On the other hand, colloidal starch levels are high enough to alter dielectric behavior of the liquid phase by the mechanism of volume exclusion as demonstrated by measurements of oil-water emulsions.

Total and Free Cation Levels

Total cation levels, as seen in Table 4, suggest that extracellular levels of sodium, magnesium and calcium are negligible. Potassium, the dominant potato cation, appears in the extracellular fluid at a level of about 57 mg%. On the other hand, intracellular cation concentrations are much higher — 390, 27, 20 and 6 mg% for potassium, sodium, magnesium and calcium, respectively. Thus, potato cations are concentrated intracellularly, with potassium as the predominant species. Whole potato projections of these levels are within 10% of values obtained in flame photometer extracts from whole potato acid digests, i.e., wet and dry ash extracts. These values also generally agree with typical cation concentrations reported by Talburt and Smith (1967) and USDA (1963).

Free cation concentrations measured by ion-selective electrodes suggest that sodium, magnesium and calcium are completely dissociated and that potassium is approximately 80% dissociated and 20% associated. The same binding level is observed in both the extracellular and intracellular fluids. Thus, binding interactions similar to those of milk are observed in the potato. But more importantly, intracellular cation concentrations are seen to be appreciably higher than extracellular levels, which suggests higher intracellular effective salts concentrations in the potato as a basis for loss behavior.

pH Levels

pH measurements of the potato gave an extracellular fluid pH of 5.2 and an intracellular fluid pH of 7.5. These results are surprising since they imply the possibility of net negative charge for intracellular surfaces and net positive charge for extracellular surfaces, depending on surface isoelectric points. They also indicate the possibility of membrane hydrolysis effects as observed by Hauser (1954) for colloidal electrolytes. These results suggest that further study of surface phenomena in food materials might be of value in analyzing relaxation and conductivity effects on low-frequency dielectric behavior as well as other phenomena. The effects of pH, per se, on dielectric behavior are considered negligible at normal levels in the potato since hydrogen and hydroxyl ion concentrations are too small to contribute directly to loss behavior through bulk conductivity.

Conductivity Levels

Measurements of cell-free extracts show effective salt concentrations in sodium chloride equivalents of about 0.13 M for the intracellular

fluid and 0.10 M for the extracellular fluid. Projected to the whole potato, a bulk liquid effective salts concentration of approximately 0.12 M sodium chloride equivalents was obtained. When the measured ash content of 1.0% wet weight was expressed in sodium chloride equivalents, a total salts level of 0.22 M was obtained. By considering the fraction of total salts bound to be 20%, as indicated by cation binding measurements, a dissolved salts concentration of about 0.18 M is obtained. The indicated bulk effective salts concentration is seen to be much lower than this. This suggests an additional complexing factor similar to that observed in milk. By dividing the difference between dissolved and effective salts concentrations in potato measurements by the total salts concentration implied by ash content, it was seen that total potato salts require an additional reduction of 26% of ash content as shown by Mudgett *et al.* (1971, 1974a). These results suggested a revised modelling basis for the potato which is discussed in the section on raw potato measurements which follows.

Proximate Analysis

Proximate analysis for the potatoes used in cell-free and whole potato extracts are summarized and compared with typical USDA values for raw potato in Table 5.

Table 5. Proximate analysis of raw potato used for cell-free and whole potato extracts

Constituent	Concentration (g/100 g wet wt)	
	USDA	Measured
Moisture	79.8	80.2
Ash	0.9	1.0
Protein	2.0	3.1
Carbohydrate	17.1	16.0
Lipid	0.2	—

Low-Frequency Potato Measurements

Dielectric properties for raw potato samples were obtained by conductivity measurements at 25°C for frequencies from 100 Hz to 18 Mhz. Dielectric constant and loss variations with frequency are shown in Fig. 1. These measurements suggest a minimum of four relaxations in the potato which are identified as regions A, B, C, and D. Both the

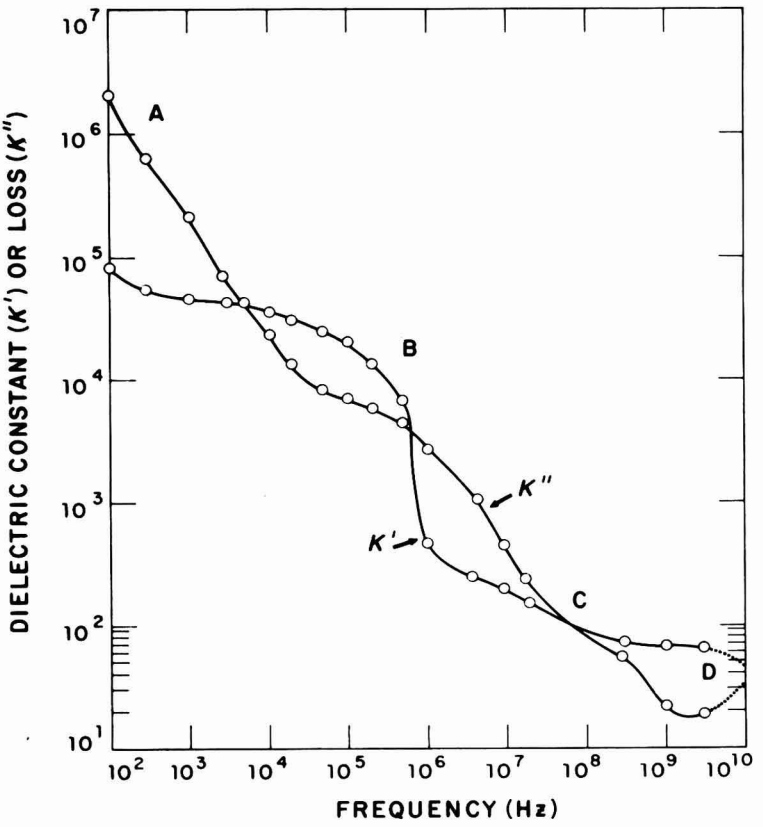

FIG. 1. MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW POTATO FOR LOW AND HIGH FREQUENCIES AT 25°C

dielectric constant and loss decrease with increasing frequency and regions of dispersion where relaxations occur are recognized as those between points of inflection on the dielectric constant curve as the frequency approaches the critical frequency of a particular constituent or group of constituents. The variation of dielectric constant in raw potato is seen to be remarkably similar to that observed for packed red blood cells as reported by Cole (1972). The comparison is shown in Fig. 2. Cole associates region A with surface effects or nonlinear behavior, region B with surface charge or conductivity and region D with the relaxation of free water. Region C appears to be omitted but is identified by Takashima (1969) and others as associated with the relaxation of bound water and dissolved proteins. It seems unlikely that regions A,

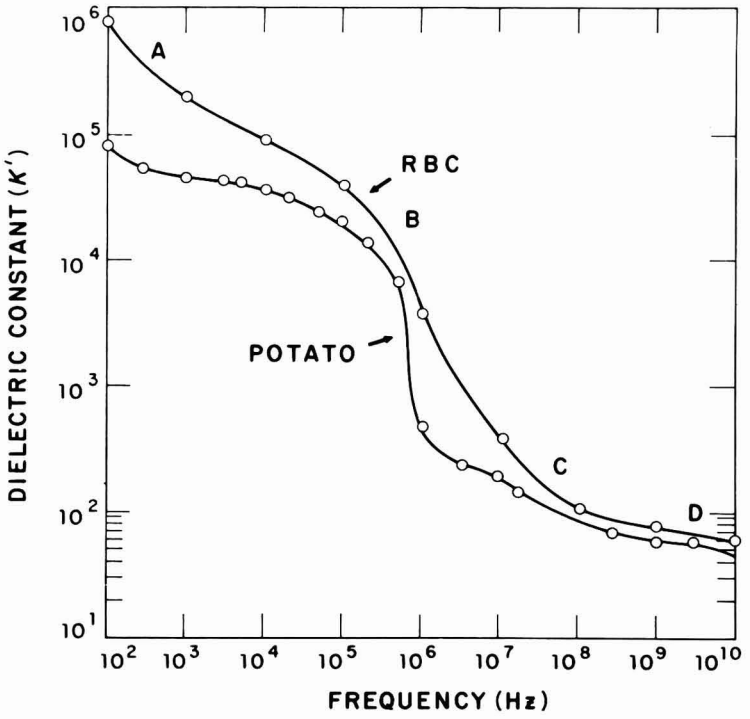

FIG. 2. COMPARISON OF VARIATIONS IN DIELECTRIC CONSTANT WITH FREQUENCY FOR RAW POTATO AND RED BLOOD CELL SUSPENSIONS (COLE, 1968) AT 25°C

B, or C contribute much to dielectric behavior at frequencies from 300 Mhz to 3 Ghz since the critical frequencies of relaxations at A and B are two decades or more below 300 Mhz and the relaxation in region C is very small, as seen for both dielectric constant and loss. These measurements suggest that the dominant mechanisms of dielectric behavior in raw potato at high frequencies are associated with the relaxation of free water, the conductivity of dissolved ions and the volumetric exclusion of aqueous ionic liquid phases by colloidal solids of low dielectric activity.

Cation binding levels and conductivities observed in cell-free extract measurements were used to obtain Distributive, Maxwell and Rayleigh model predictions by substitution of a bulk liquid phase with an effective salts concentration of 0.12 M sodium chloride equivalents based on observed binding and complexing effects of 20 and 25%, respectively. Raw potato measurements from 300 Mhz to 3 Ghz at 25°C were made for comparison with two-phase model predictions. Six or seven potato

slices were measured at each frequency to establish reproducibility of a micrometer-drive slicing fixture. Samples were a Long Island variety with a nominal moisture content of 85.0% and ash content of 0.8%. These measurements, as shown in Table 6, showed standard errors ranging from ±0.7 to 1.7 dielectric constant units and from ±0.2 to 1.4 dielectric loss units in terms of reproducibility at frequencies of measurement. Accuracy of standing wave measurements was estimated as approximately ±5% for both dielectric constant and loss. Thus, errors of measurement accuracy and sample reproducibility taken together suggest total errors of approximately 10%. Comparison of Distributive model predictions with these measurements show RMS errors on the order of 10%, indicating substantial agreement of measurements with predictions at 25°C.

Table 6. Error analysis of raw potato measurements and distributive model predictions from 300 MHz to 3 GHz at 25°C

Property	Frequency (MHz)	Predicted	Mean[a]	Standard Error	RMS Error
K′	3000	68	65	±1.7	±5.0
	915	69	66	0.7	3.0
	300	70	65	1.2	5.7
K″	3000	16	18	0.7	2.5
	915	21	21	0.2	0.6
	300	56	49	1.4	7.2

[a]Arithmetic mean of six or seven samples at each frequency

Temperature-Frequency Variations

Raw potato measurements from 300 Mhz to 3 Ghz, 5–65°C, were compared with Distributive model predictions for samples of Long Island variety as shown in Fig. 3–5, indicating substantial agreement at all frequencies and temperatures with Distributive model predictions. Similar results were obtained for California potatoes of 85.0% moisture and 0.6% ash contents.

Interpretation of Complexing Effects

The similarity in modelling results for milk and raw potato measurements in terms of observed "complexing" effects, described in electrophysical terms by Von Hippel (1965), suggests a general effect in aqueous phases of biological materials very similar to electrochemical

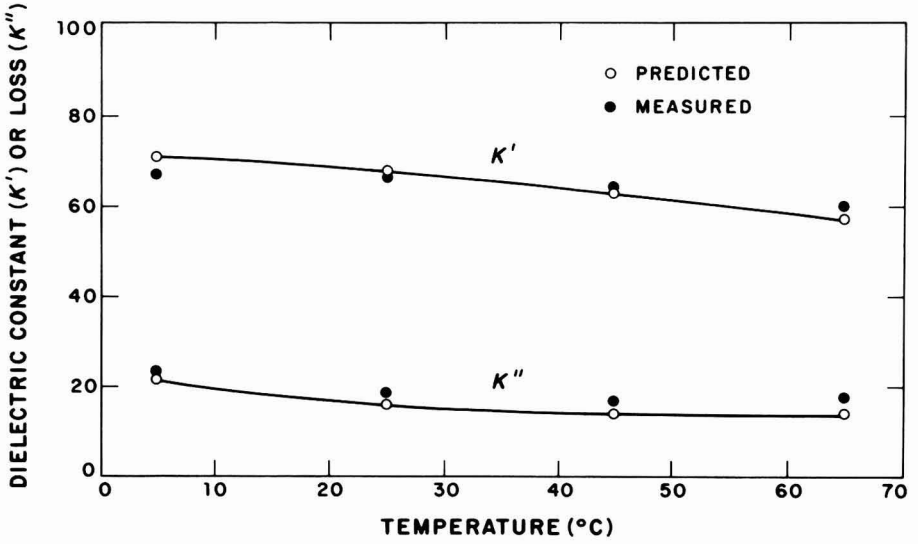

FIG. 3. COMPARISON OF MEASURED DIELECTRIC CONSTANTS, AND LOSSES IN RAW POTATO WITH DISTRIBUTIVE MODEL PREDICTIONS AT 3 GHz, 5–65°C

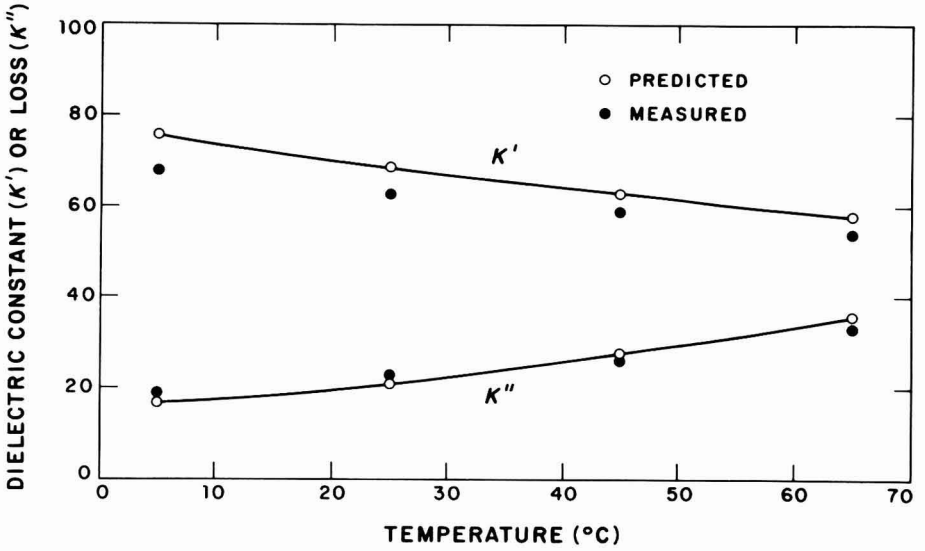

FIG. 4. COMPARISON OF MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW POTATO WITH DISTRIBUTIVE MODEL PREDICTIONS AT 915 MHz, 5–65°C

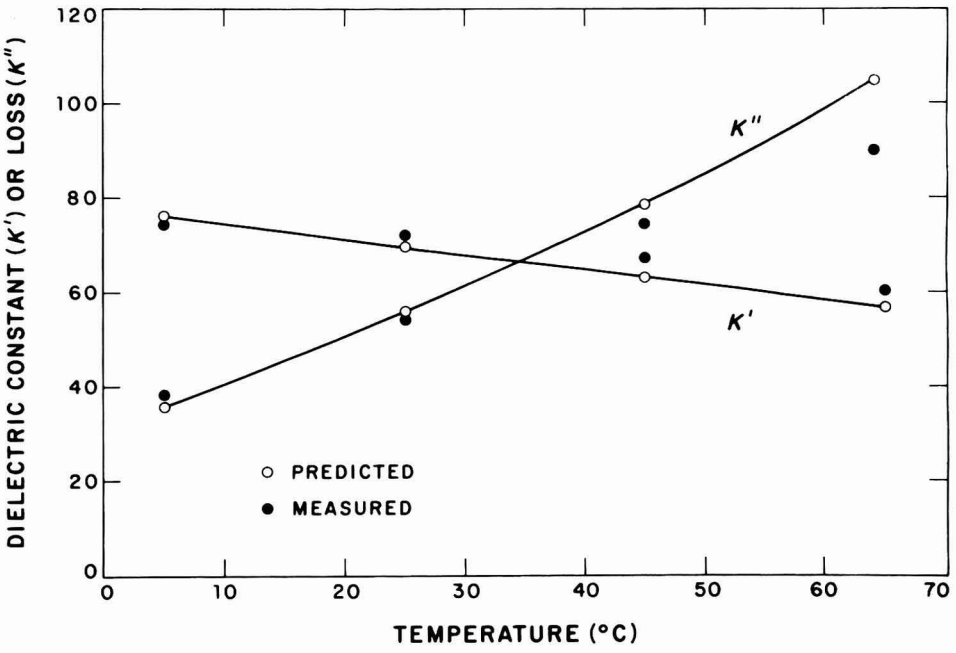

FIG. 5. COMPARISON OF MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW POTATO WITH DISTRIBUTIVE MODEL PREDICTIONS AT 300 MHz, 5–65°C

concepts of thermodynamic activity. That is, the activity of strong electrolytes below saturation levels is seen to increase nonlinearly with concentration due to reduced thermodynamic activity coefficients which are predicted for 1:1 electrolytes at dissolved salts concentrations up to 0.1 M by the Debye-Huckel equation. Thus, a possible quantitative basis is seen for observed complexing effects. To test this possibility, total salts concentrations implied by ash content in milk and potato were reduced by observed salt binding levels in cation binding studies to obtain dissolved salts concentrations. These were then multiplied by theoretical Debye-Huckel activity coefficients to obtain predictions of effective salts concentrations for comparison with those obtained by conductivity measurements. Results of this comparison, seen in Table 7 show reasonable agreement between predictions and measurements for both milk and potato. Observed effective salts concentrations are some 10% below the Debye-Huckel predictions. But the results are consistent with general concepts of thermodynamic activity and provide a quantitative approach to estimating effective salts

Table 7. Analysis of effective salts concentration in milk and potato

Concentration	Milk	Potato
Total salts[a]	0.2500	0.2200
Fraction unbound	0.64	0.84
Free salts[a]	0.1600	0.1850
Predicted activity coefficient	0.71	0.70
Effective salts — predicted[a]	0.1140	0.1300
Effective salts — observed[a]	0.1030	0.1210
Observed activity coefficient	0.64	0.65

[a]Sodium chloride equivalents per liter

concentrations in aqueous phases of liquid and solid foods, subject to additional corrections. Butler (1964) points out that predictions of thermodynamic activity coefficients at ionic strengths above 0.1, particularly for complex mixtures of electrolytes, are generally lower than observed levels due to Coulombic interactions of ionic fields at higher concentrations, which may be neglected in dilute solutions where the dissolved ions are well-separated.

High-frequency dielectric behavior of raw potato appears to be effectively determined by its physical-chemical properties in two dominant mechanisms of interaction between potato moisture and other potato constituents. Hasted-Debye model behavior is seen for a bulk liquid phase. Effective salts concentration of the liquid phase was approximated by reducing total potato salts implied by ash content by 20% to reflect ion binding effects and by an additional 25% to reflect thermodynamic activity or complexing effects. Thus, the behavior of a bulk aqueous phase, containing extracellular and intracellular regions of different conductivities, which appear to be averaged volumetrically, is determined by water and ion activities generally related to moisture and ash contents. A second dominant mechanism of interaction appears to result from volume exclusion effects in aqueous regions by inert colloidal solids which include most of the protein, carbohydrate, and lipid contents. The net effect of these mechanisms was seen to result in Distributive model behavior of the potato.

Measurements reported by To *et al.* (1974) for raw beef samples of 74% moisture and 1.1% ash contents from 300–2450 Mhz, and 5–65°C were also compared with Rayleigh model predictions for the same modelling parameters as raw potato. That is, total salts concentrations based on ash content were gratuitously reduced by 20% to reflect binding effects and an additional 25% to reflect thermodynamic

activity effects in a bulk aqueous phase and an inert solid phase was assumed to have the same properties as raw potato. The results of this comparison, as shown in Fig. 6–8, show substantial agreement between these raw beef measurements and Rayleigh model predictions at all frequencies and temperatures of measurement.

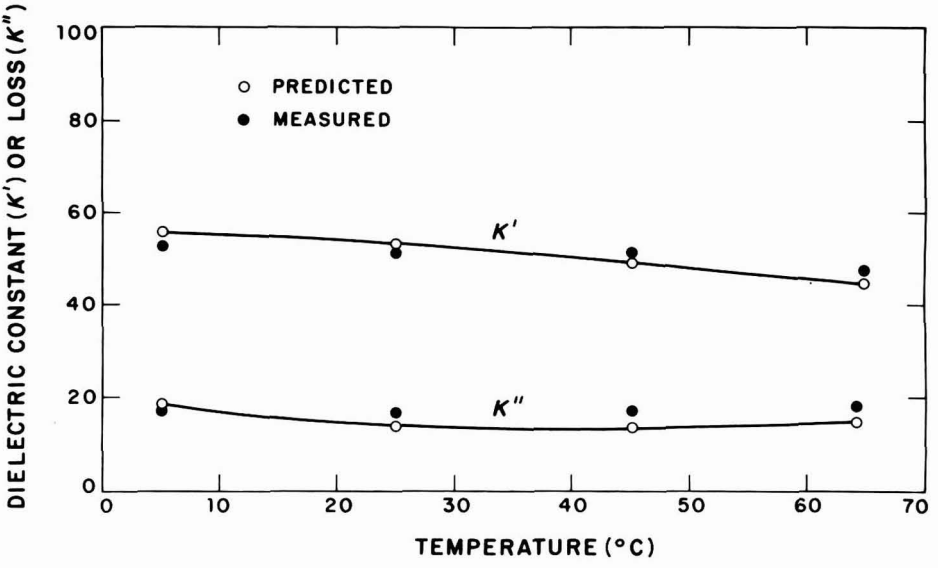

FIG. 6. COMPARISON OF MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW BEEF (TO *ET AL.* 1973) WITH RAYLEIGH MODEL PREDICTIONS AT 2450 MHz, 5–65°C

Observed correlation of measurements with the Distributive model suggests that potato solids may be concentrated in specific regions and are less disperse, than, for example, solids in raw beef whose dielectric behavior was predicted by the Rayleigh model. These differences appear to be related to biological structure. That is, the potato is composed of large cuboidal cells enclosed not only by a membrane but also by a relatively massive cell wall. Potato cells are highly interconnected by cytoplasmic bridges and pectinic materials. Thus, potato solids may be better represented by a model derived from lumped-circuit analysis based on an equivalent circuit of parallel liquid and solid phase impedances. On the other hand, the biological structure of raw beef muscle is more disperse and consists of bundles of long fibers with protein sheaths but without cell walls. Accordingly, dielectric behavior

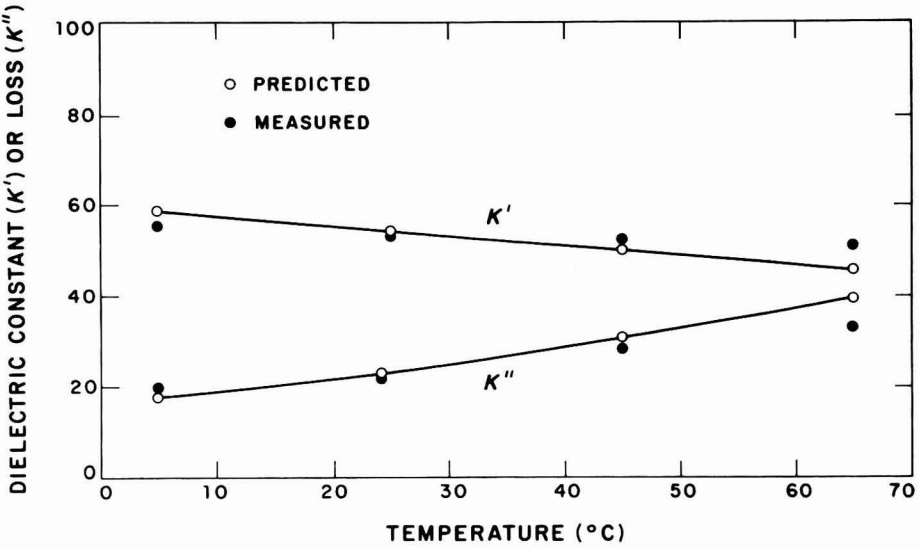

FIG. 7. COMPARISON OF MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW BEEF (TO *ET AL.* 1973) WITH RAYLEIGH MODEL PREDICTIONS AT 915 MHz, 5–65°C

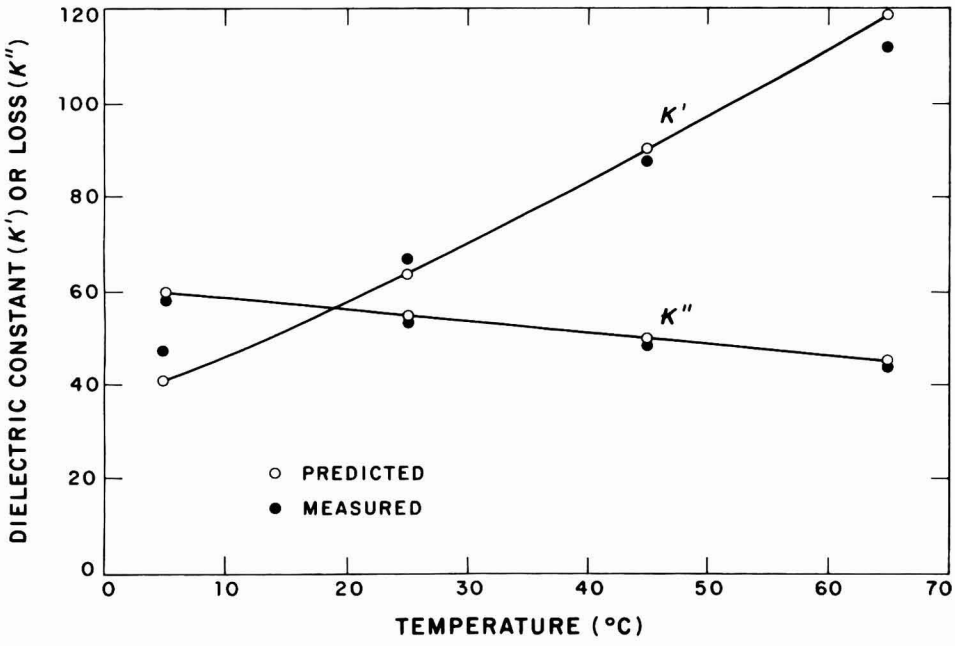

FIG. 8. COMPARISON OF MEASURED DIELECTRIC CONSTANTS AND LOSSES IN RAW BEEF (TO *ET AL.* 1973) WITH RAYLEIGH MODEL PREDICTIONS AT 300 MHz, 5–65°C

in beef muscle may be more consistent with a model based on long cylinders suspended in a continuous liquid phase. While mechanisms of interaction other than free water and salts, subject to the effects of colloidal exclusion, could contribute to dielectric behavior in solid foods, these concepts appear to offer a reasonable basis of prediction at high frequencies for a variety of solid foods.

Preliminary analysis of additional measurements reported by To *et al.* (1974) for raw turkey, chicken and pork from 300–2450 Mhz and 5–65°C suggests considerable variation in effective salts concentrations which imply essentially different binding or activity levels. The data of Bengtsson and Risman (1971) for meats, vegetables and fish at 3 Ghz could not be analyzed for loss behavior since ash contents or conductivities were not provided, however measurements of dielectric constant for selected samples show reasonable agreement with Fricke model predictions based on potato modelling parameters, as shown in Table 8.

Table 8. Comparison of measured (Bengtsson and Risman 1971) and predicted dielectric constants at 3 GHz, 25°C, for some solid foods

Food	Temp. (°C)	Model	Dielectric Constant	
			Predicted	Measured
Raw beef	0	Rayleigh	54.5	47.8
	20		53.2	47.7
	40		49.4	44.5
	60		45.4	36.5
Cooked cod	0	Rayleigh	57.7	46.0
	20		56.3	46.5
	40		52.2	44.5
	60		48.0	41.5
Raw pork	0	Rayleigh	54.8	47.3
	20		53.4	53.2
	40		49.5	52.2
	60		45.4	47.6
Mashed potato	0	Maxwell	64.6	66.7
	20		63.0	64.6
	40		58.4	59.3
	60		53.6	55.3
Cooked peas	0	Maxwell	66.5	64.3
	20		64.8	63.2
	40		60.1	58.9
	60		55.1	53.0

Thus, reasonable approximations of dielectric properties are seen to result for selected solid foods from their treatment as two-phase systems whose aqueous dielectric behavior is described by the Hasted-Debye models and whose colloidal properties are relatively constant. Structural factors related to colloidal particle shape, size and distribution and orientation in an electric field are generally seen to modify individual phase properties to give behavior which may be estimated at high frequencies by two-phase Distributive, Maxwell or Rayleigh models. While specific model behavior in solid foods cannot be predicted *a priori*, the range of model predictions may be sufficiently narrow to give reasonable approximations for most practical purposes by the Maxwell model. The Maxwell model gives values intermediate to those of the Distributive and Rayleigh models, as shown in Fig. 9 for a hypothetical solid food at 25°C, 0.3–3 GHz, with 20% solids content and an effective salts concentration of 0.1 M sodium chloride equivalents.

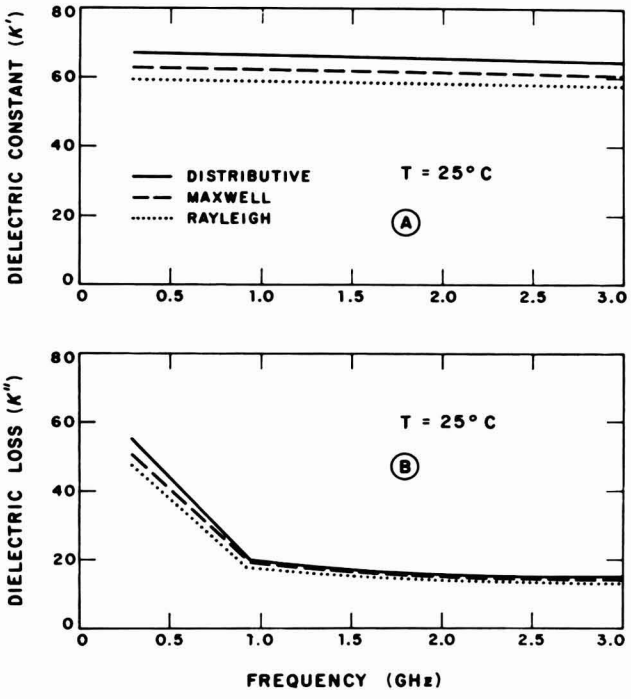

FIG. 9. COMPARISON OF VARIATIONS WITH FREQUENCY FOR DISTRIBUTIVE, MAXWELL AND RAYLEIGH MODEL PREDICTIONS OF DIELECTRIC CONSTANTS AND LOSSES AT 25°C FOR A HYPOTHETICAL SOLID FOOD

Estimating the effective salts concentrations in bulk aqueous phases of solid foods is a practical problem, particularly for meats and fish. A possible approach for these is to release the juices by uniform dielectric heating in a closed plastic container, as suggested by To (1974, personal communication) and to measure conductivities of centrifuged supernatants. For fruits and vegetables, measurements of cell-free extracts by the method used for raw potato in this work may provide reasonable estimates of bulk conductivity levels. Such methods require additional study to explore the significance of temperature-dependence in foods on ion binding and activity levels.

Based on the literature and the results of this work a generalized physical-chemical model is proposed to relate observed mechanisms of interaction between food biochemical constituents and water at high frequencies. This is shown diagrammatically for generalized aqueous and surface regions of foods in Fig. 10. In liquid and solid food systems, major dielectric activity is seen to be associated with a liquid phase, as represented in the aqueous region of the diagram. Behavior of such regions at high frequencies is believed primarily due to free water and salts concentrations subject to the effects of volume exclusion by dielectrically inert colloidal materials. The possibility of synergistic loss behavior in some foods is seen for high levels of dissolved carbohydrates, however such effects were not observed in milk or potato studies in this work or in preliminary analysis of measurements reported in the literature. The water in the model is in free or several associated states. Surface regions of dissolved proteins may bind water at various energy levels in a surface monolayer, seen in the diagram in irrotational or hindered rotational forms which depend on the number of hydrogen bonds between the molecule of bound water and reactive surface sites. Additional multilayers of bound water at successively decreasing energy levels are seen in structural regions near cell wall and membrane surfaces. Water is also seen to be bound in hydration sheaths or layers by dissolved counterions due to Coulombic interactions and by dissolved carbohydrates through hydrogen bonding between partially charged regions of water molecules and sterically available hydroxyl substituents. Total salts concentrations may be reduced by saturation effects in the aqueous region or by charge effects of dissolved proteins in the surface region, subject to control by acid-base equilibria. Surface effects may result in salts binding, particularly for polyvalent ions, or in free surface charge depending on pH levels reflected by hydrogen and hydroxyl ion concentrations, generally determined by Henderson-Hasselbach behavior of acids and bases. Aqueous and surface regions are generally seen to be electroneutral, with

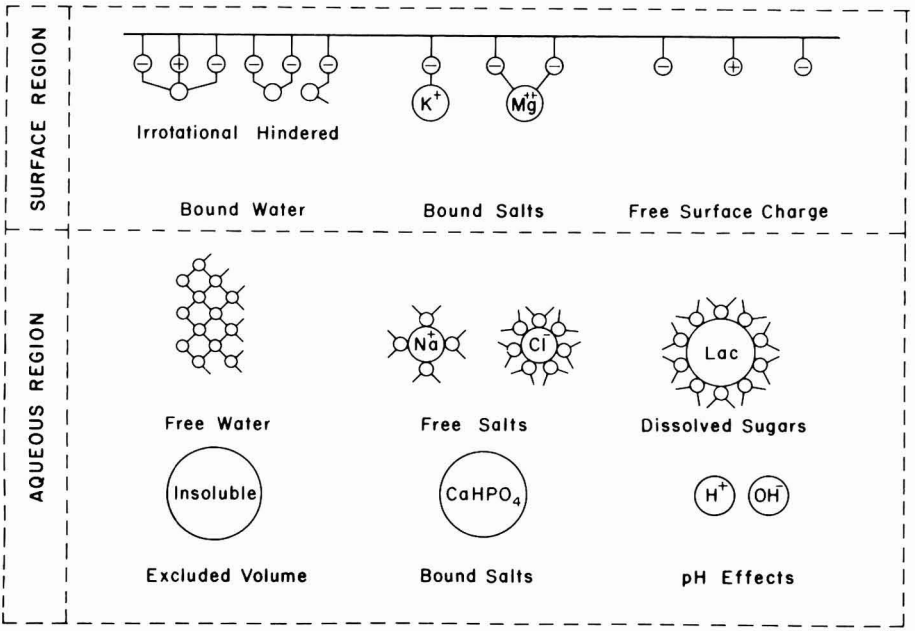

FIG. 10. A GENERALIZED PHYSICAL-CHEMICAL MODEL FOR MECHANISMS OF INTERACTION OF FOOD CONSTITUENTS AT HIGH FREQUENCIES

counterion distributions resulting from surface charge effects that give rise to surface potentials described by the Stern-Gouy double layer concepts and expressed electrokinetically as zeta-potentials. These are seen to have little effect on high-frequency dielectric behavior, per se, since dipole and ionic losses in aqueous and surface regions appear to be effectively determined by free water and ionic concentrations based on free water relaxation in terms of dipole rotation and ionic conduction in terms of free charge density. But at submicrowave frequencies, on the order of 10^8 Hz, free surface charge effects in terms of surface conductivities may result in relaxation of dissolved proteins to give twisting, bending and rotation of molecular segments in some native or denatured conformational state depending on the ionic environment. These surface effects are generally considered to be negligible in terms of dielectric behavior at high frequencies. The effects of pH, per se, are also considered negligible, because of electroneutrality and low hydroxyl and hydrogen ion concentrations in foods at normal pH levels. Other effects associated with the properties of an "excitable membrane" in terms of permeability and surface behavior appear to be

much more complex. But their relaxation and conductivity effects are observed at frequencies far below the microwave region. Extremely low or high temperature behavior associated with phase changes of water from liquid-to-solid or liquid-to-vapor states are not considered in the model and are beyond the range of the present work. But mechanisms of dielectric behavior may be considerably different at such temperatures and should be investigated further in terms of high frequency heating effects.

ACKNOWLEDGMENTS

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ANTIOXIDANT EFFECT OF SPICES, HERBS AND PROTEIN HYDROLYZATES IN FREEZE-DRIED MODEL SYSTEMS: SYNERGISTIC ACTION WITH SYNTHETIC PHENOLIC ANTIOXIDANTS

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ABSTRACT

Investigation of natural antioxidant and synergistic activity of some food ingredients included twenty spices, herbs and plant protein hydrolyzates. Of the spices tested, clove, cinnamon, sage, rosemary, mace, oregano, allspice and nutmeg were highly antioxidant and when coupled with BHA, these spices acted as strong synergists. Autolyzed yeast proteins (AYP), when combined with some of these spices, significantly extended the stability of the model food system.

Results of tests of freeze-dried model systems consisting of corn oil and carboxymethyl cellulose (CMC) stored at 65°C suggest possible applications for stabilizing oxygen sensitive foods against quality deterioration.

INTRODUCTION

To feed humanity in the near future, the food industry may have to depend more and more on engineered foods. New methods and materials will be needed to maintain high quality of these food products. At present, the accepted means of delaying the onset of oxidative rancidity is the addition of synthetic phenolic antioxidants such as BHA, BHT, TBHQ and others along with vacuum packaging. An additional technique uses palladium to catalyze the reaction of oxygen with hydrogen to form water to decrease O_2 to near zero levels. This method was shown by Tamsma *et al.* (1960) and by Bishov *et al.* (1971) to be effective in delaying the development of rancidity in dehydrated foods under this condition Tamsma *et al.* (1960) obtained foam-dried whole milk of high quality after long-time storage. Bishov *et al.* (1971) showed that dried vegetables such as carrots and spinach, which are

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extremely oxygen sensitive, retained their acceptability for one year at 37°C. Sweet potatoes, green beans, fish, chicken, beef and pork products also benefited from this treatment.

Recently, several investigators have shown that spices act as antioxidants. Lea and Swoboda (1957) found polyphenolic compounds in both green and black tea leaves. Chipault *et al.* (1951, 1955) showed that a large number of commonly used spices had antioxidant properties and more recently, Cort (1974) used a hemoglobin peroxidation test to screen a whole series of phenolic compounds for antioxidant activity. In a related study, Cort (1974a) showed that ascorbic acid and ascorbyl palmitate acted both as oxygen scavengers and as antioxidants. Chang *et al.* (1976) patented a method to separate some antioxidant compounds from rosemary and sage. Pratt and Watts (1974) and Pratt (1976) showed that flavone aglycones of some vegetable extracts had antioxidant activity and that naturally occurring plant phenolic compounds also acted as antioxidants. Watanabe and Ayano (1974) found antioxidants in water and in alcohol soluble fractions from some ground spices. Hirahara *et al.* (1974) observed antioxidant activity in a number of various spices on oils and fats. Tuomy and Fitzmaurice (1971) observed that several multicomponent freeze-dried rations, among them chili con carne, beef and rice, chicken and rice and beef stew, were unusually stable to oxidation. It was suggested that rice may be in some way involved in this stabilizing action. It is quite probable that in addition to that, protein hydrolyzates, resulting from the processing of beef and chicken meat and also the presence of the spices may have played an important role. Bishov and Henick (1967) showed the involvement of hydrolyzed vegetable proteins (HVP) derived from soy proteins in stabilizing dehydrated chicken soup powder.

The human race has used spices and herbs since antiquity to preserve scarce food supplies. While the primary effect was bacteriostatic, recent studies have shown that preservation was also accomplished by the antioxidant activity of some of the compounds present in these plant materials when these compounds acted synergistically with each other.

Webster's Dictionary defines synergism (from the Greek word "synergus") as working together. While this phenomenon has been frequently observed in nature, its mode of operation is not clearly understood. As it concerns the field of fat oxidation in foods, synergism was studied by a number of investigators. Olcott and Mattill (1936) achieved synergistic effects with several organic acids. However, Dutton *et al.* (1948) showed that much of synergism of these acids was due to their sequestering of the pro-oxidant iron and copper present as

trace metals in the oil. Dugan and Kraybill (1954) made an important observation; that when BHA and BHT were used together, the resulting stabilizing action was greater than when these antioxidants were used separately. This was called positive synergism. When BHT and PG were used, they provided greater protection when used separately. This was referred to as negative synergism. In another study, Dugan and Kraybill (1956) showed that the addition of BHA and the tocopherols beyond optimum level resulted in negative synergism. More recently, using freeze-dried model systems, Bishov and Henick (1974, 1975) found that plant protein hydrolyzates and autolyzates of yeast acted as antioxidants in stabilizing stripped corn oil; and when coupled with BHA and other phenolic antioxidants these plant products were significant synergists. The antioxidant activity of plant protein autolyzates and hydrolyzates is thought to be due to the amino acids and peptide groups.

The objective of the present research was to investigate the effectiveness of the natural antioxidants known to be present in many botanical species such as protein hydrolyzates, spices and herbs, mints and other plants. An additional objective was to determine the synergistic effectiveness of these natural antioxidants when acting in concert with BHA, BHT, α-tocopherol, caffeic acid and others.

EXPERIMENTAL

Materials

Commercial food grade spices and herbs shown below were supplied as powders[1] from McCormick Industrial Flavor Division.

Spices and Herbs

Clove	Nutmeg	Turmeric
Cinnamon	Marjoram	Paprika
Sage	Thyme	Garlic
Mace	Celery	Onion
Oregano	Red Pepper	White Pepper
Rosemary	Bay Leaf	Black Pepper
Allspice		

[1] To pass through #30 U.S. Standard Sieve.

Other plant materials which were used to test for antioxidant and synergistic activity, but which are unknown or are not normally used commercially are shown below.

Salvia triloba and *Tilia hrezentea*[2] are found in Greece and many other Mediterranean countries. Both are used in hot beverages. Their antioxidant properties are unknown. *Comptonia peregrina* (Sweet Fern) is a small bushy plant 3–4 feet high with fern-like leaves. It grows on poor soils in the temperate climates. Its antioxidant properties, commercial or other uses are unknown. The leaves of these plants and of alfalfa, black tea, peppermint, spearmint, and red clover were dried in an air oven at 65°C and pulverized to pass through U.S. Standard Sieve No. 30. The peeled orange was blended and freeze-dried.

Other materials are listed below with their commercial sources:

Other Materials	Source
Food grade autolyzed yeast protein (AYP)	Food Ingredient Division, The Nestle Company
Carboxymethyl cellulose (CMC)	Hercules Manufacturing Company
Stripped corn oil (low in tocopherols)	Distillation Products, Ind. Eastman
Butyl hydroxy anisole (BHA)	Eastman Chemical Products, Inc.
Butyl hydroxy toluene (BHT)	Eastman Chemical Products, Inc.
Tertiary butyl hydroquinone (TBHQ)	Eastman Chemical Products, Inc.
Alpha tocopherol	Eastman Chemical Products, Inc.
Plicatic acid	ITT Rayonier, Inc.
Caffeic acid	J. T. Baker Chemical, Inc.
Hydroquinone (HQ)	J. T. Baker Chemical, Inc.
Propyl gallate	Mallincrodt Chem. Works
3,5-di-tert-butyl-4-hydroxy anisole Lot #354 (Topanol)	ICI American, Inc.

METHODS

The method employed was based on a freeze-dehydrated model emulsion of sodium carboxymethyl cellulose (CMC) matrix upon which

[2] Dr. John G. Kapsalis supplied both *Salvia triloba* and *Tilia hrezentea* herbs.

the oxidizable substrate corn oil was deposited. Oxygen uptake in the flask was monitored periodically by gas chromatography.

The models, in duplicate aliquots, were prepared individually by adding in sequence (in a small blender cup 250 ml capacity) 50 ml deionized water, the antioxidant materials, i.e., spices, protein hydrolyzates and phenolic antioxidants as required, plus one gram CMC. These were blended for 2 min, followed by addition of 1 g of the corn oil. The complete mixture was then blended once more for an additional 2 min. The blended emulsion was transferred from the blender cup to a 250 ml round bottom flask, shell frozen in a bath of 95% ethanol and solid CO_2 (dry ice) and freeze-dried at 0.6 torr overnight. After breaking the vacuum with air, a serum type rubber stopper was stretched over the neck of the flask. After all the flasks were thus prepared, they were placed in a 65°C oven to accelerate oxidation. Oxygen uptake was determined by measuring the decrease of this gas in the flask by gas chromatography (Bishov and Henick 1966, 1975).

The end of the induction period (IP) was taken arbitrarily when an apparent 50% of the original oxygen had been consumed. Mean gas volume in the flasks used was 275 ± 2.6 ml. Air in the flask, with 20.9% oxygen, provides, at 27°C, 2.33 mMol of oxygen per gram of oil. At an apparent 50% depletion of oxygen, when the chromatographic determination indicates 10.45% oxygen, the actual oxygen remaining is equivalent to (0.1045) (79.1)/(1 − 0.1045) = 9.23% in the original air. The oxygen uptake at IP is then 2.33 (20.9 − 9.23)/20.9 = 1.30 mMol/g of oil. As corn oil contains ca. 50% linoleic acid, at IP, ca. 80% of the linoleate present has been oxidized.

Reproducibility of IP was determined both for control and for one antioxidant, 0.5% AYP and spice (oil basis). Eight flasks of each were prepared separately as described; all flasks were prepared and oxidized during one run. For n = 8, the mean IP for control was 28.0 ± 1.51 hr, and for AYP was 56.9 ± 1.88 hr. Over a period of several weeks, duplicate determinations of IP were made using 0.01% of seven different phenolic antioxidants. Each flask of each pair was prepared separately as described; each was prepared and oxidized in a different run. The total range of IP was 49–68 hr; the differences between duplicates were from 0–7 hr; mean difference between duplicates was 3.6 hr. It is apparent from this data that reproducibility among like samples is within ±5%.

At 65°C, 1 hr is equivalent to approximately one to two days at 20°C, assuming a Q_{10} of 2 to 3. However, the presence of antioxidants may change the Q_{10} as compared to control by 50 to 200% (Ragnarsson and Labuza 1977). The study at 65°C can still be used to

compare the systems, since the actual protection at 20°C would increase. This method enables the completion of a large number of samples with many variables within one experiment in 4–10 days. Stability factor (SF) was calculated as a ratio of hours of IP of sample to that of control.

Percent synergism was calculated as follows:

$$\text{Syn } \% = 100 \frac{(m - c) - [(p - c) + (a - c)]}{(m - c)}$$

Where m = IP of combined treatment; p = IP of the phenolic antioxidant; a = IP of spice, protein hydrolyzates, etc.; c = IP of control; IP = Induction Period — time for 50% uptake at 65°C in hours.

RESULTS AND DISCUSSION

Figure 1 stability factors show that most of the spices have significant antioxidant activities. Among those which had strong synergistic activity with BHA were clove, cinnamon, sage, mace, oregano and rosemary. Among other plants which are not known for their antioxidant activity (with the exception of black tea leaves, Lea 1957), but which had strong antioxidant activity were *Salvia triloba*, *Tilia hrezentea*, sweet fern, peeled orange, spearmint, peppermint, alfalfa and red clover. The phenolic antioxidant BHA, was chosen for these experiments on the basis of our past experience (Bishov and Henick 1975) and because of its wide use in foods. As shown in Fig. 6, it proved to be one of the best in the test model system. A positive relation between strong antioxidant activity of the spices and consequent synergism with many phenolic antioxidants was observed. The reasons for this are not clearly understood.

Spices, herbs and autolyzed yeast proteins (AYP) were used in the experiments to compare the relative antioxidant activity of these food components. It was previously shown (Bishov and Henick 1974, 1975) that plant protein hydrolyzates acted as strong antioxidants and in concert with some phenolic antioxidants, i.e., BHA, as significant synergists.

Relatively low levels (less than 2% oil basis) of spices and herbs were needed for maximum effects. This is exemplified by *Salvia triloba* (Fig. 2), an herb in the sage family and by several other spices and plants shown in Table 1. At the levels shown, there was no perceptible carry-

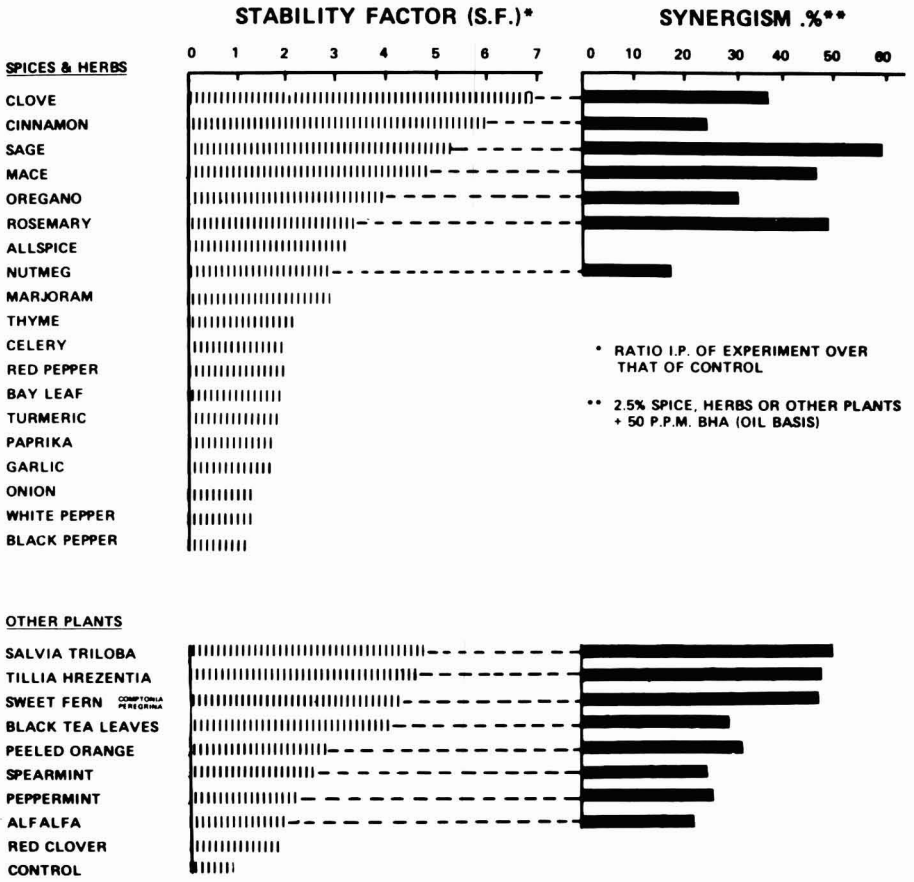

FIG. 1. ANTIOXIDANT ACTION OF SOME SPICES AND THEIR SYNERGISM WITH BHA

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

over of the aroma of the spice in the dried model. This was established by smelling and tasting the freeze-dried models before incubation. Above these levels, no further significant increase in synergistic activity was observed. In a similar manner, the synergistic activity of BHA declined with increasing levels. An increase from 50 to 100 ppm caused decline in synergism as shown by the displacement of the whole curve to lower values in Fig. 2.

Experiments with both the protein hydrolyzates and the spices showed that addition of these beyond 1–2% resulted in only small

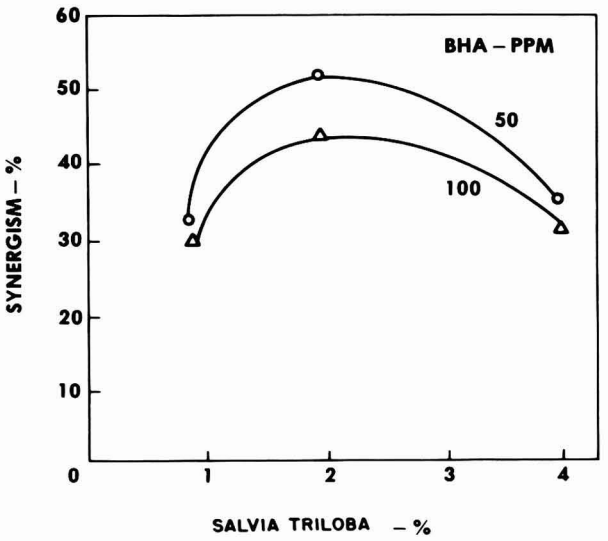

FIG. 2. SYNERGISTIC ACTION OF HERB-SALVIA TRILOBA WITH BHA

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

increase in protection (Fig. 3). Several levels, each of AYP and of *Salvia triloba* were coupled with BHA. Generally, the spices showed higher antioxidant activity than the protein hydrolyzates. It can also be seen in this figure that at levels beyond 50 ppm BHA, there was no increase in antioxidant activity. Fig. 4 confirms these results with other spices and AYP. Mean value of the antioxidant activities of the top ten spices shown in Fig. 1 were compared to control with and without hydrolyzed protein AYP. Generally, the spices were more efficient than the protein hydrolyzates at all levels. It is postulated that the spices contain, in addition to phenols, many other compounds which may interact synergistically with BHA. The breakdown products of AYP on the other hand are largely amino acids and other protein fractions. A number of amino acids were shown to have antioxidant activity (Marcuse 1962; Karel *et al.* 1966; and Bishov and Henick 1975).

Experiments to determine the solubility of the active spice antioxidants, showed that these compounds were soluble in both 95% ethyl alcohol and in water. Table 2 shows that the alcohol extracts of rosemary, sage, clove, mace and cinnamon, with the exception of allspice contained most of the antioxidants. It is worth noting that only

Table 1. Synergistic effects of some spices and other plants with BHA

		BHA—%				
		0	0.005		0.01	
	%	SF[1]	SF	Syn.[3] (%)	SF	Syn. (%)
Sage	0.0	1.0[2]	1.9	—	3.0	—
	2.5	5.2	13.5	59	13.0	32
	5.0	8.9	13.2	33	—	—
Mace	0.0	1.0	1.9	—	3.0	—
	2.5	4.8	7.3	48	7.5	14
	5.0	6.0	8.0	39	—	—
Rosemary	0.0	1.0	1.9	—	3.0	—
	2.5	3.5	7.6	50	7.0	25
	5.0	4.8	7.3	48	—	—
Salvia	0.0	1.0	1.9	—	3.5	—
triloba	2.5	4.7	9.9	48	8.6	44
	5.0	6.0	10.4	37	9.5	30
Tilia	0.0	1.0	1.9	—	2.8	—
hrezentia	2.5	4.6	9.3	46	8.0	23
	5.0	7.1	12.7	40	—	—
Comptonia	0.0	1.0	1.9	—	2.7	—
peregrina	2.5	4.3	9.9	45	8.4	57
(Sweet Fern)	5.0	6.3	11.7	42	10.9	53

[1] SF — Stability factor ratio of induction period (IP) of sample to that of control
[2] SF Control = 1; range of IP, 25–30 hr at 65° C
[3] See equation for calculating percent synergism

allspice had a lower stability factor than the other spices in the alcohol extract. Water soluble antioxidants were found in sage, oregano, thyme and clove. Strong antioxidant activity and synergism of *Comptonia peregrina* (Sweet Fern) with BHA were recorded in Table 3. Figure 5 shows that water extracts of clove contained most of the antioxidant activity of the whole clove. It is of interest to note here that the antioxidant activity of these plant materials was measured in an oil-in-water emulsion.

Figure 6 shows the synergistic activity of sage and AYP with several of the phenolic antioxidants. The data in this figure show that both the protein hydrolyzates and the spices were effective synergists with these antioxidants. Figure 6 also shows that synergism varies widely with the phenolic antioxidants. BHA and BHT are among the most effective when combined either with the spices or the protein hydrolyzates. The

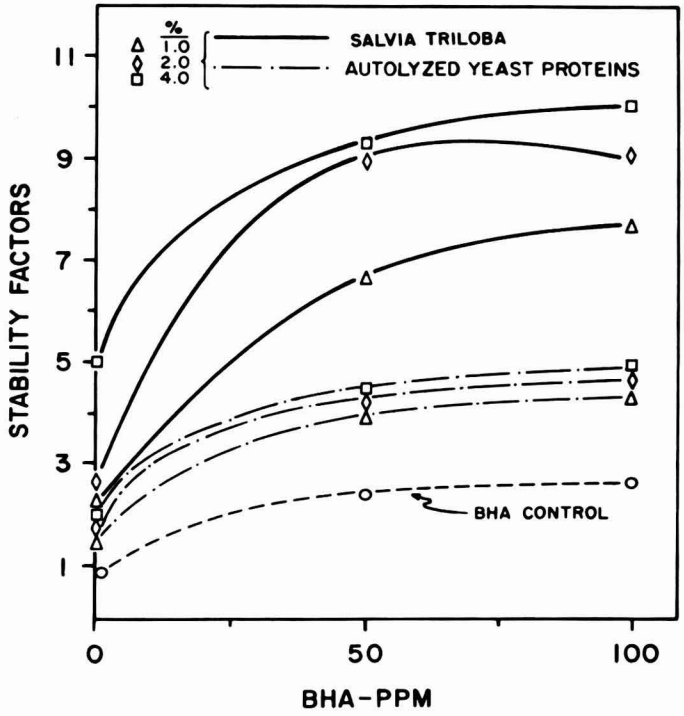

FIG. 3. ANTIOXIDANT ACTION OF SALVIA TRILOBA AND AUTOLYZED YEAST PROTEINS WITH BHA

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

elucidation of the reasons for these differences in antioxidant activity would provide an interesting investigation.

CONCLUSIONS

This work suggests that many food ingredients including spices, herbs and protein hydrolyzates may find use as antioxidants especially in low moisture food systems. In addition, these food ingredients also exhibited strong synergistic activity with many synthetic phenolic antioxidants.

The implications of these findings are that equivalent protection of food fats against rancidity could be achieved with much smaller quantities of the synthetic phenolic antioxidants such as BHA, BHT, and others when used in concert with spices, herbs, and plant protein hydrolyzates in the formulation of prepared foods.

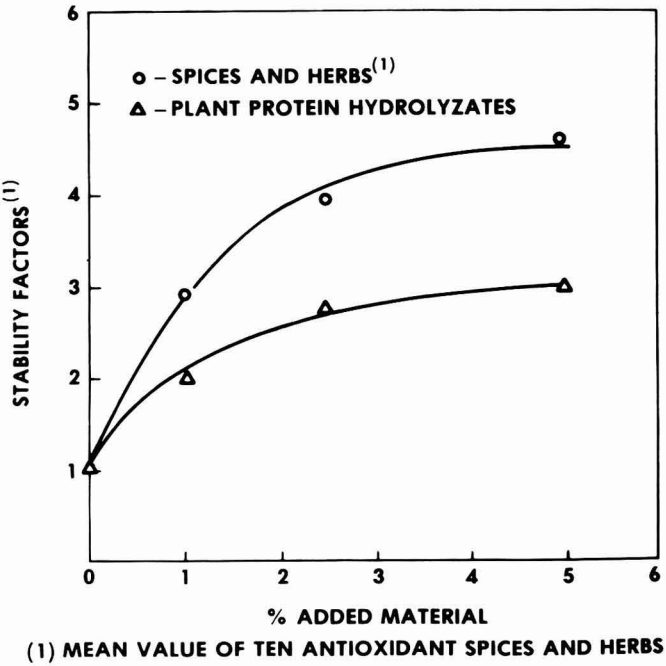

FIG. 4. ANTIOXIDANT ACTION OF SPICES AND HERBS AND PLANT PROTEIN HYDROLYZATES IN CORN OIL BASED DRY MODEL FOOD

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

Table 2. Antioxidant activity of alcohol[1] extracted spices

Spices	Soluble[2] SF	Insoluble[3] SF
Rosemary	7.0	1.2
Sage	5.7	1.2
Clove	3.8	1.2
Mace	3.4	1.3
Cinnamon	2.5	1.6
Allspice	1.2	2.1

[1] 95% ethyl alcohol
[2] Soluble fraction solids < 20% of whole spice
[3] 2.5% (oil basis)

Table 3. Antioxidants and synergism of *Comptonia peregrina*[1] with BHA

	BHA—%		
	00	0.005	
Alcohol Extract Solids[2] %	IP[3]	IP	Synergism %
0.00	30[5]	60	—
0.18	40	125	58
0.45	86	148	27
0.90	98	190	39
1.80	166	290	37
			MV[4] = 40

[1] Sweet Fern — 18% solubility in alcohol. Alcohol soluble solids
[2] Oil basis — 95% ethyl alcohol extract solids
[3] IP — induction period — hours at 65° C for 50% of O_2 uptake
[4] MV — mean value of synergism
[5] Control

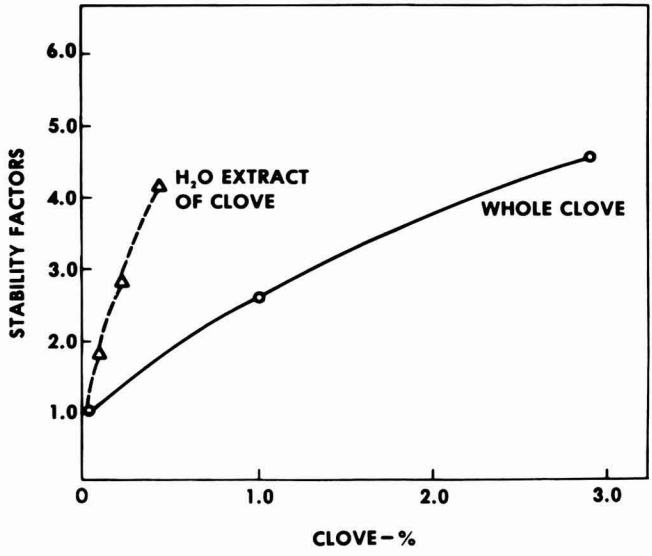

FIG. 5. ANTIOXIDANT ACTION OF CLOVE AND OF CLOVE WATER EXTRACT

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

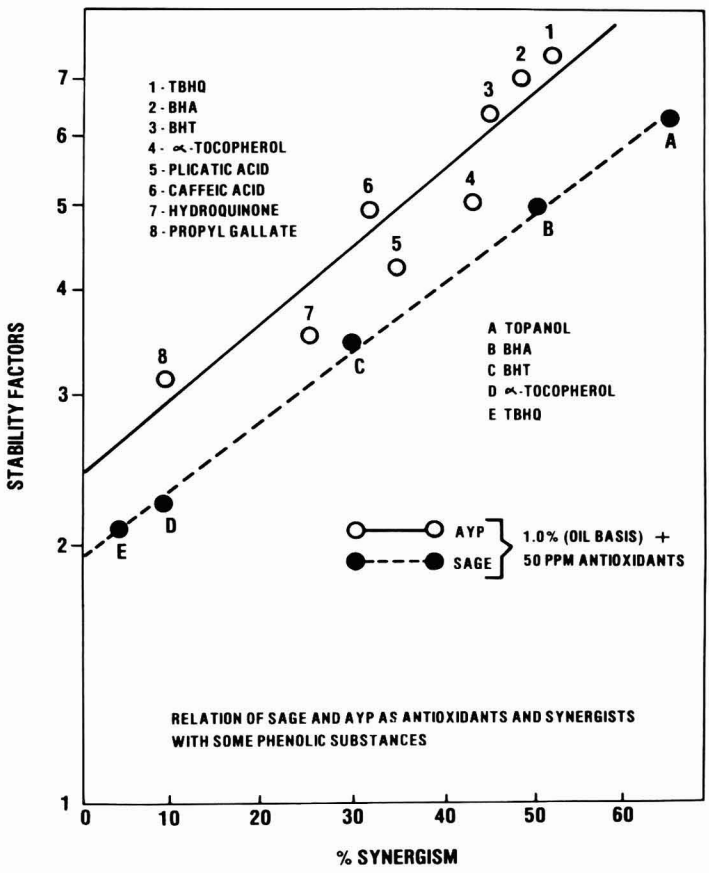

FIG. 6. RELATION OF SAGE AND AYP AS ANTIOXIDANT AND SYNERGISTS WITH SOME PHENOLIC ANTIOXIDANTS

In all cases, corn oil—CMC freeze-dried oil-in-water emulsion was used to measure antioxidant activity.

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THE PROPERTIES OF WATER IN RELATIONSHIP TO WATER BINDING IN FOODS: A REVIEW[1,2]

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Considerable evidence exists that water does not function just as an inert solvent in terms of the functional properties of food materials. In fact, water behaves as a very unusual material that contributes to the structure of foods and controls the sensory, physical and chemical properties with respect to changes in quality. This review will examine some of the properties of water with respect to both macromolecules and food systems in terms of how the properties relate to the water binding properties of foods.

Natural and synthetic foods as consumed vary in moisture content significantly. Table 1 lists various classes of foods and their moisture range. As seen, the range is large. Fresh tissue foods can contain anywhere from 60 to 98% moisture whereas dehydrated foods usually contain less than 10% water. Between these two extremes are foods that vary over the total range of moisture. Each of these foods has a texture that is unique to it. For example, processed cheeses can vary in texture from hard Brick to an extremely soft Brie, with a difference in moisture content somewhere between 20 and 60% water. This variation contributes to the difference in texture of the cheeses, but the difference may not be solely a dilution factor. In some tissue foods such as fruits and vegetables, the loss of water by wilting results in loss of the crisp texture of the food. On the other hand, a dry crisp potato chip becomes soft as it gains moisture.

The properties of water that are of interest with respect to food texture are:

(1) The water holding capacity or amount of water held in the food under various conditions.

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[1] Adapted from a presentation at the Second Annual Summer Program on Physical Properties of Food, Texturization of Food, 1973 — University of Massachusetts, Amherst, MA

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Table 1. Examples of textured foods

1. Meat and Fish (60–80% water)
2. Fruits and Vegetables (90–98% water)
3. Cheese (20–60% water)
4. Bread (20–30% water)
5. Cereals and Snack Products (2–10% water)
6. Candy (2–10% water)
7. Dehydrated Foods (2–5% water)
8. Intermediate Moisture Foods (25–40% water)

(2) The plasticity that the water creates in the food in terms of its degree of flowability.

(3) The effect that water has on binding together of the various ingredients of food to give the desired texture.

(4) The properties of water that help stabilize both foams and emulsions.

(5) The effect of dissolved solutes on the viscosity and surface tension of the water in the food system.

Many methods have been used for determining the degree of binding of water in foods which frequently give quite different values. These include:

(1) The water activity or relative vapor pressure of water in the food (Labuza 1968).

(2) Use of nuclear magnetic resonance which describes the degree of vibrational and rotational freedom that the water molecule has in a food system (Shanbhag *et al.* 1970).

(3) Calorimetric measurement, such as differential scanning calorimetry or differential thermal analysis which uses the effect of heat to measure the ease of removal of water from foods (Duckworth 1971; Sussman and Chin 1966).

(4) Dielectric measurement which measures the freedom of a water molecule to vibrate within a system at a specific frequency (Roebuck *et al.* 1972).

(5) The effect of pressure on squeezing water out of a food. This has been an age old method to determine the water holding capacity or binding of water in meat systems (Hamm 1963).

(6) Centrifugation, as in the pressure method, is the use of a force to remove water from food, in this case, spinning a sample very rapidly to create a gravitational field and measuring the amount of water lost.

(7) The measurement of the degree of syneresis. In this case, a gel is

formed using a particular macromolecule such as protein, and then it is allowed to set and the amount of water that leaks out of the gel gives a measurement of the binding capacity of the water-macromolecule interaction.

(8) The determination of the freezing curve of foods (Moy *et al.* 1971). In this calorimetric measurement, through calculation of the freezing point depression, one can measure the amount of water that is unfrozen in a food system below the normal freezing temperature. This unfrozen water is considered as bound water.

(9) The determination of water sucked out of the food by some dry inert material such as filter paper (Lewicki and Labuza 1977).

The results may be used to explain the ability of water to be held or bound in the food through water-food interactions. For example, one such phenomenon is gelation. It is possible to make food gels of less than one percent solids. For many gels very little water leaks out of the system upon standing. Calculations show that the distance between the macromolecules is very large so there is a tremendous amount of free space available for water to move about in the gel. In addition, experiments with diffusing solutes in gels show that the water has the properties of almost pure bulk water. However, the water does not leak out, due to either some unusual weak long range force or to capillary suction in the pores formed between the macromolecules. Similarly, when a fruit or a vegetable or a piece of meat is cut, not very much water leaks out. This water is held in the cells or between cells as a result of these same properties.

Hermansson (1972) concluded that all properties such as swelling, solubility, viscosity and gelation resulting from water-protein interactions seem to be dependent on the balance between attractive and repulsive forces. Hamm (1963), in reviewing the conditions necessary for immobilization of free water within colloidal systems, admitted that he could not explain the true character of the forces involved. He divided high water content foods into two classes; namely, thermoreversible secondary valence gels, where hydrogen bonds crosslink macromolecules in a three-dimensional structure, and principal valence gels, which are thermo-irreversible and bound together by multivalent cations or salt bridges. If these latter macromolecules are brought in close proximity, not enough space is left for the immobilized water, and syneresis occurs, whereas if intermolecular cohesion becomes too weak, the gel becomes a colloidal solution.

In pectin gels, hydrogen bonds must be formed between carbonyl oxygens and —OH groups of polygalacturonic acid molecules for the gels to form. The gel may also form if unesterified carbonyl groups are

ionized and bridged by divalent cations. Repulsion of negative charges in a high methoxyl gel may prevent gel formation at high pH where no calcium is present. At very high calcium ion concentrations, insoluble calcium pectinate precipitates out and the gel is destroyed. Sucrose is usually added to form gels of high methoxyl pectin at low pH. The sucrose is said to cause an orientation of the water dipoles, which attract each other mutually and cause stiffening of the gel. Sucrose is also said to form H-bonds with pectin molecules between their hydroxyl groups or to aid in gel formation by attracting water molecules at the surface of pectin molecules, thus allowing these to interact with each other to form a linked network of molecules.

Starch gels differ from pectin gels in that unless they are modified they have no ionizing groups. However, the same kind of considerations apply as regards hydrogen bonds between starch molecules to give the gel its structure and power to immobilize water. Of course the extensive internal bonding of the starch granule has to first be broken down by heating before any water can be imbibed. A certain amount of amylopectin which is branched is also required to form a three-dimensional network in the gel since amylose is linear.

Gelatin, as an example of a typical protein gel, differs from polysaccharide gels in that bonding is possible only at distinct remote sites. The greatest part of the bonding is due to the H-bonds between C—O groups and —NH groups of peptide linkages and not the electrostatic bonds. This was deduced because chemical modification of the polar groups did not greatly influence the mechanical properties of these gels, whereas gelation could be prevented by blocking the peptide groups by a biuret-like reaction with Cu^{+2} ions at low ionic strength and gelatin concentrations. Electrostatic bonds do have an effect on gelation, and too large a number of positively charged groups (pH much less than pI) or negatively charged groups (pH much greater than pI) can prevent gelation.

The amount of "free" but immobilized water in muscle tissues is strongly influenced by the spatial molecular structure of the muscle tissue. Tightening the network of proteins by attraction of charged groups (pH − pI) decreases immobilized water whereas loosening the protein structure has the opposite pH to values higher or lower than the iso-electric point (pI) resulting in mutual repulsion of like charges. As seen in Fig. 1, this results in an increase in the amount of bound water.

The above considerations show only how changes in the amount of immobilized water can be brought about by either enlarging or decreasing the space available for structured water between the surfaces of macromolecules. It does not explain what forces come into play in

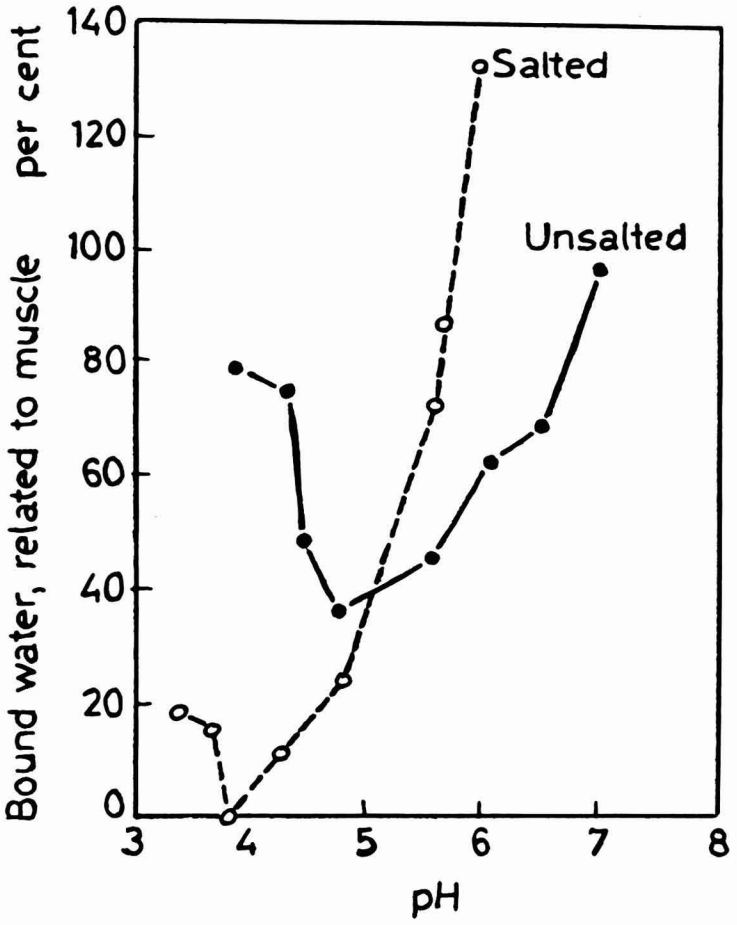

FIG. 1. AMOUNT OF WATER BOUND IN BEEF MUSCLES AS MEASURED BY PRESSURE METHOD AS A FUNCTION OF pH AND SALT (2% NaCl) FROM HAMM (196)

structuring or holding in the water and why certain macromolecule surfaces structure water more extensively than other types of macromolecules. However, it is suggested that the macromolecules need to be at concentrations high enough to form a three-dimensional network. If the solution is too dilute the long range forces in the immobilized water will be weakened by the presence of the normal bulk water forces and the remoteness of the macromolecule surfaces influencing them. As the inter-molecular nature of ordinary bulk water is itself a matter of controversy, it is necessary to review briefly some modern concepts of water.

Water is one of the very few unusual substances found in nature that under ordinary conditions exists both as a solid, a liquid, and a vapor. It is unusual in that compared to other materials that have similar molecular weight and structure, it has a much higher boiling point than expected. In the liquid state it is more dense than similar liquids. Water has an extremely high dielectric constant, one of the largest known specific heats and surface tensions and its heat of vaporization is the highest among common materials. From studies of spectroscopic techniques such as nuclear magnetic resonance (Franks 1968; Tait and Franks 1971) there seems to be evidence for a strong bond between the water molecules in a pure solution. About 40% of the water present at normal temperature is strongly bound. Measurement of other properties such as the viscosity of water or diffusion solutes within it gives evidence that water behaves as a normal low molecular weight liquid in direct opposition to the other results (Nemathy 1968).

Several theories of liquid structure have been developed based on the extensive hydrogen bonding occurring in water. Because of its tetrahedral structure, water can bond with four other water molecules or with other hydrophilic groups. The basic theories show that liquid water is not just monomers floating around in an otherwise random motion but that in solution an extensive network can exist.

There are two basic theories of liquid water structure. The continuum model theory suggests that the structure of water can be assumed to be continuous throughout the whole phase. The other theory is one in which the structure is presumed to be a mixture of various sizes of water polymers mixed in with monomers. The physicists prefer the continuum model which suggests that water is continuously bonded like ice. To explain various properties, this continuous structure may have certain defects in it to allow for the perturbations, or the bonds are proposed to have a very short relaxation time so as to account for the monomeric properties; that is, these bonds are being broken and formed very rapidly (life time 10^{-12} to 10^{-13} seconds). Based on this, water can be considered as a fast solid; in other words, the bonds are easily broken by such things as heat or shear, but they form this continuous structure with a short lifetime. An analysis by Raman spectroscopy shows that, indeed, there seems to be only one type of bonding between water molecules in the bulk giving support for this theory (Frank 1970). To firm up the theory, several authors have proposed models for the various defects in the continuous structure of water (Forslind 1971) which accurately account for the solvent and electrical properties of water. In defect models, water is presumed to be composed of either ice with bent of missing hydrogen bonds or a struc-

ture of broken down ice with holes into which monomers may fit. The number of defects is a function of temperature such that there is a change in number of hydrogen bonds per water molecule as temperature increases. Interestingly the model predicts a large change in degree of bonding at 37°C which is biologically significant temperature. Recently Stillinger (1977) has used Schroedinger wave mechanics to show that all liquid water properties can be calculated from the continuum model standpoint.

The other models are based on water being a mixture of monomers and polymers. The clathrate model for water presumes that water can structure itself like ice, but with a different configuration in such a way that its structure is not permanent. Again, this accounts for the polymeric properties of water. This theory has some strength in the fact that it has been shown that a number of non-polar molecules such as methane, ethane and fluorocarbons can, under pressure, cause clathrates to actually crystallize out into a solid form (Fennema *et al.* 1973); however, it is not generally accepted for bulk water.

The most complex of the mixture models for the structure of water is the flickering cluster, Nemethey-Scheraga mixture model (Nemethey 1968). It is based on the principle that liquid water is a mixture of both monomers and polymers of about 20–90 water molecules in size. The ratio of monomer to polymer is a function of temperature. As temperature increases the number of polymer clusters increases but the amount of water per cluster decreases as shown in Table 2. This balance between polymer-monomer can account for the usual maximum in density in water at 3.98°C as well as for some of the polymeric properties found for water; for example, the high heat of vaporization and specific heat. The life-time of these clusters is also suggested to be of very short duration, less than 10^{-11} seconds or about 1000 times larger than a molecular vibration. The forces are weak, however, so that the bonds will break under conditions where shear exists in solution. Thus, this model would also allow for the low molecular weight properties related to viscosity and diffusion.

One significant result of this model suggests that when large non-polar to weakly polar molecules such as proteins are put into an aqueous solution, they cause a structuring of the water around them to some high distance. This structuring not only protects the macromolecule from thermal denaturation effects such as unfolding, but also tends to structure the water in such a way as to give it a more polymeric nature. This structuring is evident in the dissolution of small molecular weight hydrocarbons in water. For example, many molecules such as methane, when dissolved in water, show an exothermic heat of

Table 2. Calculated cluster size and concentration for waters

T°C	Cluster Size No. Molecules/ Cluster	Cluster Concentration No./Mole Water $\times 10^2$
0	90.6	0.84
10	71.5	1.02
20	57.0	1.24
30	46.5	1.47
40	38.4	1.72
50	32.3	1.98
80	22.9	2.57
100	21.0	2.68

reaction. This exothermic heat would tend to suggest a high degree of solubility; however, solubility is extremely low. When the solution mechanism is examined thermodynamically, what is suggested is that the hydrocarbon molecules tend to order the structure of water in their vicinity, thus creating a negative total entropy which results in the low degree of solubility.

The same effect can happen with hydrophobic portions of large molecular species such as proteins, and thus a long range effect on structure of water occurs much beyond the calculated monolayer value of water. It is found that the calculated enthalpy, entropy and free energy for removal of water from various macromolecules is greater than that from pure water above the monolayer value (Masuzawa and Sterling 1968). The difference from that of pure water is not as large for agar and starch as it is for carboxymethyl cellulose, which is probably due to the three dimensional arrangement and the type of substitution on the polymer.

The work of Goto and Isemura (1964) perhaps may be used to explain some of these differences. As seen in Fig. 2, the degree of hydration of sucrose is very different from that of amino acids as a function of temperature, with sucrose losing its hydration shell of water much more rapidly as temperature increases even though it has more polar groups. This same effect must occur in the macromolecule and certainly explains part of the better gelation ability of gelatin compared to starch. What is required is an analysis of the structure-promoting or structure-destroying properties of the individual residues in macromolecules.

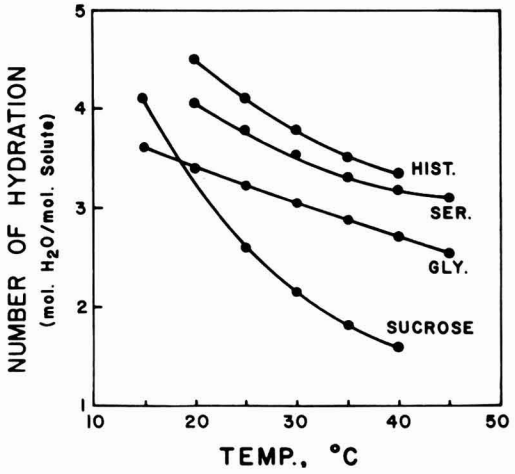

FIG. 2. DEGREE OF HYDRATION OF SUCROSE AS COMPARED TO AMINO ACIDS AS A FUNCTION OF TEMPERATURE (GOTO AND ISEMURA 1964)

A number of biologically important molecules can influence water structure, as has been illustrated by Franks (1968) and Berensen (1966). They have indicated that the oxygen spacings of some biologically significant molecules are similar to those of ice in terms of distance between hydrogen-bonding groups. Similarly, for macromolecules the distance between hydrogen-bonding groups is also very close to what may be presumed for the structure of water. It is suggested, therefore, that in biological systems such as protein and water, the interaction between the protein and the water helps stabilize the structure of the system by the development of weak long range forces. These long range forces would then promote the stability of the structure that is formed and thus contribute to the textural characteristic of the food. This structuring may also account for the protection afforded by certain cryoprotective agents such as glycerol and sugar in preventing freezing damage. These molecules not only lower the freezing point, but aid living biological systems by preventing ice formation, possibly by structuring water in such a way as to prevent the nucleation of an ice crystal.

Ionic species themselves can influence water binding. As seen in Fig. 3, different salts have different effects on the magnetic susceptibility of water protons. A shift in the positive direction usually suggests a greater amount of hydrogen bonds being broken. It is unusual that urea, which

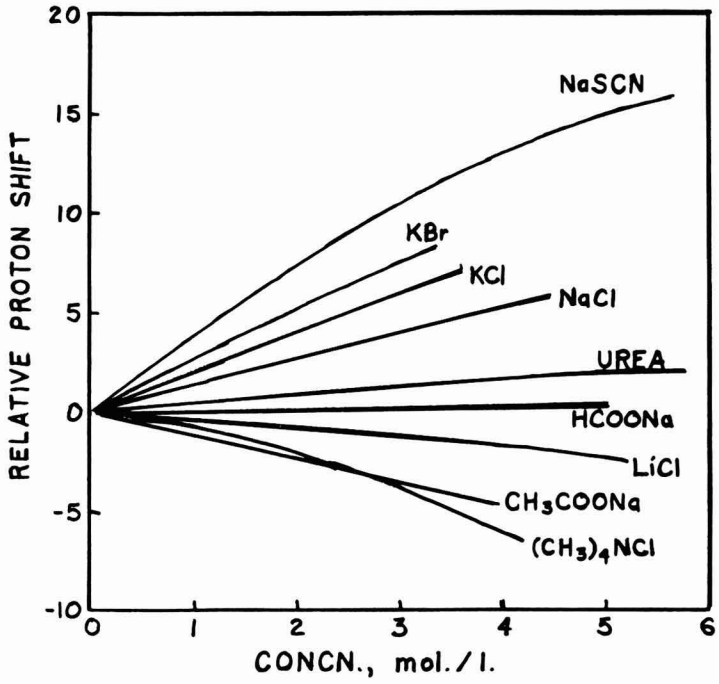

FIG. 3. RELATIVE PROTEIN DIAMAGNETIC SUSCEPTIBILITY SHIFT FOR WATER IN AQUEOUS SOLUTIONS AS A FUNCTION OF SOLUTE CONCENTRATION (GOTO AND ISEMURA 1964)

is used to precipitate protein (i.e., decreases water binding drastically), causes only a slight change in susceptibility or breakage of the water structure as measured this way. The fact that use of urea results in protein insolubilization is because urea will hydrogen bond strongly to the water near it with a different three dimensional arrangement. Thus there are two kinds of water molecules around the urea molecule, those water molecules which have broken hydrogen bonds because of the ionic field effect, and those which are very strongly bound to the urea itself. The urea thus competes with the protein for water and since the urea bonds are stronger, the protein is therefore denatured as it loses its water shell. The effect of pH as well as salts on protein solubility and water holding capacity can thus be explained by ionic and water competition effects. When the protein is in the tightest configuration, it has the least amount of ability to hold water between the pores, and a changed degree of total long range forces. The presence of salt shifts the

ionic field and thus the size of the pore changes. Unfortunately, measurement of these forces and change in pore structure has not been mathematically related to the water holding and water binding capacity of food systems. Examining Fig. 1, however, shows that there are significant interactions of ions with protein and water.

An important term used in relationship to water binding is the "water activity" of the system. The water activity, a_w, is defined as the vapor pressure of water in equilibrium with a food relative to that of the vapor pressure of pure water at the same temperature:

$$a_w = p \text{ food}/p \text{ } H_2O \tag{1}$$

Figure 4 shows a plot of moisture content versus water activity for food systems. As can be seen, for most tissue foods which have moisture contents greater than 50%, the water activity is close to that of one; that is, water binding does not seem to depend on a_w. This is the moisture range that most foods are originally in. It is only below a moisture content of about 1 g water/g solids, or 50% moisture content, that the water activity is significantly lowered. In general the water above 1 g H_2O/g solids is free water since the a_w is very high, yet as mentioned earlier it is bound in such a way as to be held in food systems quite tightly from a physical standpoint.

Many different factors are responsible for lowering water activities. First, and most important, is the effect described by Raoult's Law:

$$a_w = \frac{N_{H_2O}}{N_{H_2O} + N_{solute}} \tag{2}$$

a_w = water activity
N = moles of respective material

When a solute is dissolved in water, because of competition for water molecules, the relative vapor pressure of the water is decreased as a function of the mole fraction of water to the total moles of water and solute in solution. What would be desired is a high water content as a_w decreases; i.e., the water is present but is bound. In addition, one would want the polymer-water interaction to occur in such a way as to give the system a solid plastic-like texture rather than existing as a fluid. Most macromolecular substances do not behave according to the Kelvin equation, and indeed do hold a high amount of water as a_w decreases. This effect may be attributed to some of the long range effects of macromolecule structuring of water. With macromolecules the a_w

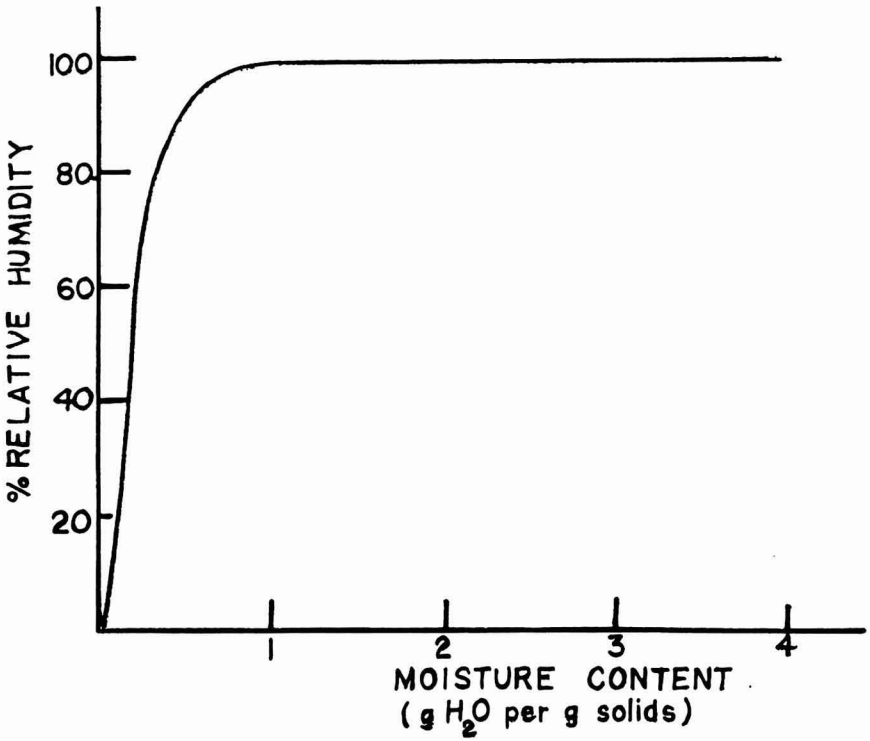

FIG. 4. EQUILIBRIUM RELATIVE HUMIDITY ($a_w \times 100$) OF A FOOD AS A FUNCTION OF MOISTURE CONTENT

lowering can be calculated by the Flory-Huggins equation (Flory 1942; and Huggins 1942a; 1942b), which is based solely on molecular volumes and small interactions:

$$\ln a_w = \ln\phi_1 + \left(1 - \frac{V_1}{V_2}\right)\phi_2 + \chi\phi_2^2 \qquad (3)$$

V_1 = molar volume of solvent
V_2 = molar volume of macromolecule
ϕ_1 = volume fraction of solvent
ϕ_2 = volume fraction of macromolecule
χ = interaction parameter

Busk (1977) recently has shown this to be a good model for prediction of a_w lowering of food gelling agents and that the interaction term can

be used to calculate some of the long range forces that help to bind water at high water concentration.

The other factor that is important in analyzing the binding of water in foods is the size of the capillary, as mentioned earlier. As predicted from the Kelvin equation, as the size of a capillary is reduced, the vapor pressure of water is significantly lowered, and thus water becomes more bound:

$$\ln a_w = \frac{-2\gamma\cos\theta\ V_m}{r\ RT} \tag{4}$$

γ = surface tension
θ = wetting angle
V_m = molar water volume
R = gas constant
T = °K
r = capillary size

Sample calculations are shown in Table 3. Most foods have pores of 10–100 μ in size, and thus the a_w lowering of the pore is small (Bluestein and Labuza 1972); however, the suction pressure in these pores is high (Lewicki *et al.* 1977). This suction pressure must be the result of the interactive forces of the surface of the pore with the water. As seen in Table 3, pores of 10–100 μ can draw water to significant heights. Conversely this means the capillary structure itself can hold this water in, and an external force equal to this would be needed to remove the water. Thus the three dimensional structure itself acts to entrap or "bind" water.

Table 3. Effects of capillaries

Pore Size	Water Activity	[a]h cm
100 μ	0.999999	150
10 μ	0.99999	1500
1 μ	0.999	1.5×10^4
0.1	0.99	1.5×10^5
0.01	0.91	1.5×10^6
0.001	0.89	1.5×10^7

[a]height to which water is drawn

Another factor controlling water binding is related to the hysteresis effect in preparing the system, that is, whether water is added to a dry system to reach the final state or is removed from the original tissue. As seen in Fig. 5, a larger amount of water is held in the desorption process. This can be attributed to several factors, as reviewed by Labuza (1968) and Labuza (1973a), including the capillary structure and supersaturation of amorphous carbohydrates which structure water internally so that it becomes more difficult to remove. The first factor is not now possible to measure. The latter factor explains partially greater water binding of sugar-containing food systems.

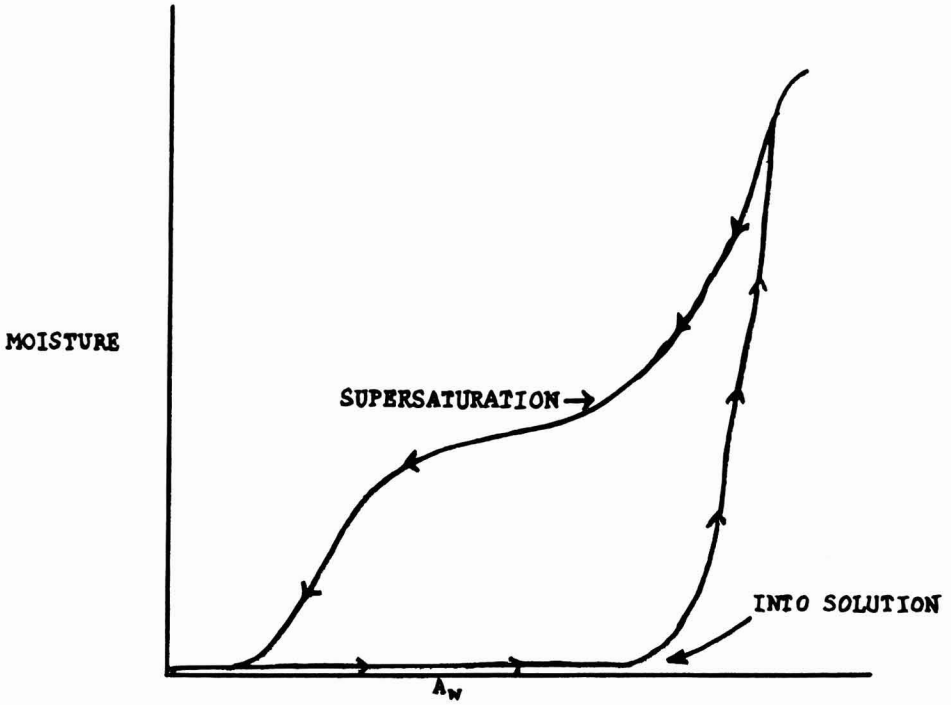

FIG. 5. HYSTERESIS EFFECT IN WATER ADSORPTION-DESORPTION FOR A FOOD SHOWING THE SUPERSATURATION EFFECT

Our last factor responsible for binding of water is the surface solute-water interactions. Table 4 lists the monolayer moisture contents for four macromolecules. It can be seen that the number of hydrophilic groups per monomer has no relationship to the amount of water held at the BET monolayer. For example, agar has about 2.5 hydrophilic

groups per monomer, yet agar holds only one molecule of water per 2.5 groups. This is due to its internal hydrogen bonding and configuration. On the other hand, gelatin being a fairly linear molecule has about one water molecule bound to each monomer group in its chain. Thus gelatin would seem to indicate that it is a better water binder; however, because of less hydrophilic groups per 100 g, it actually binds less water at the monolayer. This becomes more obvious at high moisture levels when these polymers are made into gels. From the wideline NMR data of Cope (1969) seen in Table 5, agar binds water much more tightly than does gelatin. In a 3% gel, the agar decreases the NMR peak height of deuterium oxide by almost 60%, whereas there is no decrease for gelatin. Similarly, the line width for water in the NMR spectrum for a 3% gel is extremely wide for agar and very sharp for gelatin indicating strong long range effects on water in agar. Thus, there is a significant difference between the two macromolecules, with agar seeming to be a better water binder at the high moisture levels in contrast to it being a poor water binder at the low moisture levels based on the number of hydrophilic groups per monomer. This is further supported by the suction pressure data from Lewicki *et al.* (1977). A 3% agar gel has a suction pressure of about 10^4 Newtons/m^2 whereas for a 3% gelatin gel it is only about 10 N/m^2. It is surprising, however, that the 3% agar gel shows more syneresis than does gelatin.

Table 4. Characteristics of some typical food polymers

Polymer	Monolayer Moisture Content g H_2O/100 g	Binding Energy Q_s KCal/ Mole	I Mole H_2O per Mole Monomer	II No. Hydrophilic Groups per Monomer	Ratio I/II
Agar	9.3	1.83	0.93	2.5	1/2.5
CMC	9.5	1.81	1.46	3[c]	1/2
Gelatin	7.4	1.68	0.51	0.46[a]	1/1
Starch	7.5	1.74	0.77	3[b]	1/3

[a]46% of amino acids have side chains, peptide bonds internally bound
[b]usually 2 OH groups internally bound
[c]carboxymethyl group prevents internal bonding

Flour dough formation is a practical example of the interactions of water binding and textural properties of food materials. Gur-Arieh *et al.* (1969) made adsorption-desorption isotherms for six flour samples prepared to size distributions ranging from ten to fifty microns.

Table 5. Effects on gelatin and agar on wideline NMR spectra of H_2O and D_2O[a]

Concentration of Gelatin or Agar %	Decrease in NMR Peak Height of D_2O		Line Width of NMR Spectrum of H_2O	
0			1.6	1.6
1			—	5.0
3	—	55	—	16.5
5	7	67	1.6	—
10	9	88	—	50.0

[a]Cope (1969)

At moisture contents of up to about 25% ($a_w < 0.85$) the water binding characteristics of the flours were the same. However, these flour fractions gave different cake volumes when baked, with a better volume for the flour with the larger specific solid surface area as measured by N_2 adsorption. Labuza (1968) has shown N_2 surface area to be unimportant, however, with respect to water interactions in foods. Based on alkaline water retention and microscopic examination of the starch granules, Gur-Arieh *et al.* (1969) found that as the flour is ground to a smaller size, more damage occurred to the granule so that more free amylopectin was released. This free amylopectin crosslinks forming a three-dimensional structure, thus making the dough more plastic so that it can entrap gas. At low moisture content this property is not important. Bone *et al.* (1969) reported that the viscosity of corn starch during cooking varied significantly with addition of sucrose. Most likely this was due to the effect of sugar on the structuring or interaction of water with the starch components. A drop in a_w from 0.98 to about 0.96, by adding sugar, increased the temperature and doubled the time needed to gelatinize the starch. This could mean that there is a direct competition between the starch and the sugar for water, and therefore if more water is held by the sugar, there is less gelatinization. An osmotic effect may also occur in that there could be less leaching out of the amylopectin from the starch granules as the aqueous phase concentration increases.

Wood *et al.* (1972) have found that the amount of lipid binding in a dough can critically affect its rheological characteristics. For example, as the moisture content of a dough is increased from 20 to 32%, the amount of lipid that can be bound increases significantly. Using a differential scanning calorimetric method (measures freezability), they

found there was no difference in the amount of free water in the doughs. Unfortunately, at the levels of moisture content used, one might not expect a difference to occur. It should be obvious, however, from the composition of the doughs used that there would be a difference in water activity as was shown by Lee (1970) in studying enzymatic action in doughs. Possibly the same effect as discussed above for starch pastes is taking place allowing for a difference in interaction between the starch and the lipid with a change in texture. This textural difference was indicated by a very strong relationship between the dough resistance after mixing versus the moisture content of the flour. Below 29% moisture, the dough mixture does not behave as a gel at all, but tends to be very powdery, offering no resistance. However, above 30% moisture content, dough development takes place. The protein-starch-water system then exhibits long range effects and the viscosity or resistance to mixing increases significantly as moisture increases. A well-developed dough is formed as moisture is increased until a dilution effect takes place and thereafter a decrease in dough resistance can be attributed to the lubricating action of water in the system.

Hermansson (1972) has studied the functional properties of swelling food proteins with respect to water. Some of the results of water uptake of dry proteins are summarized in Fig. 6. The swelling in casein is not very different from soy as measured with a capillary suction device initially. However, with time the water holding capacity of casein decreases after approaching a maxima. The reasons for this were not clearly pointed out, although it may be due to a solubilization of the material. Whey on the other hand has a poor water binding character. In most textured food systems, various salts, such as sodium chloride, are added. These help in developing flavor as well as texture. As seen in Fig. 7 (Hermansson 1972) the addition of a small amount of sodium chloride to the dry system causes casein to bind more water than soy. With both, water binding decreases, whereas with whey the salt has no effect. This obviously should be related to the effect of salt on the solubility of the various portions of the protein and the ionic effect on the three dimensional structure as related to both short and long range water binding forces. The concentrations used are within a reasonable range of 1–3% sodium chloride which could be utilized in various food products.

When gels were made of these various proteins, a very different effect was found. For example, with 10% protein dispersions heated in water, casein did not gel at all, whereas the soy protein and whey protein concentrate did form gels after reaching a certain temperature. As seen in Fig. 8, the soy protein formed a gel much more easily than did the

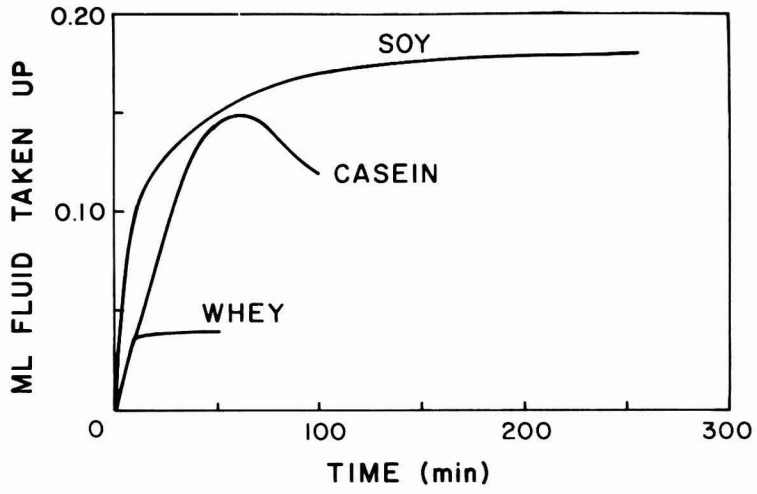

FIG. 6. WATER SWELLING OF VARIOUS PROTEINS (20 MG) AS A FUNCTION OF TIME (HERMANSSON 1972)

whey, but after reaching the temperature where whey began gelling, the viscosity of the soy protein gel decreased drastically. As noted by Hermansson (1972), however, whey protein gels were not very stable with the soy, and water could not be pressed out even under high pressure.

Another area with respect to water interactions in foods is the stability of emulsions. Johnson *et al.* (1966) have shown that there are large deviations from the Derjaguin-Landau-Verwey-Overbeck (DLVO) theory for floculation or destabilization of emulsions. The explained deviations can be postulated on the basis that there is a structuring of water next to the particles in a suspension that provides an additional repulsive potential which is particularly noticeable when the electrostatic potential on the surface becomes very small. This results in greater emulsion stability. This could be used to advantage in food systems, although it has been reliably measured only in aqueous solutions of polyvinyl-toluene or polyvinyl-alcohol (PVA) particles. The rate of floculation in these studies is much less than had been predicted by the DLVO theory, especially as particle size increases from 0.08 to 0.13 microns. There also happens to be a break in the activation energy curve at 37°C, something which was mentioned before in terms of water structure. The evidence for structured water in these systems is shown in Fig. 9 in which the NMR spin relaxation rate ($1/T_2$) of water is plotted versus interparticle separation distance for 0.08 micron PVA

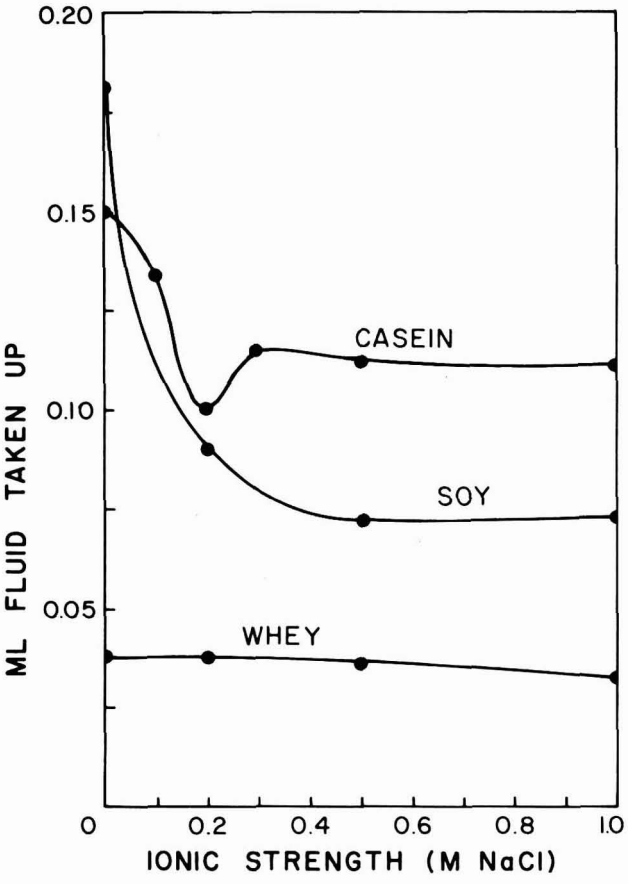

FIG. 7. EFFECT OF SALT CONCENTRATION ON EQUILIBRIUM VALUE OF SWELLING OF VARIOUS PROTEINS (HERMANSSON 1972)

particles. A higher value of $1/T_2$, (i.e., short relaxation time) indicates a greater degree of structuring of water. As seen, as particle-particle distances decrease to below 1,000 Angstroms, there is a large increase in structuring. This structuring increases as temperature decreases, which is expected based on the theories of water. These results fit in with what was discussed on the long range interactions of surfaces of macromolecules, in this case, particles in an aqueous solution. Certainly, these results may be useful in the design of an emulsion in terms of particle concentration and size.

Up to this point the effect of water structure on properties of textured food in terms of the water binding characteristics of

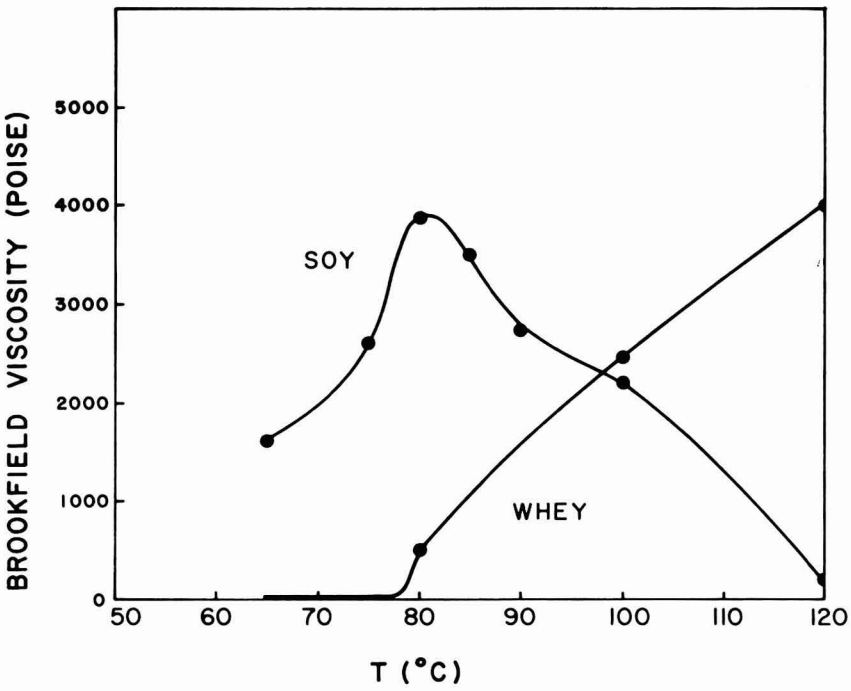

FIG. 8. GEL STRENGTH AS A FUNCTION OF TEMPERATURE (HERMANSSON 1972)

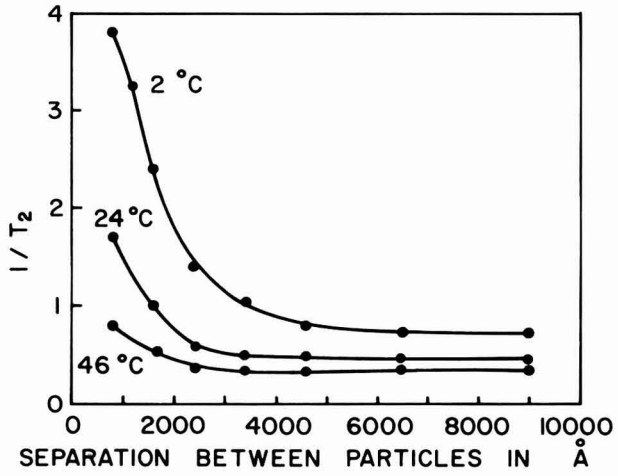

FIG. 9. DEPENDENCE OF SPIN-SPIN RELAXATION RATE ($1/T_2$) BY NMR AS A FUNCTION OF INTER-PARTICLE SEPARATION FOR 0.13 μ PVA PARTICLES (JOHNSON *ET AL.* 1966)

macromolecules has been discussed. It is also known that water affects the texture of a food during storage. Fig. 10 gives an overall picture of various reactions that can occur in foods as a function of water activity (Labuza 1970). As seen, rancidity occurs very rapidly in the dry state, decreasing as moisture increases, and then increasing again, whereas hydrolytic reactions such as enzymatic reactions and non-enzymatic browning do not begin until there is a monolayer of absorbed moisture formed. Above the monolayer moisture, reaction rates increase and may approach a maximum. The rate may decrease again due to dilution effects above the maxima. Also, at certain water activities, usually above 0.65, microbial growth can occur. Thus in processing of foods such as gels, we must either pasteurize, sterilize or add microbial inhibitors if the water activity is high.

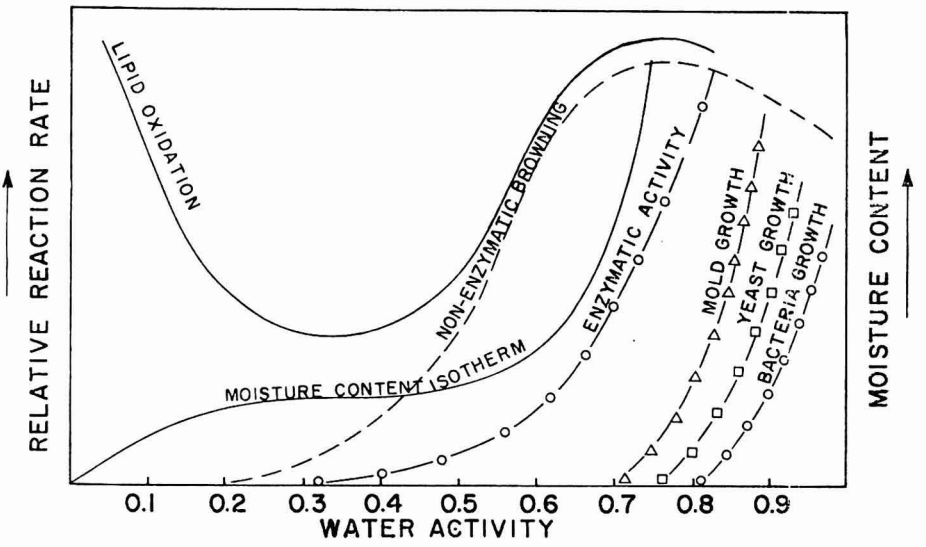

FIG. 10. STABILITY OF FOODS AS A FUNCTION OF WATER ACTIVITY (a_w)

In relationship to texture, many dehydrated or intermediate moisture foods will become tough during storage (Waletzko and Labuza 1975). The higher the a_w of the dehydrated food, the greater is the increase in hardness of the product during storage, making it more unacceptable due to increased lipid oxidation and non-enzymatic browning. Lipid oxidation is a reaction in which unsaturated fats react with oxygen to produce free radicals and peroxides that can react directly with proteins to cause them to crosslink and become insolubilized. At increasing

moisture contents, the rate of oxidation increases; however, it does reach a maximum in high moisture (30–80% water) texturized foods, probably due to dilution (Chou and Labuza 1973).

For many foods the Maillard browning reaction is the most important one with respect to deterioration. In this reaction reducing sugars react in the presence of amino acids and proteins to produce a brown pigment. The most significant factor, however, is that this brown pigment is an irreversible complex between polymers of the reducing sugar reactions and proteins, causing a loss in solubility as well as a loss in biological value and a change in the texture of the food to the point that it becomes tougher (Labuza 1973b). Formulated foods thus should be prepared under conditions to minimize the browning and therefore maximize the desired texture. The rate of browning is a function of moisture content, and a maximum occurs somewhere at intermediate moisture water activities of 0.6 to 0.9 (Labuza 1970).

Finally, changes in moisture during storage also contribute to textural changes in foods. Vickers (1976) investigated the effect of moisture loss on the decrease in crispness of fruits and vegetables. Water is lost not only from the pores but also from the cells, which reduces the turgor pressure (related to suction pressure). Dry foods, as mentioned earlier get softer if they gain moisture. Experiments in our laboratories with a variety of dry foods show this to occur almost universally at about an a_w of 0.45–0.5. At this a_w the water begins to act more as a dissolving and swelling agent lending plasticity to the food structure. Kapsalis *et al.* (1967) has done further work in this area on the Instron.

Overall, some of the interesting properties of water in relationship to its interactions with macromolecules and other food components have been explored. It is known that macromolecules, besides forming capillary structure which can hold water, also induce a long range structuring effect on the water. This can account for the good water binding that is found in some high moisture content systems. This structuring has also been proposed as a mechanism for the stabilization of water inside the cell and for the sodium pump that operates across the cell wall (Ling 1965). Whether this property has anything to do with the water activity or degree of boundness as measured by calorimetry or vapor pressure measurement is not clear. However, as solutes are added to a food to decrease the water activity effects on gel strengths, the energy required for mixing and changes in the rate of chemical interactions, are affected. These changes can increase desirable texture such as through lipid binding as well as decrease desirable texture such as through lipid oxidation and non-enzymatic browning. It is hoped that

in the future when water in a texturized food system is examined, these factors can be examined in such a way as to develop a better food system.

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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.

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

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

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

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

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

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

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

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

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

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

Description of experimental work or explanation of symbols should go below the table proper.

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

Acknowledgments: Acknowledgments should be listed on a separate page.

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

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

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