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D.B. LUNN  
EDITOR

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## **EDITORIAL**

The Journal of Food Processing and Preservation is soliciting papers for a new section entitled "Computer Codes and Their Application." These nonpeer reviewed short papers describing computer codes and their application are intended for information only. Further information on the codes can be obtained by writing directly to the author. The Journal of Food Processing and Preservation would be pleased to receive these types of papers.

We will also have a section entitled "Data Bank." Should you have some data on chemical or physical properties or on analysis applied to food processing, please forward them to me for consideration in this section. These should be short, one to two page data sets. These papers will be sent out for review. Please let me know what you think of these opportunities for publishing.

Daryl Lund  
Editor

## CONTENTS

Editorial .....	v
A Sauce Product from Enzyme-Hydrolyzed Canola Meal A.Y.M. MA and B. OORAIKUL.....	163
Characteristics and Acceptability of a Flour Prepared from Sodium Bicarbonate Soaked and Steamed Soybeans J.Y. LU and A. JASSER .....	177
Effect of Parboiling and Freezing on Quality of Three Spanish Rice Varieties R. OLALQUIAGA, J-X. GUINARD and R.P. SINGH .....	189
The Influence of Microwave Heating on the Textural Properties of Meat and Collagen Solubilization J.F. ZAYAS and J.O. NAEWBANIJ .....	203
Factors Influencing the Quality of Canned Strawberry Filling During Storage H.F. PRATT, W.A. SISTRUNK and J.R. MORRIS .....	215
Effect of Sweeteners and Stabilizers on the Structure of Ice Cream Mix as Deter- mined by Acoustic Methods D.E. SMITH and S.A. WITTINGER .....	227
Book Reviews .....	237

# A SAUCE PRODUCT FROM ENZYME-HYDROLYZED CANOLA MEAL

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## ABSTRACT

*To reduce fermentation time in canola sauce production canola meal was hydrolyzed with Alcalase 0.6L for 2 h before mixing with roasted wheat and Aspergillus cultures to form koji. After 72 h incubation 18% brine solution was added to the koji to prepare moromi, which was left to ferment for 5 weeks before canola sauce was extracted and pasteurized. The sauce was analyzed for total soluble nitrogen (TSN), amino nitrogen (AN), amino acids, total titratable acidity (TTA), organic acids, reducing sugars, total sugars, salt and color. The results were: 1.12–1.34% (w/w) TSN, 0.66–0.80 (w/v) AN, 16.65–29.95 mg/100 mL TTA, 7.43–9.67% (w/v) reducing sugars, 8.69–15.39% (w/v) total sugars, 18.72–20.37% (w/v) salt and a somewhat yellowish shade of color when diluted. Most results compare well with Kikkoman shoyu, one of the most popular soy sauces in Canada. However, amino acids, especially glutamic and aspartic acids, were less in canola sauce when compared with shoyu. Concentrations of organic acids were comparable, except for lactic and propionic acids, which were considerably less in canola sauce due to inadequate lactic acid fermentation in the moromi stage. Sensory panels rated canola sauce quality comparable to that of Chinese soy sauce, but inferior to Kikkoman shoyu and an imitation soy sauce due to a deficiency in traditional soy sauce aroma.*

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## INTRODUCTION

The feasibility of using canola meal as a soybean substituent in the production of a soy sauce-type condiment was investigated by Ooraikul, *et al.* (1980), who produced an acceptable sauce from the meal with either the traditional one-year fermentation or a shortened (one month) semi-chemical process, using a combination of HCl hydrolysis and mold fermentation. However, HCl is highly corrosive and expensive, and the acid hydrolysis process requires costly equipment, making it economically unattractive to large-scale industry. An alternative method must be found to prehydrolyze canola meal and produce high quality sauce in the shortest period of time to make canola sauce competitive against soy sauce.

Ma and Ooraikul (1986) have shown that Alcalase 0.6L, a serine type endoprotease, can effectively hydrolyze canola meal. They determined optimum hydrolytic conditions so that protein solubilization, assayed as total soluble nitrogen (TSN) yield, was maximized. The hydrolyzed canola meal was subsequently fermented with microbial cultures to produce a condiment similar to soy sauce.

However, whether or not the canola sauce is organoleptically acceptable depends on its chemical properties. Onaga *et al.* (1956) studied chemical composition of soy sauce in relation to its quality and concluded that salt, acidity and nitrogen contents were important factors influencing flavor acceptance of the sauce. Udo (1931) and Ueda, *et al.* (1958) reported that glutamic acid and glutamates were the chief ingredients responsible for the delicious taste of soy sauce, whereas lactic, acetic, succinic and phosphoric acids were related to the desirable aroma, taste, storage and color quality of the sauce. Yokotsuka (1981) stated that over 300 volatile flavor constituents, which include carbonyls, organic acids, nitrogenous compounds, esters, alcohols, S-containing compounds and phenols, have been isolated from soy sauce.

In this report, the procedure for making a condiment similar to soy sauce from canola meal with the aid of Alcalase 0.6L, chemical analyses and sensory evaluation of the condiment, in comparison with a Japanese shoyu (soy sauce), are described.

## MATERIALS AND METHODS

### Manufacturing Process for Canola Sauce

Fifty grams of defatted canola meal (Western Canada Seed Processor Ltd., Lethbridge, AB) were mixed with 210 mL of distilled water and 15.4 mL of Alcalase 0.6L (Novo Industri A/S Novo Alle, DK-2880 Dagsvaerd, Denmark) in a double-walled glass vessel. The vessel was kept at 69°C with a water bath

circulator (Braun Thermomix 1441, B. Braun Melsungen, West Germany). The pH of the hydrolyzing mash was maintained at 9 with 1M NaOH by means of a pH-stat (Metrohm AG AH-9100, Herisau, Switzerland), while constantly stirred with a magnetic stirrer. The volume of NaOH consumed was recorded over 2 h reaction time. Water was added to the mash to make up the total liquid volume to 250 mL in order to bring the meal-solvent ration to 1:5 (w/v).

The pH of the canola meal hydrolysate was adjusted to 5.5 with concentrated HCl. Approximately 140 mL of liquid was pressed from the mash and refrigerated for later use. The residue was thoroughly mixed with 50 g of coarsely-ground roasted wheat and a roux bottle of either *Aspergillus oryzae* 14895 (NRRL 1989) or *A. sojae* 16320 (ATCC, Parklawn Dr., Rockville, MD), or both. The concentration of the mold culture in the mixture was approx. 0.2% by weight. The resulting mixture, koji, was analyzed to ensure a moisture content of 40–45%. The koji was incubated at 28 °C for 72 h with occasional stirring.

The pressed liquid was mixed with an appropriate amount of salt to make up an 18% brine which was added to the mature koji (72 h old). If necessary, a few drops of concentrated lactic acid were added to lower pH to 5.5. The mixture, moromi, was fermented at room temperature and stirred three times weekly. Total soluble solids and pH were assayed every 3–4 days throughout the 5 week fermentation period.

Fermented moromi was pressed with a manual screw press. A canola sauce was, thus, extracted and pasteurized at 75 °C for 30 min in a water bath. The sauce was further clarified with a preparative ultracentrifuge (Beckman Instruments Inc., Fullerton, CA) using a JA 14 rotor at a speed of 10,000 rpm for 30 min, followed by vacuum filtration. The resultant clear canola sauce was bottled and kept refrigerated until further use.

## Analyses of Canola Sauce

**Fractionation of Soluble Components.** Canola sauce was diluted 20 times with distilled water. Ten milliliters of the diluted canola sauce was adjusted to pH 1.5–2.0 with 1M HCl and carefully poured into a cation-exchange column which contained Dowex 50w-x8, 100–200 mesh in H<sup>+</sup> form (Bio-Rad lab., Richmond, CA). Canola sauce diluent penetrated the column slowly until the liquid was level with the top of the resin. The column was washed with 70 mL water at the rate of about 1 drop/s and 70 mL was collected and passed through an anion-exchange column which contained Dowex 1-x8, 100–200 mesh in Cl<sup>-</sup> form (Bio-Rad Lab., Richmond, CA). The anion-exchange column was washed with 70 mL water, giving a total of 140 mL effluent. The effluent was evaporated in a Buchi Rotavapor-R (Foss Electric Canada Ltd., Cornwall, Ont.) and reconstituted with 5 mL distilled water to produce a solution ten times more dilute than the original sauce. The solution had neutral pH and contained compounds such as sugars.

The cation-exchange column was eluted with 140 mL of 4M  $\text{NH}_4\text{OH}$ , rapidly in the first 30 mL, then slowly at about 1 drop/s for the last 110 mL. The effluent was evaporated to dryness until free of ammonia. A few drops of  $\text{HCOOH}$  was added to the residue and re-evaporated. The dry residue was reconstituted with 5 mL distilled water, resulting in a solution ten times more dilute than the original sauce. The solution contained amino acids and other nitrogenous compounds.

The anion-exchange column was eluted with 80 mL of 4M  $\text{HCOOH}$  with a flow rate of about 1 drop/s. The effluent was evaporated to dryness, free of pungent odor, and the residue redissolved in 5mL water, resulting in a solution ten times more dilute than the original sauce. The solution contained organic acids.

**Quantitation of Amino Nitrogen (AN).** One milliliter aliquot of the cation-exchanged solution was further diluted with 10 mL of deionized water and 0.5 mL of the diluted solution reacted with ninhydrin according to Rosen (1957). AN content was assayed colorimetrically at 570 nm and the result, in mMoles leucine equivalent/100 mL sauce, was extrapolated from previously prepared leucine standard curve.

**Quantitation of Total Soluble Nitrogen (TSN).** A micro-Kjeldahl method adapted from Pearson (1976) quantitated TSN of the sauces, using less than 0.5 mL of the original sauces for the analysis.

**Amino Acid Analysis.** One milliliter of the cation-exchanged solution was further diluted with 5 mL of  $\text{HCl}$  (pH 2.2) and analyzed with a Beckman Amino Acid Analyzer Model 121 MB (Beckman Instruments Inc., Palo Alto, CA).

**Total Titratable Acidity (TTA).** TTA of the sauces was determined by titrating 1 mL aliquot of anion-exchange solution with 0.005M  $\text{NaOH}$  using phenolphthalein as an indicator.

**Organic Acid Analysis.** A High Pressure Liquid Chromatograph (HPLC; Bio-Rad Lab., Mississauga, Ont.) was used for the analysis of organic acids. The instrument was equipped with a model 1330 HPLC pump, an Aminex HPX-87 column (300x7.8 mm), a micro-guard HPLC column, a model 1305 UV detector with a standard deuterium lamp, and a flatbed chart recorder model 1321. A model 7125 injector (Rheodyne Inc., CA) was used for constant 20 L sample loading.

The anion-exchanged solution was decolorized by shaking with a small amount of charcoal, followed by centrifugation in an IEC centrifuge model NH-SII (Intern. Equip. Div., Needham Heights, MA). The following conditions were employed in the analysis: 0.012N  $\text{H}_2\text{SO}_4$  mobile phase with a flow rate of 0.8 mL/min; 65 °C column temperature; UV detector was monitored at 210 nm.

Organic acids in the sauces were identified by comparing retention times with those of pure standards which included citric, iso-citric, pyruvic, malic, trans-aconitic, glyoxylic, succinic, formic, lactic and propionic acids. The standard organic acids were of analytical grade obtained from Sigma Chemical Co., St. Louis, MO, and Terochem Laboratories Ltd., Edmonton, AB. Further confirmation of the identifications was performed using the 'spiking' technique.

**Analysis of Sugar.** One milliliter aliquots of the fraction with neutral pH were analyzed for reducing and non-reducing sugars according to the procedures described in the Official Methods of Analysis of the A.O.A.C. (1975) sections 31.026, 31.038, 31.040-41 and 52.018.

**Total Soluble Solids (TSS) and pH.** A small amount of moromi was collected every 3-4 days throughout the 5 week fermentation, pressed, and the TSS and pH of the liquid were determined with an Abbe refractometer (Carl Zeiss, West Germany) and an expanded scale Fisher pH meter, respectively.

**Salt (NaCl) Concentration.** Salt concentration of the sauces, expressed as % chloride, was determined with a Fisher Accumet Selective Ion Analyzer Model 1750 (Fisher Scientific Co., Pittsburgh, PA), equipped with a chloride electrode model 94-17 (Orion Research Inc., Cambridge, MA). Each sauce was analyzed in triplicate.

**Color Measurement.** Color of the sauce was determined with a HunterLab colorimeter model D25L-2 and expressed in terms of L, a and b values.

**Moisture Content.** Moisture contents of koji were analyzed in an Automatic Volatility Computer model AVC-MP (CEM Corp., Indian Trail, NC) using about 0.5 g koji and 12 min residence time.

### Sensory Evaluation

Three sensory evaluation sessions were conducted using preference test with four canola sauces (CS) and three brands of commercial soy sauce, viz. Kikkoman shoyu (KS), Chinese soy sauce (CSS) and China Lily (CL), an imitation soy sauce made from soy protein extract, monosodium glutamate, salt, coloring, and water.

The panelists consisted of ten staff and students in the Department of Food Science, the University of Alberta. The sauces were scored for aroma, taste and overall acceptance on a 9-point hedonic scale, 1 being the least and 9 the most preferred. The results were analyzed statistically using analysis of variance and Duncan's Multiple Range Test.

The first session evaluated CS1 (canola sauce fermented for 5 weeks with *A. oryzae* and moromi adjusted to pH 5.5 before fermentation), CS2 (as CS1 but

with *A. sojae*), CS3 (as CS1 but with a mixture of *A. oryzae* and *A. sojae*), CS4 (as CS1 but moromi adjusted to pH 6.5 before fermentation) and Kikkoman shoyu (KS).

The second and third sessions evaluated the four canola sauces together with Chinese soy sauce (CSS) and China Lily (CL), respectively.

## RESULTS AND DISCUSSION

### Manufacturing Process

Conditions for hydrolysis of canola meal were stipulated by Ma and Ooraikul (1986), except that NaOH instead of buffer solutions was used to maintain pH at 9. Possibly because of NaOH and the fact that the meal-solvent ratio in this experiment was 1:5 (w/v) instead of 1:10 (w/v), TSN yield in the hydrolysate was almost doubled to 0.85% (Table 1) as compared to 0.488% obtained by Ma and Ooraikul (1986) where the latter ratio was used. The modified hydrolysis conditions increased rate of fermentation in that the desired level of TSN in canola

Table 1. Total soluble nitrogen (TSN), nitrogen yield, amino nitrogen (AN) and AN/TSN ratio of canola sauces, Kikkoman shoyu and enzyme hydrolysate of canola meal (averages of two replicates)

Samples <sup>a</sup>	TSN (w/w) %	Nitrogen <sup>b</sup> Yield, %	AN (w/v) %	AN/TSN
CS1	1.31	71.74	0.80	61.06
CS2	1.16	63.53	0.70	60.34
CS3	1.34	73.38	0.66	48.50
CS4	1.12	61.34	0.73	65.18
KS	1.38	73.70 <sup>c</sup>	0.70	58.72
EH	0.85	46.54	0.64	75.29

<sup>a</sup> CS1 - canola sauce prepared with *A. oryzae* and initial moromi pH of 5.5

CS2 - canola sauce prepared with *A. sojae* and initial moromi pH of 5.5

CS3 - canola sauce prepared with *A. oryzae* and *A. sojae* and initial pH of 5.5

CS4 - canola sauce prepared with *A. oryzae* and initial moromi pH of 6.5

KS - soy sauce produced by Kikkoman Shoyu Co., Ltd., Japan

EH - enzyme hydrolysate of canola meal (with Alcalase 0.6L) prior to *Aspergillus* fermentation

<sup>b</sup> Nitrogen yield - TSN in sauce/total nitrogen content in raw materials (4.565 g/100g)

<sup>c</sup> From Hesseltine and Wang (1978)



sauce was attained in shorter time than it would if the buffer solutions and lower meal-solvent ratio were used.

When the koji was spread about 3–4 cm thick on enamelled trays, covered with aluminum foil and incubated at 28 °C, the moisture decreased from 40–45% to about 28% over a 72 h period. At maturity (after 72 h) the koji was covered with greenish yellow mycelia, with a sweetish and slightly ammoniacal smell.

The expressed hydrolysate provided a convenient means for the preparation of brine solution to be added to the koji to make moromi without diluting the moromi excessively. In all but one case moromi was adjusted to pH 5.5 with lactic acid at the beginning of fermentation to increase the rate of fermentation by facilitating the growth of lactic bacteria. It was anticipated that pH of the mash would quickly drop below 5.0 to attract yeast development. Spontaneous inoculation of moromi with lactic bacteria and yeasts naturally present in air was presumed. One sample of moromi was adjusted to pH 6.5 to study fermentation characteristics at a higher pH level.

### Total Soluble Solids and pH

The change in total soluble solids (TSS) in moromi over a 5 wk period is shown in Fig. 1. TSS of all moromi, except CS1, increased from about 29% to about 35%. CS1 appeared to start at a greater concentration of 32% and increased to 38%, making a similar gain of 6%. In all moromi, except CS4, pH declined very gradually from 5.5 to between 5.1 (CS1) and 5.4 (CS2), while in CS4

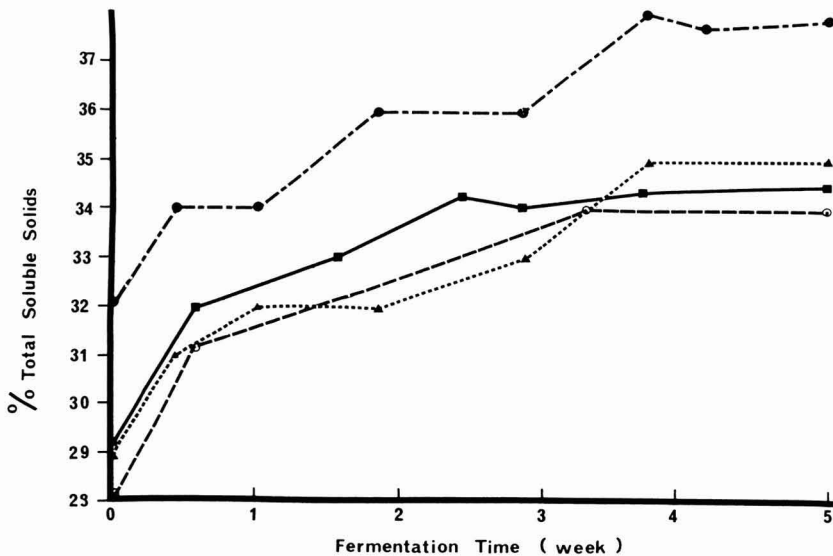


FIG. 1. CHANGE IN TOTAL SOLUBLE SOLIDS OF THE MASH DURING THE MOROMI STAGE OF CANOLA SAUCE FERMENTATION

●---● CS1, koji prepared with *A. oryzae*, initial moromi pH of 5.5; ■---■ CS2, koji prepared with *A. sojae*, initial moromi pH of 5.5; ○---○ CS3, koji prepared with a mixture of *A. oryzae* and *A. sojae*, initial moromi pH of 5.5; ▲---▲ CS4, koji prepared with *A. oryzae*, initial moromi pH of 6.5

pH dropped quickly in the first 2 wk from 6.5 to 5.6 then gradually to about 5.5 at the end of 5 weeks (Fig. 2). These changes in TSS and pH were attributed to the conversion of proteins to amino acids, and starch to sugars and organic acids.

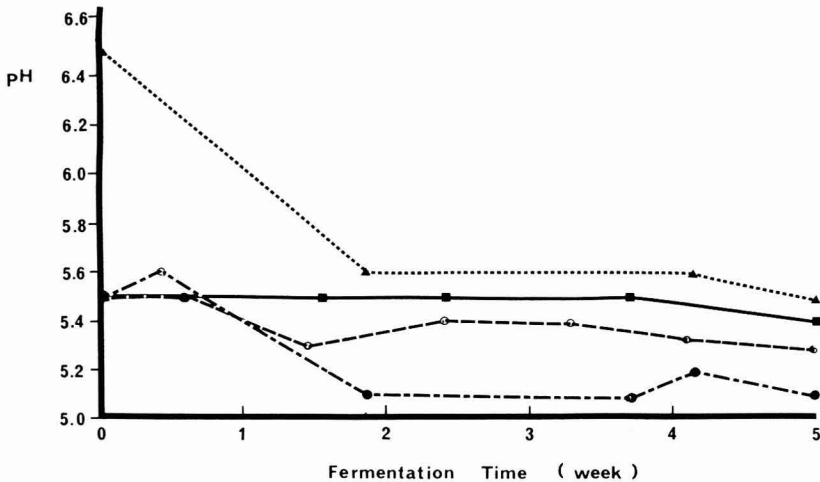


FIG. 2. CHANGE IN pH OF THE MASH DURING THE MOROMI STAGE OF CANOLA SAUCE FERMENTATION (Legends are same as in Fig. 1.)

Ooraikul *et al.* (1980) reported a two-step increase of TSS in the conventional one-year fermentation. They associated the first increase with bacterial fermentation, and the second with yeast. In the present experiment the second increase of the TSS was not detected since yeast fermentation could not be initiated at pH above 5.0 (Yong and Wood 1977).

The concentration of TSS in canola sauces (35–38%) compared well with that of Kikkoman shoyu (36%). However, pH of the commercial sauce at 4.9 was less than that of the canola sauces, indicating a greater degree of acid fermentation in Kikkoman shoyu.

### Chemical Composition

The Japanese Government recognizes three grades of soy sauce, i.e. special, upper and standard, which are differentiated by organoleptic quality, % TSN, TSS content other than NaCl, and color of the sauce (Fukushima 1979). TSN, nitrogen yield (the ratio of TSN in the sauce to nitrogen content in raw materials) and the ratio of amino nitrogen (AN) to TSN are regarded as quality indices of soy sauce and dictate its price. High nitrogen yield denotes an effective enzymatic conversion of proteins in raw materials to soluble forms.

Table 1 lists TSN, nitrogen yield, AN, and AN/TSN of various canola and soy sauces and an enzyme hydrolysate (EH) of canola meal. Canola sauces have values quite comparable to those of Kikkoman shoyu (KS). Hesselstine and Wang (1978) reported that an AN/TSN ratio of 50% was regarded as evidence of good quality soy sauce. Most canola sauces had an AN/TSN ratio greater than that of KS, suggesting that canola sauces prepared with the aid of Alcalase 0.6L compared favorably with commercial soy sauce in quality.

CS3 prepared with a combination of *A. oryzae* and *A. sojae* produced the greatest TSN (1.34%) and nitrogen yield (73.38%) among the canola sauces, and were the closest to those of KS at 1.38% and 73.70%, respectively. However, AN of 0.66% was the smallest, indicating great concentrations of other nitrogenous compounds which do not contribute significantly to the taste and aroma of the sauce. Thus, AN/TSN of 48.50% was the lowest among the sauces.

The TSN and nitrogen yield of the enzyme hydrolysate (EH) of canola meal were 0.85% and 46.54%, respectively. Comparing these values with the TSN and nitrogen yield of the canola sauces revealed that over 60% of nitrogen yield occurred in the hydrolysis by Alcalase 0.6L prior to the fermentation.

Concentrations (mMoles/mL sauces) of amino acids in canola sauces, Kikkoman shoyu and EH are shown in Table 2. Two major amino acids, glutamic and aspartic acids, were reported by Udo (1931) to be responsible for the delicious taste of soy sauce. Comparing the concentrations of amino acids in canola sauces and EH revealed that free amino acids in canola sauce were produced during both koji and moromi fermentations. The sequence of events concerning proteins in canola sauce manufacturing process may be as follows: Alcalase 0.6L broke down proteins in raw materials to small peptides and amino acids. The peptides and remaining proteins were further hydrolyzed by mold proteases during the koji and moromi stages to free amino acids and other soluble nitrogenous compounds.

The total titratable acidity (TTA) in canola sauces was relatively low (17–30 meq. NaOH/100 mL sauce) when compared to 33 meq. NaOH/100 mL sauce in KS (Table 3). This was reflected in the slow decline of pH in the moromi stage. It appeared that short fermentation time did not allow proper development of lactic acid and, subsequently, yeast fermentation.

The HPLC chromatograms of canola sauces and KS revealed 11 organic acids, 9 of which were identified. Yokotsuka (1981) stated that the delicate acidic bouquet of soy sauce was attributed to lactic, acetic, succinic and phosphoric acids. Acetic and phosphoric acids were not analyzed in the present experiment, but out of the 11 organic acids detected lactic and formic acids appeared to be the most prominent. Lactic acid was characteristically the most important organic acid in naturally-fermented soy sauce in terms of quantity and contribution to taste, while levulinic and formic acids were distinctive traits of

Table 2. Amino acids in canola sauces, Kikkoman shoyu and enzyme hydrolysate of canola meal (mMole/mL, average of triplicate determinations)

Amino acid	CS1	CS2	CS3	CS4	KS	EH
Aspartic acid	24.40	34.50	35.50	39.50	65.70	4.35
Threonine	14.40	16.70	24.25	19.85	30.60	1.95
Serine	16.45	29.45	31.40	31.50	52.35	4.30
Glutamic acid	61.85	83.40	87.75	76.80	86.55	9.65
Proline	20.80	25.25	34.25	21.85	52.00	6.35
Glycine	20.35	25.10	28.90	28.90	37.05	3.40
Alanine	37.05	40.00	47.75	43.95	55.65	6.75
Cystine	6.20	7.35	8.75	7.00	3.40	4.90
Valine	29.80	38.35	43.50	38.80	55.95	6.70
Methionine	13.60	12.10	17.15	11.90	10.05	5.85
Isolucine	19.05	23.55	26.55	23.90	41.30	4.70
Leucine	34.45	41.75	29.10	40.15	65.10	8.15
Tyrosine	11.20	13.75	16.15	13.15	6.90	1.70
Phenylalanine	16.45	18.65	21.55	17.00	31.05	3.95
Lysine	19.35	23.85	29.10	24.05	49.05	1.45
Arginine	17.65	25.70	30.55	25.75	21.75	2.00

Table 3. Total acidity (meq. NaOH/100 mL) and organic acid concentration (mg/100 mL) of canola sauces. Kikkoman shoyu and enzyme hydrolysate of canola meal (averages of two replicates)

	CS1	CS2	CS3	CS4	KS	EH
Total acidity	24.40	16.65	23.45	29.95	33.35	16.30
Citric acid	8.60	6.50	12.46	4.60	7.13	1.48
Isocitric acid	2.65	0.66	2.18	1.71	3.03	-
Pyruvic acid	0.14	0.07	0.15	0.13	0.18	-
Unknown 1	++	+	++	+	-	-
Malic acid	2.98	1.44	3.07	2.51	4.75	-
Trans-aconitic acid	0.03	+	0.02	0.02	-	-
Unknown 2	++	+	++	++	++	-
Glyoxylic acid	0.08	-	-	-	-	-
Succinic acid	0.74	-	0.74	0.52	+	-
Formic acid	14.12	16.76	U/E	6.86	16.87	0.57
Lactic acid	36.01	63.78	U/E	74.58	87.86	-
Propionic acid	10.90	2.20	3.80	7.60	48.49	-

++ - significant amount

+ - present

- - absent

U/E - unable to estimate due to overlap



chemical soy sauce (Fukushima 1981). The canola sauces contained relatively large concentrations of lactic and formic acids, but no levulinic acid. Therefore, based on qualitative and quantitative characteristics of organic acids in the sauces, canola sauce quality appeared to fall between the quality of naturally-fermented and chemical soy sauces. In general, the concentrations of organic acids in canola sauces were less than those in KS.

Salt and reducing sugar contents in canola sauces (Table 4) compared well with those in KS. However, nonreducing sugars and, consequently, total sugar contents in CS were greater than in KS. Use of different raw materials as well as shorter fermentation probably resulted in a greater quantity of unfermented sugar in canola sauces than in KS.

Table 4. Sugar and salt contents of canola sauces, Kikkoman shoyu and enzyme hydrolysate of canola meal (averages of two replicates)

Sample	Reducing Sugar (% Glucose)	Non-reducing Sugar (% Sucrose)	Total Sugar (%)	Salt (% NaCl,w/v)
CS1	8.81	6.58	15.39	19.22
CS2	7.43	1.26	8.69	18.72
CS3	8.77	2.08	10.85	19.85
CS4	9.67	0.49	10.16	20.37
KS	6.76	0.95	7.71	18.12
EH	trace	trace	trace	trace

Canola sauces appeared to be more yellowish and slightly darker than KS, which was more reddish. Color of the sauces was attributed to oxidation products of Amadori compounds and intermediates from reactions among amino acids and sugars. Therefore, different conditions, especially temperature and time, employed in the production and pasteurization of the sauces might explain color differences between CS and KS. The light reddish color of KS may be due to favorable fermentation conditions in relation to color formation (Okuhara and Saito 1970). Furthermore, different constituents in raw materials would certainly affect the colorization of the sauces (Hashiba *et al.* 1981).

## Sensory Evaluation

Results of sensory evaluation were inconclusive and difficult to interpret. The preferences of untrained panelists varied widely. There was significant inconsistency within individual panelists in scoring the same sauce samples over the three sessions, and no sample among the canola sauces was positively identified as the best. Nevertheless, average scores of the overall acceptance suggested that canola sauces produced in this experiment were inferior to Kikkoman shoyu and China Lily, but comparable to Chinese soy sauce. General comments from panelists revealed that canola sauce and soy sauce were quite similar in taste. However, canola sauce lacked reminiscence of the myriad aromas typical of high quality soy sauce. The difference in aromas between canola and soy sauces, again, indicated the deficiency in acid and sugar fermentations during the moromi stage of canola sauce. Further modification in the fermentation process of canola sauce is, therefore, necessary to bring about improvement in organoleptic quality.

## ACKNOWLEDGMENT

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# CHARACTERISTICS AND ACCEPTABILITY OF A FLOUR PREPARED FROM SODIUM BICARBONATE SOAKED AND STEAMED SOYBEANS

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## ABSTRACT

*A soyflour was prepared by soaking the beans in 9 volumes of 0.5% NaHCO<sub>3</sub> overnight (pH 8.5), steamed at 100°C for 30 min, dried at 50–60°C under vacuum, then ground to flour.*

*The flour had a light tan color and no objectional beany odor. The composition of the flour was 48% protein, 19% flour, 5% ash, and 3.5% moisture. No lipoxxygenase nor antitrypsin activity was detected in the flour. The phytate content was 0.75%.*

*Protein solubility, emulsifying capacity, foaming capacity, oil and water absorption of the flour was determined. The results showed that the flour prepared from bicarbonate soaked and steamed soybeans possessed desirable functional characteristics except protein solubility was decreased.*

*Two products, noodles and bread, were prepared by using the soyflour as an ingredient. Sensory evaluation of the products revealed that both products were well accepted.*

## INTRODUCTION

Soybeans, one of the major crops in this country, are high in protein and oil and have a high quality protein. However, the use of whole soybeans in food is still much limited in scope because of the problems such as beany odor, bitter taste, and tough texture, which often associate with improperly processed soybeans. In addition, soybeans also contain antinutritive factors such as trypsin inhibitor (T.I.) (Smith *et al.* 1963; Rackis 1965), hemagglutinin, flatulence factors, and phytic acid (Leiner 1977, 1979). Elimination or reduction of these factors is desirable from a nutritional point of view.

Alkaline treatment of beans appears to enhance inactivation of lipoxygenase, which is the primary cause for beany odor of soybeans (Wolf and Cowan 1971). Soaking and cooking in alkaline solution improved palatability and rehydration of peas and beans (Dawson *et al.* 1952; Badenhop and Hackler 1970). A bland taste soy milk was prepared from soybeans soaked and cooked in 0.5% sodium bicarbonate solution (Nelson *et al.* 1976). Recently, Ashraf and Snyder (1981) reported that soaking soybeans in 0.1M NaOH resulted in a bland tasting soy milk with no painty flavor.

The purpose of the present study was, therefore, to prepare a flour from bicarbonate soaked whole soybeans. The processing condition, composition, properties of flour, and acceptability of food products containing soyflour were determined.

## EXPERIMENTAL

### Soy Flour

The Bragg variety soybeans were purchased from a local seed store. The soybeans were first cleaned, then soaked in 9 volumes of 0.5% sodium bicarbonate solution overnight at 25–30 °C, maintaining the pH at 8.5 by adjusting with 1 N NaOH. pH was monitored by a pH meter. After draining, the beans were steamed at 100 °C for various times, then dried in a vacuum oven at 50–60 °C. The dried beans were dehulled and then ground into flour at 60 mesh or less. The flour was packaged in a plastic pouch and stored in a refrigerator before use.

### Analysis

**Proximate Composition.** Oil, moisture, protein, and ash were determined according to AOAC (1980). Amino acid composition was analyzed according to Liu and Chang (1971) at the Army Natick Laboratory, Natick, Mass. Trypsin inhibitor (Kadade *et al.* 1974), lipoxygenase (Tappel 1962), and phytic acid (Wheeler and Ferrel 1971) were also determined.

**Functional Properties.** Bicarbonate treated soy flour samples were analyzed for protein solubility (Albracht *et al.* 1966), emulsion capacity (Swift *et al.* 1961), foaming and gelation (Coffman and Garcia 1977), and oil and water absorption capability (Beuchat 1977). An Oswald viscometer was employed to determine relative viscosity of 1 and 5% soy flour solution at 25 °C. A Gardner XL-10A colorimeter was employed to measure the color of flours.

### Acceptability of Soy Products

Two products, noodles and bread, were prepared using soy flour as an ingredient. The composition of noodles was 20% soy flour and 80% wheat flour



(Siegel *et al.* 1975). The formula of bread was the same as the one reported by Klein *et al.* (1980), replacing 13.6% of wheat flour with soy flour. The noodles were cooked and served with a small amount of tomato sauce. A slice of bread with a cup of water was served to panelists for evaluation. Twenty six students and faculty at Tuskegee University, Alabama, participated in the test. A 9-point hedonic scale was employed to determine flavor intensity and overall acceptability (ASTM 1968), with nine being the highest score, and one being the lowest score. The difference in mean scores was computed statistically (Steel and Torie 1980).

## RESULTS AND DISCUSSION

Figure 1 shows the relationship between the T.I. activity of bicarbonate treated soybeans and heating time. The T.I. activity decreased with time and dropped rapidly after 15 min at 100 °C. At 30 min, 95% of TI was inactivated. Many studies have shown the effect of heat processing on T.I. activity. Borchers *et al.* (1947) reported that the T.I. of solvent extracted soybean meal was inactivated at 100 °C (steam) and 60 min. Jackson (1981) reported that more than 90% of T.I. was inactivated when soy milk was heated at 99 °C for 60 min. The present study indicates that 95% of T.I. was inactivated after 30 min at 100 °C steam. This relatively shorter time could be the effect of bicarbonate soaking.

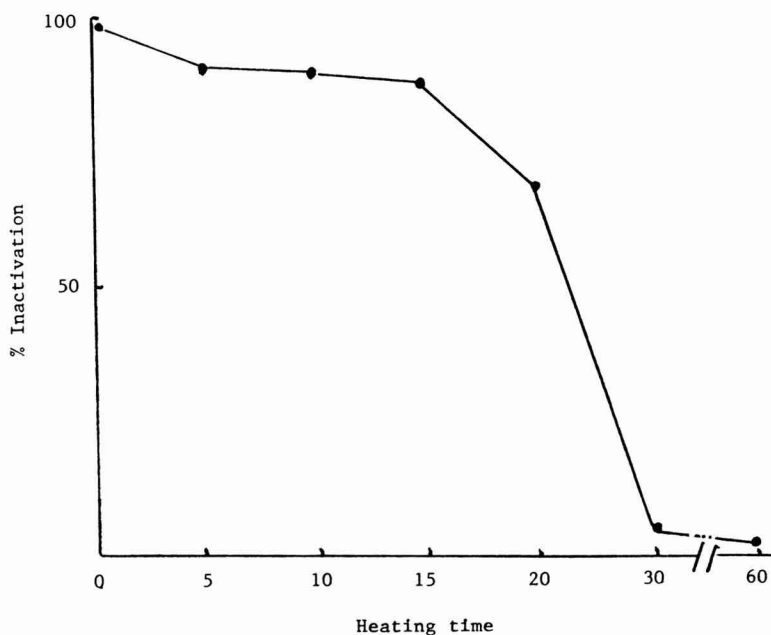


FIG. 1. EFFECT OF HEATING ON TRYPSIN INHIBITOR ACTIVITY

Heating is an effective way to destroy lipoxygenase, which is responsible for beany odor of soybeans (Nelson *et al.* 1976; Wilkens *et al.* 1967; Mustakas *et al.* 1969). The result (Fig. 2) showed that lipoxygenase is very sensitive to moist heat. At 100°C steam, nearly 100% activity was lost at 15 min.

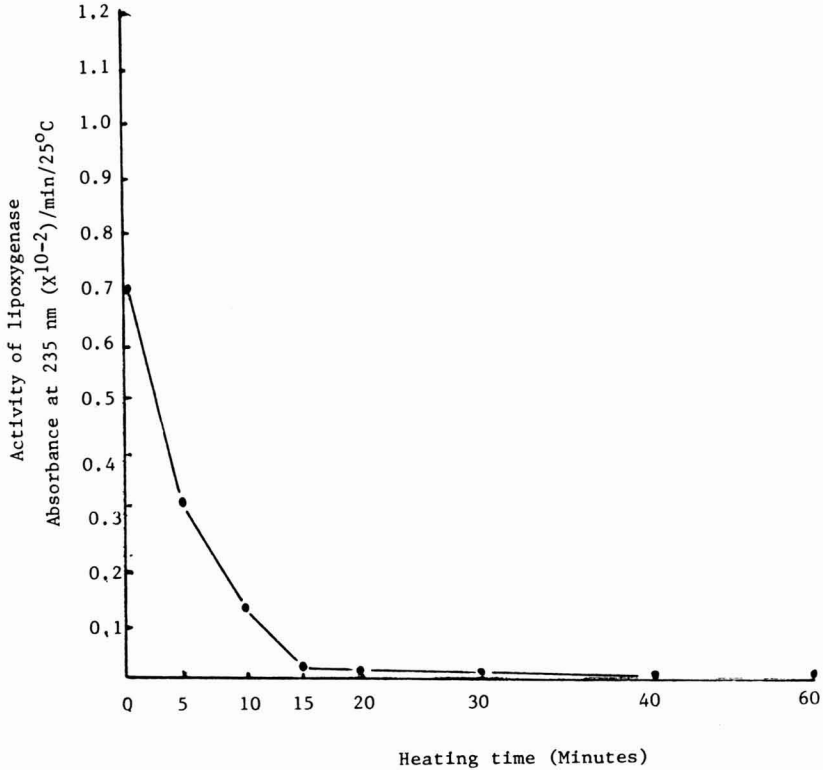


FIG. 2. EFFECT OF HEATING TIME ON LIPOXYGENASE ACTIVITY

The major form of phosphorous in soybeans is phytic acid. Lolas *et al.* (1976) reported that the Bragg variety contained 1.11% phytic acid. Our results (Table 1) indicated that the raw soybeans contained 1.0% but phytic acid concentration decreased to 0.73–0.76% after bicarbonate soaking and steaming. Since phytic acid is stable to heating at 100°C steaming, the decrease of phytate in the bicarbonate soaked flour was most likely caused by leaching out of phytic acid in the soaking solution.

Table 1. Phytic acid content of soy flours

Sample	Heating Time (minutes)	Phytic Acid % <sup>a</sup> (dry weight basis)
Untreated Soy Flour	0	1.00
Treated Soy Flour <sup>b</sup>	0	0.75
	5	0.73
	10	0.74
	15	0.75
	20	0.76
	30	0.74
	60	0.75

<sup>a</sup>Average of 3 determinations

<sup>b</sup>Treated soyflour (soaked in 0.5% NaHCO<sub>3</sub> and heated at 100 C)

Based on the results of T.I. and lipoxygenase inactivation and phytic acid content of soybeans, it was decided that the best condition for preparation of flour from bicarbonate treated soybeans was steam heating for 30 min. At this condition, lipoxygenase activity was not detected, 95% of T.I. was inactivated, and there was a 25% decrease in phytic acid content of soybeans. The flour thus prepared had a light tan yellow color and no beany odor. The soyflour was, therefore, analyzed for composition, functional properties, and acceptability.

Table 2 shows the composition of soyflour. Protein, oil, and ash content of the treated soyflour were lower than that of the raw soyflour. This is probably a result of soaking beans in bicarbonate solution, which caused the leaching out of some composition into the solution. The treated soyflour had lower moisture content of 3.5%, because the soaked and steamed beans were dried in a vacuum oven.

Soybeans are high in protein (40-50%). The soyprotein is known for its well-balanced amino acid pattern, except for slight deficiency in methionine (Smith and Circle 1972). The amino acid composition of the bicarbonate soaked soyflour is shown in Table 3. The flour contained all the essential amino acids; however, the methionine content was 0.6%. The low methionine content of the flour could be due to alkaline treatment of soybeans followed by steam heating that might have caused some losses of methionine. Supplementation with a

methionine-rich protein may be necessary in order to improve protein quality of the flour.

Table 2. Proximate composition of bicarbonate treated soy flour

Soy flour	Protein <sup>c</sup>	Oil <sup>c</sup>	Ash <sup>c</sup>	Moisture
			%	
Untreated <sup>b</sup>	52.4 ± 0.9	20.7 ± 0.0	6.7 ± 0.0	8.5 ± 0.5
Treated	49.7 ± 0.5	19.6 ± 0.2	5.1 ± 0.2	3.5 ± 0.1

<sup>a</sup>Mean ± S.E. of three determinations

<sup>b</sup>Untreated - raw soybean flour

<sup>c</sup>based on a dry weight

Table 3. Amino acid composition of bicarbonate treated and steamed soy flour

Amino Acid	% <sup>a</sup>
Lysine	4.7
Histidine	2.0
Arginine	5.2
Aspartic acid	10.6
Threonine	3.6
Serine	5.4
Glutamic acid	16.2
Proline	5.8
Alanine	5.8
Glycine	3.3
Valine	4.6
Methionine	0.6
Isoleucine	3.9
Leucine	7.1
Tyrosine	2.1
Phenylalanine	3.1

<sup>a</sup>Calculated as g amino acid/100 g protein

Mean of two determinations

Heating is effective in destroying lipoxygenase and T.I., but it also denatures other soy proteins and causes the loss of functionality. Table 4 shows a comparison of functional properties of soyflour from raw and from bicarbonate soaked and heated soybeans. The most significant difference was a decrease in solubility, as expected. The solubility of the bicarbonate soaked flour was 38% while that of the raw soybean was 88%. It is well known that if meal or flour is treated with moist heat, the proteins become insoluble (Wolf 1970). The lower solubility of the flour from treated soybeans was due to denaturation of soy protein by steam heating.

Table 4. Functional properties of bicarbonate treated soy flour

	Untreated	Treated	*Soyflour
Solubility %	88	38	
Emulsion Capacity $\frac{\text{ml oil}}{\text{g sample}}$	200 $\pm$ 0.07	180 $\pm$ 0.07	142.4
Foaming capacity (ml)			
Volume after whipping	110.0	108.0	
Weight after whipping	100.35	101.0	
Specific volume $\frac{\text{ml oil}}{\text{g sample}}$	1.10	1.08	
Relative viscosity			
1% solution	1.02	1.04	
5% solution	3.47	4.21	
Gelation			
Heating for 10 min.	-	+	
Water absorption $\frac{\text{g water}}{\text{g flour}}$	1.70 $\pm$ 0.31	1.60 $\pm$ 0.00	1.70
Oil absorption $\frac{\text{ml oil}}{\text{g flour}}$	1.00 $\pm$ 0.0	1.08 $\pm$ 0.0	0.96
Color			
L	50.2	48.6	
a	- 3.4	- 3.5	
b	9.3	8.1	

\*Sathe and Salunkhe 1981.

Mean of three determinations

There was a slight decrease in emulsion and foaming capacity, and some increase in viscosity and oil absorption capability for the treated samples. Gelation reaction was negative for raw but was positive for treated product. The water and oil absorption capacity of the treated flour was similar to that of commercial soyflour reported by Sathé and Salunkhe (1981). However, emulsion capacity appeared higher. The result indicates that bicarbonate soaked and heated soyflour retained much of the functional properties of soybeans, except solubility.

Addition of 20% soyflour resulted in darker color and loss of elasticity of noodles. This was probably due to lack of gluten in the soyflour. The results of sensory evaluation of noodles are shown in Fig. 3 and 4. The mean scores for flavor intensity were 6.84 for the soyflour added noodles and 6.80 for the control; overall acceptability scores were 8.04 for the soyflour added noodles and 7.96 for the control. The difference was not significant ( $P > 0.05$ ), indicating that addition of soyflour did not impair overall acceptability or the flavor of the product.

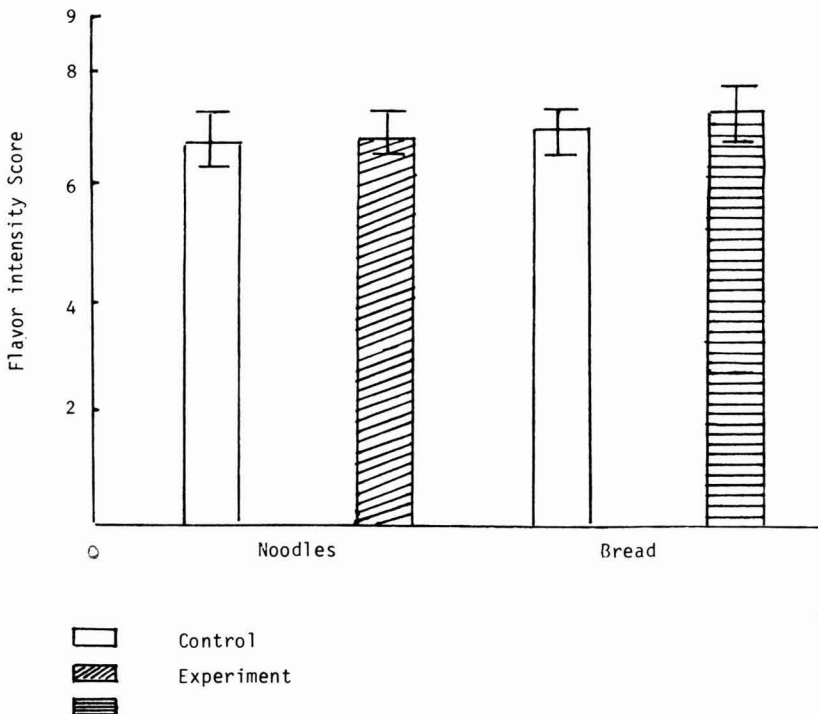


FIG. 3. FLAVOR INTENSITY OF NOODLES AND BREAD

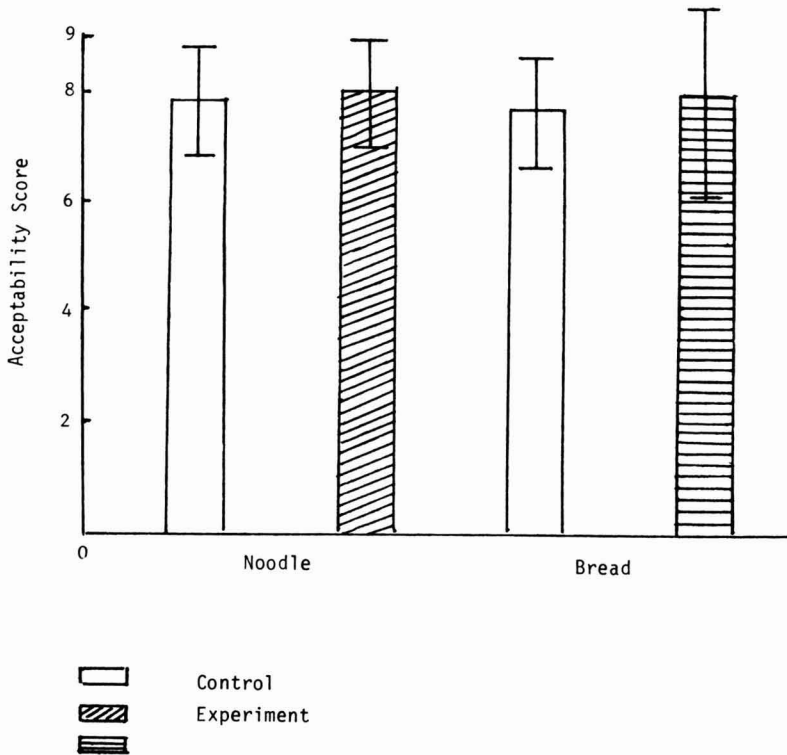


FIG. 4. OVERALL ACCEPTABILITY OF NOODLES AND BREAD

One obvious problem observed in bread was that soyflour added bread had a smaller loaf volume. The average loaf volume was 1709 mm<sup>3</sup> for the control and 1570mm<sup>3</sup> for the soyflour added bread. The reason probably is a lack of gluten in soyflour, which is responsible for stretching and increasing the volume of bread. The soyflour bread also had a slightly darker color; however, it looked like a rye bread and was not objectionable. The results of sensory evaluation are shown in Fig. 3 and 4. The mean score of flavor intensity of soyflour added bread was 7.20, while that of the control was 6.96. The mean scores of overall acceptability were 8.0 for the soyflour added bread and 7.77 for the control. The values were slightly higher for the soyflour added bread than the control; however, the difference was not significant ( $P > 0.05$ ).

It was concluded that both noodles and bread that contained soyflour were highly acceptable in terms of flavor and taste. It appears that the bicarbonate treated and steamed soyflour could be used as an ingredient to improve nutritive quality as well as functional property of various food products.

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# EFFECT OF PARBOILING AND FREEZING ON QUALITY OF THREE SPANISH RICE VARIETIES

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## ABSTRACT

*The effects of parboiling and of freezing at  $-18$ ,  $-40^{\circ}\text{C}$  or in liquid nitrogen on quality of cooked rice was studied for three Spanish varieties, Bahia, Sequal and Italpatna, using instrumental and sensory measurements. Parboiling prior to cooking and then freezing reduced rice quality. Freshly cooked samples differed from frozen cooked samples for firmness as measured instrumentally but not for any of the sensory parameters. Principal component analysis did not separate samples on the basis of their varietal quality.*

## INTRODUCTION

Rice is found in many dishes from the Mediterranean countries. Preparation of ready-to-eat dishes with frozen cooked rice would be of considerable interest to the industry. However, the nature and extent of the changes in rice quality due to the freezing process are not well known.

Several methods for rice freezing have been patented. The most recent ones were developed by Mutoh *et al.* (1978) and Akesson *et al.* (1984). In the former, cooked rice was frozen by exposure to liquid refrigerant in a rotary drum containing blades which helped separate the kernels during the freezing process. In the latter, boiled rice was frozen in a freezer operating between  $-25$  and  $-50^{\circ}\text{C}$ . Prior to freezing, the rice was cooled and intimately mixed with 20–80% w/w of already frozen, free-flowing boiled rice. A capsule-packed freezing procedure (CPF) was developed by Mitsuda *et al.* (1983) which accelerated the freezing rate during the period of maximum crystal formation ( $-1$  to  $-5^{\circ}\text{C}$ ) in order to set smaller ice crystals and better quality.

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Yet, relatively few studies looked at the effect of freezing rate on rice quality. Boggs *et al.* (1951) reported that a sensory panel found no difference between rice frozen at  $-12$ ,  $-18.5$ , or  $-23$  °C, stored for 1, 8 or 32 weeks and freshly cooked rice. According to Mitsuda *et al.* (1983), CPF and deep ( $-20$  to  $-50$  °C) frozen samples were harder than freshly cooked samples. They retained their stickiness in contrast to refrigerated ( $0$  °C) samples, and gradually became softer upon storage.

Extensive literature is available on instrumental (Kumar *et al.* 1976; Perez and Juliano 1979; Bhattacharya *et al.* 1982; Fellers *et al.* 1983) and/or sensory (Civille and Szczesniak 1973; Juliano *et al.* 1981; Juliano *et al.* 1984) measurements of cooked rice texture. Using some of these instrumental and sensory techniques for quality evaluation, the purpose of the present investigation was to study the effects that parboiling, cooking and freezing at  $-18$  °C,  $-40$  °C or by immersion in liquid nitrogen (LN) had on three commercial rice varieties grown in Spain as related to their suitability for use in commercial rice dish preparation. American and Spanish panels were used to account for cultural differences when sensory evaluation was made.

## MATERIALS AND METHODS

### Materials

Three Spanish varieties Bahia, Sequial (short grain) and Italtatna (long grain) were tested. Thirty 500 g-cardboard boxes of each variety were shipped by air from Spain for this study.

### Parboiling

For each variety, half the samples were parboiled in a steam retort. Fifty gram samples were placed in metallic mesh screen bags inside the retort. Samples were steeped in recirculating hot water with an overriding air pressure and steamed with saturated steam. Temperature reached  $107$  °C during steaming, as determined by a thermocouple placed in the upper part of the retort. The bags were then removed and placed on a bench where the rice dried overnight, without any supplemental airflow, at room temperature ( $25$  °C). Upon drying, the samples had a moisture content of 12–13%. Dried samples were removed from the metallic mesh bags and stored in polyethylene bags.

### Cooking

Rice (regular or parboiled) was cooked 100 g at a time in an automatic rice cooker in twice the equivalent volume of distilled water (about 110 mL). Cooked samples were then placed in polyethylene bags to prevent dehydration.

## Freezing

Immediately after cooking and one day before instrumental or sensory testing, sample bags were either placed in the freezer at  $-18$  or  $-40$  °C, respectively, or immersed in liquid nitrogen (LN) and kept at  $-40$  °C once frozen. Freezing rates were determined by a thermocouple installed inside a hypodermic needle. The needle was inserted into a rice kernel in the middle of the 100 g-sample. By means of a data acquisition system (Hewlett Packard 3050B) connected to a desk-top computer, temperature was recorded every minute and plotted against time. It took about 2 h for the rice samples to approach their respective freezing temperature ( $-18$  or  $-40$  °C).

## Sensory Measurement of Rice Quality

**Design.** Using a partially balanced block design, the effects of variety, precooking treatment (parboiled versus regular), and freezing temperature (fresh,  $-18$ ,  $-40$  °C, LN) on rice quality were evaluated in duplicate by two panels.

**Panels.** The Spanish panel consisted of 10 Spanish students in temporary residence at University of California, Davis, aged between 23 and 46, with no previous experience in sensory testing. The American panel consisted of 8 students, aged between 23 and 29, and experienced in sensory testing. Both panels were similarly trained prior to evaluation of the samples.

**Sample Preparation.** After keeping 5 h in a room maintained at  $-20$  °C, frozen samples were placed on aluminum trays, covered with aluminum foil to prevent dehydration and thawed in an electric oven at  $200$  °C one hour before evaluation. The rice layer in the tray was 2 cm thick. Freshly cooked samples were prepared in a rice cooker 100 g at a time in twice their volume of distilled water immediately before testing.

**Sample Evaluation.** Descriptive analysis (Stone *et al.* 1980) and deviation-from-reference (Pangborn 1984) techniques were used. In the first session, using four of the experimental samples chosen by the experimenters for their distinct characteristics, descriptors and definitions of the rice sensory attributes were selected separately by both panels in their respective language (Table 1). During the following two sessions judges were trained to consistently and reproducibly evaluate the intensity of each attribute selected by their panel using a deviation-from-reference technique. The reference used was a freshly cooked sample of California Silver Pearl rice. The performance of all judges during training was satisfactory as assessed by the fact that their ratings of identical samples did not differ significantly between the first and the second session. In each of the following six sessions held on different days, 4 of the 24 experimental samples were evaluated in duplicate by each panel using the same deviation-from-

Table 1. Descriptors and definitions of rice sensory attributes developed by each panel

Descriptors	Definitions
----- American panel	
Firmness	Force exerted by the molar teeth to prepare the sample for swallowing. It is evaluated by placing a spoonful of rice in the mouth and chewing it twice.
Stickiness	Force required to separate the teeth joined by the rice paste, once the sample is compressed by the molars. It is evaluated by placing a spoonful of rice in the mouth and chewing it twice.
Appearance	Rough-smooth aspect of the kernel surface. It is evaluated by visual examination of the sample.
Fracturability	Degree to which kernels break down and size of the particles in which they break down when sheared with the teeth. It is evaluated by placing a spoonful of rice in the mouth and chewing it twice.
Rice aroma intensity	It is evaluated by sniffing the sample.
----- Spanish panel	
Dureza	Same as firmness
Pegajosidad	Same as stickiness
Soltura	Degree to which the kernels move separately or not when a force is applied to them. It is evaluated by visual examination of the rice moved around with a spoon.
Elasticidad	Degree of recovery of their original shape by the rice kernels upon deformation by the teeth. It is evaluated by placing a spoonful of rice in the mouth and chewing it twice.
Uniformidad	Degree to which the rice appears fully cooked without uncooked or grainy spots. It is evaluated by placing a spoonful of rice in the mouth and chewing it twice.
-----	

reference technique. Hedonic evaluation of the samples was also required by including 'degree of liking' ('grado de aceptacion') in both score sheets. Each session was about 30 min long. All testing was conducted in isolated booths under white light illumination and at room temperature ( $22 \pm 2^\circ\text{C}$ ). Samples were served in styrofoam cups coded with three-digit numbers. The cups were kept at  $60^\circ\text{C}$  in water baths installed in each booth.

### Instrumental Measurement of Rice Quality

**Firmness.** Firmness was measured using an Instron model 1140 Food Tester with a modified Ottawa Texture Measuring System (OTMS) cell (with perforated plate), a 2.5 x 2.6 cm plunger and a 0–20 kg load cell. Crosshead and chart speeds were set at 100 mm/min. Firmness was defined as the maximum force required to compress 16 g of rice through the perforated plate (average of three replicates).

**Stickiness.** Stickiness was defined as the work required to lift a 50 cm<sup>2</sup> OTMS plunger from the platform with the rice pressed in between for 10 s at 0.4 mm clearance (average of three replicates). Crosshead and chart speeds were set at 5 and 1000 mm/min, respectively (Perez and Juliano 1979).

**Data Analysis.** Individual analyses of variance (AOV) were run on each attribute rated by the panels and on firmness and stickiness as measured by the Instron. When significant differences were found, Fisher's Least Significant Difference (LSD) was used for mean separation. Correlation coefficients were calculated to determine relationships among sensory attributes. Sensory and instrumental measurements were then analyzed by Principal Component Analysis (PCA). Whereas AOV allows one to test for significant differences among samples over one attribute, PCA enables the determination of the number of dimensions over which differences among samples lie.

## RESULTS

### Effect of Parboiling Prior to Freezing on Rice Quality and Acceptance

Samples parboiled prior to freezing were significantly ( $p < 0.001$ ) less firm, less sticky and less elastic than the regular samples as judged by the Spanish panel (Table 2). The American panel found that parboiled samples were significantly ( $p < 0.001$ ) less firm, less fracturable and less smooth, and had a significantly ( $p < 0.001$ ) lower rice aroma than regular samples (Table 3). Parboiled rice received significantly ( $p < 0.001$ ) lower hedonic ratings (degree of liking and 'aceptacion') than regular rice (Tables 2 and 3). Instron measurements showed that parboiled samples were significantly ( $p < 0.001$ ) less firm and sticky than regular samples (Table 4).

### Effect of Freezing on Rice Quality

Figure 1 shows quality of fresh samples and samples frozen at  $-18$ ,  $-40^{\circ}\text{C}$  or in LN across all three varieties. No significant difference was found among freezing temperatures ( $-18$ ,  $-40^{\circ}\text{C}$  or LN) or between frozen and freshly

Table 2. Analyses of variance of sensory attribute ratings by the Spanish panel (10 judges): Degrees of freedom (d.f) and Mean Squares

Source of variation	d.f.	Mean Squares						
		Dureza	Pegajosidad	Soltura	Elasticidad	Uniformidad	Aceptacion	
Judge (J)	9	233.65	812.52***	829.55***	1267.57***	2405.24***	2173.20***	
Pre-treatment (P)	1	16473.63***	2434.50***	20790.17***	21373.35***	539.75	20163.17***	
Freezing rate (F)	3	994.82***	128.49	899.57***	1521.20***	116.04	1565.16***	
Variety (V)	2	1154.65**	1077.10**	2164.64***	652.02*	373.22	988.75***	
Replications (R)	1	69.01	401.50	174.00	76.00	22.97	0.60	
J*P	9	188.18	410.32	1031.94***	331.98	1004.26***	828.44***	
J*F	27	193.35	220.96	322.21**	240.41	286.58*	335.53***	
J*V	18	317.69*	256.96	419.56***	337.45*	230.92	153.53	
J*R	9	59.25	97.22	78.86	48.95	103.38	138.63	
P*F	3	849.64**	224.28	130.54	509.04*	511.72*	315.05	
P*V	2	817.78**	103.26	60.10	542.59*	561.34	599.18**	
P*R	1	161.01	3.17	13.00	27.55	98.10	214.67	
F*V	6	2027.67***	638.76**	518.23**	558.36**	438.68*	415.98**	
F*R	3	54.55	143.23	44.32	108.36	12.54	142.00	
V*R	2	43.85	82.66	11.26	12.59	95.20	29.79	
Error	383	175.18	195.78	159.47	175.60	186.97	126.96	

\*, \*\*, \*\*\* Significant at  $p < 0.05$ ,  $0.01$ , and  $0.001$ , respectively.



Table 3. Analyses of variance of sensory attribute ratings by the American panel (8 judges): Degrees of freedom (d.f.) and Mean Squares

		Mean Squares						
Source of variation	d.f.	Firmness	Stickiness	Fracturability	Rice aroma	Smoothness	Liking	
Judge (J)	7	519.39***	437.81**	370.51*	8753.78***	488.50***	1463.95***	
Pre-treatment (P)	1	2614.59***	21.57	1921.57***	9401.04***	27135.38***	7288.88***	
Freezing rate (F)	3	652.74**	395.84	275.82	647.01*	63.75	574.05**	
Variety (V)	2	728.79**	22.83	382.65	718.34*	5424.77***	706.18**	
Replications (R)	1	25.01	13.13	382.00	590.04	222.04	89.13	
J*P	7	73.53	550.81**	186.12	800.51***	404.35***	758.15***	
J*F	21	244.74*	479.76***	277.73*	401.95**	92.29	160.16	
J*V	14	248.12*	218.72	430.29***	357.79*	199.21*	206.93	
J*R	7	159.14	133.78	57.68	30.59	41.60	68.90	
P*F	3	547.20**	160.57	52.13	432.81	298.88*	639.73**	
P*V	2	2348.73***	179.39***	1128.36***	153.83	3541.71***	1.86	
P*R	1	9.38	7.32	9.07	15.84	0.01	685.34*	
F*V	6	834.04***	279.04	476.32**	241.67	77.03	105.03	
F*R	3	177.34	22.65	91.21	25.75	40.02	90.47	
V*R	2	25.13	126.33	271.10	87.32	68.46	54.32	
Error	80	130.48	157.53	154.08	193.17	93.41	126.79	

\*,\*\*,\*\*\* Significant at p<0.05, 0.01, and 0.001, respectively.

cooked samples for stickiness, fracturability, smoothness, 'pegajosidad' (stickiness) and 'uniformidad' (uniformity). Freshly cooked samples were significantly less firm (Instron) than frozen samples ( $p < 0.05$ ). Both panels rated samples frozen in liquid nitrogen (LN) significantly ( $p < 0.05$ ) less firm (sensory firmness and 'dureza') than the other samples, but this was not confirmed by Instron measurement of firmness. Samples frozen at  $-40^{\circ}\text{C}$  had a significantly ( $p < 0.05$ ) higher 'soltura' (looseness) than the other samples. Freshly cooked samples were liked significantly better than samples frozen at  $-18^{\circ}\text{C}$  or in LN ( $p < 0.05$ ), and as much as samples frozen at  $-40^{\circ}\text{C}$  by both panels.

Table 4. Analyses of variance of instrumental measurements of firmness and stickiness: Degrees of freedom (d.f) and Mean Squares

Source of variation	d.f.	Mean Squares	
		Firmness	Stickiness
Pre-treatment (P)	1	389.67 <sup>***</sup>	38.30 <sup>***</sup>
Freezing rate (F)	3	21.91 <sup>***</sup>	4.88 <sup>**</sup>
Variety (V)	2	1.32	1.82
Replications (R)	2	0.19	0.28
P*F	3	15.17 <sup>***</sup>	7.92 <sup>***</sup>
P*V	2	31.32 <sup>***</sup>	9.65 <sup>***</sup>
P*R	2	1.13	0.34
F*V	6	6.59 <sup>***</sup>	2.03
F*R	6	0.30	0.83
V*R	4	0.41	0.44
Error	40	1.15	0.96

<sup>\*\*\*</sup>, <sup>\*\*</sup>, Significant at  $p < 0.01$  and  $0.001$ , respectively.

### Differences Among Varieties

No significant difference was found among varieties for sensory stickiness, fracturability and 'uniformidad' (uniformity) (Tables 2 and 3). Bahia samples were rated significantly ( $p < 0.05$ ) less firm than Sequial and Italtatna samples by the Spanish panel, whereas they were rated significantly ( $p < 0.05$ ) firmer by the American panel. Bahia samples had a significantly ( $p < 0.05$ ) lower rice

aroma than Sequial and Italtatna samples. Smoothness of the kernels increased significantly ( $p < 0.05$ ) in the order Bahia - Sequial - Italtatna. Sequial and Italtatna were liked significantly ( $p < 0.05$ ) better than Bahia by the Spanish panel, whereas only Sequial was significantly preferred to Bahia by the American panel.

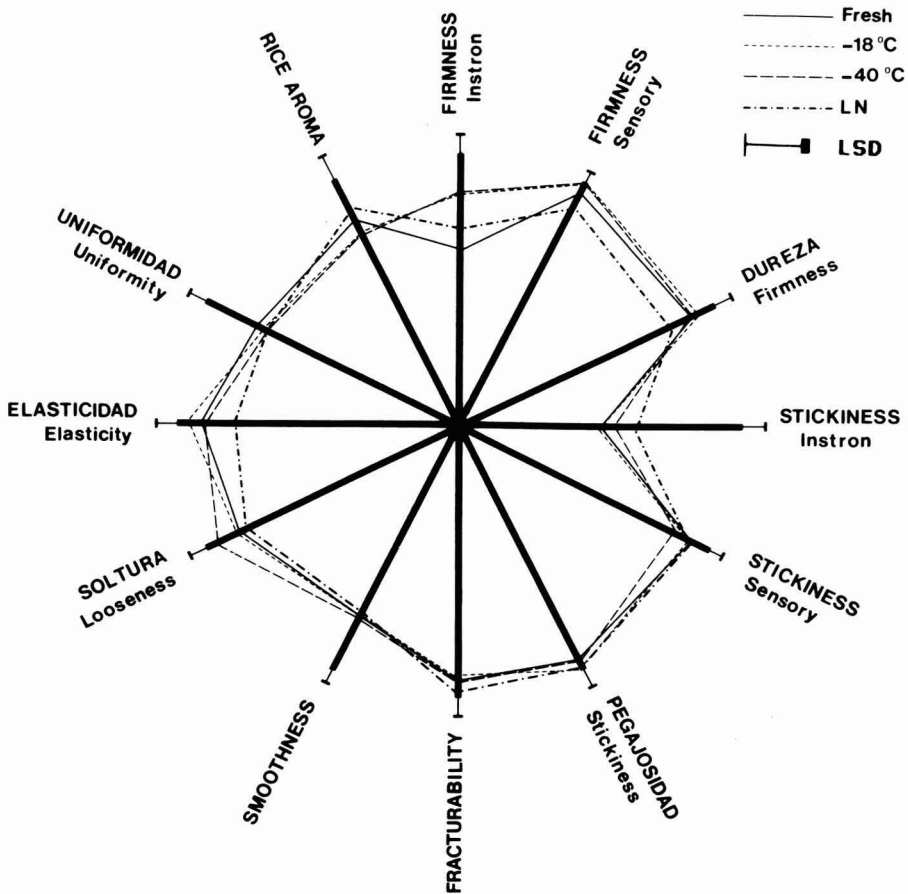


FIG. 1. MEAN RATINGS OF QUALITY ATTRIBUTES OF FRESHLY COOKED RICE AND COOKED RICE FROZEN AT  $-80$ ,  $-40^{\circ}\text{C}$  OR IN LIQUID NITROGEN (LN) ACROSS THREE SPANISH VARIETIES, WITH CORRESPONDING LEAST SIGNIFICANT DIFFERENCES AT  $P < 0.05$

### Principal Component Analysis

The first and second principal components accounted for 41 and 14% of the total variance, respectively. Figure 2 shows sensory and instrumental variables plotted with the rice samples on the first two principal components (PC). The largest difference among the attributes of the rice samples is due to variation along the first PC in degree of liking, 'aceptacion', 'elasticidad' and 'dureza', although 'soltura' and 'pegajosidad' also contribute. Degree of liking and 'aceptacion' were highly correlated with each other as shown by their proximity on the first two PC, indicating that degree of liking did not differ between the Spanish and the American panels. Appearance and aroma might be important factors in determining hedonic ratings since they were highly correlated with degree of liking and 'aceptacion'. Good correlation was found between Instron and sensory firmness ('dureza') as shown by their proximity on the first two PC. However, this was not true of Instron and sensory stickiness ('pegajosidad'). Most parboiled rice samples (P) are located on the left side of the plane formed by the first two PC, probably because they received lower hedonic ratings than the regular rice samples (R). No clustering of the rice samples can be made as a function of the variety or freezing rates. This is consistent with the significant interaction of variety by freezing rates encountered in most of the analyses of variance of the sensory and instrumental data. The lower left quadrant includes many of the Bahia rice samples, suggesting that this variety received low hedonic scores and was firm (sensory firmness) and sticky ('pegajosidad').

### Comparison Between the Spanish and American Panels

The panels differed in their selection of sensory attributes. The five attributes selected by the Spanish panel were related to rice texture, whereas the American panel included visual (smoothness) and olfactive (rice aroma) attributes. Firmness ('dureza') and stickiness ('pegajosidad') were selected by both panels. Replications were not a significant source of variation for any of the sensory ratings by either panel (Tables 2 and 3), indicating that both panels were reproducible in their evaluations. Correlation coefficients among descriptors are shown in Table 5. There were several significant correlations among American descriptors and among Spanish descriptors. Therefore, both panels might have selected several descriptors to describe the same characteristic (i.e., firmness and fracturability,  $r = -0.81$ ,  $p < 0.001$ ; 'dureza' and 'soltura',  $r = 0.78$ ,  $p < 0.001$ ; 'soltura' and 'elasticidad',  $r = 0.74$ ,  $p < 0.001$ ; 'dureza' and 'elasticidad',  $r = 0.83$ ,  $p < 0.001$ ). This is illustrated on the PCA by the proximity of some Spanish or American descriptors, respectively. Correlation between Spanish and American descriptors of the same attribute was significant for firmness ( $r = 0.42$ ,  $p < 0.05$ ) but not for stickiness.

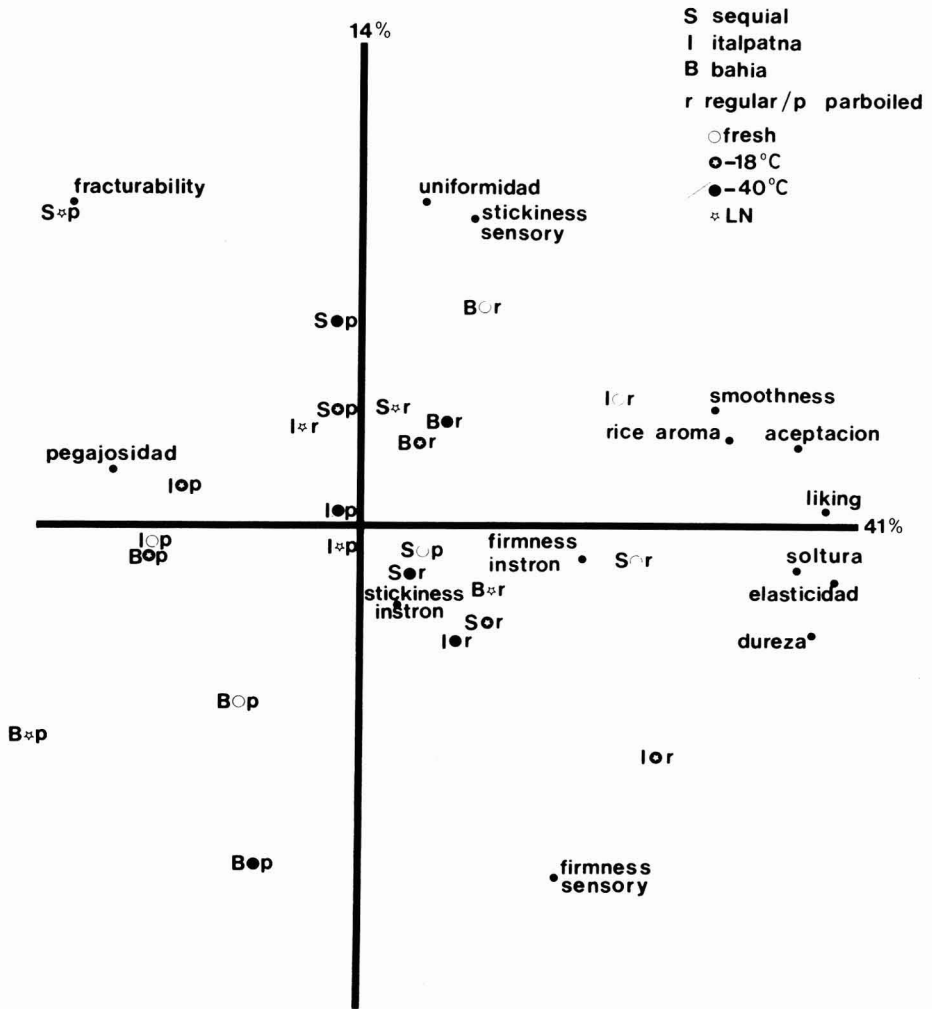


FIG. 2. PRINCIPAL COMPONENT ANALYSIS OF SENSORY AND INSTRUMENTAL RATINGS ACROSS 24 RICE SAMPLES

Table 5. Correlation matrix among the sensory descriptors (d.f. = 22)

	1	2	3	4	5	6	7	8	9	10
1. Firmness	1.00									
2. Stickiness	-0.40*	1.00								
3. Fracturability	-0.81***	0.29	1.00							
4. Rice aroma	0.19	0.28	-0.36	1.00						
5. Smoothness	0.06	0.13	-0.30	0.66***	1.00					
6. Dureza	0.42*	0.15	-0.62**	0.49*	0.59**	1.00				
7. Pegajosidad	-0.33	0.15	0.26	-0.33	-0.27	-0.43*	1.00			
8. Soltura	0.46*	0.03	-0.62**	0.68***	0.64***	0.78***	-0.51**	1.00		
9. Elasticidad	0.42*	0.15	-0.62**	0.61**	0.71***	0.83***	-0.43*	0.74***	1.00	
10. Uniformidad	-0.28	0.47*	0.27	0.19	0.21	-0.19	-0.09	0.13	0.00	1.00

\*,\*\*,\*\*\* Significant at  $p < 0.05$ ,  $0.01$  and  $0.001$ , respectively.

## DISCUSSION

Descriptive analysis allowed to determine the nature and the extent of some changes in cooked rice quality upon freezing. The correlation matrix among sensory descriptors and the PCA indicated that both panels selected some redundant descriptors, that is, a significant percentage of the variance for one attribute could be accounted for by the variance for another one. This study also confirmed that firmness and stickiness (measured objectively or subjectively) are the best discriminants among samples for rice quality (Juliano *et al.* 1981, 1984). A past collaborative study (Juliano *et al.* 1981) indicated that instrument indexes for hardness and stickiness were more sensitive than the corresponding taste panel scores in discriminating among rice samples. In this study, Instron measurements of firmness and stickiness were respectively less and more sensitive than the corresponding sensory measurements in discriminating among samples (Tables 2, 3, 4).

Parboiling prior to cooking and then freezing reduced rice quality. Parboiled samples were less firm, less sticky and less smooth than regular samples as indicated by sensory and instrumental measurements. In addition, they received lower hedonic ratings than regular samples as indicated by the analyses of variance of 'degree of liking' and 'grado de aceptacion' (Tables 2 and 3), and by the PCA of sensory and instrumental data (Fig 2). PCA suggests that this quality decrease is unidimensional along the first principal component, which is a linear combination of 'elasticidad' (elasticity), 'dureza' (firmness), 'soltura' (looseness) and 'pegajosidad' (stickiness). This quality decrease is mostly due to gelatinization of the starch during the parboiling process.

Freshly cooked samples were significantly different from frozen (-18, -40°C, LN) cooked samples for Instron firmness (Fig. 1) but not for any of the measured sensory parameters. Similarly, frozen cooked long grain (Texas Patna) and short grain (California Pearl) rice samples were reported by Boggs *et al.* (1951) to be fully equal to the freshly cooked samples in every sensory parameter after reheating. As mentioned by Mitsuda *et al.* (1983), reheating often produces undesirable flavor in foods containing volatile and fresh materials. However, no significant difference was found between fresh and frozen samples for rice aroma, the only flavor attribute measured in this study. Yet, freshly cooked samples received significantly higher hedonic ratings than frozen cooked samples, and further investigation is needed to determine why freezing of cooked rice lowers its acceptance. It can be speculated that it is because they affected 'soltura' (looseness), 'elasticidad' (elasticity) and 'dureza' (firmness) to different extents that freezing rates received different hedonic ratings. Indeed, these three attributes were strongly correlated with degree of liking and 'grado de aceptacion' as shown by PCA (Fig. 2) and their intensity differed among freezing rates as shown by AOV (Table 2).

Differences among varieties were not as pronounced as differences between parboiled and regular samples or differences among freezing temperatures (Tables 2, 3, and 4), and principal component analysis did not separate samples as a function of their varietal quality. Selection of one variety over the others for commercial dish preparation should be made in relation to the quality attributes most likely to affect overall acceptance of the dish.

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# THE INFLUENCE OF MICROWAVE HEATING ON THE TEXTURAL PROPERTIES OF MEAT AND COLLAGEN SOLUBILIZATION<sup>1</sup>

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## ABSTRACT

*Quality characteristics, textural properties, and solubility of collagen were determined for two different methods of heating, duration of heating, and end temperature of the product. Properties of connective tissue and collagen solubilization were studied by determining shear cohesiveness, shear firmness and hydroxyproline solubilized. The microwave heating of meat samples solubilized more collagen than did the conventional method of heating. The data showed significant variations among the samples for percent hydroxyproline solubilized by microwave heating. Differences in shear cohesiveness and firmness were established for microwave and conventionally heated samples. Water holding capacity and moisture content of meat heated by microwave were similar to those heated by boiling.*

## INTRODUCTION

Conventional heat treatment of meat and meat products is a lengthy, labor-consuming process with yield and quality factors leaving much to be desired. Processing meat products by microwave energy may provide better quality control (shrinkage, flavor) and greater retention of nutrients than conventional processes. Microwave processing offers a means of rapidly providing uniform heat energy throughout the product. There are numerous opportunities in the meat processing industry in which industrial microwave units can be applied. Commercial microwave processing systems included precooking chicken and bacon,

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tempering frozen meat, coagulation of extruded reformed meat, sterilization and freeze-drying. A Swedish manufacturer of microwave equipment has installed several meat pattie cooking systems in Europe. The system is completely automated from beginning to end with the ground meat formulation being formed into patties (Decaru 1984). The process of heat treatment of sausages with diameter 50–60 mm was developed (Zayas *et al.* 1978). Applications of microwave can be expected to continue to expand as cost of labor and fuel escalate.

Microwaves, if properly applied, will accomplish heating requirements better than other forms of heating. In addition to obvious benefits, certain microwave applications in food processing have halved energy requirements and have reduced labor time and space needs by 75% (Smith 1977).

Many comparisons of the effects of microwave versus conventional heating methods upon food product quality can be found in the literature (Armbuster and Haefele 1975). However, most of these studies have been carried out using domestic microwave ovens whose heat distribution patterns are generally less uniform than those of industrial units (Zayas and Rebane 1973).

A great deal of early work on microwave cooking was devoted primarily to studies on nutrient retention (Campbell and Proctor 1958). These studies showed that retention of nutrients was greater with microwave heating because of relatively uniform heat distribution in the food plus reduced leaching-out of nutrients.

Most processed meat products contain a relatively high amount of connective tissue, the most important structural component affecting quality characteristics of muscles. Muscle fibers are toughened by extensive heating, while the collagenous connective tissue requires a moist atmosphere and long cooking to soften it by conversion of collagen to gelatin. During thermic contraction of collagen less collagen was solubilized in the meat of older than younger animals, when heated for 10 min at 65 °C. As temperature increased from 60 to 80 °C the amount of labile collagen in the epimysium increased from 35 to 54%. Labile collagen influences the tenderness of meat. Relationship between histological characteristics of muscle and connective tissue fibers and sensory tenderness of the muscle was established (Chambers *et al.* 1982).

Rate of heat penetration has been shown to affect collagen conversion and differs with the cooking medium (Cover and Hostetler 1960). The rapid rate of heat production by microwave energy has complex effects on the contractile and connective tissue fibers.

Changes in the collagen during heat treatment influenced significantly the tenderness and juiciness of meat. No differences were found in fragmentation of turkey muscle cooked conventionally or by microwave (Bowers and Heier 1970). However, moderate disintegration of muscle fibers was observed in beef semitendinosus muscle cooked to 50–65 °C by microwave (Goertz and Ross

1973). Greater histological changes occurred in collagenous tissue when beef was cooked by microwave than when it was cooked in a conventional oven by either moist or dry heat (McCrae and Paul 1974). Several researchers have indicated that meat is generally less desirable in tenderness and has higher cooking losses when cooked in the microwave oven than when prepared in a conventional oven (Ream *et al.* 1974). Existing parameters of microwave heating do not allow to produce a finished product of a sufficiently high quality. Particularly, it is due to a lack of completed studies of connective tissue properties after microwave heat treatment.

The goal of this research was to establish the influence of microwave heating on the quality characteristics of meat, particularly textural properties and solubilization of collagen, depending on the end temperature of the product and time of heating.

## MATERIALS AND METHODS

### Muscle Source

Beef Bicep femoris muscles from 8 animals were chosen for this study. Those muscles contain appreciable amounts of collagen and elastin. The outer fat covering was stripped off and each muscle was cut into eight 200 g pieces. Samples obtained from each muscle were individually wrapped in aluminum foil, ground into sets of four, and stored at  $-22^{\circ}\text{C}$  until used. Geometry and dimensions of samples are presented at Fig. 1.

### Heat Treatment of Meat Samples

Before heating, a set of muscles was thawed at  $20^{\circ}\text{C}$  for 3 h, then for 18 h at  $4^{\circ}\text{C}$ . Temperature of the thawed samples ranged from  $-1^{\circ}\text{C}$  to  $4^{\circ}\text{C}$ . Weights of samples were recorded before and after heating to calculate heating losses. Heating times were also recorded.

Meat samples within the group were randomly assigned to heat treatment. The order of treatment was also randomized. Heating of meat samples was carried out inside heat resistant plastic bags by microwave or by boiling (control samples) to end-point temperatures of  $65^{\circ}$  and  $80^{\circ}\text{C}$ . Cooking bags used for experiments allowed for a closed environment and created a vaporous atmosphere while cooking. This method reduced the evaporative cooling that occurred in early tests on meat products.

The samples were assigned to a microwave oven (Sharp, Model NR 8200 with carousel turntable system) operating at 525–550 W cooking power and a frequency of 2450 MHz. The oven was set at "Roast power" to heat the samples, and power level calibrated by water load measurements was 400–420 W.

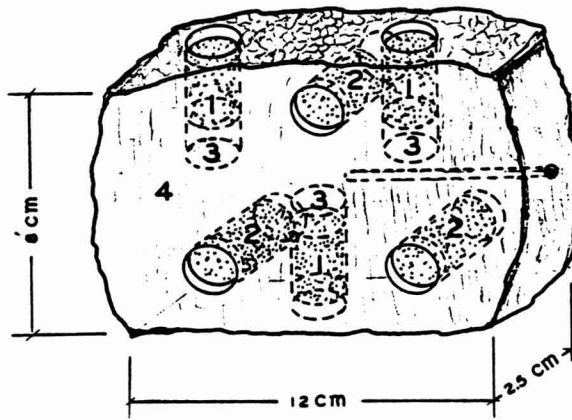


FIG. 1. SAMPLING PLAN FOR BICEP FEMORIS MUSCLE

1. Perpendicular to fiber shear sample
2. Parallel to fiber shear sample
3. Sample for water holding capacity
4. Ground for moisture and hydroxyproline determinations

A second series of experiments was carried out with samples cooked to internal end-point temperatures of 85 and 95 °C as measured by an alcohol thermometer. End-point temperatures were measured in the geometrical center of the samples.

Measurements related to quality characteristics of cooked meat included water holding capacity (WHC), textural characteristics, moisture content, and the percentage of hydroxyproline solubilized. They were measured following a fixed position sampling plan (Fig. 1). Measurements of WHC were made on cooked meat samples (300 mg) using the press method. The ratio of the pressed meat area to the expressed liquid area was designated as the expressible liquid index (ELI). WHC was obtained by subtracting ELI from 1.0, arbitrarily chosen as the maximum WHC. Moisture content was determined using AOAC method (1980).

The effect of two methods of heating on the connective tissue of meat samples was measured by the determination of hydroxyproline solubilized before and after cooking, using 3 g raw or 2 g cooked meat samples. The amount of hydroxyproline that solubilized during the cooking process was determined as the difference between the total hydroxyproline in raw meat sample and that in the water-washed, cooked samples. Ground raw meat sample was introduced into a

50 mL ampoules and hydrolyzed by adding 30 mL 6N HCl. Ratio of sample to acid 1:10. The samples were sealed in 50 mL ampoules and hydrolyzed for 20 h at 107 °C. Cooked ground samples were washed two times with warm distilled water (40 °C). This was done to remove all the adhering solubilized OH-proline and water soluble proteins (Paul 1973). After washings they were separated from the meat residue by centrifugation at 4000 rpm for 10 min. The water washed meat residues were transferred into the ampoules and hydrolyzed in the same way as the raw samples. Ampoules were opened and contents were decanted into beakers. Hydrolyzates were neutralized to pH 6.5–7.0 with 2.5N NaOH and made up to 250 mL in volumetric flasks with distilled H<sub>2</sub>O. Diluted hydrolyzates were filtered through charcoal to obtain a colorless solution for colorimetric assay of OH-proline. Hydroxyproline was assayed using a modified method of Bergman and Loxley (1961). Solubility of collagen, which reflects degree of collagen gelatinization, was correlated to the different methods of heating, time of heating, and the end-point temperature of the product.

Textural characteristics, shear cohesiveness, and firmness were determined both perpendicular and parallel to the muscle fibers. The measurements were based on the assumption that the force required to shear muscle perpendicular to its fibers reflects the extent of muscle fiber toughening, whereas that required to shear muscle parallel to its fiber reflects the extent of collagen solubilization.

The Instron Universal Testing Machine, Model 1122 was used for shear measurements. Circular cores (diameters 1.2 cm) of cooked muscles were sheared using a Warner-Bratzler attachment (D-372-26) with a crosshead load of 20 kg travelling at the speed of 50 mm/min. A shear deformation curve was recorded in a chart moving at the speed of 100 mm/min and was used to evaluate shear cohesiveness and shear firmness of the cooked muscle samples. Shear cohesiveness was the peak force (kg) on the shear deformation curve. Shear firmness was measured as a slope of the line drawn from the origin of the curve to the peak and expressed in kg/min (Larmond and Petrasovits 1972).

Duncan multiple range tests (DMRT) were used to compare the means of the measurements significantly affected by treatment (Snedecor and Cochran 1980). Correlation coefficient analysis was utilized to evaluate the relationship among selected paired measurements.

## RESULTS AND DISCUSSION

Comparing microwave and conventionally heated samples to the end-point temperature 65 °C and 80 °C indicated no significant differences in their textural characteristics (Table 1). Shear cohesiveness and shear firmness measured perpendicular to the fibers of the samples heated by microwave were similar to those heated by boiling. This was established despite the significantly shorter

time of microwave heating and larger heating losses. At the same time, shear cohesiveness and firmness measured parallel to fibers were slightly lower for samples heated by microwave. This reflects a difference in the level of collagen solubilization during conventional and microwave heat treatments.

Increasing the end-point temperature from 65 °C to 80 °C caused an insignificant increase of the textural characteristics, shear cohesiveness, and firmness measured perpendicular to the fibers. It is quite evident that the time-temperature combinations had an effect on the tenderness of the meat, probably causing both physical and chemical changes in the connective tissue and in muscle fibers. The tenderness of cooked meat is generally considered to be controlled by the heat-induced changes in collagenous connective tissue and in the contractible proteins. The number of crosslinkages between the collagen molecules within the connective tissue is associated with collagen solubility.

Table 1. Mean values obtained for bicep femoris muscle heated by microwave and convectional method

Measurement	Method of heat treatment			
	Microwave 65 °C	Conventional 65 °C	Microwave 80 °C	Conventional 80 °C
Shear cohesiveness, kg (perpendicular to fibers) <sup>c</sup>	6.33 <sup>a</sup>	6.10 <sup>a</sup>	7.01 <sup>a</sup>	6.70 <sup>a</sup>
Shear cohesiveness, kg (parallel to fibers) <sup>c</sup>	5.09 <sup>a</sup>	6.01 <sup>b</sup>	5.36 <sup>a</sup>	5.85 <sup>a</sup>
Firmness, kg/min (perpendicular to fibers) <sup>c</sup>	25.87 <sup>a</sup>	25.98 <sup>a</sup>	29.36 <sup>a</sup>	31.31 <sup>a</sup>
Firmness, kg/min (parallel to fibers) <sup>c</sup>	17.77 <sup>a</sup>	20.38 <sup>b</sup>	16.46 <sup>a</sup>	19.54 <sup>b</sup>
%Solubilized hydroxyproline	27.61 <sup>a</sup>	18.52 <sup>b</sup>	28.32 <sup>a</sup>	20.30 <sup>b</sup>
% Moisture	61.28 <sup>a</sup>	63.49 <sup>a</sup>	58.15 <sup>a</sup>	60.94 <sup>a</sup>
% Heating losses	38.78 <sup>a</sup>	27.01 <sup>b</sup>	40.61 <sup>a</sup>	30.64 <sup>b</sup>
Water holding capacity	0.73 <sup>a</sup>	0.76 <sup>a</sup>	0.67 <sup>b</sup>	0.73 <sup>a</sup>
Heating time, min	7.53 <sup>a</sup>	14.25 <sup>b</sup>	7.94 <sup>a</sup>	19.31 <sup>b</sup>

<sup>a, b</sup>Means bearing the same letter in row are not significantly different (P<0.05)

<sup>c</sup>Average from 4 muscles; measurements were done in triplicates. Determined from shear force deformation curve.

There were nonsignificant differences in moisture content between microwave and conventionally heated samples on the one hand, and between samples heated to end-point temperatures of 65 °C and 80 °C on the other. Method of heating and end-point temperature did not influence water holding capacity of the samples.

Data presented in Table 1 illustrate a tendency for heating losses to be greater with microwave than with conventional heat treatment. When comparing samples heated to an end-point temperature of 65 °C with those heated to 80 °C, it was found that total heating losses were greatly increased by the longer time and higher temperature.

It was not reflected on the remaining moisture content of the cooked samples suggesting that losses included other meat components. Evidently, greater fat losses and solidified particles were noted in the drippings of microwave heated samples, than in control samples. The larger cooking losses among the microwave samples may possibly account for the greater percentage of hydroxyproline solubilized obtained for the above samples.

The rapid rate of heat generation by microwave had complex effects on the connective tissue fibers. Microwave heat treatment solubilized more collagen from the meat samples than did the conventional heating method (Table 2). Microwave heating resulted in a higher percentage of hydroxyproline solubilization: 27.65% for end-point temperature 65 °C and 28.53% for end-point temperature 80 °C. For control samples, percentage of hydroxyproline solubilization was 19.18% for end-point temperature 65 °C and 18.97% for end-point temperature 80 °C. The data on 65 °C and 80 °C internal temperatures of the samples showed variations among the samples (Table 1). Collagen solubility should be considered when attempting to biochemically explain the toughness of meat. Collagen solubilized was higher for microwave at the end-point temperature 65 °C and at the end-point temperature 80 °C.

Effect of microwave heating on the collagen of connective tissue of beef makes an important contribution to the quality of the finished product. Previous work had indicated an increase in amount of free sulfhydryl groups after microwave heating and unfolding of the proteins polypeptide chains (Zayas *et al.* 1974).

Correlation analysis of the measurements suggested that the moisture content in the finished product had a low effect on the textural characteristics, particularly shear firmness and shear cohesiveness measured perpendicular to fibers (Table 3). However, moisture content in the finished product influenced shear firmness measured parallel to fibers. Moisture content in the product after heat treatment also affected water holding capacity. Correlation coefficients between textural properties and percent solubilized hydroxyproline were insignificant. On the other hand, heating losses affected water holding capacity.

Table 2. Solubilized hydroxyproline of beef (biceps femoris) heated by microwave and conventional methods<sup>a</sup>

Treatment	Percentage of solubilized hydroxyproline, N of sample										Mean	P-value
	21	41	52	63	64	78	78	64	63	52		
Microwave (65°C)	28.04	27.84	32.17	21.49	27.04	29.33	29.33	27.04	21.49	32.17	27.65	0.019
Conventional (65°C)	18.65	11.52	26.02	10.31	31.01	17.57	17.57	31.01	10.31	26.02	19.18	
Microwave (80°C)	18.36	36.11	27.82	27.67	26.26	34.97	34.97	26.26	27.67	27.82	28.53	0.054
Conventional (80°C)	14.17	10.72	22.53	19.77	29.58	17.06	17.06	29.58	19.77	22.53	18.97	

<sup>a</sup>Mean value from 3 replications.



Table 3. Significantly correlated measurements across samples heated by microwave and conventional methods to end point temperatures of 65 and 80°C

Paired measurements, df = 30	Correlation coefficient	P. value
% Moisture vs.		
Shear cohesiveness (perpendicular to fibers)	0.38	0.030
Shear firmness (perpendicular to fibers)	0.41	0.020
Shear firmness (parallel to fibers)	0.52	0.002
Water holding capacity	0.59	0.009
% Heating losses vs:		
Water holding capacity	-0.50	0.030

Textural characteristics, shear cohesiveness, and shear firmness measured perpendicular to the fibers were similar for microwave and conventionally heated samples to end-point temperatures of 85°C and 95°C (Table 4). Shear cohesiveness measured perpendicular to fibers was lower for samples heated conventionally to end-point temperature of 95°C than samples heated to 85°C, but there was no difference in shear cohesiveness measured parallel to fibers. Firmness of the samples measured parallel to the fibers after heating to the end-point temperature of 85°C was higher for conventional method than for microwave. This means that increasing the end-point temperature to 95°C caused more tender samples as a result of higher level of collagen gelatinization and an increase in disintegration of muscular fibrils.

Table 4. Mean values obtained for bicep femoris muscle heated by microwave and conventional method

Measurement	Method of heat treatment			
	Microwave 85°C	Conventional 85°C	Microwave 95°C	Conventional 95°C
Shear cohesiveness, kg (perpendicular to fibers) <sup>c</sup>	5.44 <sup>a</sup>	5.54 <sup>a</sup>	5.15 <sup>ab</sup>	4.60 <sup>b</sup>
Shear cohesiveness, kg (parallel to fibers) <sup>c</sup>	4.42 <sup>a</sup>	5.39 <sup>a</sup>	4.75 <sup>a</sup>	4.74 <sup>a</sup>
Firmness, kg/min (perpendicular to fibers) <sup>c</sup>	19.99 <sup>a</sup>	19.80 <sup>a</sup>	21.07 <sup>a</sup>	17.59 <sup>a</sup>
Firmness, kg/min (parallel to fibers) <sup>c</sup>	14.93 <sup>a</sup>	17.42 <sup>b</sup>	15.99 <sup>a</sup>	16.11 <sup>a</sup>
% Solubilized hydroxyproline	32.96 <sup>a</sup>	27.68 <sup>b</sup>	30.84 <sup>a</sup>	27.42 <sup>b</sup>
% Moisture	56.76 <sup>a</sup>	58.69 <sup>a</sup>	56.95 <sup>a</sup>	55.70 <sup>a</sup>
% Heating losses	32.93 <sup>a</sup>	34.79 <sup>b</sup>	33.57 <sup>a</sup>	35.41 <sup>a</sup>
Water holding capacity	0.68 <sup>a</sup>	0.70 <sup>a</sup>	0.65 <sup>a</sup>	0.66 <sup>a</sup>
Heating time, min	1.78 <sup>a</sup>	11.43 <sup>b</sup>	1.81 <sup>a</sup>	13.65 <sup>b</sup>

<sup>a,b</sup> Means bearing the same letter in row are not significantly different ( $P < 0.05$ )

<sup>c</sup> Determined from shear force deformation curve as average from 4 muscles

This could be explained by fragmentation of muscle proteins increased with increasing end-point temperature and by higher level of collagen solubilization. Hearne *et al.* (1978) observed an increase in the muscle fibrillar disintegration in samples heated from 60 to 70°C. Davey and Gilbert (1974) obtained decreased shear force values in meats cooked above 80°C.

Firmness measured parallel to fibers for samples heated to the end-point temperature 85 °C was higher for conventionally heated samples, but there was no difference between samples at the end-point temperature 95 °C.

No other apparent differences were observed between microwave and conventionally heated samples, despite the differences in heating time and end-point temperature (Table 4). Water holding capacity, moisture content and heating losses were similar or statistically they were not different for microwave and conventionally heated samples to end-point temperature 85 °C and 95 °C.

The microwave energy solubilized more collagen from the meat samples than did conventional heat energy (Table 4). The data showed an increase in percent solubilized collagen by microwave heating of the meat samples.

Differences in solubilized hydroxyproline were also established for microwave and conventional heating to end-point temperatures of 65 °C, 80 °C, 85 °C, and 95 °C (Table 2 and Table 4).

## CONCLUSION

Research has focused on studying the influence of two methods of heat treatment, microwave heating and boiling, on quality characteristics of meats. Solubility of collagen and textural properties were studied dependent on the different methods of heating, heating time, and the end-point temperature of the product. Comparing microwave and conventionally heated samples has indicated differences in their characteristics. The rapid heat production by microwave had complex effects on the contractile and connective tissue fibers. The microwave energy solubilized more collagen from the meat samples than did conventional heat energy. The data show an increase in percent solubilized collagen by microwave heating and increasing internal temperature of the samples. At the same time the data on the 65 °C, 80 °C, 85 °C, and 95 °C internal temperature samples showed highly significant variations among the samples from 8 animals for percent hydroxyproline solubilized by microwave heating. Some of the textural properties of meat heated by microwave were different than those heated by boiling, despite the differences in heating time and heating losses. Correlation analysis of measurements suggested that the moisture content of the cooked samples affected textural characteristics, shear cohesiveness and shear firmness ( $r = 0.52$ ), and water holding capacity ( $r = 0.59$ ).

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# FACTORS INFLUENCING THE QUALITY OF CANNED STRAWBERRY FILLING DURING STORAGE<sup>1</sup>

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## ABSTRACT

*Two cultivators, Sunrise and Cardinal were used to prepare strawberry filling from frozen sliced 5 + 1 fruit to sugar to assess the effects of sweetener, cultivar, added color and flavor, and storage temperature and time on quality parameters. Glucose corn syrup caused less color degradation in the filling than fructose syrup. The cultivar Sunrise was rated better in color acceptance than Cardinal after storage when the temperature and time were increased, primarily due to the lighter initial color. The addition of FDC #40 food color improved color, which was especially beneficial to the Sunrise fruit after 2 and 3 months storage. The addition of flavors had no effect on any of the quality parameters. Higher storage temperatures (24°, 32°C) for 2 and 3 months storage were detrimental to quality. Both cultivars were acceptable for filling, but refrigeration was required, and the addition of color was necessary to extend shelf-life beyond 2 months. Color addition was more beneficial for the lighter-colored cultivar, Sunrise.*

## INTRODUCTION

Quality of strawberries is influenced by many factors including environmental factors, pH, cultivar, maturity, method of harvesting, post-harvest storage, method of processing and storage of the processed product (Kertesz and Sondheim 1948; Lukton *et al.* 1956; Markakis 1974; Meschter 1953; Morris *et al.* 1978; Sistrunk and Moore 1971; Sistrunk and Morris 1978; Sistrunk *et al.* 1982; Sistrunk *et al.* 1983; Spayd and Morris 1981; Wrolstad and Abers 1979).

<sup>1</sup>Published with the approval of the Director of Arkansas Agricultural Experiment Station.

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Strawberries are harvested mechanically once-over when 15 to 40% of the fruit is immature (Morris *et al.* 1978). There is a need to develop products that can utilize the total crop of machine harvested fruit. Low-sugar products such as toppings, fillings and spreads are one type of manufactured product in which the immature fruit can be blended with ripe fruit. These products can be manufactured with sucrose, corn syrup sweeteners, additional colors and flavors, starches, and gums to attain a desirable product.

In strawberry preserves and jams, Sistrunk and Morris (1978) demonstrated that neither green fruit nor cultivar influenced the color and flavor changes in the products. However, storage temperature and time significantly affected the quality. The greatest deterioration in quality occurred at 24° and 35°C during storage compared to 2°C. The commercial preserves purchased in supermarkets were usually lower in quality than samples stored 9 months or longer at 24°C (Morris *et al.* 1979). When highly colored cultivars were used to manufacture either preserves or jam, as much as 50% green fruit could be used without influencing the color and flavor of the product. Green fruit puree had a dilution effect on color but not a synergistic effect in strawberry jam (Spayd and Morris 1981).

Strawberry spread (38% soluble solids) that was manufactured from 75% ripe fruit and 25% green 'Cardinal' strawberries deteriorated rapidly in quality (Sistrunk *et al.* 1982). After 4 month's storage, the spread was rated low in color and flavor except when stored at 2°C. Therefore, there is a rapid loss of color in low sugar strawberry products such as spreads. Concurrently, a change in color occurred when measured by the Color Difference Meter and total anthocyanins. The anthocyanins are converted to red-brown and brown polymeric pigments by a number of different mechanisms (Cash and Sistrunk 1971; Lukton *et al.* 1956; Meschter 1953; Sistrunk and Cash 1970; Poesi-Langston and Wrolstad 1981).

The objectives of this study were to develop an acceptable formula for strawberry filling, and assess the changes in quality attributes as affected by sweetener, cultivar, flavor, and color additives, and storage temperature and time.

## MATERIALS AND METHODS

### Preparation for Freezing

One hundred and fifty pounds of Cardinal strawberries were picked at the main Agricultural Experiment Station at Fayetteville, Arkansas, and the same amount of Sunrise fruit was picked on the farm of a commercial grower. The strawberries were capped by hand and washed in a small-fruit cleaning system (Morris *et al.* 1978). The cleaned fruit was sliced in 3/8 in. slices by a commercial slicer and filled into polyethylene bags. Sucrose was added at the rate of 500 g of strawberries to 100 g of sugar (5:1 ratio) and frozen at -28°C.

### Statistical Design

The experiment was designed as a factorial with 2 cultivars (Cardinal, Sunrise); 2 colors (FDC #40 food color, no color); 3 flavors (control, pistachio, lemon); 3 storage temperatures (2 °, 24 ° and 32 °C); and 3 storage times (0, 2, 3 months). Two replications of each lot were prepared for sensory evaluation and laboratory analyses.

### Preparation of Filling

Before being processed into topping, the frozen strawberries were allowed to defrost overnight at 2 °C. Thawed fruit was drained in a plastic collander, then 500 g of Staleys "Dura-Jell" corn starch was mixed with 12.6 g of xanthan gum, 12.6 g guar gum and 10 g glucose. After mixing, the dry ingredients were dispersed in 4200 mL of water and the juice from drained berries. Four batches were prepared for each cultivar. The starch mixture was transferred to a 22 L steam-jacketed kettle, antifoam was added, and the batch heated to 85 °C. Either fructose or 42 DE glucose corn syrup (2800 g) was added to individual batches and the temperature was brought back up to 85 °C. The drained fruit (12,150 g) was added and the temperature was maintained at 85 °C. Half the batch was poured into 211 x 400 fruit enamel cans containing 3 drops of either French's pistachio, lemon flavoring or no flavoring, and the other half of the batch containing 20 mL of 2% FDC No. 40 red dye was added to the cans containing the flavorings to a fill of 6 mm headspace. The cans were closed in a semi-automatic closing machine, then processed 10 min in a steam exhaust box and cooled. The cans were placed at storage temperatures of 2 °, 24 ° and 32 °C for 0, 2, and 3 months.

### Sensory Evaluation

The filling was subjected to a sensory panel of 10 to 12 panelists, using a 10 point scale with ten being the highest rating and one the lowest rating. Each sample was rated against a control for color, consistency and flavor. The control was assigned a score for each quality factor in accordance with initial quality. The control was a sample stored at 2 °C with no color added, but containing either no flavor, pistachio or lemon. The control was rated in the middle of the score range at 5 to 7 for flavor, color, and consistency since no color was added.

### Analytical Tests

These included measurements of soluble solids, pH, titratable acidity, color and consistency. Before the tests were begun, the topping was blended for 10 s in an Osterizer blender at 24 °C to obtain a uniform macerated sample. The soluble solids were measured by a Bausch and Lomb refractometer. Five g of topping were mixed with 45 mL of water for the measurement of pH, and then titrated with 0.1 N NaOH to an end point of 8.3. The titratable acidity was calculated as

citric acid. The color was measured on a Gardner Color Difference Meter standardized against a standard red plaque ( $L = 24.4$ ,  $a = 25.2$  and  $b = 11.8$ ). The consistency was recorded on a Bostwick consistometer at 20 s of flow.

Data were analyzed by analysis of variance by the University of Arkansas computer system. Means of main effects are separated by Duncan's Multiple Range test at 5% significance level. Means of interactive effects are separated by Least Significant Difference at 5% level.

## RESULTS AND DISCUSSION

The pH, percent citric acid and Bostwick consistency were not significantly affected by any of the main effects. Therefore, the data are not shown in the main effect tables. In the initial samples, analyzed during the first week after processing, neither soluble solids nor any of the color measurements were affected by sweetener (Table 1). After three months storage, filling made with fructose syrup was darker (lower L), less red and more blue (lower  $a$  and  $b$ ) than filling made with glucose (Table 2). Also, soluble solids and chroma were lower in filling made with fructose syrup. The difference in soluble solids between sweeteners was due to formulation, while the lower chroma in filling made with fructose was due to more color degradation.

Table 1. Effects of sweeteners, cultivar, temperature, added color and flavors on quality of strawberry filling prior to storage

Main effects	Soluble Solids %	Color L <sup>1/</sup>	Difference $\pm a$	Meter $\pm b$	Hue (a/b)	Chroma ( $a^2+b^2$ ) <sup>1/2</sup>
Sweetener						
Fructose	27.3a <sup>2</sup>	28.5a	26.6a	10.2a	2.6a	28.5a
Glucose	27.2a	28.7a	26.4a	10.3a	2.6a	28.4a
Cultivar						
Cardinal	26.9b	25.8b	26.8a	9.5b	2.8a	28.4a
Sunrise	27.6a	31.5a	26.2a	10.9a	2.4b	28.4a
Color						
Color	27.5a	27.0b	27.7a	10.9a	2.9a	29.3a
No color	27.0b	30.3a	25.3b	9.6b	2.4b	27.5b
Flavors						
1 none	27.3b	28.4a	26.2a	10.1a	2.6a	28.1a
2 pistachio	27.5ab	28.6a	26.6a	10.2a	2.6a	28.5a
3 lemon	27.6a	28.9a	26.7a	10.3a	2.6a	28.7a

1/ CDM - Color Difference Meter dark red plate  $L=24.4$ ,  $a=25.6$ ,  $b=11.8$

2/ Means separated in columns by main effects by Duncan's Multiple Range test at 5%. Values followed by different letters are significantly different ( $P<0.05$ ).



The filling made from the cultivar, Cardinal, was initially lower in  $L$  and  $b$  and higher in hue values than that made from Sunrise (Table 1), and these differences were maintained at 3 months storage even though there were drastic changes in color (Table 2).

Table 2. Effects of sweetener, cultivar, temperature, added color and flavors on quality of strawberry filling after three months storage

Main effects	Soluble Solids %	Color L <sup>1</sup> /	Difference + <u>a</u>	Meter + <u>b</u>	Hue (a/b)	Chroma (a <sup>2</sup> +b <sup>2</sup> ) <sup>1</sup> / <sub>2</sub>
Sweetener						
Fructose	28.8b <sup>2</sup>	24.6a	20.7b	9.2b	2.3a	22.8b
Glucose	29.4a	23.8b	22.8a	9.9a	2.3a	24.9a
Cultivar						
Cardinal	28.8b	21.9b	22.1a	8.7b	2.5a	23.7a
Sunrise	29.4a	26.5a	21.4a	10.5a	2.1b	24.9a
Color						
Color	29.5a	22.4b	24.3a	9.1b	2.7a	26.1a
No color	28.7a	25.9a	19.1b	10.0a	1.9b	21.8b
Flavors						
none	29.3a	23.9a	21.9a	9.5a	2.3a	24.0a
pistachio	29.2a	24.2a	21.6a	9.6a	2.3a	23.7a
lemon	28.7a	24.5a	21.7a	9.6a	2.3a	23.7a
Storage Temperature						
2°C	28.9a	26.5a	28.2a	11.3a	2.5a	30.4a
24°C	29.2a	23.1b	19.2b	8.9b	2.2b	21.3b
32°C	29.3a	22.9b	17.7c	8.6b	2.1c	19.8c

1/ CDM - Color Difference Meter dark red plate  $L=24.4$ ,  $a=25.6$ ,  $b=11.8$

2/ Means separated in columns by main effects by Duncan's Multiple Range test at 5%. Values followed by different letters are significantly different ( $P<0.05$ ).

The addition of color produced color differences as compared to the control (Table 1). The  $a$ ,  $b$ , hue and chroma values were significantly higher than those of the control (no color). At 3 months storage, wider differences in  $a$ , hue, and chroma values occurred between the addition of color and no color because of the improved stability in the presence of the FDC #40 color (Table 2).

Although preliminary studies indicated that the addition of pistachio and lemon flavors to strawberry filling rated higher in flavor than the control and other added flavors, the present study did not reflect these differences either at 0 or 3 months storage (Table 3).

Table 3. Sensory evaluation of the color, consistency and flavor of strawberry filling

Treatment	Color	Initial			Three months storage		
		Consistency	Flavor	Color	Consistency	Flavor	
<b>Sweetener</b>							
Fructose	7.9a <sup>2/</sup>	7.6a	7.1a	5.2a	6.0b	5.3a	
Glucose	7.4a	7.6a	7.0a	5.1a	6.4a	5.5a	
<b>Cultivar</b>							
Cardinal	8.4a	7.8a	7.4a	5.1a	6.0b	5.3a	
Sunrise	6.4b	7.4a	6.7b	5.1a	6.3a	5.4a	
<b>Color</b>							
Color	8.6a	7.8a	7.3a	6.7a	6.3a	5.6a	
No color	6.7b	7.4a	6.8b	3.6b	6.0b	5.2b	
<b>Flavor</b>							
1 none	7.5a	7.7a	7.0a	5.1a	6.2a	5.4a	
2 pistachio	7.8a	7.7a	7.0a	5.1a	6.2a	5.5a	
3 lemon	7.5a	7.4a	7.1a	5.2a	6.1a	5.2a	
<b>Storage Temperature</b>							
2°C	--	--	--	6.6a	6.3a	6.1a	
24°C	--	--	--	4.5b	6.1b	5.1b	
32°C	--	--	--	4.2c	6.1b	5.0b	

1/ Rated on a scale of 1-10, with 1=very poor, 5=acceptable, and 10=excellent. Each sample was rated against a 2°C NC Glucose sample.

2/ Means separated in columns by main effects by Duncan's Multiple Range Test at 5%. Values followed by different letters are significantly different ( $P < 0.05$ ).

Storage temperature had a very important effect on color of the filling at 3 months storage (Table 2). Color was lost rapidly at 24 °C, and even more rapidly at 32 °C as indicated by the decrease in L, a, b, hue and chroma values compared to those at 2 °C.

Sensory tests showed no difference between sweetener for color, consistency and flavor at 0 storage, but at 3 months storage, filling made with glucose was rated higher in consistency (Table 3). The panel rated Cardinal higher on color and flavor prior to storage, although at 3 months storage, there was no difference between Cardinal and Sunrise. Because of the much darker red color in Cardinal, color degradation was greater during storage, and therefore, panel ratings decreased more rapidly on Cardinal compared to Sunrise. Panel ratings for consistency were rated significantly higher on filling made from Sunrise at 3 months storage.

The addition of color to the filling improved the ratings for color, consistency and flavor at both 0 and 3 months storage (Table 3). The more intensive red color of fillings with added color might have influenced the ratings for consistency and flavor, since the fillings were evaluated under nonmasked conditions. The addition of pistachio and lemon flavor had no effect on the sensory tests for quality. Sensory tests reflected the rapid changes in quality during storage, similar to the results of the physical tests.

The significant interaction between sweetener and cultivar on soluble solids showed that there were lower soluble solids in Cardinal filling at 3 months storage when fructose was added as sweetener compared to glucose (Table 4). This was possibly related to the greater losses in color with fructose, especially at 32 °C (Table 6). Also, much greater differences in L and a values occurred between cultivars when glucose was used as the sweetener (Table 4). The lower L and higher a values on Cardinal filling with glucose sweetener than with fructose is surprising since fructose has been shown to be a more reactive sugar (Markakis 1974; Meschter 1953).

Table 4. The interactive effects of sweetener and cultivar on quality of strawberry filling at three months storage

Three months Variables	%	Color <sup>1</sup>	
		Soluble Solids	Difference Meter L <u>a</u>
<u>Fructose</u>			
Cardinal	27.9	23.3	21.0
Sunrise	29.7	26.0	20.5
<u>Glucose</u>			
Cardinal	29.6	20.6	23.2
Sunrise	29.1	27.0	22.3
LSD @ 5% <sup>2/</sup>	0.7	0.9	1.0

<sup>1/</sup> Color Difference Meter L=24.4, a=25.6, b=11.8.

<sup>2/</sup> Means of the interactive effects separated in columns by Least Significant Difference at 5% level.

The interaction between cultivar and added color on physical expression of color indicated that the difference in color (L, a, b, hue and chroma) of filling made from Sunrise was more pronounced when color was added (Table 5). Since Sunrise has a lighter red color one would expect the addition of color to improve the color more than in Cardinal, a dark red cultivar.

The interaction of sweetener and storage temperature on viscosity was caused by the significant decrease in viscosity (increase in cm flow/20 s) with fructose as the temperature of storage was increased compared to no change with glucose (Table 6). Apparently, glucose had more effect on color at the higher temperatures of storage as shown by the change in L and b values. Filling made with glucose was darker (lower L) than that made with fructose when stored at 32 °C. However, the hue of filling made with glucose was higher than that made

with fructose when stored at 32 °C. The sensory color ratings decreased with an increase in storage temperature in filling sweetened with fructose, while there was a reverse change in ratings with glucose. This could have been caused by a more rapid degradation of pigment and loss of red color at higher temperatures with fructose as a sweetener, whereas in filling with glucose the darkening of color was more acceptable.

Table 5. The interactive effects of cultivar and added color on color quality of strawberry filling at three months storage

Variables	Color Difference Meter <sup>1/</sup>				
	L	+a	+b	Hue (a/b)	Chroma (a <sup>2</sup> +b <sup>2</sup> ) <sup>1/2</sup>
<u>Cardinal</u>					
Color	21.7	23.6	8.6	2.74	25.10
No color	22.6	20.6	8.8	2.31	22.40
<u>Sunrise</u>					
Color	23.2	25.1	9.7	2.59	26.94
No color	29.3	17.7	11.2	1.56	21.04
LSD @ 5% <sup>2/</sup>	0.9	1.0	0.4	0.06	1.04

<sup>1/</sup> Color Difference Meter L=24.4, a=25.6, b=11.8.

<sup>2/</sup> Means of the interactive effects separated in columns by Least Significant Difference at 5% level.

The interaction of cultivar and storage temperature on color demonstrates the higher rate of loss of color with an increase in storage temperature in the darker colored cultivar, Cardinal, compared to the light colored Sunrise (Table 7). The decrease in b (blueness) was greater between 2° and 32 °C storage temperatures in Cardinal filling. Chroma and sensory color ratings reflected similar differences in color between cultivars. The panel rated filling made from Sunrise significantly higher than Cardinal in consistency only when stored at 2 °C.

Color added to the filling interacted with storage temperature on sensory color and hue (Table 8). Although there were significant differences in color at each storage temperature between samples of filling with and without added color, there was a greater decrease in sensory color and hue when no color was added and temperature was increased.

Table 6. The interactive effects of sweetener and storage temperature on quality of strawberry filling at three months storage

Variables	Bostwick consistency (cm flow)	Color Difference Meter <sup>1/</sup>			Sensory <sup>2/</sup> color
		L	+b	Hue (a/b)	
<u>Fructose</u>					
20°C	8.5	26.4	10.7	2.57	6.53
24°C	9.9	23.4	8.4	2.20	5.82
32°C	10.1	23.9	8.5	2.07	5.65
<u>Glucose</u>					
20°C	9.6	26.7	11.8	2.50	6.14
24°C	9.6	22.8	9.3	2.27	6.40
32°C	9.8	21.8	8.7	2.19	6.58
LSD @ 5% <sup>3/</sup>	0.6	1.1	0.6	0.08	0.43

1/ Color Difference Meter L=24.4, a=25.6, b=11.8.

2/ Rated on a scale of 1-very poor to 10-excellent.

3/ Means of the interactive effects separated in columns by Least Significant Difference at 5% level.

Table 7. Interaction of cultivar and storage temperature on quality of strawberry filling after three months storage

Variables	+b	Color Difference Meter <sup>1/</sup>			Sensory <sup>2/</sup>	
		Hue (a/b)	Chroma (a <sup>2</sup> +b <sup>2</sup> ) <sup>1/2</sup>	Color	Consistency	
<u>Cardinal</u>						
20°C	10.9	2.7	31.4	6.9	6.1	
24°C	7.7	2.5	20.6	4.4	6.1	
32°C	7.5	2.4	19.3	4.0	6.0	
<u>Sunrise</u>						
20°C	11.6	2.4	29.5	6.3	6.6	
24°C	10.0	2.0	22.1	4.6	6.2	
32°C	9.7	1.9	20.4	4.4	6.3	
LSD @ 5% <sup>3/</sup>	0.6	0.1	1.3	0.4	0.4	

1/ Color Difference Meter L=24.4, a=25.6, b=11.8.

2/ Rated on a scale of 1-very poor to 10-excellent.

3/ Means separated in columns by Least Significant Difference at 5% level.

Table 8. The interactive effects of added Color and Storage temperature on sensory color and hue of strawberry filling at three months of storage

Variables	Sensory <sup>1/</sup> Color	CDM <sup>2/</sup> Hue (a/b)
<u>Added Color</u>		
2°C	7.7	2.8
24°C	6.4	2.6
32°C	5.9	2.6
<u>No Added Color</u>		
2°C	5.5	2.3
24°C	2.7	1.8
32°C	2.5	1.7
LSD @ 5% <sup>3/</sup>	0.4	0.1

1/ Rated on a scale of 1-very poor to 10-excellent.

2/ Color Difference Meter L=24.4, b=25.6, a=11.8.

3/ Means separated in columns by Least Significant Difference at 5% level.

Storage of the processed filling for 3 months had a significant effect on all quality parameters measured (Table 9). The pH decreased during the storage period, while titratable acidity as citric increased slightly. The viscosity decreased with storage time as shown by the increase in rate of flow (Bostwick) and by the decrease in sensory ratings. The increase in soluble solids during storage was possibly related to hydrolysis of polysaccharides since corn syrup contains intermediate polysaccharides. Color as measured by L and hue values decreased at each successive storage period, while a, b and chroma values decreased during the first two months of storage, and then increased at three months, probably as a result of the rapid onset of browning that occurred. Similar results have been reported on other strawberry products (Sistrunk and Morris 1978; Sistrunk *et al.* 1982). However, the decrease in sensory ratings for color and flavor was similar to the color values L and hue. The greatest change in a, b and chroma of the strawberry filling occurred by the two month storage period.

Table 9. The effect of storage time on quality parameters of strawberry filling

Storage Time	pH	% Citric acid	Bostwick consistency cm flow	% soluble solids	Color Difference Meter <sup>1/</sup> L	<u>+a</u>	<u>+b</u>
<b>Months</b>							
0	3.40a <sup>3</sup>	.47b	9.3c	27.3c	28.6a	26.5a	10.3a
2	3.37b	.48a	9.4b	28.3b	26.6b	19.7c	8.3c
3	3.29c	.48a	9.6a	29.1a	24.2c	21.8b	9.6b
Storage Time	Hue (a/b)	Chroma (a <sup>2</sup> +b <sup>2</sup> ) <sup>1/2</sup>	Sensory ratings <sup>2/</sup>				
			Color	Consistency	Flavor		
<b>Months</b>							
0	2.63a	28.5a	7.62a	7.61a	7.03a		
2	2.40b	21.2c	5.50b	6.51b	5.97b		
3	2.30c	23.9b	5.11c	6.20c	5.37c		

1/ Color Difference Meter L=24.4, a=25.6, b=11.8.

2/ Rated on a scale of 1-very poor to 10-excellent.

3/ Means separated in columns by quality parameters by Duncan's Multiple range test at 5%. Values followed by different letters are significantly different (P<0.05).

In conclusion, filling made with glucose was more viscous after 3 months of storage than that made from fructose. Color degradation was greater in Cardinal than in Sunrise filling during 3 months of storage as measured by objective and sensory tests. Sensory color was always rated higher on Sunrise with added color than on all other samples after 3 months of storage. Since Sunrise is low in anthocyanins, the addition of FDS #40 greatly improved color of filling made from this cultivar. Although filling made with fructose retained color comparable to that made with glucose when stored at 24 °C, the color loss at 32 °C was more rapid with fructose. Color and flavor losses in canned strawberry fillings were rapid during storage, especially at 32 °C. A lighter-colored cultivar such as Sunrise with added FDC color appears to offer the better potential for filling that is to be stored at approximately 24 °C.

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# EFFECT OF SWEETENERS AND STABILIZERS ON THE STRUCTURE OF ICE CREAM MIX AS DETERMINED BY ACOUSTIC METHODS

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## ABSTRACT

*Various ratios of guar gum to locust bean gum were used with nine combinations of sucrose, 36 DE corn syrup and 42 high fructose corn syrup (42 HFCS) in a standard ice cream formulation. It was demonstrated that differences in the structure (defined as change in adiabatic compressibility) of the mixes could be determined by measuring the acoustic parameters of sound velocity and density. From these parameters adiabatic compressibility was calculated. Data indicated an increase in mix structure when the guar to locust bean gum ratio was increased. This occurred with each of the nine sweetener combinations. With respect to changes in sweetener, the amount of corn syrup had little effect on structure. An increase in 42 HFCS, however, increased the structure. Differences in structure were attributed to the changes in the structure of water contained in the mix.*

## INTRODUCTION

Each type of solute molecule affects differently the structure of a solution. Solute molecules can be classed as either "net structure breakers" or "net structure formers." This has been discussed by Fennema (1985) with respect to different classes of molecules. For example, small ions by virtue of their polarizing power cause a definite solution structure to form.

In food systems, structure development is often attributed to the macromolecules present in the food. Proteins such as gluten in bread and casein in cheese are major contributors to the structure of these foods. Another group of macromolecules which affects the structure of foods are the polysaccharides.

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The type and degree of gelatinization of starch will produce foods of varying characteristics. Of the polysaccharides used in foods, no group has been more studied than those classed as gums or stabilizers. Rees (1981) studied various polysaccharides in aqueous systems and developed structural models for their interaction. Labuza and coworkers (1981, 1983) studied the effect of various gums on water activity and their water binding properties. Rheological properties of locust bean and guar gum solutions, with and without added sucrose and glucose have been studied (1977, 1979).

Polysaccharides, disaccharides and monosaccharides have been studied as to their structuring properties or behavior. One area of extensive study has been to determine the hydration of these molecules. The usual approach has been to assay by various techniques (Shiio 1958; Franks *et al.* 1973) the number of water molecules bonded to the carbohydrate molecule. Recently, Smith 1981 and Smith and Winder 1983 used acoustic techniques to study the structure of various aqueous solutions containing monosaccharides and disaccharides. Data from these studies were used to explain the differences noted in the viscosity of ice cream mixes (Smith *et al.* 1984).

While model systems have been used to study the interaction of gums and monosaccharides and disaccharides, only limited studies have focused on their interaction or effect on the structure of foods. In ice cream, for example, studies to date have centered on the differences in mix viscosity caused by various stabilizer-sweetener combinations (Cottrell *et al.* 1979, 1980). One possible way to study interactions of these types in ice cream mix is to use the previously mentioned acoustic methods to assess the structure formed. This study was undertaken to determine (1) if acoustic methods could detect differences in the structure of ice cream mixes containing various stabilizer-sweetener combinations (structure being defined as a change in adiabatic compressibility) and (2) if differences, once detected, could be used to evaluate the structuring properties of the stabilizers and sweeteners.

## METHODS AND MATERIALS

### Mix Formulation and Processing

A mixture design (Cornell 1981) was used to determine the effect of selected sweeteners and stabilizers on the structure of ice cream mix. The three sweeteners chosen, sucrose, 36 DE corn syrup (36 DE CS), and 42 high fructose corn syrup (42 HFCS) were combined in ice cream mixes in nine different ratios as shown in Table 1. Each of the nine mixes was made with three different ratios (25:75, 50:50, 75:25) of guar gum to locust bean gum, giving a total of 27 different mixes. Mix 3, with a 50:50 ratio of stabilizers, was produced seven times to determine variability in processing. Mixes were processed in random order.

Table 1. Combinations of sweeteners used for making ice cream mix

Mix	% of Sweetener		
	Sucrose	36 DE CS	42 HFCS
1	20	30	50
2	45	30	25
3	70	30	0
4	10	40	50
5	35	40	25
6	60	40	0
7	0	50	50
8	25	50	25
9	50	50	0

Sixty eight kilogram batches of each ice cream mix were prepared. Mixes consisted of 10% milkfat, 10% milk solids-non-fat, 17% sweetener solids (at 9 ratios mentioned above), 0.15% guar/locust bean gum (Continental Colloids, Chicago, IL), 0.105% carrageenan (Continental Colloids, Chicago, IL), 0.10% mono and diglycerides (Continental Colloids, Chicago, IL) and 63% water. All mixes were batch-pasteurized (68 °C for 30 min), passed through a double stage homogenizer ( $1.38 \times 10^4$  kPa first stage and  $3.45 \times 10^3$  kPa second stage, respectively), and then cooled to 5 °C. Milkfat and total solids of each mix were determined in duplicate using the Mojonnier fat test (Case *et al.* 1985) and oven solids method (A.O.A.C. 1980), respectively.

### Instrumentation

The instrument used for the acoustic studies was the Solution Analyzer (SA) (Model 2200, Hartel Corporation, Fort Atkinson, WI) previously used to study the physical structure of fats and fatty acids (Hustad *et al.* 1971) as well as to analyze various liquids for gross composition. The operation of the instrument has been detailed in other papers (Winder *et al.* 1970; Hustad *et al.* 1971).

An indirect measurement of sound velocity as a triggering frequency value ( $f$ ) is obtained from the SA. Sound velocity can be calculated from triggering frequency values with the following formula, if the path length of the pulse in the test cell and the time delay of the electronic circuit are known at a specific temperature:

$$\frac{1}{f} = \frac{1}{c} = [L + \tau]$$

where:  $f$ =triggering frequency in cycles/second;  $1/f$ =time in seconds for one pulse to traverse the sample and electronic circuit;  $c$ =velocity of sound through the sample in centimeters/second;  $L$ =path length of the pulse through the sample in centimeters;  $\tau$ =time delay in the electronic circuit in seconds.

Fifteen triggering frequency values for pure water were collected over the temperature range used in the experiments. These frequency values, temperatures at which they were collected and  $c$  values from published data of Del Grosso and Mader (1972) were used to calculate the path length and time delay. With these values known at each temperature, sound velocity through any liquid could be calculated by inserting the  $f$ -value for the liquid at a specific temperature and solving the equation for  $c$ .

### Sample Testing in Solution Analyzer

Preliminary studies showed that it was necessary to de-gas samples prior to testing in the SA. To do this, 150 ml of mix were placed in a 500 ml round bottom flask. A vacuum was applied sufficient to cause the sample to boil. Boiling was allowed to occur for 30 s. Samples were then tested in the SA by first flushing the test cell with 25 mL of sample, later discarded. The cell was then completely filled with sample. To avoid loss of moisture through evaporation, the cell was closed by insertion of a Teflon-plug-type cover. The sample was allowed to equilibrate to 45 °C. Temperature was monitored with a thermistor probe immersed in the sample (Model 5810, Digitec Thermistor Thermometer, United Systems Corp., Dayton, OH). When a stable  $f$ -value was displayed, it was recorded and testing repeated with another portion of the same mix. The mean value for the determinations was used to calculate the sound velocity through the mix. The standard deviation based on the duplicate determinations of the seven replications of mix three was  $\pm 0.15$  m/s.

### Density Determination

The density of each mix was measured with a vibrational densitometer (Mettler DMA 35). After calibration with water, the instrument had an accuracy of  $\pm 0.001$  g/cm<sup>3</sup>.

### Adiabatic Compressibility Calculation

Using the experimentally obtained density and sound velocity values, adiabatic compressibility was calculated by employing the La Place relationship (1816):

$$v = \frac{1}{\sqrt{\rho \cdot \beta_{ad}}}$$

rearranged to:

$$\beta_{ad} = \frac{1}{\rho \cdot v^2}$$

where:  $v$  = sound velocity in cm/s;  $\rho$  = density in g/mL;  $\beta_{ad}$  = adiabatic compressibility in cm<sup>2</sup>/dyne. The standard deviation based on the duplicate determinations of the seven replications of mix three was  $\pm 0.01$  cm<sup>2</sup>/dyne.

## RESULTS AND DISCUSSION

Sound velocity values for the 27 mixes at 45 °C are presented (Table 2). From these data it can be seen that increases in the ratio of guar gum to locust bean gum resulted in increases in the sound velocity through the mixes. Sound velocities ranged from 1572.73 m/s for mix 6, containing a 25:75 gum ratio, to 1581.16 m/s for mix 1, containing a 75:25 gum ratio. The density value for all the mixes at 45 °C was the same, i.e., 1.083 g/cm<sup>3</sup>. Using this value for density and the sound velocity data in Table 2, the adiabatic compressibilities of the mixes were calculated. These calculated values are presented in Table 3. The compressibility values ranged from a high of 37.33 x 10<sup>-12</sup> cm<sup>2</sup>/dyne for mix 6, containing a 25:75 gum ratio to a low of 36.93 x 10<sup>-12</sup> cm<sup>2</sup>/dyne for mix 1, containing a 75:25 gum ratio.

Based on the small standard deviations for both the measured sound velocity ( $\pm 0.15$  m/s) and the calculated adiabatic compressibility ( $\pm 0.01 \times 10^{-12}$  cm<sup>2</sup>/dyne) and the range of data presented in Tables 2 and 3, it can be stated that acoustic methods of analysis can be used to detect differences in the structure of ice cream mixes. These differences in structure being defined as changes in adiabatic compressibility. This ability to detect differences is not unexpected. For example, Hustad *et al.* (1971) were able to detect an increase in the number of double bonds present in the C<sub>18</sub> series of fatty acids using acoustic methods of determination. Also, one of the authors (Smith 1983) reported differences in the sound velocity and adiabatic compressibility of aqueous solutions of various monosaccharides. These differences were attributed to changes in the conformation of the various monosaccharides such as changing an equatorially positioned hydroxyl group to an axial position.

The data in Tables 2 and 3 show that an increase in guar to locust bean gum ratio within a sweetener system causes an increase in sound velocity and a decrease in adiabatic compressibility. These changes can be interpreted as

Table 2. Sound velocity (m/s) through ice cream mixes made with three ratios of guar gum to locust bean gum and nine sweetener combinations

mix number	sweetener Suc:CS:HFCS	Guar to Locust Bean Gum Ratio		
		25:75	50:50	75:25
1	20 : 30 : 50	1578.10	1579.59	1581.16
2	45 : 30 : 25	1576.67	1577.55	1579.08
3	70 : 30 : 0	1573.51	1573.89	1576.43
4	10 : 40 : 50	1579.46	1580.07	1579.46
5	35 : 40 : 25	1575.86	1576.77	1578.10
6	60 : 40 : 0	1572.73	1575.62	1576.77
7	0 : 50 : 50	1577.01	1579.49	1580.31
8	25 : 50 : 50	1576.47	1577.82	1580.07
9	50 : 50 : 0	1575.18	1575.82	1575.86

Std. Dev.  $\pm 0.15$  m/sec; based on duplicate determination from seven replications of mix 3.

reflecting an increase in the structure of the ice cream mixes, as more highly structured systems enhance sound velocity through them. This is analogous to the fact that sound travels faster through water than through air. That guar gum contributes to a more highly structured system is supported by other research. Wallingford and Labuza (1983) reported that guar binds about four times as much water as locust bean gum. Viscosity data reported by Blanchard and Mitchell (1979) indicated that solutions of guar gum have much greater viscosities than locust bean solutions. Lastly, measurements of the electrical energy needed to freeze ice cream mixes have shown that increases in the amount of guar gum cause increases in the electrical energy needed to freeze the mix (Smith *et al.* 1985). Thus based on data presented here and from other researchers cited, it can be stated guar gum based on its inherent characteristics and interactions with mix components creates a more structured system.

As noted above, an increase in guar gum within a sweetener system results in an increase in structure. However, if one looks at the effect of changes in sweetener, it appears the sweetener is also important in defining the structure of the ice cream mix. Data in Table 3 indicate that, as the amount of HFCS is increased, the structure of the mix increases (as evidenced by the decrease in compressibility). This fact is most readily apparent from data expressing the average

Table 3. Adiabatic compressibility ( $\text{cm}^2/\text{dyne} \times 10^{-12}$ ) of ice cream mixes made with three ratios of guar gum to locust bean gum and nine sweetener combinations

% HFCS	Guar: Locust Bean Ratio	Adiabatic Compressibility ( $\text{cm}^2/\text{dyne}$ ) $\times 10^{-12}$ for 3 levels of Corn Syrup Solids		
		30	40	50
50	75:25	36.93	37.01	36.97
	50:50	37.01	36.98	37.01
	25:75	37.08	37.01	37.13
	Ave.*	37.01	37.00	37.04
25	75:25	37.03	37.08	36.98
	50:50	37.10	37.14	37.09
	25:75	37.14	37.18	37.15
	Ave.*	37.09	37.13	37.07
0	75:25	37.16	37.08	37.18
	50:50	37.27	37.20	37.20
	25:75	37.29	37.33	37.21
	Ave.*	37.24	37.22	37.20

\* Ave. is for 3 gum ratios for a particular sweetening system.

Std. Dev.  $\pm 0.01 \times 10^{-12}$   $\text{cm}^2/\text{dyne}$ ; based on duplicate determinations from seven replications of mix 3.

(for the three gum ratios) compressibility for each sweetener. It should be noted that 36 DE CS does not appear to be a major factor in the structuring of the mixes, since the average for compressibility does not change very much when 36 DE CS levels are increased from 30% to 50% of the sweetener at constant HFCS level in the mix. Thus, the changes in structure under consideration in this instance can be attributed to changes in the ratio of HFCS to sucrose. Model system work with these sugars (Smith 1981; Smith and Winder 1983) has shown that fructose and glucose (the components of HFCS) have similar structuring abilities at 45 °C, whereas sucrose structures to a lesser degree. Previous study of changes in mix viscosity has also shown that a mix with HFCS as the sweetener will have a greater viscosity than a mix containing sucrose. The most probable explanation for these observations is that, HFCS and sucrose bond with water differently. The result being a more structured (less compressible) mix when HFCS versus sucrose is used as the sweetener.

In summary, acoustic methods can be used to note differences in ice cream mix structure (changes in adiabatic compressibility). Changes in mix structure can be attributed to changes in stabilizers and sweeteners. Finally these structural changes can be related to other properties of mix or model systems (viscosity, water binding and energy requirements to freeze the mix) containing these mix components.

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## BOOK REVIEWS

**Gums and Stabilizers for the Food Industry. 3.** 1986. G.O. Phillips, D.J. Wedlock and P.A. Williams, eds. Elsevier Applied Science Publishers, London. Hardbound, pp. 675. \$99.00.

This book is the Proceedings of the 3rd International Conference on Gums and Stabilizers for the Food Industry, held at Wrexham, Clwyd, Wales in July 1985. The 65 papers contained in the book are mostly research-type presentations although a few are reviews. Figures, tables and references are provided in appropriate abundance. A wide range of hydrocolloids are discussed from a variety of viewpoints including molecular characteristics, physical properties, analytical methodology, functionality, stability, physiological effects, polymer-polymer interactions, applications, new types and future trends. Papers are almost without exception of high quality.

The book will be of greatest value to food researchers and technologists, of moderate value to technical sales personnel and, not surprisingly, of little value as a textbook (except as a reference).

The cover, binding and paper are of acceptable quality. The print is legible but not attractive since the book was prepared directly from manuscripts submitted by the authors. A further consequence of this undesirable, but justifiable cost-reducing practice, is that the style varies somewhat among the chapters. The index is ten pages in length—clearly too short for a book of nearly 700 pages.

In summary, anyone desiring reasonably current, high-quality technical information on food hydrocolloids will find this book of great value.

Owen Fennema  
University of Wisconsin-Madison

**Functional Properties of Food Macromolecules.** 1986. J.R. Mitchell and D.A. Ledward, eds. Elsevier Applied Science Publishers, London. Hardbound. pp. 433. \$86.00.

The trend toward increased consumption of foods fabricated by "trial and error manipulation of ingredients with little understanding of underlying science" provided the stimulus for this book. Attention is given to the functional properties of two important ingredients in fabricated foods—proteins and polysaccharides—with special emphasis directed to their effects on physical properties. Major topics include the contributions of proteins and polysaccharides to rheological properties, gelation, water holding, fat binding and the formulation and stabilization of foams and emulsions. In addition, protein texturization and the functional properties of protein-polysaccharide mixtures are discussed.

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In each of the nine chapters an in-depth scientific approach is used. The subject is of high quality and the book will be of great value to persons involved in fundamental research on the functional properties of proteins and polysaccharides, and to persons involved in food process and product development.

The quality of the cover, binding, paper and printing is very good. Tables, illustrations and references are abundant, but the references, unfortunately, are presented in an abbreviated style so titles of articles are not given. The index of 16 pages is very adequate.

This book is a high-quality addition to an important area of food science and is highly recommended for the groups previously mentioned.

Owen Fennema  
University of Wisconsin-Madison

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

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CONTENTS

Editorial ..... v

A Sauce Product from Enzyme-Hydrolyzed Canola Meal  
**A.Y.M. MA and B. OORAIKUL** ..... 163

Characteristics and Acceptability of a Flour Prepared from Sodium Bicarbonate  
Soaked and Steamed Soybeans  
**J.Y. LU and A. JASSER** ..... 177

Effect of Parboiling and Freezing on Quality of Three Spanish Rice Varieties  
**R. OLALQUIAGA, J-X. GUINARD and R.P. SINGH** ..... 189

The Influence of Microwave Heating on the Textural Properties of Meat and  
Collagen Solubilization  
**J.F. ZAYAS and J.O. NAEWBANIJ** ..... 203

Factors Influencing the Quality of Canned Strawberry Filling During Storage  
**H.F. PRATT, W.A. SISTRUNK and J.R. MORRIS** ..... 215

Effect of Sweeteners and Stabilizers on the Structure of Ice Cream Mix as Deter-  
mined by Acoustic Methods  
**D.E. SMITH and S.A. WITTINGER** ..... 227

Book Reviews ..... 237

10-10-23