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# Journal of FOOD PROCESSING and PRESERVATION

Edited by
T. P. LABUZA

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

VOLUME 7, NUMBER 1 MARCH 1983

JOURNAL OF FOOD PROCESSING AND PRESERVATION



All articles for publication and inquiries regarding publication should be sent to Prof. T. P. Labuza, University Minnesota, Department of Food Science and Nutrition, St. Paul, MN 55108 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., 155 Post Road East, Suite 6, Westport, Connecticut USA.

One volume of four issues will be published annually. The price for Volume 7 is $65.00, which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $77.00 per year via surface mail and $85.00 per year via airmail.

Subscriptions for individuals for their own personal use are $45.00 for Volume 7, which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $57.00 per year via surface mail, and $65.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

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

The *Journal of Food Processing and Preservation* (ISSN: 0149-6085) is published quarterly by Food & Nutrition Press, Inc.—Office of Publication is 155 Post Road East, Suite 6, Westport, Connecticut 06880 USA.

Second class postage paid at Westport, CT 06880.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., 155 Post Road East, Suite 6, Westport, CT 06880.

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Food & Nutrition Press, Inc.
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ISSN: 0145-8892

Printed in the United States of America

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# DISINFESTATION OF DRIED FOODS BY FOCUSED SOLAR ENERGY

T.O.M. NAKAYAMA, JON M. ALLEN[1], SCOTT CUMMINS, and Y.Y. DAVID WANG

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Received for Publication October 18, 1982
Accepted for Publication December 16, 1982

## ABSTRACT

*Temperatures in excess of 50° produced by focused solar dryers can be used for disinfestation of dried foods. All life stages of the hide beetle, Dermestes maculatus, were eliminated from dried mullet in a single exposure of 30-60 min. The same results were obtained with the Indian meal moth, Plodia interpunctella with dried peaches and with the merchant grain beetle, Oryzaephilus mercates on oatmeal. Among packaging materials, black plastic was shown to be most effective followed by clear plastic. Aluminum foil was found to be reflective and resulted in lower temperatures within the container. The lethality of oven, microwave, and infrared heating units were also tested on the life stages of the hide beetles.*

## INTRODUCTION

The postharvest loss of food to insects continues to be a problem. Chemical techniques such as insecticides besides being technologically demanding, have drawn concern by consumers and environmentalists with respect to residue problems.

Physical treatments such as microwave, infrared and ionization radiation, have found certain degrees of success (Kirkpatrick *et al.* 1972; Cogburn 1967), but are also expensive. Conventional solar drying used by man since antiquity while effective for preservation of foods has not eliminated insects from dried fish. *Dermestes* beetles are particularly damaging and have given rise to the traditional belief that "dried fish must have beetles" (Anon. 1978). A modified plastic tent type of solar dryer was used by Doe *et al.* (1977) to kill all stages of blowfly in 20 h at 45°. Reinfestation is always a problem and thus attention must be given to proper packaging and storage conditions.

[1] Minority Research Apprenticeship Program, SEA, U.S. Dept. of Agriculture

It was the purpose of the study reported here to explore the feasibility of using a focused solar dryer to disinfest dried food products. The methodology involves low cost and simple procedures which may allow its use in areas with abundant sunshine.

## MATERIALS AND METHODS

### Maintenance of Insect Cultures

The species used in this study were the hide beetle (*Dermestes maculatus* Degeer), a cosmopolitan pest infesting foods containing animal proteins (Osuji 1972), the Indian meal moth (*Plodia interpunctella* Hubner), which infests dried fruits, and the merchant grain beetle (*Oryzaephilus mercates*), which infests grain products.

The cultures were obtained from the USDA Stored Product Insects Research and Development Laboratory, Savannah, GA, and maintained according to the procedures used at the Laboratory (Miller *et al.* 1969). They were incubated at 27°, 80% R.H. and a 12 h photoperiod.

### Construction of the Focused Solar Dryer

A low-cost, solar food dryer incorporating a concentrating reflector was constructed according to the instructions provided by U.S. Citrus and Subtropical Products Laboratory (Coleman *et al.* 1979). The dryer utilizes a parabolic reflective surface formed by weaving aluminum foil between strings fastened to the wooden end pieces. The shelves are located on the foci of the parabola and the entire dryer is covered with clear plastic. Cool air enters the lower front of the dryer, and the hot and humid air is vented out the upper back side.

A temperature recorder (Electronik 16, Honeywell Industrial Division, Fort Washington, PA) equipped with remote thermocouple sensors was used to record the temperature changes in and out of the solar dryer. Gainesburgers® dog food, which was used to maintain the beetles, was cut into 1½ in. squares and was placed at different spots to simulate foods for drying. The thermocouples were placed in the center of the samples or in the center of the food package when internal temperatures were recorded.

### Preparation of Dried Foods

Mullet fish (*Mugil cephalus*) were prepared into fillets and placed skin side down on the drying tray of the solar dryer for 2-3 days. Local peaches were similarly washed, machine pitted, and the halves were dipped in a solution composed of 1 tablespoonful of Vege-Fresh® antioxidant (Morton Quality Products, Chicago, IL) per gallon of water

for 3-4 min. The halves were placed on the tray of the solar dryer with the flesh side up and dried in 4-5 days. The oatmeal (Old Fashioned, Quaker® Oats, Chicago, IL) was purchased and used as is. In the case of foods in polyethylene or aluminum foil, they were packaged into sizes approximately 1.6 × 10.0 × 6.3 cm. The foil was 3.5 mil and the plastic films were 2 mil thick.

## Measurement of Lethality of Oven, Microwave, and Infrared Heating

The hide beetles at various life stages i.e., eggs, larvae, pupae and adults, were picked from cultures and subjected to heat treatments. From 5 to 10 beetles were tested in each treatment.

For the oven-heat experiments, a Blue M. Stabiltherm Lab Oven (Model OV-490, Blue M. Electric Co., Blue Island, IL) equipped with an air-blast fan was used. The oven was preset at a designated temperature; the beetles were placed in aluminum cups with lids and placed inside the oven. One at a time, the cups were withdrawn from the oven and the beetles were transferred into plastic petri-dishes containing dog food and were incubated in the environmental room and observed for survival for 1-2 weeks.

For experiments with microwave and infrared heatings, the beetles were placed in petri-dishes and treated without the lids. The treatments were performed on individual dishes for each designated exposure time period. A solidstate Kenmore 2450 MHz 1450W microwave range (Sears, Roebuck and Co., Chicago, IL) was used for the microwave heating experiment. The range was turned off at preset time periods because of the difficulty of measuring the temperatures of the insects. After the treatments, the lids of the petri-dishes were replaced and the cultures were incubated for further observation. For infrared heating, a 250W General Electric infrared heat reflector was stationed 6.5 cm above a metal platform where the petri-dishes were placed. The lamp was turned on for a designated exposure time, and then turned off. The rise of temperature during the heating period was recorded by reading the mercury thermometer placed on the platform under the lamp at 10 s intervals and showed linear relation with time. The temperature of the insects was not measured.

## Infestation and Treatment of Dried Foods

The dried mullets were torn into pieces and placed into four 1-qt fruit jars. Three of the jars were allowed to become infested with all life stages of the hide beetles. After one-week of incubation, the contents of one of the insect infested jars were poured onto an aluminum tray (22 × 30 × 3 cm) and the tray was placed on the dryer tray inside the solar dryer. The disinfestation process was performed during the 2-3 h

period, from mid-morning till noon. The jars were then incubated for assessing growth of any survivors.

Similar procedures were applied to dried peaches, except that the peaches were not torn into pieces and Indian meal moth eggs were used to infest the peaches. The disinfestation process was performed after 2-3 weeks of incubation. All life stages of Indian meal moth grown on the rearing medium were also treated in a similar way with the solar dryer.

The merchant grain beetle was used to infest oatmeal which was treated in flexible packages of clear and black plastic. Similarly plastic wrapped cultures of hide beetles in burgers were disinfested.

## RESULTS AND DISCUSSION

It appears that oven heating for 30 min at 50°C effectively eliminated all life stages of the hide beetles. The temperatures appear critical as high percentages of the eggs and pupae survived 45°C for 120 min (Table 1).

Microwave heating under these conditions appeared to be a much quicker way to kill the insects. Within 40 s of exposure the hide beetles in the stages of larvae, pupae and adults were killed. The eggs seem more resistant to microwave heating, they were not killed until exposed for 150 s or longer (Table 2). Temperatures were not measured.

The use of infrared heating appears to be similar to that of microwaves. All life stages were eliminated within 55 s of exposure time (Table 2).

Table 1. Effect of oven-heating on the survival of the hide beetles

| Preset temp. | Holding Time (min) | % of survivals | | | |
|---|---|---|---|---|---|
| | | Eggs | Larvae | Pupae | Adults |
| 40°C | 10 | 20 | 100 | 100 | 100 |
| | 30 | 10 | 100 | 100 | 100 |
| | 60 | 20 | 100 | 100 | 100 |
| 45°C | 10 | 60 | 100 | 80 | 100 |
| | 30 | 80 | 100 | 40 | 100 |
| | 60 | 100 | 0 | 100 | 100 |
| | 90 | 40 | 0 | 80 | 0 |
| | 120 | 100 | 0 | 40 | 0 |
| 50°C | 10 | 0 | 10 | 90 | 10 |
| | 30 | 0 | 0 | 0 | 0 |
| | 60 | 0 | 0 | 0 | 0 |

Table 2. Time of exposure (seconds) required to completely eliminate hide beetle

| | Eggs | Larvae | Pupae | Adults |
|---|---|---|---|---|
| Microwave | 150 | 15 | 40 | 25 |
| Infrared | 45 | 25 | 55 | 25 |

## Temperature Changes

The changes in temperature of Gainesburger® at various spots on the drying tray of the solar dryer and the ambient air during a typical day is shown in Fig. 1. The day was hazy and partly cloudy (July 3, 1980; Georgia Experiment Station weather record: Max. temp. 36.9°, min

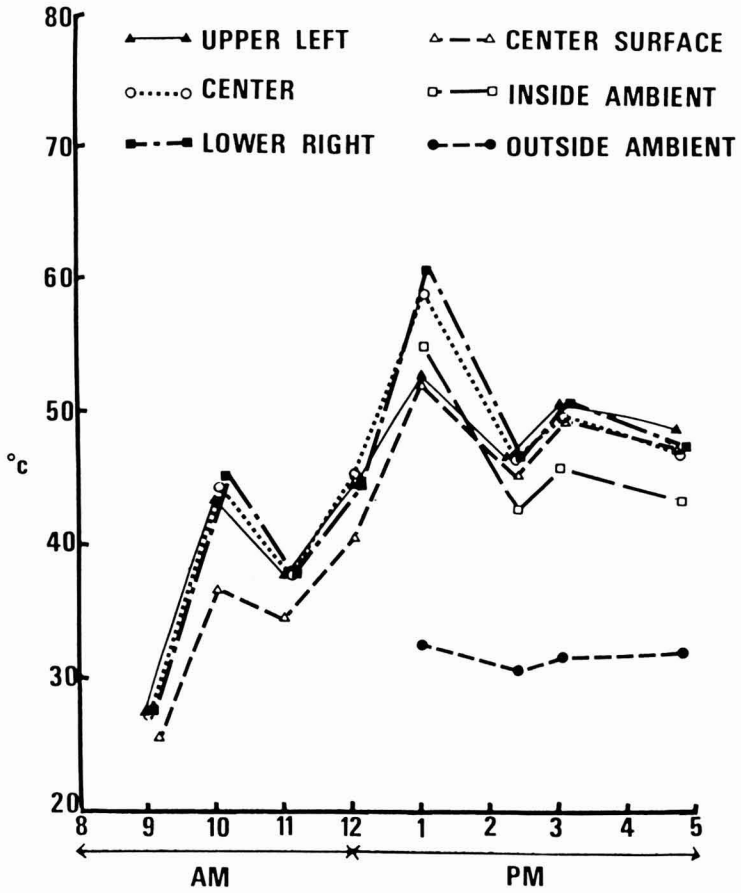


FIG. 1. TEMPERATURE CHANGES IN DIFFERENT PARTS OF THE DRYER

23.8°, wind 62 km/24 hr; rainfall 0 cm; evap. 1.0 cm/24 h). The food temperature reached 50° after 12 noon and reached its maximum temperature of 61° at about 1 p.m. No large differences in the temperature at various positions of the tray were observed. The temperature of the Gainesburger® was near that of the ambient air inside of the dryer which was much higher than the temperature of the ambient air outside the dryer. On the other hand as shown in Fig. 2, when the burgers were wrapped in plastic, the internal temperature reached 50° rather readily, even on a day which was partly cloudy. Aluminum foil on the other hand showed reflective qualities. Figure 3 shows similar results with oatmeal. On these particular days, the maximum outside air temperatures were 34-35°. The effects of the package material were black plastic > clear plastic > aluminum foil.

The fish and peaches which were disinfested under these conditions (30 min or more at > 50°C) showed no signs of infestation at the end of 2 weeks while the controls were clearly infested. Further incubation for six months confirmed the observations.

The results indicate that although the conditions given by Cruess (1948) for disinfesting fruits, namely exposure at a temperature of

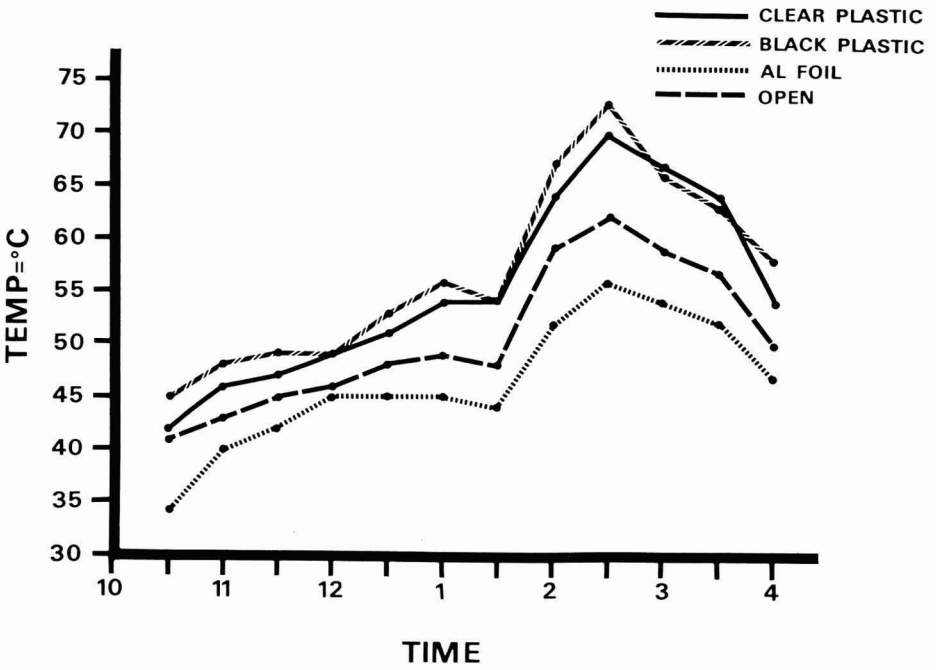


FIG. 2. EFFECTS OF TIME AND PACKAGE MATERIAL ON TEMPERATURE OF SEMI-SOLID FOOD (GAINESBURGER®) IN A SOLAR DRYER

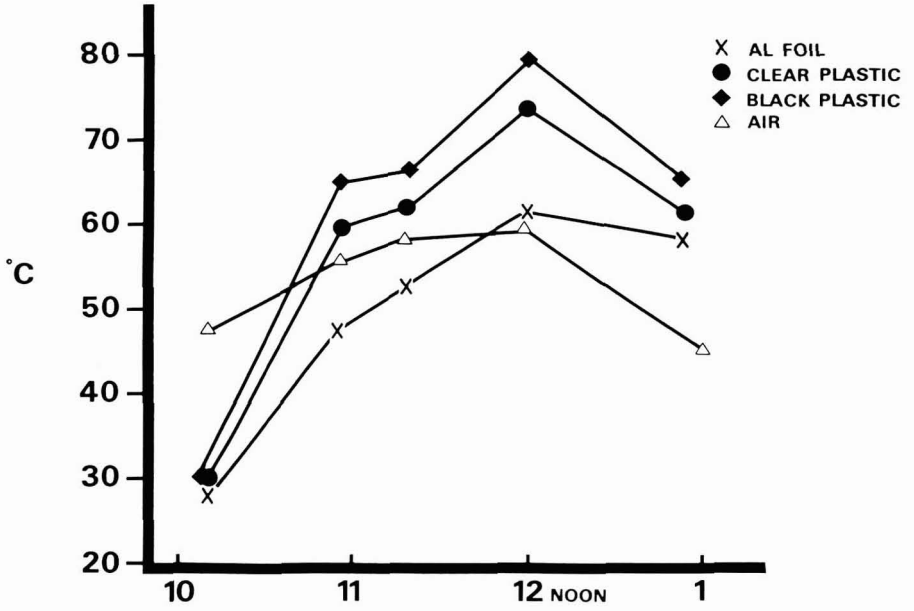


FIG. 3. EFFECTS OF TIME AND PACKAGE MATERIAL ON TEMPERATURE OF OATMEAL IN A SOLAR DRYER

70-74° for a period of several hours at the end of the drying cycle were not achieved, disinfestation was achieved by use of the focused solar dryer in this case. The conditions for disinfestation used here, 30 min at 50°C, would be a minimum and further trials should be carried out to establish the requiste margin of safety.

## CONCLUSION

This study reveals that food products may be disinfested by focused solar radiation. The use of packaging not only prevents reinfestation, but in the case of plastic enhances the heating while aluminum foil decreases it. An additional merit of disinfestation using solar energy would be the application of this process in countries where sophisticated equipment and electricity may not be readily available and where the insect problem is most notorious.

## ACKNOWLEDGMENT

The authors acknowledge the assistance of Dr. J.E. Baker of USDA Stored-Product Insects Research and Development Laboratory for

providing the insect cultures and rearing instructions. Appreciation is also extended to R. Koons for his efforts in construction of the solar dryer.

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# DISTRIBUTION OF THE MAJOR MINERALS BETWEEN SOLUBLE AND COLLOIDAL PHASES OF BUFFALO MILK AS AFFECTED BY pH

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Received for Publication October 13, 1982
Accepted for Publication December 5, 1982

## ABSTRACT

*The influence of alteration in milk pH (in the range of 5 to 9) on the distribution of seven major minerals, namely, Calcium, Magnesium, Sodium, Potassium, Phosphate, Citrate and Chloride between the soluble and colloidal phases of buffalo milk was determined through ultracentrifugation technique. Study revealed that lowering in pH progressively increased the concentration of calcium, magnesium, phosphate and citrate in the soluble phase. However, the increase in soluble citrate was found to be statistically nonsignificant. On the other hand, the increase in pH above normal caused a progressive decrease in the soluble magnesium. The shift in the distribution of calcium, phosphate, and citrate was irregular. The influence of pH on the distribution of three monovalent minerals, sodium, potassium and chloride, could be studied effectively only in the case of potassium which remained almost unchanged between pH 5 to 7 but increased slightly between pH 8 to 9.*

## INTRODUCTION

Inspite of the extensive studies on the partitioning of minerals between the soluble and colloidal phases in milk, information on the influence of alteration in pH on such partitioning is quite limited (De Kadt and Van Minnen 1943; Zittle *et al.* 1958; Sebela and Klicnik 1977; Brule and Fauquant 1978). No such information on the effect of alteration in pH of milk on the partitioning was available for buffalo milk and even for cows' milk having developed wide pH variation for one reason or another. A knowledge of the effect of pH on the status of major minerals is likely to help in evolving more suitable technolog-

ical processes for treating buffalo milk to yield products having better taste and longer shelf-life. Consequently, an investigation with buffalo milk was conducted, results of which are communicated here.

## Materials and Methods

### Collection of Milk Samples

Ten samples of herd bulk milk from not less than twenty-five buffalo of the Murrah breed of the Institute herd were collected, in adequately cleaned aluminum containers during the morning milkings. The samples were stored at refrigerator temperature (4-6°C) for 1-2 h between the period of collection and starting of analysis.

### Measurements Performed on the Samples

**Partitioning of Minerals**. Partitioning of minerals between the soluble and the colloidal phases of milk was affected by separation of phases through ultracentrifugation (at a speed of 185,000 × g, for 30 min) of milk at 20°C as described earlier (Sindhu and Roy 1976a). The serum was drained off and collected.

**Determination of Milk Constituents**. Proteins, lactose and seven major minerals, namely, calcium, magnesium, sodium, potassium, phosphate, citrate and chloride were determined according to earlier description (Sindhu and Roy 1973; 1976a). Determination of fat was done volumetrically by the Gerber method as per ISI specification (IS:1958) and total solids was done by the gravimetric method (IS:1961).

**Determination of Milk pH**. Milk pH was determined with a mains operated pH meter, Elico Model LI-10 at room temperature (18-20°C) using a combination of glass and saturated calomel electrodes.

**Plan of Work**. Each sample was divided into six lots of 175 ml each, while the first lot was used as control, the second, third, fourth and fifth lots were used for pH adjustment respectively, to pH 5,6,7,8 and 9 with addition of either 1 N hydrochloric acid or 1 N sodium hydroxide solution. The aliquots, after bringing to the desired pH, were kept at 18-20°C for one hour to attain equilibrium, pH was measured again, and adjusted to the nearest desired value by further addition of small volumes of acid or alkali. It was maintained at room temperature again for 15 min and the final pH measured. All the pH adjusted samples and original milk (control) were subjected to ultra-centrifugation for the separation of soluble and colloidal phases. Portions of milk and whey were used for the determination of seven minerals respectively, for total concentration and soluble proportions.

## RESULTS AND DISCUSSION

### Composition and pH of Milk Samples

Results of the concentration of total solids, fat, proteins, lactose and seven major minerals in composite samples of buffalo milk are given in Table 1 along with the average values and standard errors for each of the constituents. Values of pH for individual samples and average ± standard error are also given in Table 1. The pH, fat and proteins were determined in all ten samples, while the lactose and three monovalent minerals were determined only in the first five samples and the four divalent minerals in the last eight samples (as indicated in the Table 1). The present values for total solids, fat, proteins, lactose and minerals were comparable to the values observed by other workers (Laxminarayanan and Dastur 1969).

### Partitioning of Minerals at Normal pH of Milk

Results for the dissolved proportions of seven major minerals determined in the ultracentrifuged whey along with their total concentrations in buffalo milk are given in Table 2. The present values for the dissolved proportions of different minerals in buffalo milk were in agreement with the values reported earlier (Sindhu and Roy 1976a; 1976b). On the other hand, the present values for the dissolved proportions of divalent minerals were much lower than those reported in cows' milk (Davies and White 1960; De Mann 1962). However, the values for the dissolved proportions of three monovalent minerals (sodium, potassium and chloride) observed now in buffalo milk were comparable to the values reported for the same in cows' milk by the earlier workers. The difference in the extent of partitioning of divalent minerals in the milks from two species may be due to the difference in the concentration of casein in these milks. Buffalo milk contains more casein compared to cows' milk. Furthermore, most all the casein in it is present in the micellar form (Ganguli 1973) while in the cows' milk about 10% of the casein is present in the dissolved phase (Rose 1968). Phosphorus forms an integral part of the casein molecule and calcium, magnesium and citrate are associated with it. Therefore, a higher concentration of casein, more so in the micellar form in buffalo milk, may be responsible for the presence of higher proportions of these minerals in the colloidal phase leaving lower proportions in the dissolved phase. One additional reason for relatively higher proportions of calcium and magnesium in the colloidal phase in buffalo milk may be the presence of higher amounts of these cations in it. About 44 mM of calcium and 9 mM of magnesium are present in buffalo milk (Sindhu

Table 1. Concentrations of total solids, fat, lactose and total major minerals, at normal pH of buffalo milk[a]

| Sample No. | pH | Total solids (g/100g) | Fat[b] (g/100ml) | Lactose (g/100g) | Total minerals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ca | Mg | P | Citric Acid | Na | K | Cl |
| 1. | 6.75 | 19.437 | 8.80 | 5.55 | — | — | — | — | 57.74 | 107.65 | 74.33 |
| 2. | 6.90 | 18.061 | 8.50 | 5.60 | — | — | — | — | 44.98 | 99.50 | 70.25 |
| 3. | 6.80 | 17.568 | 7.50 | 5.01 | 163.43 | 26.18 | 130.89 | 279.88 | 41.10 | 101.24 | 66.08 |
| 4. | 6.66 | 18.866 | 9.00 | 5.76 | 169.93 | 17.90 | 168.81 | 242.11 | 40.61 | 113.76 | 57.81 |
| 5. | 6.75 | 17.480 | 8.10 | 5.46 | 174.85 | 16.52 | 171.26 | 222.54 | 40.37 | 112.54 | 57.81 |
| 6. | 6.73 | 18.373 | 8.23 | — | 173.96 | 24.94 | 156.58 | 210.40 | — | — | — |
| 7. | 6.75 | 19.604 | 8.60 | — | 184.89 | 17.47 | 154.13 | 205.51 | — | — | — |
| 8. | 6.75 | 18.165 | 7.90 | — | 173.08 | 15.95 | 132.11 | 193.76 | — | — | — |
| 9. | 6.85 | 16.725 | 8.00 | — | 181.94 | 12.95 | 171.26 | 241.13 | — | — | — |
| 10. | 6.80 | 17.450 | 7.80 | — | 184.12 | 12.95 | 156.28 | 249.55 | — | — | — |
| Average ± Std. error | 6.77 ± 0.021 | 18.173 ± 0.291 | 8.24 ± 0.15 | 5.48 ± 0.01 | 175.78 ± 2.64 | 18.11 ± 1.76 | 155.17 ± 5.72 | 230.61 ± 9.90 | 44.96 ± 3.30 | 106.94 ± 2.88 | 65.26 ± 3.31 |

[a] Results pertain to 10 samples except for lactose and minerals (only 8 samples were analysed for divalent minerals and 5 samples for lactose and monovalent minerals)

[b] Fat was determined volumetrically by the Gerber method

Table 2. Effect of pH on the concentrations of minerals in the soluble phase of buffalo milk[a]

| Sr. No. | Mineral | Concentration in mg/100 g Milk ± Standard Error | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Whole Milk | Soluble Phase | | | | | |
| | pH[b] | 6.76±0.053 | 5.02±0.011 | 5.97±0.010 | 6.76±0.053 | 7.01±0.017 | 7.99±0.016 | 8.97±0.011 |
| 1. | Calcium | 175.78±2.64 | 139.31±5.59 (79.25) | 66.39±1.72 (37.77) | 40.28±1.70 (22.92) | 37.74±1.18 (21.46) | 32.13±2.25 (18.28) | 44.75±4.82 (25.46) |
| 2. | Magnesium | 18.11±1.76 | 13.62±0.749 (75.21) | 10.96±0.88 (60.52) | 8.95±0.73 (49.42) | 8.33±0.711 (46.00) | 7.02±0.733 (38.76) | 6.60±0.653 (36.44) |
| 3. | Sodium[c] | 44.96 | 45.83±2.76 (101.94) | 44.38±4.04 (98.79) | 43.43±4.61 (96.60) | 49.70±2.72 (110.5) | 72.32±1.33 (160.9) | 85.40±4.71 (189.94) |
| 4. | Potassium | 106.94±2.88 | 103.28±2.87 (96.58) | 105.65±3.29 (98.79) | 105.68±3.36 (98.82) | 104.05±2.81 (97.30) | 97.27±2.96 (90.96) | 94.49±2.36 (88.36) |
| 5. | Phosphorus | 155.17±5.72 | 107.24±4.62 (69.11) | 70.49±2.03 (45.43) | 53.00±2.70 (34.16) | 55.11±2.24 (35.52) | 48.76±3.22 (31.42) | 51.56±4.74 (33.23) |
| 6. | Citrate | 230.61 | 217.11±7.55 (94.15) | 202.32±8.35 (87.73) | 199.92±8.31 (86.69) | 199.98±8.21 (86.72) | 199.76±7.31 (86.62) | 203.40±8.15 (88.20) |
| 7. | Chloride[d] | 65.26±3.31 | 270.57±17.72 (414.6) | 137.96±3.43 (211.4) | 67.66±3.98 (103.7) | 65.48±3.47 (100.3) | 65.65±3.49 (100.6) | 61.56±3.09 (94.33) |

[a] Results pertain to the analysis of 8 samples of herd milk for 4 polyvalent and 5 samples for 3 monovalent minerals

[b] pH expressed as average ± standard error

[c] Higher concentration of sodium and chloride in the soluble phases than the whole milk is due to their extraneous addition to milk

[d] Figures in parenthesis are for the percentage of the consitutents in the soluble phase

and Roy 1978) compared to 30 mM of calcium and 5 mM of magnesium in cows' milk (Davies and White 1960). Due to higher concentrations of these cations in buffalo milk, its aqueous phase may be saturated with respect to these minerals exceeding their solubility which leads to an excess of these cations which can associate with the casein micelles.

## The Influence of pH on Partitioning

Results for the dissolved proportions of the above mentioned seven minerals in milk samples adjusted to different pH are also presented in Table 2.

**Influence on divalent minerals.** It is evident from the results in Table 2 that acidification of milk by decreasing its pH from 6.76 (original) to about 6 and 5 in general caused a progressive and significant shift in calcium, magnesium and phosphate from the colloidal to the dissolved phase. However, such shifts in the case of citrate due to decrease in pH was comparatively less and statistically nonsignificant. The cause for such a shift of divalent cations (calcium and magnesium) due to decrease in pH may be the decreased binding of these cations to the casein and other proteins (which carry a net-negative charge at normal pH of milk) due to the decrease in electric charge on them. On the basis of the parallel between binding of cations and net-negative charge on casein micelles, it was suggested by Zittle *et al.* (1958) that a favourable negative charge is an important factor in the binding of calcium to casein. On the other hand, the reason for decreased binding of divalent anions (phosphate and citrate) due to increase in net-negative charge on proteins may not hold valid as the charge on both of them (proteins and anions) is alike. However, it was established by Zittle *et al.* (1958) that no phosphorus is bound to casein below pH 6 because at this pH the phosphate is not present in its bivalent form (which is the form that must be present for complex formation). Therefore, a decrease in pH accompanied by a decrease in bivalent phosphate may lead to a decrease in the colloidal phosphate. This is evident from the findings in the present study. The decrease in milk pH may also act similarly on the colloidal citrate because it may also be associated in a comparative manner along with calcium to the casein micelles probably as suggested by Ter Horst (1963) for calcium and phosphate. He suggested that calcium and phosphate are present in the form of a complex with the $NH^{3+}$ groups of lysine or arginine in a structure as, (Casein-$NH^{3+}$)-$PO_4^{3-}$-$Ca^{2+}$. Further evidence for cooperative association of calcium and citrate with casein is suggested by the fact that the formation of the above complex ion [(Casein-$NH^{3+}$)-$PO_4^{3-}$-$Ca^{2+}$] is enhanced by citrate ions. Therefore, the decrease in colloidal calcium and phosphate

may be accompanied with a decrease in colloidal citrate. The present findings on the influence of alteration in pH of milk on the partitioning of minerals may be considered unique as no such report on buffalo milk was there in the literature. However, Ismail *et al.* (1971) from their studies on the influence of variations in the original pH of fresh milk on the partitioning of four divalent minerals reported "a nonsignificant reverse correlation between pH and Ca+Mg ions" and "between pH and ratio of Ca and Mg ions to total Ca+Mg". Reports on this aspect of cows' milk were also neither numerous nor comprehensive. However, the present findings are comparable in quality to the results reported by other workers (De Kadt *et al.* 1943; Zittle *et al.* 1958; Davies and White 1960; Brule and Fauquant 1978) for cows' milk. Davies and White (1960) observed that the decrease in pH from 6.77 to 5.6 (1.17 unit) caused the maximum decrease (37.6%) in the colloidal phase of calcium which was followed by (21.6%) in phosphate and (13.0%) in magnesium. The decrease was much less (4%) in the citrate. In the present study also a similar trend of a decrease in the colloidal form of the above four divalent minerals was observed. On the basis of this trend in the shift of minerals due to a decrease in pH, it can be concluded that solubilization of the four divalent minerals was proportionate to the extent of their association with the casein micelles.

Addition of alkali to milk to increase its pH caused an increase in the concentrations of colloidal calcium and magnesium except at about pH 9 where a decrease in the colloidal calcium was observed. Reason for increased binding of these cations with proteins particularly to caseins may be an increase in the net-negative charge on them due to an increase in pH and also due to combination of a portion of added alkali to them as suggested by Pyne (1962). Studies by other workers (Chanutin *et al.* 1942; Ramsdell and Whittier 1944; Carr 1953; Zittle *et al.* 1958) also revealed an increase binding of calcium to casein due to increase in pH. However, it is difficult to explain the lower binding of calcium at pH 9 observed in the present study (Table 2). Changes caused upon increasing the pH were comparatively less in anions (phosphate and citrate) than those in the cations. While the citrate remained almost unchanged, the change in phosphate was not regular (a slight increase at pH 7 but decrease at pH 8 and 9 in the soluble phosphate). The lower and irregular decrease in soluble phosphate and negligible change in citrate caused by the increase in pH may be due to two factors which exerted the influence which was opposite to each other. A decrease in pH was affected by the addition of alkali (NaOH), probably a portion of which could have combined with casein and thus had competed with phosphate and citrate for the positive sites on it. Therefore, a decrease was caused in the binding of phosphate and

citrate to the casein micelles. On the other hand, due to an increase in the net negative charge on casein, a greater amount of calcium was bound. Thus an increased binding of calcium to casein in turn might have caused an increase in the phosphate and citrate bound to casein due to the cooperative binding of these ions. Therefore, the influence of these two opposite factors working simultaneously may lead to very little or no shift in the phosphate and citrate during the increase in pH.

**Effect on Monovalent Minerals**. The influence of alteration in pH of buffalo milk on the partitioning of the three monovalent minerals (sodium, potassium and chloride) could be studied effectively only in the case of potassium (Table 2). The decrease in pH from 6.76 to 5 caused a progressive decrease in soluble sodium but had little or no effect on the soluble potassium. On the other hand, due to increase in pH from 6.76 to 9 there was a progressive decrease in soluble potassium. However, the influence of increase in pH could not be determined in the case of sodium due to its extraneous incorporation into milk. For comparison with the present findings no other report was available on the effect of pH on partitioning of monovalent minerals in buffalo milk. However, in cows' milk Davies and White (1960) observed that a decrease in pH caused a progressive increase in soluble potassium while the increase in soluble sodium was irregular. A similar shift in the colloidal sodium and potassium was suggested by Pyne (1962) on the basis that when alkali is added to milk a portion probably combines with casein thus increasing its cation binding capacity somewhat by virtue of the increase in pH produced and thus slightly raising the proportion of monovalent to divalent cations bound. In the present study the decrease in pH was obtained with the addition of dilute hydrochloric acid (which furnished additional chloride ions to milk) hence its influence on the partitioning of chloride could not be elucidated. However, when alkali was added to milk to increase the pH, decrease of about 6% was obtained in the soluble chloride at pH 9. No other report was available in literature on the influence of pH on chloride either in cows' or buffalo milk.

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# EXTENDING ONION STORAGE LIFE BY GAMMA-IRRADIATION

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Received for Publication November 23, 1982
Accepted for Publication January 3, 1983

## ABSTRACT

*Gamma irradiation (0.03 kGy ± 25%) effects on onions bulbs from local cultivar were studied. The treatment proved to be effective in reducing weight loss and increasing the percentage marketable bulbs. Radiation-induced darkening in marketable irradiated bulbs had no influence on their inner aspect.*

## INTRODUCTION

It is known that storage life of onions is limited mainly by sprouting and rotting. Relatively low doses of gamma radiation, through sprout inhibition, give a satisfactory method for extending their storage period (Baraldi 1975). The Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (1981) has recommended approval of onions irradiated at an average dose of up to 0.15 kGy for human consumption.

Sprout inhibition of onions by gamma irradiation was found to be influenced by the physiological state of the bulbs at the time of irradiation, the radiation dose, and the storage temperature after irradiation (Thomas 1975). It has been observed that the sprout radioinhibition process promotes darkening phenomena inside the bulbs (Temkin-Gorodeiski *et al.* 1972; Mennitti 1977; Grünewald 1978; Lustre 1979).

In our preliminary studies, onions of the variety "Valenciana Sintética 14", the one most cultivated in this region, were irradiated 40 days after harvest at 0.03 kGy ± 25% of Co-60 gamma rays. After seven months' storage under the environmental laboratory conditions, not one of them showed external sprouts (Curzio *et al.* 1980). This paper shows the results obtained on onions harvested in early March and stored in a commercial warehouse after irradiation. Observations of

weight loss, marketable bulbs, and darkening of samples at laboratory scale were made. A trial of samples at pilot scale was checked for marketable bulbs.

## MATERIALS AND METHODS

Onions of the variety "Valenciana Sintética 14" grown in S.E. of the Buenos Aires province and cured naturally in the field were used in these studies. About 400 kg of sound and even bulbs were packed in cartons of $28 \times 30 \times 35$ cm in size and irradiated 40 days after harvest at the facilities of Comisión Nacional de Energía Atómica. The samples were treated with a dose of 0.03 kGy of $^{60}Co$ gamma rays at the rate of 2.44 $kGy-h^{-1}$ with a Dmax/Dmin ratio of 1.25.

After treatment, the irradiated samples and the control ones were arranged so as to count with two experiments, at laboratory and pilot scales. Both groups were stored in a commercial warehouse for 330 days at temperatures ranging from 6°C to 32°C (R.H. 50-90 per cent).

For the experiment at laboratory scale, 20 samples of 1,000 ± 100 g were packaged in perforated paper bags, and the cumulative percentage weight of each sample was taken with a Bosch P115 monoplate balance. Furthermore, from 60 samples (20 bulbs each) three samples were tested monthly for external sprouting, rotting, and firmness. Those bulbs that showed external sprouting, rotting, or little firmness, were discarded and the remaining ones recorded as marketable bulbs percentage. Afterwards the marketable bulbs were cut open longitudinally and observed for the incidence of inner sprouting and the appearance of radiation-inducing darkening. The criterion adopted for inner sprouting was the presence of a green coloration.

About 300 kg of piled-out bulbs were considered the pilot scale sample. Every 60 days, 50 bulbs were drawn out at random and records of the marketable bulbs percentage were made.

## RESULTS

The cumulative percentage of physiological losses in weight (i.e. losses due to transpiration and respiration) along the 270 days' storage period for both control and irradiated onion bulbs is shown in Table 1. While control samples suffered an average decrease of 43.3% of their weight, the irradiated ones lost only 22.8% at end of the 270 day test period.

Percentage of marketable bulbs during the 330 days' storage period for both control and irradiated onions is shown in Table 2. Until 210

Table 1. Cumulative percentage physiological losses in weight in control and irradiated onions stored at 6-32°C (R.H. 50-90%)

| Treatment | Percentage Weight Losses After (Days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 |
| Control | 2.9±0.7 | 4.3±1.2 | 5.2±1.0 | 9.2±1.3 | 14.8±2.0 | 20.2±3.4 | 26.3±4.0 | 43.3±7.2 |
| Irradiated | 1.4±0.5 | 2.5±0.9 | 3.5±0.8 | 5.1±1.0 | 7.4±0.7 | 10.1±1.6 | 14.9±2.0 | 22.8±3.3 |

Table 2. Percentage of marketable bulbs in control and irradiated onions stored at 6-32°C (R.H. 50-90%)

| Treatment | Percentage of Marketable Bulbs After (Days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | 330 |
| Control | 100.0 | 100.0 | 100.0 | 98.0 | 93.0 | 80.0 | 44.5 | 38.0 | 18.0 | 0.0 |
| | — | — | — | ±1.0 | ±6.3 | ±3.2 | ±5.5 | ±8.5 | ±9.9 | — |
| Irradiated | 100.0 | 100.0 | 96.0 | 97.0 | 96.0 | 92.0 | 97.0 | 95.0 | 60.0 | 26.0 |
| | — | — | ±3.5 | ±2.0 | ±3.8 | ±2.9 | ±2.9 | ±4.8 | ±11.0 | ±13.0 |

days of storage, there are not appreciable differences between the irradiated and nonirradiated samples. After this period the marketable bulbs percentage was greater in the irradiated samples. The discarded bulbs in the control samples were due to sprouting and rotting while in the irradiated samples was due only to rotting. (Comparable results were obtained in a separate experiment with garlic.)

At checking the marketable bulbs for the green inner sprout along the control period, it was seen that in the non-irradiated bulbs development began about 120 days after harvest while it was absent in the irradiated ones.

Radiation-induced darkening in the marketable bulbs appeared in two forms: a) Small brown spots were observed in the growth zone after 90 days post-harvest; the size of the spots remained constant with time. b) Yellowish-brown stem plates began to appear 180 days after-harvest. They reached a maximum of 2 cm in length by the end of the test period.

## DISCUSSION

A dose of 0.03 kGy was effective to prevent the sprouting of onion bulbs variety "Valenciana Sintética 14" when they were storaged under commercial warehouse conditions. The lower weight loss shown by the irradiated bulbs is closely connected with the sprout inhibition.

The small brown spots on the growth zone of the treated bulbs began to appear at about the time when the non-treated bulbs began to sprout internally. The yellowish-brown stem plates observed after irradiation may be connected with the continued elongation of the meristem cells despite the treatment, as was reported by Temkin-Gorodeiski *et al.* (1972). These two forms of darkening observed in the marketable irradiated onions had no influence on their inner aspect. Similar results were found by Grünewald (1978).

The onion variety selected had a satisfactory behaviour in the presence of treatment, since this decreased the weight loss and extended the storage life of the bulbs. These results clearly show that "Valenciana Sintética 14" onion variety is suitable for irradiation treatment towards commercial purposes.

## ACKNOWLEDGMENTS

Thanks are given to the Comisión Nacional de Energía Atómica for irradiation process commissioning, and to CORFO-Río Colorado for

onion samples. This work was supported, in part, by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires.

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# THE INFLUENCE OF MAILLARD BROWNING AND OTHER FACTORS ON THE STABILITY OF FREE TRYPTOPHAN[1]

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Received for Publication November 19, 1982
Accepted for Publication January 14, 1983

## ABSTRACT

*The influence of various factors on the stability of free tryptophan was evaluated in several reaction systems. After treatment, tryptophan was measured by high performance liquid chromatography. Factors such as heating, protein, and glucose were responsible for tryptophan losses in a system undergoing Maillard browning. Hydrogen peroxide caused extensive losses while pH and light had minimal effect on tryptophan stability in solution. When the stability of tryptophan was compared to lysine and threonine during browning, threonine had the highest retention. At low water activity (0.22), tryptophan had the lowest retention (42%) while at a high water activity (0.75) lysine had the lowest retention (5%). When fortified corn tortillas were made using the typical heat treatment involved in preparation, retention of added lysine and tryptophan was 85-90%.*

## INTRODUCTION

Amino acid fortification of cereal grains has been suggested as one means of improving the protein quality of a limited diet. In most cereal grains, lysine is the first-limitng amino acid and threonine is second-limiting except in corn in which the second-limiting amino acid is tryptophan (Jansen 1974). Fortification of certain cereal grains with lysine has been shown in many instances to improve either the protein efficiency ratio in rats or nitrogen balance in humans. Further improvement in protein quality has been demonstrated when a second-limiting amino acid is added in conjunction with lysine to a cereal product. Rosenberg *et al.* (1960) found optimal growth to occur in rats fed a bread diet when fortified with both lysine and threonine. Studies by

[1] Paper No. 13,066, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul Minnesota 55108

Scrimshaw *et al.* (1958) and Bressani *et al.* (1958) in young children indicated that increased nitrogen retention occurred when corn masa was fortified with both lysine and tryptophan.

The stability of amino acids used in fortification of a protein source should be considered in order to achieve optimal improvement of protein quality. A major reaction likely to lead to destruction of added amino acids in foods is Maillard browning. Free amino groups of the amino acids react with carbonyl compounds, resulting in loss of biological availability of the amino acids. The stability of added lysine in breads has been studied alone and in conjunction with the stability of added threonine (Ericson *et al.* 1961; Murata *et al.* 1979). The extent of loss of added lysine and threonine varied among these studies, with the primary loss occurring in the crust.

Little or no information is available on the stability of free tryptophan in foods, although it is known that tryptophan will participate in the Maillard reaction (Heyns and Noack 1964; Rooney *et al.* 1967; Lee *et al.* 1979), will undergo photodegradation (Yoshida and Kato 1954), is unstable to peroxidizing methyl linoleate (Yong *et al.* 1980) and cannot withstand the usual conditions for acid hydrolysis of a protein (Friedman and Finley 1971).

The nutritional advantage of supplementation of cereals or cereal products with the second-limiting amino acids, threonine and tryptophan, in conjuction with lysine has previously been demonstrated. However, little work has focused on the stability of these amino acids relative to each other or on other factors which would contribute to the loss of free tryptophan. The purpose of this research was to evaluate the effects of pH, heat, reducing sugar, protein, hydrogen peroxide and light on the stability of tryptophan and to determine the relative stabilities of added lysine, threonine and tryptophan in reaction mixtures prone to Maillard browning. The stability of added lysine and tryptophan in corn tortillas was also determined.

## MATERIALS AND METHODS

### Preparation and Treatment of Model Systems

Part I of the research consisted of two experiments where reaction systems and treatments were used to determine the effect of pH, heat, reducing sugar, protein, hydrogen peroxide, and light on the stability of free tryptophan. Because of the nature of the variables, different reaction systems were used for the experiments. In the first experiment, the effects of pH and heat on tryptophan stability were studied

in buffered solution at different levels of solids. The levels of pH were chosen to encompass those typically found in foods. The first system consisted of 0.1 mg/ml solutions of DL-tryptophan in buffers of pH 3.0, 4.5, 6.0 and 7.5. Citrate-phosphate buffers (0.1M) were used to achieve pH 3.0, 4.5 and 6.0 while phosphate buffer (0.1M) was used to achieve pH 7.5. Five ml quadruplicate samples were placed in 10 mm × 85 mm screw top test tubes, capped with Teflon-lined screw caps and then heated for 12 h in a water bath at 95°C. This heat treatment was used for a number of experiments throughout the study so that a uniform treatment could be used for comparison. In addition, this heating time and temperature caused sufficient tryptophan losses so that relative differences between the experimental variables could be determined. Quadruplicate control samples received no heat treatment.

A second system with a higher solids content was designed to have a slurry-like consistency and yet have the pH controlled by added buffer solutions. The purpose of the slurry systems was to make the reaction system more similar to a food system than the pure solutions described above. If the loss of tryptophan in solution was due to tryptophan-tryptophan interactions, the addition of a relatively inert material such as cellulose may have an influence on tryptophan stability by acting as a diluant of the solids or as an interfering material. The second system consisted of 0.5% DL-tryptophan, 75% buffer solution at pH 3.0, 4.5, 6.0 and 7.5 and 24.5% microcrystalline cellulose (MCC, Avicel, FMC Corp., Type 101). Three-gram portions were sealed in retortable foil pouches. Samples were heated for 12 h in a 95°C water bath. Control samples received no heat treatment. Four replicates were used for each treatment. After removal from the water bath, samples were cooled in cold water and stored in the freezer (−18°C) until the time of extraction and analysis.

The effects of heat, glucose and casein on the stability of tryptophan were evaluated with four model systems. The first system was used to study the effect of heat alone on the stability of tryptophan and was composed of 0.5% tryptophan and 99.5% MCC. The second system was used to study the effect of a reducing sugar on tryptophan stability. This system was composed of 0.5% tryptophan, 4.0% glucose and 95.5% MCC. The third system was used to study the effect of a protein on tryptophan stability and contained 0.5% tryptophan, 15.0% casein and 84.5% MCC. The fourth system represented a combination of all of these factors and was composed of 0.5% tryptophan, 4.0% glucose, 15.0% casein and 80.5% MCC.

Each system was dry-blended for 2 min in an Osterizer blender, placed in desiccators containing saturated calcium nitrate ($a_w = 0.56$) and allowed to equilibrate (Rockland 1960). This $a_w$ was used because it

is in the range that is most likely to promote Maillard browning (Eichner and Karel 1972). Three-gram portions were sealed in retortable foil pouches. Quadruplicate samples of each system were heat processed in a water bath at 95°C for 12 h. Quadruplicate control samples of each system received no heat treatment. After heat processing, samples were cooled in cold water and stored at −18°C until analyzed.

The influence of an oxidizing agent, hydrogen peroxide, on free tryptophan stability was evaluated in buffered solution at pH 6.0. A citrate-phosphate buffer system containing 0.1 mg/ml of tryptophan was heated with varied levels of diluted hydrogen peroxide (Mallinckrodt). Final hydrogen peroxide concentrations ranged from 0.17 to 170 ppm. Five ml samples were placed in screw top test tubes (13 mm × 100 mm Corning No. 9826) with Teflon-lined screw caps, and these were held in a 95°C water bath for 12 h. The hydrogen peroxide concentrations and heat treatment were selected to provide a range of tryptophan destruction. Eight replicates of each treatment were prepared and analyzed. Control samples without hydrogen peroxide were held at 95°C. Additional control samples containing the varied levels of hydrogen peroxide were held at room temperature without heat treatment.

The effect of light on free tryptophan stability was determined by exposing tryptophan solutions to light for varied lengths of time. Citrate-phosphate buffer solutions at pH 3.0, 5.0 and 7.0 containing 0.1 mg/ml of tryptophan were placed in screw cap test tubes (13 mm × 100 mm Corning No. 9826). The volume placed in each tube was 4 ml which was half the tube capacity. The tubes were placed horizontally under 300 ft candles of light furnished by Cool White™ General Electric fluorescent bulbs (F15T8-CW, 15 Watt). The samples were held at room temperature and analyzed for tryptophan every 2 days for 14 days. Control samples were held at the room temperature without light exposure. Duplicate treatment and control samples were analyzed each time.

Reaction mixtures conducive to Maillard browning were prepared in order to evaluate the stability of free lysine, threonine and tryptophan in model systems undergoing this reaction. Fortification with amino acids was on an equimolar basis. On a weight basis, the reaction mixture was composed of 0.50%L-lysine HCl, 0.55% DL-tryptophan, 0.33% DL-threonine (all Sigma Chemical Co.), 8.0% D-glucose, 15.0% casein (technical grade, Sigma Chemical Co.), and MCC to make 100%. The mixture was dry-blended for 2 min in an Osterizer blender at top speed. The mixture was then split into two lots. One portion was placed in a desiccator over a saturated salt solution of potassium acetate ($a_w$ = 0.22) while the other portion was placed over sodium chloride ($a_w$ = 0.75) (Rockland 1960). The samples were held at room temperature for

5 days to permit equilibration. After obtaining the appropriate $a_w$, 3-gram samples were placed in retortable foil pouches which were then evacuated and heat sealed to prevent changes in $a_w$ due to moisture loss. Four unheated control samples at each water activity were held at −18°C. Quadruplicate samples at each water activity were heated in a water bath at 95°C for 4, 8, 12 and 16 h. After heating, samples were cooled in cold water and held at −18°C until analyzed.

Part III of this research investigated the stability of free lysine and tryptophan in a real food, corn tortillas. Masa Harina (Quaker Oats Co.), a product of corn treated with lime water and specially ground, was used in making the tortillas. This product was fortified with 0.1% of both L-lysine HCl and DL-tryptophan. The mixture was dry-blended for 2 min in an Osterizer blender. Tortillas were made according to the directions on the package. Two hundred sixty-six ml of water was mixed with 286 g of the Masa Harina, the dough was shaped into tortillas, and the tortillas were cooked about 1 min on each side in a frying pan at medium heat. Control samples consisted of the unheated fortified corn masa. Two-gram portions of six tortilla samples and four control samples were ground and extracted as described below. The extracts were then analyzed for free lysine and tryptophan.

**Amino Acid Extraction**

The method of extracting tryptophan alone and in combination with lysine and threonine was similar to that described by Schleske and Warthesen (1982). The extraction procedure requires 2 g samples of the reaction mixture which were added to 20 ml of distilled water and mixed for 1.5 min in a Sorvall Omni Mixer. Samples were quantitatively transferred to 50 ml plastic centrifuge tubes. Fifty μl of glacial acetic acid was added to the supernatants in the centrifuge tubes to adjust the pH of the samples to 4.5. The samples were centrifuged at 500 × g for 10 min. The supernatants were then transferred to 100 ml volumetric flasks. The pellets were washed and centrifuged twice with 10 ml of distilled water. The supernatants from each washing were added to the volumetric flasks. For analysis of tryptophan alone, the supernatants in the volumetric flasks were brought to volume with water. Tryptophan content could then be analyzed directly by high performance liquid chromatography (HPLC) because the indole ring absorbs well at 280 nm. Dansyl derivatives of the amino acids were made to increase the sensitivity of detection for lysine and threonine when all three amino acids were present. In extracts containing lysine, threonine and tryptophan, the supernatants were adjusted to pH 9.0 with 0.1N NaOH and brought to volume with 0.1M borate buffer, pH 9.0 in preparation for dansyl derivative formation.

## Reaction with Dansyl Chloride

The reaction with dansyl chloride was adapted from the procedure of Bayer *et al.* (1976) for amino acids. A mixture of 0.75 ml of sample extract, 0.75 ml of pH 9.0 borate buffer, and 0.75 ml of 10 mM dansyl chloride (Sigma Chemical Co.) in acetonitrile were reacted for 45 min at 40°C to form the dansyl derivatives of lysine, threonine and tryptophan. The sample was then filtered through a 0.45 μm membrane filter. Separation and quantification of these amino acid derivatives were accomplished by HPLC.

## HPLC of Amino Acids

A Waters Associates HPLC system consisting of two Model 6000A pumps, a Model 660 solvent programmer and Model 440 absorbance detector was used for amino acid analysis. A Rheodyne injection unit Model 7010 equipped with a 10 μl sample delivery loop was used. Detector output was monitored by a Hewlett Packard 3380A recorder-integrator. For tryptophan analysis, a procedure similar to that of DeVries *et al.* (1980) was used. For analysis of the dansyl derivatives of the amino acids, an HPLC method adapted from O'Keefe and Warthesen (1978) was used.

A μBondapak $C_{18}$ column (Waters Associates) was used for both analyses. For the analysis of tryptophan alone, an isocratic solvent system of 70% water, 29% methanol and 1% glacial acetic acid was used. Detection wavelength was set at 280 nm. Tryptophan eluted in 6.0 min at a flow rate of 1.5 ml/min. For the analysis of the dansyl derivatives of the amino acids, a solvent gradient beginning with 80% 0.01M phosphate buffer (pH 7.0) and 20% acetonitrile and ending at 60% 0.01M phosphate buffer (pH 7.0) and 40% acetonitrile over a 10 min linear gradient was used to achieve separation. The dansyl derivatives of threonine, tryptophan and lysine eluted at 5.8, 10.3 and 17.0 min, respectively. Detection wavelength was set at 254 nm.

The HPLC response of external standards was used to quantify the amino acid content of the samples. In the case of the dansyl derivatives of the amino acids, external amino acid standards were reacted with dansyl chloride under the same conditions as the samples. The peaks produced by the dansyl derivatives of L-lysine HCl, DL-threonine and DL-tryptophan co-chromatographed with the respective standard dansyl amino acids (Sigma Chemical Co.).

All treated samples in any one experiment were heated at one time and analyzed within a week. Samples were always held at −18°C until the time of extraction and analysis. Samples were generally extracted

one day and analyzed by HPLC the next. Preparation of the dansyl derivatives occurred on the same day as analysis. In all experiments, untreated samples were used as controls, and the loss of each free amino acid due to treatments was represented as a percent of each respective amino acid in the untreated control. This accounts for errors in fortification level (due to weighing and blending) as well as inefficiencies in the extraction procedure.

## Detection of Tryptophan Degradation Products

In the tryptophan systems subjected to hydrogen peroxide, attempts were made to characterize the tryptophan degradation products by HPLC. To better detect these compounds, the absorbance detector was set at 254 nm instead of the 280 nm used for tryptophan analysis. Other HPLC operating conditions were the same. Yong *et al.* (1980) reported that in a system with tryptophan and peroxidizing methyl linoleate, kynurenine and N-formylkynurenine were likely tryptophan breakdown products. To gather qualitative evidence about the presence of these compounds in the hydrogen peroxide treated samples, L-kynurenine (Sigma) and N-formyl-L-kynurenine (Calbiochem) standards were used to determine if there was co-elution with the degradation products.

## Moisture Analysis

Analysis of moisture was performed on both the fortified masa and the tortilla samples of Part III of this research, in order to determine the retention of added lysine and tryptophan on a dry basis. For moisture analysis, a procedure similar to that of Reineccius and Addis (1973) was used. Two-gram samples were blended with 2 g of anhydrous methanol in 4-dram screw top vials. One-half gram of anhydrous ethanol was added as an internal standard. The sample was allowed to mix for 30 min on a gyratory shaker (Model G10, New Brunswick Scientific Co.). The moisture content of methanol was determined using gas chromatography. The system consisted of a Hewlett Packard thermal conductivity gas chromatograph (Model 7620A) and a Hewlett Packard Model 3380A recorder-integrator. Separation of water, methanol and ethanol was achieved using a 1 m $\times$ 0.32 cm OD stainless steel column packed with Porapak Q (Waters Associates) operated isothermally at 110°C with a helium carrier gas flow of 35 ml/min. Quantitation of the water in the methanol was achieved using ethanol as an internal standard.

### Statistical Analysis

In the experiment to evaluate the effect of pH and heat on the stability of tryptophan of Part I, a split plot $2 \times 4$ factorial design was employed. A $3 \times 2$ factorial design was used in the experiment which examined the effect of heat, reducing sugar and protein on the stability of tryptophan. Multiple regression analysis of variance was used to analyze the data in each of these experiments (Steel and Torrie 1980). A split plot $3 \times 5$ factorial design was used in Part II of this research. In Part III of this research, statistical analysis was accomplished using two-tailed unpaired comparison t-tests.

## RESULTS AND DISCUSSION

HPLC was chosen for determining the free amino acid because it is fairly rapid, sensitive, and is capable of quantifying free tryptophan (DeVries *et al.* 1980; Yong and Lau 1979) and dansyl derivations of amino acids (Bayer *et al.* 1976). The HPLC method is also specific for the biologically available form of free tryptophan. Alterations of the tryptophan molecule such as the attachment of carbonyl groups during Maillard browning or oxidation of the indole ring would be expected to give altered retention times or absorbance characteristics when compared to free tryptophan. Tryptophan extraction efficiency from reaction mixtures containing the single free amino acid tryptophan was 98.8 ± 3.6% (mean ± standard deviation). Extraction efficiencies in reaction mixtures containing all three amino acids were 95 ± 1.0%, 95 ± 1.0% and 82 ± 1.2% for threonine, tryptophan and lysine, respectively. While the recovery of lysine was somewhat lower than the other amino acids, the method was sufficiently reproducible to determine lysine loss based on difference from a control sample.

Part I of this research examined the stability of tryptophan to pH, heat, reducing sugar, protein, hydrogen peroxide and light. In the first experiment, the effects of pH and heat on tryptophan stability were investigated. There was no significant difference in tryptophan stability for the two systems (slurry or solution) at a 10% level of significance or less. Individual effects of pH (3.0-7.5) and heat (95°C, for 12 h) were significant at a 5% level of significance or less. Interactions between pH and heat were significant at levels of 5% or less. Interactions between system and heat, system and pH, or system, pH and heat were not significant at levels of 5% or more. These results indicate that pH and heat and the interactions of pH and heat have a significant influence on the stability of tryptophan in either a liquid or slurry system. Since the

multiple regression analysis of variance showed no significant difference between the liquid and slurry systems, values for each pH for the two systems were combined to determine significant differences among mean values for the retention of tryptophan after heat treatment. A one-way analysis of variance was then performed and Tukey's W procedure was used to determine significant differences among means (Steel and Torrie 1980). Mean retention values for pH levels from 3 to 6 were 90-96% and were not significantly different from each other. At pH 7.5 the mean retention was 79.9 ± 4.9% and this was significantly lower than the means at each other pH ($P = 0.05$). The pH range studied required that the composition of the buffer salts be varied and this could have had some effect on tryptophan stability. All treatments contained phosphate buffer but citrate was not present at 7.5 where losses were somewhat greater. The greater loss at pH 7.5 could also be due to a difference in the stability of the ionic forms of tryptophan. The results indicate tryptophan in a solution or a slurry without other reactive compounds is relatively unaffected by the heat treatment of 95°C for 12 h, at the pH levels found in most foods.

The second experiment investigated the effects of heat, glucose and casein on the stability of tryptophan. These factors, along with a $a_w$ of 0.56, were chosen because they represent conditions and a composition that would promote Maillard browning and loss of added amino acids. (Wolf *et al.* 1981). Glucose was chosen as a common reducing sugar, and casein was chosen as a representative protein. The results indicated that individual effects for casein, glucose and heat were significant. Mean values for the retention of tryptophan for the various conditions are listed in Table 1.

Table 1. Retention of tryptophan for conditions of heat (95°C, 12 h), glucose and casein[a]

| Factors Studied[b] | | | |
|---|---|---|---|
| Heat | Glucose | Casein | Retention of Tryptophan(%)[c] |
| − | + or − | + or − | 100.0 ± 1.8 |
| + | − | − | 101.7 ± 1.1 |
| + | + | − | 70.8 ± 1.2 |
| + | − | + | 83.0 ± 0.9 |
| + | + | + | 61.0 ± 0.6 |

[a] initial $a_w = 0.56$

[b] + present, − absent

[c] mean of 4 replicates ± standard deviation

One would expect an interaction to occur with glucose and heat in this system. These conditions are conducive to the participation of tryptophan in the Maillard reaction. An interaction between casein and heat also occurred when measuring tryptophan recovery. This interaction is more difficult to explain, as one would not predict any major reaction to occur in the presence of tryptophan, casein and heat. A protein-amino acid interaction is possible and some type of adsorption of tryptophan onto casein may have occurred. The loss of free tryptophan was greatest in the presence of all three factors.

The effects of varied hydrogen peroxide levels on tryptophan stability are shown in Table 2. Without heating, none of the treatments containing hydrogen peroxide showed destruction during the 12 h period. When heated at 95°C, tryptophan losses were apparent even at hydrogen peroxide concentrations less than 1 ppm. The hydrogen peroxide levels used for the treatments represent concentrations that are considerably less than the 1000-3000 ppm that might be used in a food system (Rasekh *et al.* 1972; Cuq *et al.* 1973; Sreedhara and Subramanian 1981). However, hydrogen peroxide tends to be reactive in complex food systems so that the effective concentration available for reaction with amino acids would be less than the amount added. The data does suggest that tryptophan is sensitive to even low levels of hydrogen peroxide and this could have nutritional implications in food systems containing peroxidizing compounds.

When the samples were analyzed using detection at 254 nm, several peaks in addition to tryptophan appeared in the chromatogram. The major degradation peak co-eluted with the standard N-formylkynurenine implicating this as a breakdown product, although no further confirming studies or quantitation was done. Yong *et al.* (1980) demonstrated that N-formylkynurenine was the major degradation product of the reaction of tryptophan with peroxidizing methyl linoleate. It appears that both peroxidizing lipids and hydrogen peroxide can lead to tryptophan loss, perhaps along the same reaction pathway.

Table 2. Effect of hydrogen peroxide on tryptophan retention in buffered solution at pH 6.0 heated at 95°C for 12 h

| $H_2O_2$ Concentration (ppm) | Tryptophan Retention (%)[a] |
|---|---|
| 0.17 | 95 ± 1.0 |
| 1.7 | 85.5 ± 0.9 |
| 17.0 | 49.6 ± 2.0 |
| 170.0 | 14.0 ± 5.2 |

[a] Mean of 8 replicates ± standard deviation

When tryptophan solutions in glass containers were exposed to regular fluorescent light, there was very little degradation. The losses were less than 12% after two weeks, and there was no apparent influence of pH on the slight loss. No evidence of increases in kynurenine or N-formylkynurenine were observed, although Karel and Yong (1979) suggested that these would be expected products of tryptophan photooxidation. It is likely that the wavelengths and intensity of normal fluorescent lighting through glass is not going to result in extensive tryptophan loss. Photodegradation of free tryptophan in fortified foods is probably not a major factor in assessing the stability of this amino acid.

Part II of this research examined the stabilities of lysine, threonine and tryptophan in a reaction mixture prone to Maillard browning. Two systems, $a_w$ 0.22 and $a_w$ 0.75, were used, along with 5 different heating times to study the stabilities of these three amino acids. Lysine was included because of its role as a first-limiting amino acid and threonine and tryptophan were of interest because of their roles as second-limiting amino acids in cereal grains.

Individually, the effects of water activity, length of heating time and amino acid type had a significant effect on the percent retention of an amino acid (Fig. 1 and 2). For all three of the amino acids, retention

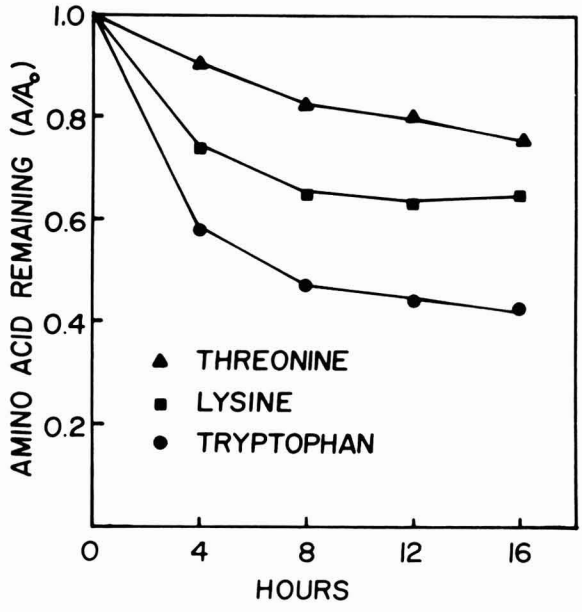


FIG. 1. RETENTION OF LYSINE, THREONINE, AND TRYPTOPHAN, HEATED AT 95°C (INITIAL $a_w$ 0.22)

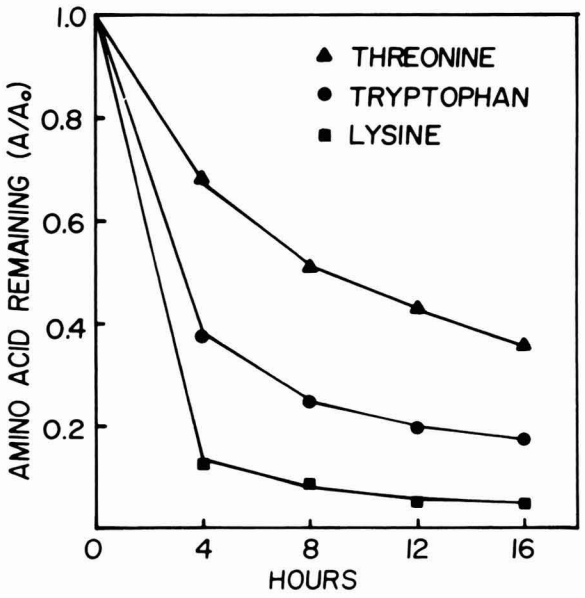


FIG. 2. RETENTION OF LYSINE, THREONINE, AND TRYPTOPHAN, HEATED AT 95°C (INITIAL $a_w$ 0.75)

was much greater at $a_w$ 0.22 than at $a_w$ 0.75. This is probably because the loss reactions involve Maillard browning which will generally proceed at a high rate in the $a_w$ range of 0.6 to 0.8 (Labuza 1970). The retention of threonine was higher than the retention of tryptophan or lysine in both systems. Threonine has only one amino group available for reaction in Maillard browning, and the other functional groups of the molecule would not be expected to react under these experimental conditions. Lysine has two free amino groups and tryptophan has one amino group and a somewhat reactive indole ring. The retention of tryptophan is greater than lysine at $a_w$ 0.75 but lysine retention is greater than tryptophan in the system at 0.22. One reason for the apparent difference in relative stability is that at the lower water activity, browning is not as extensive and other reactions such as those involving the indole ring could become significant. However, at a $a_w$ of 0.75, Maillard reactions predominate and lysine, with two amino groups, undergoes very rapid loss. The results suggest that lysine losses will be greater than tryptophan loss in situations where Maillard browning is extensive. In other circumstances, lysine may be relatively more stable than tryptophan.

Reaction rate constants were determined for the loss of each amino acid at both $a_w$'s. In each case, the data fit a first order reaction better than a zero order as determined by regression analysis. Thus, loss of each amino acid was found to fit the equation

$$\ln A = \ln A_0 - k\theta$$

where A = concentration of the amino acid
$A_0$ = initial concentration of the amino acid
$\theta$ = time
k = temperature dependent rate constant

The rate constants were calculated by treating multiple data points at a given time as separate points. Rate constants and correlation coefficients to a first order plot are shown in Table 3. The data indicates, again, that when compared under the same conditions, threonine was most stable and lysine or tryptophan was most unstable, depending on the $a_w$.

Other researchers who have studied the stability of free lysine and threonine in food systems have results that are somewhat different than those described above. Ericson *et al.* (1961) studied a fortified bread during baking and found through the use of rat growth experiments and microbiological assays that the loss of threonine was 20-40% and the loss of lysine of 5-15%. However, Murata *et al.* (1979) found that added lysine and threonine losses in bread were about equal, 14-15%. In the latter case, lysine was analyzed colorimetrically and threonine was analyzed by microbiological assay. It appears that the composition of the system, the heat treatment and the method of analysis could all be factors in comparing the relative stability of two amino acids such as lysine and threonine. Where Maillard browning is the major degradation reaction, threonine would be expected to show considerably higher retention than lysine or tryptophan as indicated in Fig. 1 and 2.

Part III of this research investigated the stability of added tryptophan and lysine in corn tortillas. Masa Harina was fortified at a level of 0.1% with each amino acid and the torillas were made in a typical

Table 3. Rate constants and correlation coefficients for the loss of free threonine, tryptophan, and lysine during browning at 95°C

| | Threonine | | Tryptophan | | Lysine | |
|---|---|---|---|---|---|---|
| $a_w$ | k ($h^{-1}$) | $r^2$ | k ($h^{-1}$) | $r^2$ | k ($h^{-1}$) | $r^2$ |
| 0.22 | 0.018 | 0.93 | 0.050 | 0.79 | 0.026 | 0.67 |
| 0.75 | 0.063 | 0.96 | 0.108 | 0.86 | 0.175 | 0.79 |

manner. Retentions of lysine and tryptophan after heating were 85.3% and 89.5%, respectively. The most probable cause of loss was due to Maillard browning although tryptophan may be involved in other reactions. Although significant losses of the two free amino acids occurred, 85% retention could still be considered very acceptable. Losses of free lysine and free tryptophan during tortilla preparation would not appear to be a major fortification problem.

## ACKNOWLEDGMENT

This work supported in part by Minnesota Agricultural Experiment Station Project No. 18-87.

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# OPTIMIZATION OF TEXTURAL AND MORPHOLOGICAL PROPERTIES OF A SOY-GELATIN MOZZARELLA CHEESE ANALOG

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Received for Publication November 4, 1982
Accepted for Publication January 14, 1983

## ABSTRACT

*Imitation mozzarella cheese was made using soy isolate and gelatin as the protein source instead of caseinate, and its textural and ultrastructural properties were studied. Of all the hydrocolloids evaluated, GFS gum (a mixture of xanthan, locust bean and guar gum) was found to give the best textural properties and melting quality to the analog. Optimum processing conditions were 5 min each of dry blending and wet blending of ingredients at 80° C. Studies done on the effect of heat treatment (time/temperature of processing) and addition of mercapto-ethanol, urea and emulsifiers suggests that hydrogen bonds, disulfide bonds and hydrophobic interactions appear to be involved in stabilizing the gel. Processing and ingredient combinations are flexible and could be applied to manufacture of many other imitation cheeses.*

## INTRODUCTION

Mozzarella cheese analogs have successfully been made from soy protein, gelatin, fat and gum arabic and shown to exhibit textural and stretching properties similar to those of natural low moisture part-skim mozzarella cheese (Yang and Taranto 1982). The gum arabic functions as a stabilizer and thickener, inducing a pseudoplastic flow behavior in the melted analog which was apparently responsible for its stretchability. However, gum arabic at the concentration used (19% w/w) is not only costly but it also induces a "stickiness" on the surface of the cheese gel, and thus it is desirable to find other gums that can be substituted for gum arabic in the formulation.

Textural properties of protein gels are closely related to their microstructure (Stanley and Tung 1976) which in turn is determined by chemical composition and physico-chemical forces. Thus it is important to understand the effects of molecular interactions and the microstructure of the colloidal system on the textural properties of the product, especially as affected by composition and process treatment. The existence of disulfide bridges as an intra- and inter-molecular cross-link is quite common in proteins from all living matter. It is, however, virtually absent in gelatin (Clark and Courts 1977) and therefore, disulfide bonds are not responsible for gelation of gelatin. On the other hand, soy protein gels, especially those made from 11S globulins, are dominated by disulfide bonds. Hydrophobic interactions of the nonpolar groups of the proteins to form an associated network may also be involved in the formation of the progel from a soy protein paste, since this type of bonding is favored by a rise in temperature (Némethy *et al.* 1963). On the other hand, hydrogen bonds may be the main source of the increased hardness observed during progel-gel transition, since this type of bonding is favored by cooling (Furukawa *et al.* 1979).

Phosphates are widely used emulsifying agents because of their versatility, low cost and buffering activity. Other process cheese emulsifiers such as sodium citrate (trisodium citrate) have also been used for years in cheese processing, although there is conflicting data regarding the comparative effects of citrate and phosphates on cheese quality (Templeton and Sommer 1936; Kiermeirer and Möhler 1960; Bohac 1962). The consensus is that they are both very satisfactory emulsifiers when used properly.

This paper describes a study of the effect of various hydrocolloids, heat treatment and addition of selected emulsifiers and "bond disrupting" reagents on textural and morphological properties of an imitation mozzarella cheese. The objective was to measure selected rheological properties and morphological characteristics using scanning electron microscopy (SEM), and attempt to optimize the processing parameters vis-a-vis the textural properties.

## MATERIALS AND METHODS

### Raw Materials

Soy protein isolate (Promine-D) was obtained from Central Soya Company (Fort Wayne, IN). Type-B gelatin (128 Bloom) was obtained from Baker Chemical Company (Phillipsburg, N.J.). A partially hydro-

genated coconut shortening, Hydrol-100, was obtained from Durkee Industrial Foods Group (Chicago, IL). GFS, a food grade xanthan-locust bean-guar gum mixture, was obtained from Kelco Company (Rahway, N.J.). Guar gum (Jaguar A-40-F) was obtained from Celanese Plastics & Specialities Company (Louisville, KY).

Urea was purchased from Mallinckrodt, Inc. (Paris, KY) and mercaptoethanol from Eastman Organic Chemicals (Rochester, N.Y.). The emulsifiers used were monosodium phosphate monobasic (Baker & Adamson Company, New York, N.Y.), sodium pyrophosphate (Fisher Scientific Company, Fair Lawn, N.J.), sodium hexametaphosphate-69% $P_2O_5$ (Victor Chemicals, Chicago, IL) and sodium citrate (Baker & Adamson Co., Morristown, N.J.).

The commercial sample, used as a basis of comparison, was part-skim mozzarella cheese from Kroger Foods, containing 17.4% fat (dry basis).

## Standard Processing Procedure

Dry ingredients (soy isolate, hydrocolloid and gelatin) were mixed in a Hobart mixer (Kitchen Aid, The Hobart Mfg. Company, Troy, OH) with a 3-quart mixing bowl and a dough bar at speed #1 for 5 min. The mixing bowl was immersed in a water bath held at 80°C to aid dispersion. Water and the fat (both preheated to 80°C) were then added and the slurry was mixed at speed #4 for 5 min with the mixing bowl immersed in the 80° water bath. Finally, the melted product was poured into an aluminum mold and cooled in an ice-water bath (4.5°C) followed by tempering at 4.5°C for 24 h. When the "bond disrupting" reagents (mercaptoethanol and urea) or emulsifiers were used, they were added in the wet-blending stage, and processing conditions were 5 min dry mixing/5 min wet blending and temperature was 80°C.

## Variables

The variables investigated and their levels are shown below:

- Mixing time at 80°C processing temperature (min dry mixing/min wet blending): 1/1, 1.5/1.5, 2.5/2.5, 5/5, 10/10 and 15/15.
- Processing temperature at 5 min dry mixing/5 min wet blending time (°C): 40,60,80.
- Concentration of urea (M, moles/liter added water): 0.1, 1.5, 3.0.
- Concentration of mercaptoethanol (M, moles/liter added water): 0.01, 0.02, 0.1.
- Concentration of emulsifiers (% w/w dry basis): 1, 5.

## Evaluation of rheological properties

An Instron Universal Testing Machine (Model TM) with a CCM compression load cell was used to measure the texture profile analysis (TPA). A plunger with a diameter of 0.64 cm was attached to the moving crosshead. The speed of the crosshead was set at 2 cm/min in both upward and downward directions. The recording chart speed was set at 5 cm/min. The sample was obtained from a sample block by using a cork borer with a 2.3 cm inside diameter, shaped with a sharp knife to a 2 cm height. The penetration of the plunger into the sample was set for 1.6 cm (80% deformation). A full-scale load range of 2 kg was used and two consecutive bites were taken. Seven parameters (Table 1) were obtained from the resulting curve (Fig. 1). The measurements were conducted at 20 ± 1°C.

Table 1. Definitions of rheological terminology in Instron curve

1. *Fracturability* – force with which the material fractures; it is the force at the first significant break in the curve; ($H_1$).
   (crumby → crunchy → brittle)

2. *Hardness* – the force necessary to attain a given deformation; is the peak force during the first compression cycle (first bite); ($H_2$).
   (soft → firm → hard)

3. *Adhesiveness* – the work necessary to overcome the attractive forces between the surfaces of the food and the other materials with which the food comes in contact (e.g. tongue, teeth, palate, etc.); is the negative force area for the first bite; ($A_3$).
   (sticky → tacky → gooey)

4. *Cohesiveness* – the strength of the internal bonds making up the body of the product; is the ratio of the positive area during the second compression to that during the first compression; ($A_2/A_1$).

5. *Springiness* – the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite.
   (plastic → elastic)

6. *Gumminess* – the energy required to disintegrate a semi-solid food product to a state ready for swallowing. It is related to primary parameters of hardness and cohesiveness; is the product of hardness × cohesiveness; ($H_2 \times A_2/A_1$).
   (short → mealy → pasty → gummy)

7. *Chewiness* – the energy required to masticate a solid food product to a state ready for swallowing. It is related to primary parameters of hardness, cohesiveness, and springiness, or a product of gumminess × springiness; ($H_2 \times A_2/A_1 \times$ Springiness).
   (tender → chewy → tough)

Source: Yang and Taranto (1982)

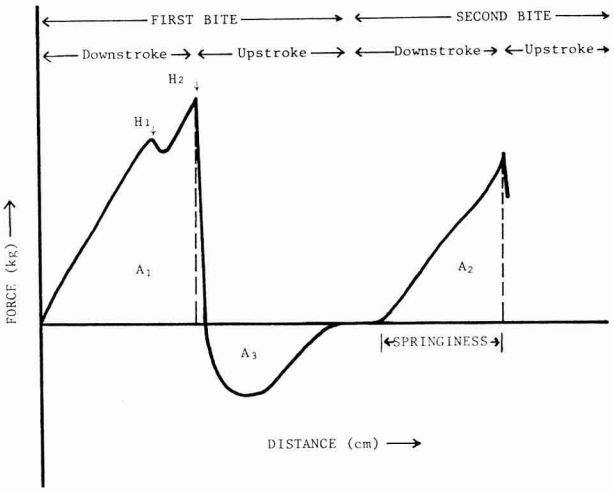



FIG. 1. TYPICAL INSTRON TEXTURE PROFILE CURVE FOR SOYBEAN CHEESE ANALOGS($H_1$: fracturability; $H_2$: hardness; $A_1$: area of first downstroke; $A_2$: area of second downstroke; $A_3$: adhesiveness).

A Weissenberg Test was used to measure the stretchability of the melted cheese samples. The experiment design is such that the liquid sample is sheared in a gap between an outer vessel rotated with a constant angular velocity and rigidly fixed inner rod. As the vessel rotates, the sample undergoes a stationary laminar shearing movement because of the combined actions of the shear imposed at the boundaries and the forces of gravity and inertia (centrifugal forces). The developed stress is distributed in various directions in space, generating a pull along the lines of flow. The lines of flow are closed circles in this experiment design. The pull along these lines compresses the liquid and forces it inwards against the centrifugal forces and upwards against the forces of gravity. By taking the initial height of the sample on the rod and the final height of the climbing sample after rotating at a fixed RPM for a certain period of time, a comparison of the stretchability among various samples can be obtained. For this experiment, a 50 ml beaker lined with a $4 \times 4$ wire screen containing 30 gm of sample was equilibrated to 80°C over a water bath. The initial submerging depth of the rod which is wrapped with a No. 2 filter paper, was 1 cm. Both the screen and the filter paper were used to reduce the lubricating effect of the exuded sample fat. The final climbing height was recorded after 1 min rotating at 60 RPM.

**Melting Quality.** The Schreiber Melting Test was used, as described by Kosikowski (1978). Essentially, this consisted of preparing a disc of the

sample of 0.5 cm height with 2.3 cm diameter, placing it in the center of a clean glass thin-walled, 15 × 100 mm Petri dish with cover. This was then placed in a preheated 232°C electric oven for exactly 5 min. The sample melted into a flat circle. The size of the melted circle was measured by centering the melted sample over a concentric numbered target-type graph (the distance between concentric lines was 0.65 cm). Data for melting quality are reported numerically as the outer edge of the flow line.

Triplicate measurements were made to each sample for all the rheological evaluations.

**Sample Preparation for Scanning Electron Microscopy.** All samples were prepared for SEM according to Kalab (1978). Samples were fixed, frozen in dry ice-acetone bath, freeze dried, fat-extracted with chloroform and dry fractured. Samples were then mounted on SEM stubs and coated with gold, and were then examined with a JEOL-JSM-U3 Scanning Electron Microscope operated at 10 Kv with a 200 $\mu$m aperture and a 13 mm working distance at a 40° tilt.

### Statistical Analysis

For the experiments with only one independent variable, a fixed-constant model with single classification was applied, and a one-way ANOVA with 5% significance level and a F-test were used to analyze the data. A Tukey's Omega procedure at the 5% level of significance was used to rank the data (Steel and Torrie 1960). In the experiment with two independent variables, i.e. amount of gelatin and amount of GFS gums, a 2 × 3 factorial design was used to evaluate the effects of individual variables and the interaction between these two variables on the dependent variable (i.e. TPA parameters, etc.). If the interaction was not significant and the effects of individual variables were significant, a polynomial regression model was selected using a computer program (SPSS, Computing Service, University of Illinois) to detect the magnitude of the individual variables on that particular parameter.

## RESULTS AND DISCUSSION

### Effect of hydrocolloids and gelatin

The composition of samples made from a combination of gelatin, fat, soy isolate, water and different types and levels of gums is shown in Table 2. Samples 1 and 2 are control samples with high levels of gum arabic and difference levels of soy isolate, similar to the analogs described by Yang and Taranto (1982). Samples 3 and 4 compare the

effect of level of GFS, while samples 4 and 5 compare the effect of relative concentrations of soy isolate and gelatin at the same level of GFS gum. Sample 6 is identical to sample 5 except it is made up with another gum, guar gum.

In order to get the same viscosity with GFS that we obtained with gum arabic in the control samples 1 and 2, we would need only about 0.5-1 g per 100 g water in the formulation (Yang 1982), i.e. about 0.28-0.55% w/w, as shown for samples 3 and 4. However, texture data (Table 3) show that these analogs generally had inferior properties compared to the control samples 1, 2 and commercial samples. However, fracturability, hardness, adhesiveness, gumminess, chewiness, stretchability and melting quality could be improved by a small increase in the gelatin concentration and a decrease in the soy isolate concentration (compare samples 4 and 5), although this had no effect on other properties. A difference in stretchability was observed between 0.28% and 0.55% GFS (samples 3 and 4) indicating the importance of an adequate amount of GFS gum. Guar gum had much higher viscosity than other gums at equivalent concentrations (Yang 1982) and thus the higher fracturability and hardness (compared to GFS gum) are expected. However, there was almost no measurable stretchability with guar gum analogs (Table 3, Sample 6).

These differences can also be observed in the scanning electron micrographs. Compared to sample 1 (Fig. 2), GFS analogs showed no visible soy isolate or gum particles (Fig. 3 and Fig. 4) and their surfaces were not "tacky". Some "chunks" were observed in the lower GFS analog, sample 3 (Fig. 3, arrows) that were not visible at the higher GFS concetration (Fig. 4). Apparently there was insufficient GFS in sample 3 to assist gelatin and soy proteins in forming a gel network. These "chunks" of condensed protein matrices also prevented the alignment of molecules during stretching, thus resulting in lower stretchability readings. Figure 5 shows the ultrastructure of the analog

Table 2. Composition of mozzarella cheese analogs (% w/w)

| Sample | Hydrocolloid | | Gelatin | Fat | Soy Isolate | Added Water |
|---|---|---|---|---|---|---|
| | Type | Concentration | | | | |
| 1 | Gum arabic | 19.0 | 19.0 | 4.8 | 9.5 | 47.7 |
| 2 | Gum arabic | 17.4 | 17.4 | 4.3 | 17.4 | 43.5 |
| 3 | GFS | 0.28 | 11.1 | 11.1 | 22.2 | 55.4 |
| 4 | GFS | 0.55 | 11.1 | 11.1 | 22.1 | 55.3 |
| 5 | GFS | 0.55 | 16.6 | 11.1 | 16.6 | 55.3 |
| 6 | Guar | 0.55 | 16.6 | 11.1 | 16.6 | 55.3 |

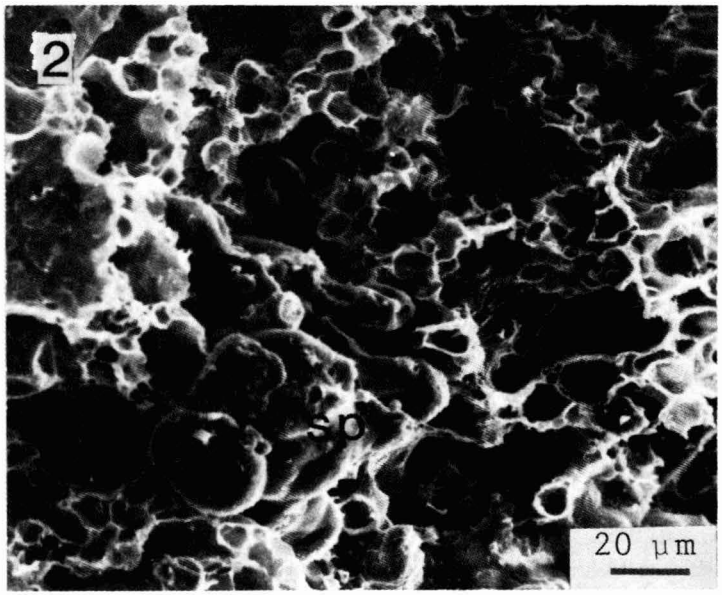



FIG. 2. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG CONTROL PREPARED WITH 19% GUM ARABIC (SAMPLE 1)Note soy proteins (SP) adhering to a gum particle.

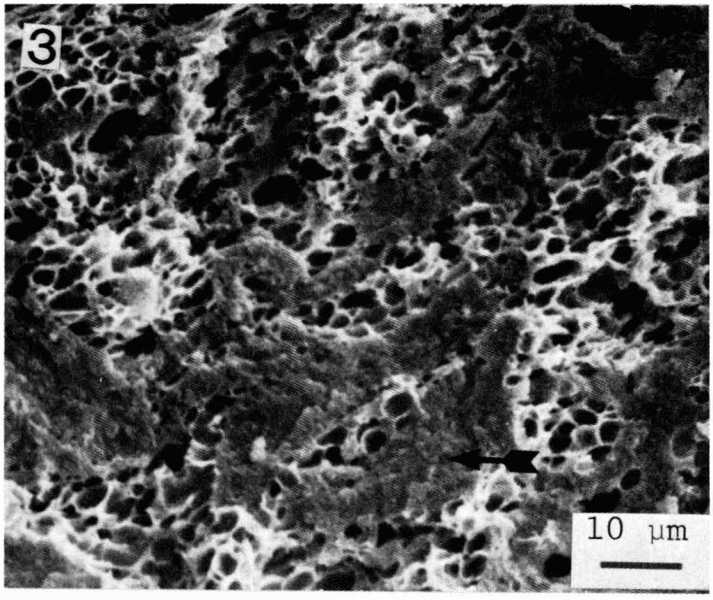



FIG. 3. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED WITH 0.28% GFS (SAMPLE 3)Note "chunks" (indicated by the arrows).

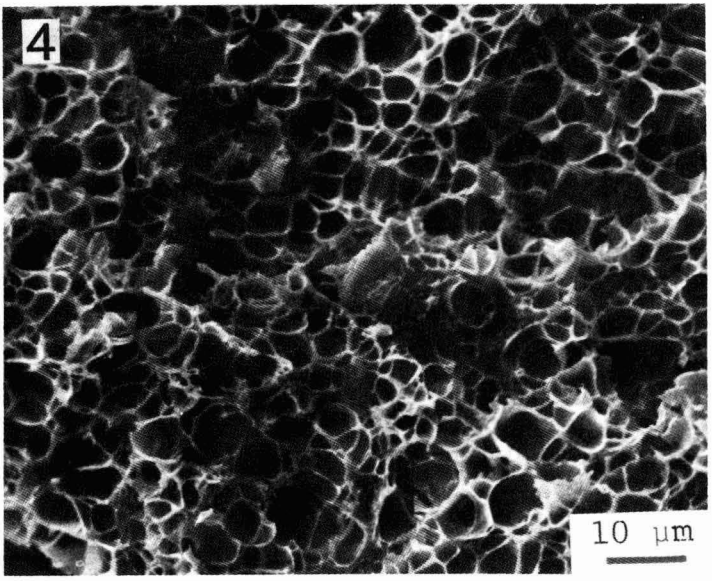



FIG. 4. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED WITH 0.55% GFS (SAMPLE 4) Note the honey-combed network and there are no "chunks."

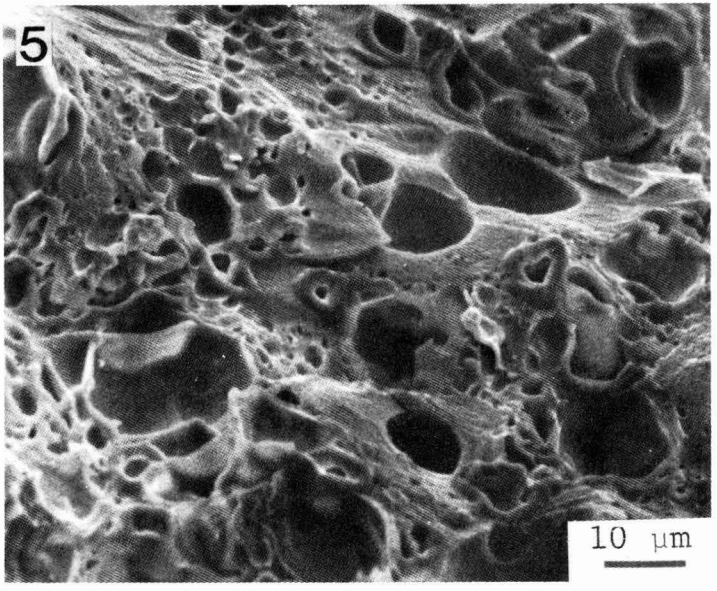


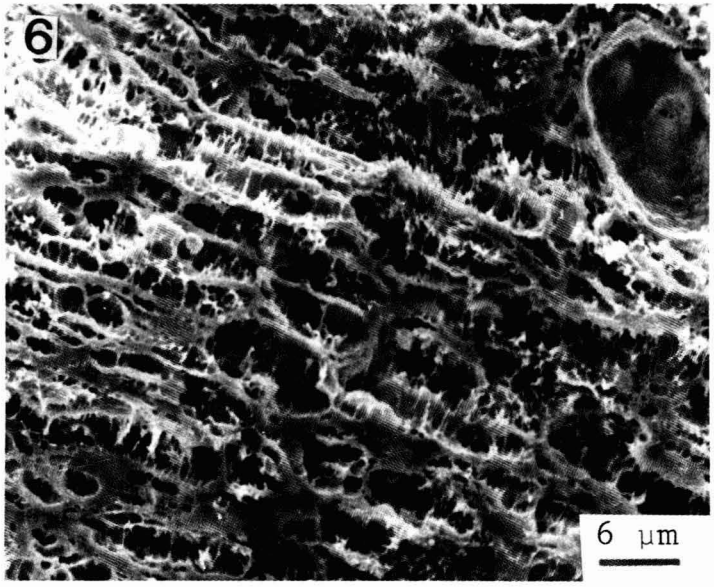

FIG. 5. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED WITH 0.55% GUAR GUM (SAMPLE 6)No clear honey-combed protein network can be observed.

made with guar gum (sample 6); no clear honeycombed network can be observed as with sample 4. The highly viscous guar gum tends to bind soy protein and gelatin into a densely packed protein matrix, which results in much less flexible structure, which in turn results in poor stretchability.

Figures 6 and 7 demonstrate the stretchability concept. Figure 6 is a sample of natural mozzarella cheese in a stretched state; the alignment of the fiber-type bundle and the air cells are visible. Figure 7 is analog #5 in its stretched state. Both showed a similar fibular alignment.

As shown in Table 3 the higher gelatin levels in sample 5 imparted properties similar to those of the commercial sample. Samples had almost twice the fracturability, hardness, adhesiveness and stretchability as the analog prepared with lower gelatin content (sample 4). This clearly indicates that gelatin contributes more than soy protein to gel fracturability, hardness and adhesiveness, and a certain ratio (e.g. 1:1) of gelatin and soy isolate should be maintained to obtain a good progel stretchability. Higher gelatin resulted in higher gumminess and chewiness but had no effect on springiness. The higher melting quality of sample 5 compared to sample 4 could be due to the greater meltability character of gelatin gels compared to soy protein gels.

FIG. 6. SCANNING ELECTRON MICROGRAPH OF NATURAL MOZZARELLA CHEESE (LOW MOISTURE PART-SKIM)This micrograph was prepared from sample in the stretched state and dry fractured. Note the clear, fiber-type alignment in the stretched bundle and compare to those in Fig. 7.



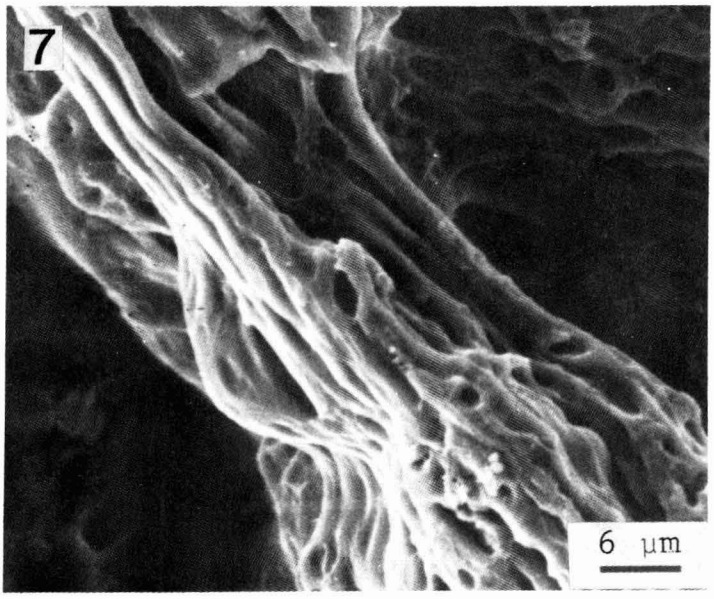



FIG. 7. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED WITH 0.55% GFS (SAMPLE 5)This micrograph was prepared from sample in the stretched state. Note the clear, fiber-type alignment in the stretched bundle.

## Effect of Gelatin and GFS Concentration

The data so far appeared to suggest that both concentration of the gum and gelatin are important in developing textural and stretching properties. Thus a study was then conducted on the effects of varying GFS and gelatin concentration (Table 4). The analysis of variance had indicated that only main effects were important (not shown here; see

Table 3. Textural properies and melting quality of soybean mozzarella cheese. Sample formulations shown in Table 2

| Property | 1 | 2 | 3 | 4 | 5 | 6 | Commercial |
|---|---|---|---|---|---|---|---|
| Fracturability (kg) | 1.12 | 0.98 | 0.70 | 0.58 | 1.05 | 1.37 | 0.90 |
| Hardness (kg) | 1.13 | 1.33 | 0.78 | 0.69 | 1.11 | 1.48 | 1.15 |
| Adhesiveness (kg-cm) | 0.16 | 0.28 | 0.18 | 0.20 | 0.35 | 0.33 | 0.38 |
| Cohesiveness | 0.51 | 0.55 | 0.52 | 0.51 | 0.42 | 0.49 | 0.49 |
| Springiness (cm) | 1.56 | 1.58 | 1.54 | 1.53 | 1.54 | 1.53 | 1.52 |
| Gumminess (kg) | 0.57 | 0.72 | 0.40 | 0.35 | 0.47 | 0.74 | 0.57 |
| Chewiness (kg-cm) | 0.89 | 1.13 | 0.62 | 0.54 | 0.72 | 1.11 | 0.86 |
| Stretchability (cm) | 0.58 | 1.44 | 0 | 0.55 | 1.07 | 0 | 1.10 |
| Melting Quality | 2.15 | 1.60 | 1.54 | 1.74 | 2.65 | 2.15 | 2.49 |

Table 4. Texture Profile Analysis of soybean-gelatin mozzarella cheese analogs prepared with different levels of gelatin and GFS gum. amples contained 16.7-17.6% soy isolate, 11.8-12.5% fat and 55-58% added water. Processing temperature = 80°C, time = 5/5 min[1] (dry/wet lending)

| elatin oncentration (%) | GFS Concentration (%) | Fract. (kg) | Hard. (kg) | Adhes.[2] (kg-cm) | Cohes. | Spring. (cm) | Gumm. (kg) | Chew. (kg-cm) | Stretch.[2] (cm) |
|---|---|---|---|---|---|---|---|---|---|
| 11.8 | 0.55 | $0.558^{d}$ | $0.592^{cd}$ | 0.114 | $0.411^{b}$ | $1.54^{a}$ | $0.243^{d}$ | $0.373^{d}$ | 0.41 |
| | 0.83 | $0.554^{d}$ | $0.640^{c}$ | 0.126 | $0.481^{a}$ | $1.54^{a}$ | $0.308^{c}$ | $0.474^{c}$ | 0.43 |
| | 1.10 | $0.441^{e}$ | $0.545^{d}$ | 0.204 | $0.495^{a}$ | $1.51^{a}$ | $0.270^{cd}$ | $0.408^{cd}$ | 0.48 |
| 16.7 | 0.59 | $1.053^{a}$ | $1.113^{a}$ | 0.348 | $0.421^{b}$ | $1.54^{a}$ | $0.469^{b}$ | $0.724^{b}$ | 1.07 |
| | 0.88 | $0.966^{b}$ | $1.133^{a}$ | 0.347 | $0.478^{a}$ | $1.55^{a}$ | $0.540^{a}$ | $0.834^{a}$ | 0.51 |
| | 1.16 | $0.807^{c}$ | $1.032^{b}$ | 0.275 | $0.475^{a}$ | $1.56^{a}$ | $0.490^{b}$ | $0.762^{ab}$ | 0.53 |

Data in the same column with different letters are significantly different ($P < 0.05$)
Analysis of variance indicated both main effects and interactions were significant (Yang 1982)

Table 5. Effect of processing temperature on textural properties and melting quality of mozzarella cheese analog (sample 5; Table 2). rocessing time = 5 min/5 min dry blending/wet blending

| emp. °C) | Fract. (kg) | Hard. (kg) | Adhes. (kg-cm) | Cohes. | Spring. (cm) | Gumm. (kg) | Chew. (kg-cm) | Stretch. (cm) | Melting Quality |
|---|---|---|---|---|---|---|---|---|---|
| 40 | $0.335^{c}$ | $0.445^{c}$ | $0.155^{c}$ | $0.452^{a}$ | $1.46^{b}$ | $0.202^{c}$ | $0.295^{c}$ | $1.06^{a}$ | $4.66^{a}$ |
| 60 | $0.412^{b}$ | $0.579^{b}$ | $0.217^{b}$ | $0.444^{a}$ | $1.49^{b}$ | $0.257^{b}$ | $0.383^{b}$ | $1.09^{a}$ | $3.04^{b}$ |
| 80 | $1.053^{a}$ | $1.113^{a}$ | $0.348^{a}$ | $0.421^{a}$ | $1.54^{a}$ | $0.469^{a}$ | $0.724^{a}$ | $1.07^{a}$ | $2.65^{c}$ |

Data in the same column with different letters are significantly different ($P < 0.05$)

Yang 1982). Thus multiple linear regression equations for the four parameters of importance could be developed as follows:

$$\text{Fracturability} = -0.275 + 0.090 \text{ (amount of gelatin)} - 0.325 \text{ (amount of GFS)}:$$

$$r = 0.978, \text{ where } r \text{ is the correlation coefficient}$$

$$\text{Hardness} = -0.533 + 0.103 \text{ (amount of gelatin)} - 0.113 \text{ (amount of GFS)}:$$

$$r = 0.984$$

$$\text{Gumminess} = -0.302 + 0.046 \text{ (amount of gelatin)} + 0.044 \text{ (amount of GFS)}:$$

$$r = 0.949$$

$$\text{Chewiness} = -0.484 + 0.072 \text{ (amount of gelatin)} + 0.067 \text{ (amount of GFS)}:$$

$$r = 0.950$$

In general, the models above indicate gelatin had a positive effect on these parameters while the level of GFS had a negative effect on fracturability and hardness and a positive effect on gumminess and chewiness. The magnitudes of the coefficients in the above correlations are similar except GFS has 3-4 times the effect on fracturability as did the gelatin concentration. This may have occurred because the GFS may have helped to build a hydrogen-bonded network with a more evenly dispersed aqueous phase, which can reduce case-hardening on the surface, thus lowering fracturability readings.

Higher gelatin concentrations, regardless of GFS levels, increased the values of all properties measured, except for cohesiveness and springiness, confirming earlier results regarding the effect of gelatin. However, part of this difference in texture could be due to the difference in moisture content of these two samples; the 11.8% gelatin analog contained 58% added water while the 16.7% gelatin analog had 55% added water. Stretchability of these formulations, which was found to be affected by gelatin, GFS and the interaction of both, was much lower than for the commercial natural mozzarella cheese or the formulations with higher soy protein concentration, indicating that soy protein also plays a role in stretchability.

## Effect of Heat Treatment

The Instron TPA in Table 5 show that, except for cohesiveness, all texture profile parameters increased with increasing processing temperature, suggesting that since the rate of bond formation is apparent-

ly favored by a rise in temperature, hydrophobic interactions may be involved. However, unfolding of the protein also occurs with an increase in temperature, which provides more sites for hydrogen bond formation, which also could play a role in the increased fracturability, hardness and adhesiveness observed during the transition from progel to gel, since this type of bonding is favored by cooling (Furukawa *et al.* 1979).

Cohesiveness was unaffected by processing temperature in agreement with the data of Furukawa *et al.* (1979). Since stretchability tests were conducted after tempering each sample at 80°C for 30 min., the soy protein molecules in each sample were unfolded to the same extent and thus the similar values in Table 5 are not surprising.

Melting quality is also a complex property. It is possible to have high melting point yet only moderate gel strength (Stainsby 1977). On warming a gel, the least stable, short links in the fine network are the first to become disordered, or melt. The strongest links remain intact to the melting point of the whole gel, and some persist in the viscous solution for a few degrees above the melting temperature (Stainsby 1977).

Gels stabilized by secondary forces frequently exhibit a relatively sharp melting temperature which depends on the relative number of bonds involved in each set interacting chain segments. Increasing the number of bonds per segment increases the melting temperature and the melting point becomes more sharply defined (Veis 1964). A significant increase in the melting quality of these analogs was observed as processing temperature decreased (Table 5). This indicates a sharp decrease in the number of bonds per segment, probably due to incomplete network formation. In practice, the melting quality of the cheese analogs should be maintained in a range close to that of commercial samples. Too high or too low a melting quality would downgrade the products.

Figures 8 through 10 show the SEM micrographs of mozzarella cheese analogs prepared at different temperatures. There was a distinct difference in the microstructure of the gels formed at various temperatures. The SEM image of the gel formed at 80°C (Fig. 8) revealed a porous structure with thin membraneous walls that could exhibit a high strain at a given stress. This translates to relatively high values of fracturability and hardness. In the 60°C-induced gel (Fig. 9), network formation was looser. In the 40°C-induced gel (Fig. 10), the development of the porous structure was not yet completed. This resulted in a paste-like structure with all the water, fat and air bubbles loosely entrapped in the system. This weak and imcomplete system may have

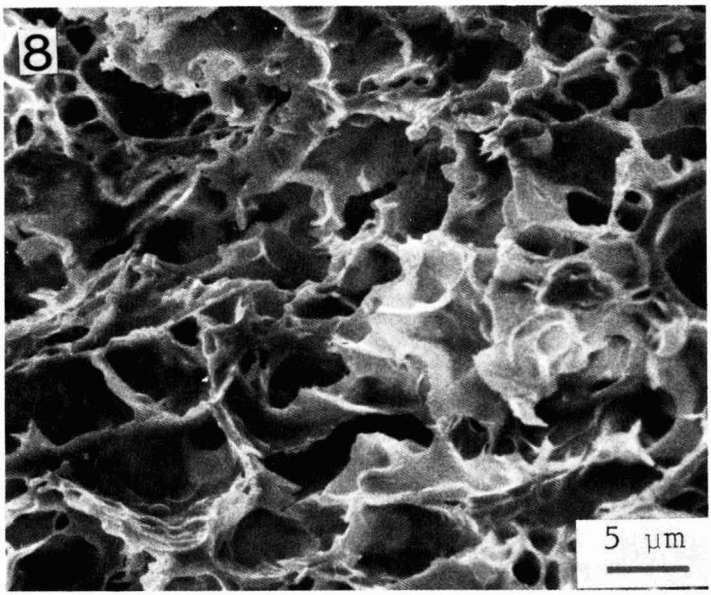



FIG. 8. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED AT 80°C

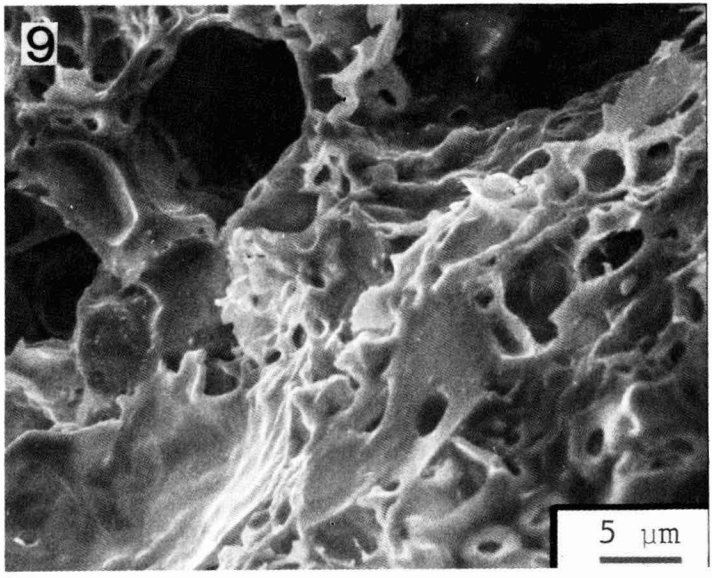



FIG. 9. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED AT 60°C

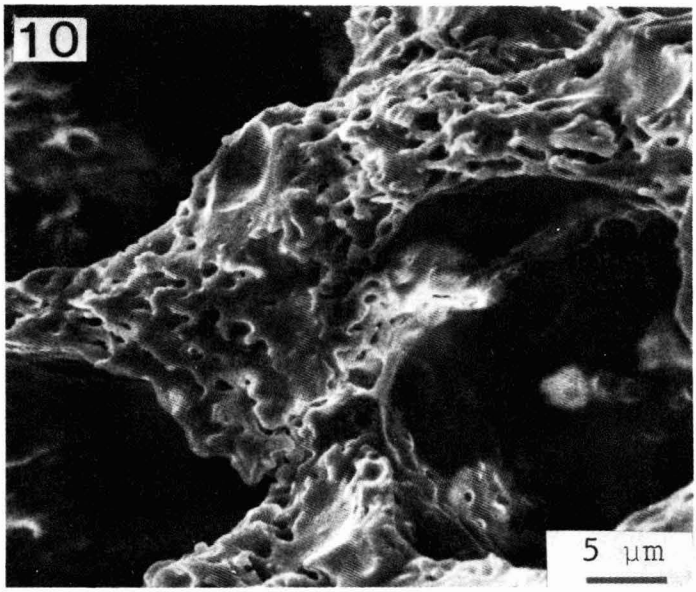



FIG. 10. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED AT 40°C

been responsible for the relatively low values of fracturability, hardness and adhesiveness. Thus it appears that the formation of a porous, "honeycombed" network is necessary for the production of a firm gel.

Table 6 shows the effect of processing (mixing) time on textural properties. The effect of heat is a function of time and temperature and thus it is not surprising that trends similar to the temperature effects were observed here also. Time, however, has another important effect. As a gelatin molecule is placed in a large volume of water, it will swell and its density decreases to approach a new equilibrium value. This state of equilibrium is considered as a balance between the tendency of the molecules to imbibe solvent and to resist elastic distention of the structure (Ferry 1948). A sufficient time is required in order to achieve this equilibrium. Soy proteins apparently need even longer time to be completely dispersed. The ultrastructures shown in Figures 11 and 12 illustrate this phenomenon. Figure 11 shows an insufficient swelling of the molecules. The system imbibed little solvent when wet blended for 1 min at 80°C. Hence, an underdeveloped network resulted which possessed less strength to resist the exerted strain. Samples wet blended for 5 min (Fig. 4) show a fine network with solvent evenly imbibed. This caused considerable distention of the structure. Figure 12 shows a gel that was wet blended for 15 min. However, the moisture

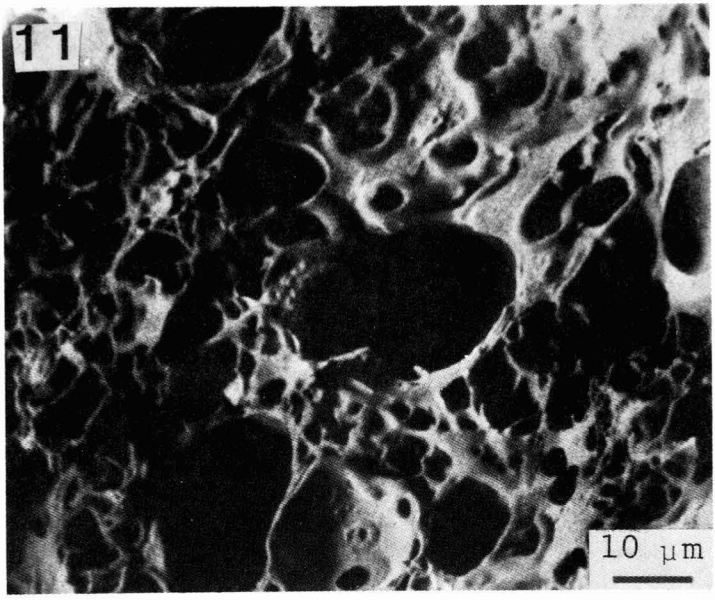



FIG. 11. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED AT PROCESSING TIME OF 1 MIN/1 MIN (DRY MIXING/WET MIXING)

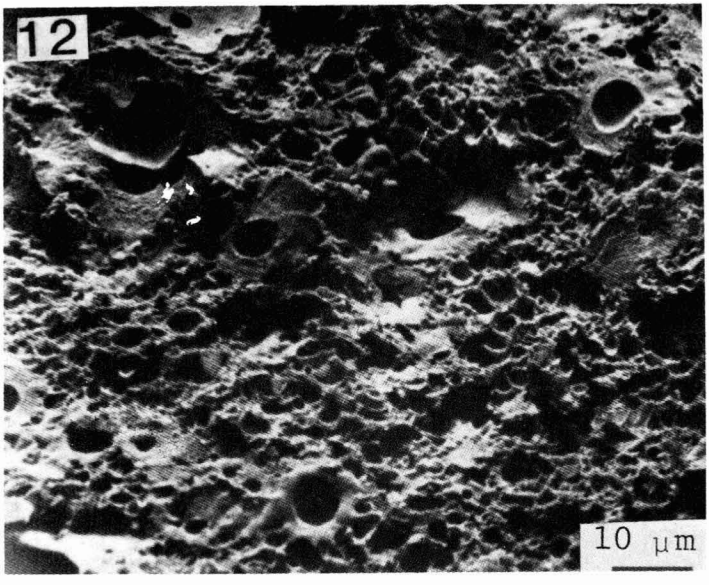



FIG. 12. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG PREPARED AT PROCESSING TIME OF 15 MIN/15 MIN (DRY MIXING/WET MIXING)

Table 6. Effect of processing time (min dry blending/min wet blending) on textural properties and melting quality of mozzarella cheese analog (sample 5; Table 2). Processing temperature = 80°C[1]

| Time (min/min) | Moisture (%) | Fract. (kg) | Hard. (kg) | Adhes. (kg-cm) | Cohes. | Spring. (cm) | Gumm. (kg) | Chew. (kg-cm) | Stretch. (cm) | Melting Quality |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/1 | 55.18$^{a}$ | 0.657$^{c}$ | 0.840$^{d}$ | 0.251$^{e}$ | 0.470$^{c}$ | 1.54$^{a}$ | 0.394$^{d}$ | 0.607$^{d}$ | 0.25$^{e}$ | 2.78$^{a}$ |
| 1.5/1.5 | 54.29$^{ab}$ | 0.664$^{c}$ | 0.902$^{d}$ | 0.296$^{d}$ | 0.492$^{bc}$ | 1.52$^{a}$ | 0.444$^{d}$ | 0.672$^{d}$ | 0.57$^{d}$ | 2.80$^{a}$ |
| 2.5/2.5 | 53.73$^{b}$ | 0.826$^{b}$ | 0.946$^{d}$ | 0.313$^{d}$ | 0.456$^{c}$ | 1.55$^{a}$ | 0.431$^{d}$ | 0.669$^{d}$ | 0.89$^{c}$ | 2.72$^{a}$ |
| 5/5 | 52.20$^{c}$ | 1.053$^{a}$ | 1.113$^{c}$ | 0.348$^{c}$ | 0.421$^{c}$ | 1.54$^{a}$ | 0.469$^{c}$ | 0.724$^{c}$ | 1.07$^{b}$ | 2.65$^{a}$ |
| 10/10 | 48.04$^{d}$ | 1.124$^{a}$ | 1.518$^{b}$ | 0.403$^{b}$ | 0.527$^{ab}$ | 1.52$^{a}$ | 0.797$^{b}$ | 1.212$^{b}$ | 1.16$^{a}$ | 2.66$^{a}$ |
| 15/15 | 44.32$^{e}$ | 1.276$^{a}$ | 1.798$^{a}$ | 0.458$^{a}$ | 0.554$^{a}$ | 1.52$^{a}$ | 0.997$^{a}$ | 1.516$^{a}$ | 1.19$^{a}$ | 2.64$^{a}$ |

[1] Data in the same column with different letters are significantly different ($P < 0.05$)

data in Table 6 show a considerable decrease in moisture as processing time increased. Thus it is possible that the high texture readings were caused by escape of the imbibed solvent which in turn caused the network to shrink, pack together and form a highly condensed system which resulted in high fracturability and hardness values. High readings in adhesiveness and cohesiveness were also noted at longer processing times. The significant difference these parameters contributed to the gel resulted in higher gumminess and chewiness values after 5 min wet mixing.

The critical processing time at 80°C seems to be between 2.5/2.5 and 5/5 min., since most changes were observed between these two times. Stretchability data showed significant effects of processing time up to 10/10 min. Melting quality, on the other hand, was found to be relatively unaffected by processing time despite a significant difference in moisture content. A similar result was reported by Arnott *et al.* (1957), who found that melting quality was related to the free tyrosine content rather than moisture content in process cheese. Thus, since these analogs had identical proportions of gelatin and soy protein, melting quality was relatively unaffected in our samples.

## Effect of Mercaptoethanol and Urea on Textural Properties

The addition of mercaptoethanol and urea was found to decrease the gel hardness (Table 7), indicating that both disulfide bonds and hydrogen bonds, in addition to hydrophobic bonds as suggested earlier, are involved in gel formation. This interpretation agrees with the findings of Catsimpoolas and Meyer (1970), Furukawa *et al.* (1979), Aoki (1970) and Kuwahata and Nakahama (1975). Both mercaptoethanol and urea lowered the adhesiveness significantly except at 0.1M urea.

The cohesiveness results were not fully understood. Cohesiveness values increased with increase in mercaptoethanol levels whereas they initially increase and then decrease with urea. There were no significant differences in springiness for all samples except the one treated with 3M urea which indicated severe structural destruction. Gumminess and chewiness generally show an initial increase and then decrease with urea level. Stretchability showed little sign of being affected except at the higher urea concentration, indicating that this property was probably controlled neither by hydrogen bonding nor hydrophobic interactions. An increase in the melting quality was also found in the reagent-treated samples, especially the urea-treated ones.

The microstructure of the analog treated with 0.1M mercaptoethanol is shown in Fig. 13. Numerous, large alveoli were observed with a very fine mesh-like network squeezed at the junction among alveoli. The

Table 7. Effect of bond modifiers (mercaptoethanol and urea) on textural properties and melting quality of mozzarella cheese analog (sample 5; Table 2)[1]

| Bond Modifier | Conc. (M) | Hard. (kg) | Adhes. (kg-cm) | Cohes. | Spring. (cm) | Gumm. (kg) | Chew. (kg-cm) | Stretch. (cm) | Melting Quality |
|---|---|---|---|---|---|---|---|---|---|
| Control | — | 1.113$^{a}$ | 0.348$^{a}$ | 0.421$^{c}$ | 1.54$^{a}$ | 0.469$^{c}$ | 0.724$^{c}$ | 1.07$^{ab}$ | 2.65$^{c}$ |
| Mercap. | 0.01 | 0.926$^{b}$ | 0.185$^{b}$ | 0.608$^{b}$ | 1.53$^{a}$ | 0.563$^{b}$ | 0.859$^{b}$ | 1.11$^{a}$ | 2.73$^{c}$ |
| | 0.02 | 0.765$^{c}$ | 0.168$^{b}$ | 0.616$^{b}$ | 1.56$^{a}$ | 0.472$^{c}$ | 0.733$^{c}$ | 1.09$^{a}$ | 2.78$^{c}$ |
| | 0.10 | 0.551$^{d}$ | 0.127$^{b}$ | 0.686$^{a}$ | 1.54$^{a}$ | 0.378$^{d}$ | 0.581$^{d}$ | 1.07$^{ab}$ | 3.41$^{b}$ |
| Urea | 0.10 | 0.984$^{b}$ | 0.305$^{a}$ | 0.640$^{ab}$ | 1.52$^{a}$ | 0.630$^{a}$ | 0.957$^{a}$ | 1.06$^{ab}$ | 2.68$^{c}$ |
| | 1.50 | 0.532$^{d}$ | 0.177$^{b}$ | 0.491$^{c}$ | 1.52$^{a}$ | 0.262$^{e}$ | 0.396$^{e}$ | 1.12$^{a}$ | 3.50$^{b}$ |
| | 3.00 | 0.174$^{e}$ | 0.077$^{c}$ | 0.382$^{d}$ | 1.29$^{b}$ | 0.066$^{f}$ | 0.085$^{f}$ | 0.99$^{b}$ | 3.77$^{a}$ |

[1] Data in the same column with different letters are significantly different ($P < 0.05$)

Table 8. Effect of emulsifiers on the textural properties and melting quality of mozzarella cheese analogs (Sample 5; Table 2)[1]

| Emulsifier | Conc. (%) | pH | Fract. (kg) | Hard. (kg) | Adhes. (kg-cm) | Cohes. | Spring. (cm) | Gumm. (kg) | Chew. (kg-cm) | Stretch. (cm) | Melting Quality |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sodium Phosp. | 1 | 6.18 | 0.706$^{d}$ | 0.971$^{c}$ | 0.321$^{ab}$ | 0.480$^{ab}$ | 1.53$^{a}$ | 0.466$^{b}$ | 0.710$^{b}$ | 0.66$^{d}$ | 2.68$^{a}$ |
| | 5 | 5.79 | 0.778$^{bcd}$ | 1.037$^{abc}$ | 0.362$^{a}$ | 0.491$^{ab}$ | 1.53$^{a}$ | 0.509$^{b}$ | 0.776$^{b}$ | 0.28$^{g}$ | 2.68$^{a}$ |
| Sodium Pyrophos. | 1 | 6.97 | 0.732$^{cd}$ | 1.025$^{bc}$ | 0.280$^{b}$ | 0.466$^{bc}$ | 1.53$^{a}$ | 0.476$^{b}$ | 0.728$^{b}$ | 0.94$^{c}$ | 2.65$^{a}$ |
| | 5 | 8.30 | 0.873$^{b}$ | 1.042$^{abc}$ | 0.354$^{a}$ | 0.486$^{a}$ | 1.54$^{a}$ | 0.507$^{b}$ | 0.778$^{b}$ | 0.38$^{f}$ | 2.64$^{a}$ |
| Sodium HMphos. | 1 | 6.60 | 0.798$^{bcd}$ | 1.047$^{abc}$ | 0.317$^{ab}$ | 0.460$^{bc}$ | 1.53$^{a}$ | 0.481$_{b}$ | 0.734$^{b}$ | 1.13$^{b}$ | 2.68$^{a}$ |
| | 5 | 6.82 | 0.835$^{b}$ | 1.090$^{abc}$ | 0.319$^{ab}$ | 0.433$^{d}$ | 1.53$^{a}$ | 0.472$^{b}$ | 0.719$^{b}$ | 0.53$^{e}$ | 2.63$^{a}$ |
| Sodium Citrate | 1 | 6.73 | 0.792$^{bcd}$ | 1.152$^{a}$ | 0.330$^{ab}$ | 0.512$^{a}$ | 1.52$^{a}$ | 0.589$^{a}$ | 0.897$^{a}$ | 1.53$^{a}$ | 2.68$^{a}$ |
| | 5 | 7.17 | 0.843$^{b}$ | 1.119$^{ab}$ | 0.342$^{ab}$ | 0.440$^{cd}$ | 1.54$^{a}$ | 0.492$^{b}$ | 0.759$^{b}$ | 0.12$^{h}$ | 2.64$^{a}$ |
| None | — | 6.58 | 1.053$^{a}$ | 1.113$^{ab}$ | 0.348$^{ab}$ | 1.421$^{d}$ | 1.54$^{a}$ | 0.469$^{b}$ | 0.724$^{b}$ | 1.07$^{b}$ | 2.65$^{a}$ |

[1] Data in the same column with different letters are significantly different ($P < 0.05$)

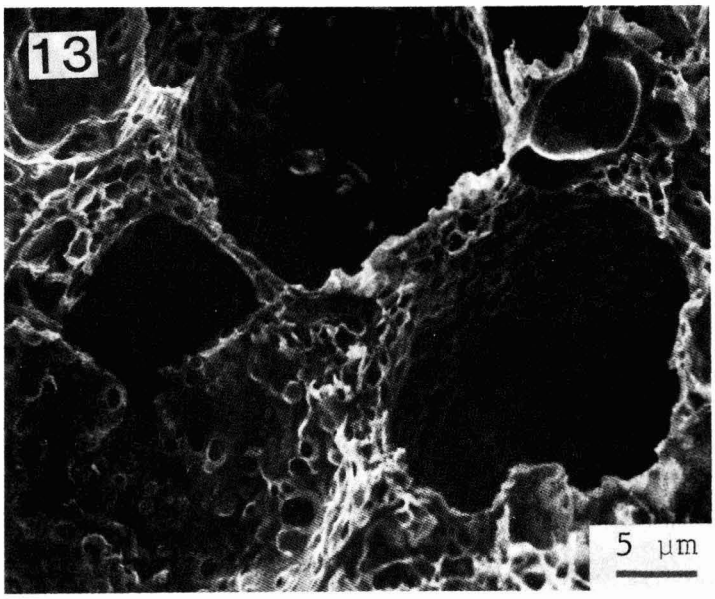



FIG. 13. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG TREATED WITH 0.1M MERCAPTOETHANOL

higher amount and larger size of the alveoli for the mercaptoethanol-treated sample compared to the control (Fig. 4) may be the reason for the low rigidity of the entire system. The finer and more compacted network may be the cause for the higher values of cohesiveness.

On the other hand, samples treated with urea showed a looser network (Fig. 14). The compactness of network decreased with increasing concentration of urea. The loose network in the gel systems may explain the low rigidity and cohesiveness, and accordingly, gumminess and chewiness. A tacky gel was observed when the urea concentration increased up to 3M (Yang 1982). This is similar to that reported by Furukawa *et al.* (1979) on the same treatment to a soy protein gel system.

## Effect of Various Emulsifiers

Table 8 shows the effect of emulsifiers on the analog. Orthophosphate lowered the pH whereas pyrophosphates and sodium citrate raised the pH of the analogs. The fracturability and hardness of the cheese analogs at 1% and 5% emulsifier levels (except for 5% sodium pyrosphate) showed no difference in the response of cheese gels to orthophosphate versus polyphosphate. In contrast, Scharpf (1971) observed

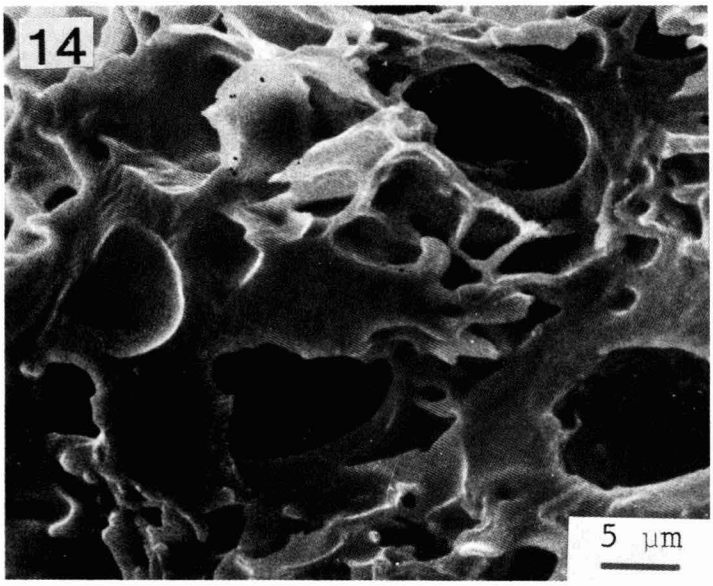



FIG. 14. SCANNING ELECTRON MICROGRAPH OF MOZZARELLA CHEESE ANALOG TREATED WITH 3M UREA

changes in the rheological properties of natural cheese when orthophosphate emulsifiers were substituted for polyphosphate emulsifiers. On the other hand, Templeton and Sommer (1936) and Kiermeier and Mohler (1960) reported that cheese became increasingly harder as the chain length of the emulsifier increased from ortho to pyro to long-chain polyphosphate. This was also not observed in this study. The hardness of the gel in the presence of phosphates was found to be equal to that of the control sample, indicating little influence on complex formation between phosphate and the other ingredients. Sodium citrate was also found to have no effect on analog fracturability and hardness.

The most significant observation was that the phosphate emulsifiers, except 1% sodium hexametaphosphate, caused a large depression in the stretchability of the cheese analogs, especially at the higher level of emulsifiers. Either phosphates formed a complex with gelatin and soy isolate, or the steric hindrance of phosphate molecules might prevent the formation of long-chain polymers which tend to elongate during stretching. On the other hand, samples treated with sodium citrate at the 1% level showed a significantly higher stretchability than the control sample, indicating a much stronger chemical interaction with the analog system than was shown for the phosphate emulsifiers. No differences in the melting quality were detected.

## CONCLUSIONS

The morphology and texture of mozzarella cheese analogs prepared using different gums were compared. The fracturability, hardness and adhesiveness of the cheese analog gels were found to be related to the amount of gelatin and concentration and type of gums. Stretchability of the cheese analog progel was also governed by the relative amounts of gelatin, gum and soy protein in the formulation. Microstructural studies indicated that GFS gum formed a uniform and delicate gel network while guar gum tended to form clumps in gel network which might retard the alignment of molecules in the progel state and hence, adversely affect the stretching properties of the analogs. Analogs made with GFS were not "sticky" on their surfaces and should cost much less than the formulaton requiring high amounts of gum arabic.

A soybean mozzarella cheese analog prepared at a high temperature was harder than those prepared at lower temperatures, whereas the cohesiveness and stretchability were unaffected by preparation temperature. Processing time and temperatures are important for obtaining similar hardness as commercial cheese while still maintaining good cohesiveness and stretchability. A processing temperature of 80°C and 5 min/5 min (dry mixing/wet mixing) processing time is considered optimum based on comparison of textural parameters in analogs with those in commercial mozzarella.

The analog system appears to be stabilized mainly by hydrogen bonds, hydrophobic interactions and disulfide bonds. Commonly used cheese emulsifiers were found to have no significant effect on hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness and melting quality except that 1% sodium citrate was found to enhance gumminess. Phosphate emulsifiers, especially at the higher levels, depressed the stretchability of the cheese analog progel whereas 1% sodium citrate enhance effect on stretchability. Therefore, emulsifier is not an essential ingredient in the manufacture of the mozzarella cheese analogs as developed here inasmuch as the gel texture and progel stretchability are concerned.

## ACKNOWLEDGMENT

This work was supported in part by the Illinois Agricultural Experiment Station, University of Illinois, Urbana. This material forms part of a doctoral dissertation submitted by C.S.T. Yang to the Graduate College, University of Illinois.

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