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COMMERCIAL PECTINASES AND THE YIELD AND QUALITY OF STANLEY PLUM JUICE[1]

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ABSTRACT

Five commercial pectinases were used to investigate efficacy for improvement of juice yield and quality from Stanley plums. Pectinases, to a varying degree, improved the yield, color-assayed as release of anthocyanins, and clarity of the juice. A significant increase in the effectiveness of pectinases was observed as the concentration was increased from 0.01 to 0.60% v/w. However, at concentrations >0.20% they tended to impart a bitter flavor in the juice. Among five pectinases, Clarex L at 0.20% produced higher yield and a sediment-free clear juice.

INTRODUCTION

Plums are grown in several areas of the United States and are important to the Michigan fruit industry as well. An active plum variety program presently underway in Michigan presents good prospects for new cultivar introductions that will enhance the viability of the plum industry. Presently, one of the major needs within plum industry is development of new processed plum products to utilize plums. One such utilization maybe in the beverage industry. Since the mid-1980s, soft drink processors have increasingly developed and marketed fruit juice based healthy beverages. As a result the juice consumption has increased about 10% in the last 2–3 years alone. A number of companies are beginning to target expressly for the juice business and the trend in beverage flavors has begun to shift to more Northern fruit flavors such as plum and raspberry (Kortbech-Olesen 1991; Sfiligoj 1992).

[1]Reference to the use of commercial pectinases in this study is not an endorsement of these products by Michigan State University.
[2]For correspondence.

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The leading plum cultivars grown in Michigan are the Italian purple plums, Blufre and Stanley. Stanley plums, used in this study, are a large, partially freestone plum with firm greenish-yellowish flesh and a dark blue-black outer skin. Pectic enzymes are usually used to extract, liquefy and clarify juice to increase yield and reduce viscosity (Cheetham 1985). The objectives of this study were to (a) investigate the effect of selected commercially available pectinases on yield and quality of plum juice, and (b) optimize processing conditions for plum juice production.

MATERIALS AND METHODS

Plums

Stanley plums grown at Michigan State University's Northwest Horticultural Experiment Station were used in this study. The plums were harvested in 1992 at commercial maturity and stored at −20C until processed.

Pectinase Enzymes

Five commercially available pectic enzymes, Clarex L and Clarex ML (Solvay Enzymes, Inc., Elkhart, IN) and Klerzyme L200, Rapidase C80L and Rapidase Press (Gist-Brocades Food Ingredients, Inc., King of Prussia, PA), were used for extraction and processing plum juice.

Plum Juice Extraction

Plums were processed into juice with some modification to a procedure previously developed in our laboratory (Siddiq *et al.* 1994). About 360 g of frozen plums were thawed for 8–12 h at 4C. The plums were crushed, macerated and heated to 80C in a stainless steel steam jacketed kettle. The macerated plums were put into 600 ml stainless steel pans. The commercial pectic enzymes, were added to aid in liquefaction of plums in concentrations of 0.05, 0.10, 0.20, 0.40 or 0.60% (v/w) . After holding for 4 h at 49C, the macerated fruit was pressed through several layers of cheesecloth to obtain plum juice. Juice yield was determined in duplicate as g juice/100 g plums.

Physicochemical Analysis

Plum juices were analyzed for yield, clarity, color, soluble solids (°Brix), titratable acidity, pH, °Brix/Acid ratio, sugars, total anthocyanins, total phenolics and enzymatic browning.

Clarity

Juice clarity or turbidity was measured according to the methods of Krop and Pilnik (1974) and Ough *et al.* (1975). The juice was shaken and 10 ml portions of juice were centrifuged at 360 × g for 10 min to remove pulp and coarse cloud particles. Percent transmittance was determined at 660 nm by Spectronic-70 spectrophotometer (Milton Roy Co., Rochester, NY). The percent transmittance was considered a measure of juice clarity.

Color

Color was assessed using a Hunter Color Difference Meter (D25 DP-9000 System, Hunter Associates Lab., Reston, VA). After standardization with a pink tile ($L^* = 73.49$; $a^* = 17.34$; $b^* = 10.28$), 50 ml of juice were placed in a standard optical cell. This color assessment system is based on the Hunter L*, a* and b* coordinates. L* representing lightness and darkness, +a* redness, −a* greenness, +b* yellowness and −b* blueness.

Soluble Solids and pH

Percent soluble solids, expressed as °Brix, were determined with an Abbe-3L refractometer (Bausch & Lomb Optical Co., Rochester, NY) at 20C. The pH of juice was determined with a digital pH meter (Model 601A, Corning Glass Works, Medfield, MA).

Titratable Acidity

A 10-gram portion of juice in 100 ml distilled water was titrated to pH 8.0 with a 0.1N NaOH solution. End point was determined with a pH meter. Titratable or total acids of the sample were expressed as percent malic acid using the following formula:

$$\text{\% malic Acid} = \text{ml NaOH} \times \text{N NaOH} \times 0.067\ \text{meq} \times 100/\text{wt of sample.}$$

Sugars Analysis

Glucose, fructose, sucrose and sorbitol are the main sugars in plums (Richmond *et al.* 1981). The separation of glucose and sorbitol by HPLC is difficult because of structural similarities (Shaw 1988). This problem of separation of glucose and sorbitol by HPLC was solved by combining the results of two different analytical techniques: HPLC and YSI analysis (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). HPLC was used to separate fructose, sucrose and a combined glucose and sorbitol peak according to Guyer *et al.* (1993).

The YSI analyzer was used to determine the glucose content. A 10-ml portion of centrifuged plum juice was diluted to 20 ml with distilled water. A standard glucose solution containing 1.8 g glucose/L was run as a standard for calibrating YSI analyzer. The YSI analyzer uses immobilized enzyme membranes for glucose determination and automatically injects 0.5 ml of liquid for analysis. Sorbitol content was determined by subtracting the glucose content, determined by YSI, from HPLC combined peak for glucose and sorbitol.

Total Phenolics

Total phenolics of plum juice were determined by the method of Singleton and Rossi (1964). Results are reported as mg tannic acid/100 ml juice.

Total Anthocyanins

Total anthocyanins in the plum juice were determined according to the method of Skalski and Sistrunk (1973). A 5-ml portion of juice was mixed with 45 ml of acidified ethanol (1.5N HCl: 95% ethanol = 15:85, v/v). The pH of the solvent was adjusted with 1N HCl as required to obtain a final pH of 1.0 in the plum extract. The diluted extract was stored in the dark for 2 h and filtered through #2 paper before reading absorbance at 535 nm. The total anthocyanins were calculated using the formula: Total Anthocyanins = [(Absorbance × dilution factor)/E] × (100/5 ml). Extinction Coefficient (E) of 98.2 was used for unidentified anthocyanins in acidified ethanol (Fuleki and Frances 1968; Francis 1982).

Enzymatic Browning

Enzymatic browning was determined according to the method of Sapers and Douglas (1987). Plum juice was poured into a glass cylinder, and the L* value was measured with a Hunter Color Difference meter. The degree of

browning was expressed as the L* value difference (ΔL*) that occurred during 1 h.

Statistical Analysis

The experiment was designed as a three factor, replication × pectinase × concentration, randomized model with balanced data. Determinations were in duplicate, except for color, which was determined in triplicate. Mean, standard errors, mean square errors, one and two factor ANOVA, correlation and interaction of main effects were performed using the Super ANOVA software (Abacus Concepts, Inc., Berkeley, CA).

RESULTS AND DISCUSSIONS

Juice Yield

The yield of plum juice significantly ($p \leq 0.05$) improved with increasing concentrations of pectinase enzymes from 0.05% to 0.6% (Table 1). Juice yield of the untreated controls was about 38% (w/w) as compared to the yields of 56.7–73.2% from the pectinase treated plum macerate. Except for Klerzyme L200 treated macerate, with a yield of 56.7%, pectinases resulted in significantly greater yields of 68–73%. Clarex ML, Rapidase C80L and Klerzyme L200 at 0.4% and 0.6% produced the best yield. Concentrations of pectinases greater than 0.2% tended to impart a slightly bitter flavor in the juice. Therefore, the addition of 0.2% pectinases to the macerated plum pulp was considered the optimal concentration. Also, above 0.2% pectinase concentration, the increases in juice yield were not substantial.

Juice Clarity

The percentage transmittance (% T) was selected as a measure of juice clarity and juice with 60% T was classified as clarified (Amir-uz-Zaman 1985). The pectinase treated plum juice was free from sediments and consistently clear as indicated by significantly greater percentage transmission compared to control plum juice. Beyond 0.1% enzyme concentration, no significant effect on juice clarity was obtained for Clarex L and Rapidase C80L (Fig. 1). Plum juice treated with enzyme L200 exhibited the largest percentage transmission, whereas Rapidase Press treated juice exhibited the smallest transmittance values. Addition of pectinases not only reduced the viscosity but also improved juice filtration. In grapes pectinase enzymes increased the average juice clarity four fold and filterability by 100% (Brown and Ough 1981).

TABLE 1.
COMMERCIAL PECTINASES AND THE JUICE YIELD

	Pectinase Concentration (% v/w)					
Pectinases	Control	0.05	0.10	0.20	0.40	0.60
			Yield (g juice/100 g fruit)			
Clarex L	37.76a[1]	60.39b	64.29c	69.29d	69.54d	71.29d
Clarex ML	37.76a	66.19b	67.91cd	68.12d	73.16e	73.18e
Rapidase Press	37.76a	61.28b	63.95b	68.03d	67.79cd	67.84cd
Rapidase C80L	37.76a	66.01bc	63.81b	67.70c	71.03d	73.00e
Klerzyme L200	37.76a	43.92b	49.01b	50.68c	55.37d	56.70e

[1] Values with the same letters in the horizontal rows are not significantly different at 5% level of significance

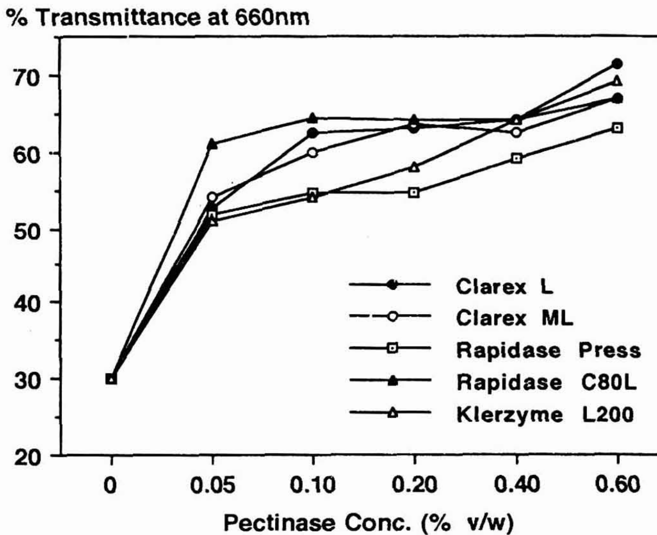

FIG. 1. COMMERCIAL PECTINASES AND THE CLARITY OF STANLEY PLUM JUICE

Color

Hunter color difference measurement values (L*, a* and b*) of pectinase treated plum juice were significantly different from control juice and the addition of pectinases resulted in a decrease in the Hunter color values (Fig. 2). Rapidase

C80L treated juice exhibited somewhat higher L* and a* values as pectinase concentration increased, resulting in a redder juice than other pectinase treated plum juice. Conversely, Clarex L treated juice exhibited the smallest color values of the plum juices and the result was a darker, purple colored juice. Clarex L, Clarex ML, Rapidase Press, and Rapidase C80L treated juice exhibited a negative linear relationship between % T and Hunter L* values (Correlation Coefficient or r values for these four pectinases were 0.98, 0.94, 0.93, and 0.97, respectively). This is probably due to release of anthocyanins as a result of enzyme addition, because the Hunter L* values were significantly ($r = 0.34$) related to the amount of anthocyanins in the juice. The correlation suggests that the larger the concentration of anthocyanins, the darker the juice. Ough *et al.* (1976) concluded that pectinase treated red grape juice was darker than untreated red grape juice, and was more red as assayed by Hunter hue.

Hue angle of pectinase treated juice was not influenced by enzyme concentration but hue angles of control juice were larger than hue angles of pectinase treated plum juice. This indicates that plum juices became more blue-green, but were still red because the hue angle was positive, than untreated juice. The hue angle was significant and negatively correlated to the Hunter L* values of juice. Hunter color results indicate that plum juice was darker and more purple after pectinases were added.

Soluble Solids

While the average soluble solids content of the pectinase treated plum juice ranged from 14.5 to 16.T Brix, soluble solids content of untreated plum juice was 14.4° Brix (Table 2). Soluble solids of Clarex ML and Rapidase Press treated juice were significantly greater than the untreated juice. The greater degree of tissue breakdown, releasing more components which contribute to soluble solids, reportedly resulted in larger Brix levels in pectinase treated juices from apple, pears, apricots and carrots (Pilnik *et al.* 1975; McLellan *et al.* 1985).

Titratable Acidity (TA) and pH

Titratable acidity of pectinase treated juice was larger than untreated juices (Table 2), which maybe due to enzymatic deesterification and degradation of pectin resulting in an increase of total acid. Pectinase concentration significantly ($p \leq 0.05$) influenced titratable acidity, and as pectinase concentrations increased so did TA values for Clarex ML and Rapidase C80L treated juice, as compared to other pectinases.

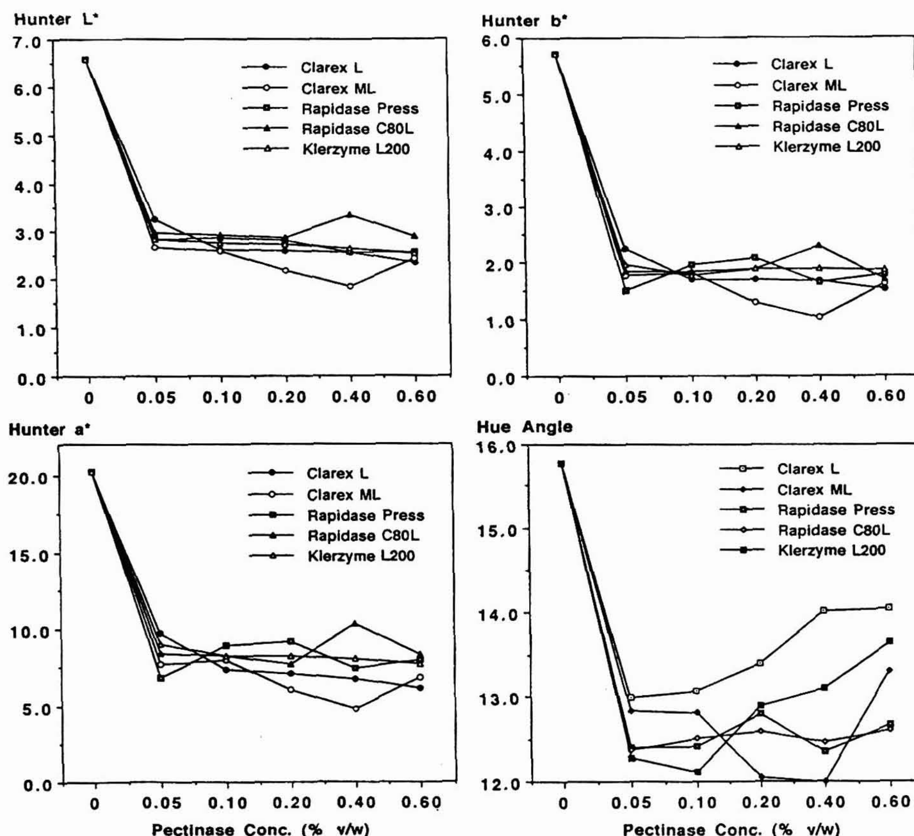

FIG. 2. COMMERCIAL PECTINASES AND THE HUNTER L*, a* AND b* VALUES AND HUE ANGLE OF STANLEY PLUM JUICE

The pH of plum juice ranged from 3.12 to 3.35 with the pectinase treated juice showing a decrease in pH values. The decrease in pH was directly related to increasing pectinase concentration. The pH of juice extracted by adding 0.1% Clarex L, Clarex ML and Rapidase Press was significantly smaller (3.17-3.26) than the control juice (3.34). Klerzyme L200 treatment did not result in a significant change in juice pH as compared to other pectinases.

°Brix/Acid Ratio

The °Brix/acid ratio is the major analytical measurement for quality in citrus and several other juices. The larger the ratio the better the flavor of the juice (Fellers 1991; Fellers *et al.* 1988). The °Brix/acid ratio of plum juice was significantly decreased ($p \leq 0.05$) by addition of Clarex L, Rapidase C80L and Klerzyme L200 (Table 2). Unlike Clarex L, Rapidase C80L and Klerzyme

L200, the use of Clarex ML and Rapidase Press showed no significant effect on the °Brix/acid ratio. In sweet cherries, °Brix/acid ratio was shown to be significantly correlated with sweetness but not so closely with flavor (Guyer *et al.* 1993).

Sugar Concentration

Glucose, sorbitol and fructose concentration increased significantly as the pectinase concentration was increased (Table 3). The concentrations of glucose, fructose and sorbitol ranged from 3.37 to 6.06, 1.66 to 3.60, and 0.53 to 1.69 g/100 ml plum juice, respectively. Wrolstad and Shallenberger (1981) reported that not only cultivar differences can contribute to differences in the amount of sucrose but processing can also result in a decrease in the sucrose content. These

TABLE 2.
COMMERCIAL PECTINASES AND SOLUBLE SOLIDS, pH, TITRATABLE ACID AND °BRIX/ACID RATIO OF STANLEY PLUM JUICE

	Pectinase Concentration (% v/w)					
	Control	0.05	0.10	0.20	0.40	0.60
Soluble Solids			(°Brix)			
Clarex L	14.47a[1]	14.73a	14.87a	15.28a	15.17a	14.55a
Clarex ML	14.47a	15.82b	15.20b	15.85b	16.02b	15.51b
Rapidase Press	14.47a	15.55b	15.21b	16.40c	16.73c	16.11c
Rapidase C80L	14.47a	14.55a	14.88a	15.08a	14.92a	14.93a
Klerzyme L200	14.47a	15.55ab	15.70b	15.81b	15.82b	15.97c
Titratable Acid			(% malic acid)			
Clarex L	1.09a	1.23b	1.26bc	1.33c	1.30c	1.34c
Clarex ML	1.09a	1.26b	1.29b	1.37c	1.34c	1.35c
Rapidase Press	1.09a	1.19b	1.21bc	1.29c	1.23bc	1.25bc
Rapidase C80L	1.09a	1.28b	1.31bc	1.35c	1.36c	1.37c
Klerzyme L200	1.09a	1.13a	1.13a	1.22b	1.24b	1.31c
°Brix/Acid Ratio						
Clarex L	13.22a	11.99b	11.76b	11.51b	11.66b	10.90c
Clarex ML	13.22a	12.59a	11.78b	11.54b	11.98b	11.45b
Rapidase Press	13.22a	13.09a	12.55a	12.74a	13.63a	12.87a
Rapidase C80L	13.22a	11.41b	11.37b	11.14b	10.97b	10.92b
Klerzyme L200	13.22a	13.75a	13.92a	12.96ab	12.78ab	12.19b

[1] Values with the same letters in the horizontal rows are not significantly different at 5% level of significance

differences are more likely due to invertases in the juice as reported by Gorsel *et al.* (1992). The sucrose content of plum juice increased with the addition of Clarex L, Clarex ML and Klerzyme L200, but above the 0.2% pectinase concentration, the sucrose concentration decreased. The specific activity of invertase in Stanley plums during ripening, processing and storage is worthy of investigation because of the changes in the physical and chemical properties of plums resulting from sucrose hydrolysis.

TABLE 3.
COMMERCIAL PECTINASES AND SUGAR CONTENTS OF STANLEY PLUM JUICE

	Pectinase Concentration (% w/w)					
	Control	0.05	0.10	0.20	0.40	0.60
Glucose			(g/100 ml)			
Clarex L	3.37a[1]	3.75c	4.12d	5.32e	4.14d	3.57b
Clarex ML	3.37a	4.61c	4.16b	5.22d	4.33bc	4.63c
Rapidase Press	3.37a	3.76b	4.67bc	4.88c	4.96c	5.26d
Rapidase C80L	3.37a	3.93b	4.63c	4.79c	6.06e	5.15d
Klerzyme L200	3.37a	4.02b	5.01c	5.51d	4.65c	5.03d
Fructose			(g/100 ml)			
Clarex L	1.66a	1.90b	1.93b	3.06d	2.22c	1.74ab
Clarex ML	1.66a	2.68c	2.15b	3.00d	2.71c	2.82cd
Rapidase Press	1.66a	1.96b	2.53b	2.65bc	3.04c	3.14c
Rapidase C80L	1.66a	2.21b	2.60c	2.61c	3.60d	3.16cd
Klerzyme L200	1.66a	1.89ab	2.23c	2.16bc	2.47c	2.59c
Sorbitol			(g/100 ml)			
Clarex L	0.53a	0.81a	0.71a	1.55b	1.63b	0.72a
Clarex ML	0.53a	1.49c	0.95b	1.69c	1.57c	1.30bc
Rapidase Press	0.53a	1.06ab	1.05ab	1.15b	1.36c	1.24c
Rapidase C80L	0.53a	0.86ab	1.05b	0.94ab	1.32b	1.18b
Klerzyme L200	0.53a	0.50a	0.52a	1.20b	0.61a	0.41a
Sucrose			(g/100 ml)			
Clarex L	3.47a	3.83b	3.78ab	5.27c	5.26c	3.24a
Clarex ML	3.47a	4.97c	4.35b	6.20d	3.33a	3.58a
Rapidase Press	3.47bc	4.05c	3.12b	3.09b	2.95b	2.16a
Rapidase C80L	3.47c	3.07c	3.14c	2.57bc	1.81b	1.13a
Klerzyme L200	3.47b	3.55b	5.06c	4.88c	3.32ab	2.67a

[1] Values with the same letters in the horizontal rows are not significantly different at 5% level of significance

Total Phenolics, Total Anthocyanins and Enzymatic Browning

Total phenolics in the enzyme treated plum juice ranged from 88 to 142 mg tannic acid/100 ml as compared to 70 mg tannic acid/100 ml in untreated juice. Pectic enzymes extract not only the pigments but also other phenolic compounds, such as tannic acid which gives a bitter flavor in the fruit juice (Marshall 1954). Brown and Ough (1981) and Ough and Crowell (1979) indicated that the influence of pectinases on total phenolics released in grape juice was related to grape varieties, enzyme types and concentration of enzymes.

Total anthocyanins generally increased with the addition of pectinases. Pectic enzymes aid in the release of pigments from plant cell (Reed 1975). A twofold increase in total anthocyanins was observed as a result of enzyme L200, whereas Rapidase C80L addition had little effect on anthocyanin release. Arnold (1992) and Rommel *et al.* (1992) reported a release of anthocyanins by pectinases in plums and blackberry juice, respectively. The average increase in total anthocyanin content of pectinase treated juice was about 30% as compared to the control juice. The correlation between total anthocyanins and browning index of juices was significant ($r = 0.37$, $p \leq 0.05$).

In conclusion, among the five commercial pectinases investigated, Clarex L at 0.2% concentration improved overall quality of plum juice. Clarex L addition resulted in optimum juice yield, compared to Klerzyme 200L, a more stable color, compared to Clarex ML and Rapidase Press, higher ACY content, better flavor, lower phenolic content, compared to Rapidase C80L, and a sediment free, clear juice, compared to Rapidase Press. Pectinases concentrations above 0.2% increased juice yields but resulted in bitter flavored plum juice, which was not acceptable.

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REFERENCES

AMIR-UZ-ZAMAN, M. 1985. Stabilization of orange juice cloud by enzymic inhibition. Ph.D. dissertation. Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI.

ARNOLD, J. 1992. Inactivation of polyphenol oxidase in Stanley plum juice using an immobilized protease enzyme. M.S. Thesis. Dept. of Food Science & Human Nutrition, Michigan State University, East Lansing, MI.

BROWN, M.R. and OUGH, C.S. 1981. A comparison of activity and effects of two commercial pectic enzyme preparations on white grape musts and wines. Am. J. Enol. Vitic. *32*, 272–276.

CHEETHAM, P. S. J. 1985. The applications of enzymes in industry. In *Industrial Enzymology*, (Godfred, T. and Reichelt, J. ed.) pp. 274-291, The Nature Press, London, U.K.

FETTERS, P.J. 1991. The relationship between the ratio of degrees Brix to percent acid and sensory flavor in grapefruit juice. Food Technol. *45*(7), 68–75.

FELLERS, P.J., CARTER, R.D. and DEJAGER, G. 1988. Influence of the ratio of degrees Brix to percent acid on consumer acceptance of processed modified grapefruit juice. J. Food Sci. *53*, 513–515.

FRANCIS, F.J. 1982. Analysis of anthocyanins. In *Anthocyanins as Food Colors*, pp. 181–207 (P. Markakis, ed.), Academic Press, New York.

FULEKI, T. and FRANCIS, F.J. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanins in cranberries. J. Food Sci. *33*, 72–77.

GORSEL, H., LI, C., KERBEL, E.L., SMITS, M. and KADER, A.A. 1992. Compositional characterization of prune juice. J. Agric. Food Chem. *40*, 784–789.

GUYER, D.E., SINHA, N.K., CHANG, T.-S. and CASH, J.N. 1993. Physicochemical and sensory characteristics of selected Michigan sweet cherry(*Prunus avium* L.) cultivars. J. Food Quality *16*, 355–370.

KORTBECH-OLESEN, R. 1991. Spectacular growth in major markets for fruit juices. Int. Trade Forum *3*(4–9), 34.

KROP, J.J.P. and PILNIK, W. 1974. Effect of pectic acid and bivalent cations on cloud loss of citrus juice. Lebensm. Wiss. Technol. *7*, 62–63.

MARSHALL, R.E. 1954. *Cherries and Cherry Products*, InterScience Publications, New York.

MCLELLAN, M.R., KIME, R.W. and LIND, L.R. 1985. Apple juice clarification with the use of honey and pectinase. J. Food Sci. *50*, 206–208.

OUGH, C.S. and CROWELL, E.A. 1979. Pectic-enzyme treatment of white grapes: Temperature, variety and skin-contact time factors. Am. J. Enol. Vitic. *30*, 22–27.

OUGH, C.S., NOBLE, A.C. and TEMPLE, D. 1975. Pectic enzyme effects on red grapes. Am. J. Enol. Vitic. *26*, 195–200.

PILNIK, W., VORAGEN, A. G. J. and DEVOS, L. 1975. Enzymic liquefaction of fruits and vegetables. Flussigesn Obst. *42* (11), 448–451.

REED, G. 1975. Pectic Enzymes. In *Enzymes in Food Processing*, pp. 73-87, Academic Press, New York.

RICHMOND, M.L., BRANDAO, S.C.C., GRAY, J.I., MARKAKIS, P. and STINE, C.M. 1981. Analysis of simple sugars and sorbitol in fruit by high performance liquid chromatography. J. Agric. Food Chem. *29*, 4-7.

ROMMEL, A., WROLSTAD, R.E. and HEATHERBELL, D.A. 1992. Blackberry juice and wine: Processing and storage effects on anthocyanin composition, color and appearance. J. Food Sci. *67*, 385-391.

SAPERS, G.M. and DOUGLAS, F.W. Jr. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. J. Food Sci. *62*, 1258-1262.

SFILIGOJ, E. 1992. The beverage market index for 1992. Beverage World *1515*, 30-41.

SHAW, P.E. 1988. *Handbook of Sugar Separations in Foods by HPLC*, pp. 31-42, CRC Press, Boca Raton, FL.

SIDDIQ, M., ARNOLD, J.F., SINHA, N.K. and CASH, J.N. 1994. Effect of polyphenol oxidase and its inhibitors on anthocyanin changes in plum juice. J. Food Processing Preservation *18*, 75-84.

SINGLETON, V.L. and ROSSI, J.A. Jr. 1964. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. *16*, 144-158.

SKALSKI, C. and SISTRUNK, W.A. 1973. Factors influencing color degradation in Concord grape juice. J. Food Sci. *38*, 1060-1064.

WROLSTAD, R.E. and SHALLENBERGER, R.S. 1981. Free sugar and sorbitol in fruits. A compilation from the literature. J. Assoc. Off. Anal. Chem. *64*, 91-103.

INACTIVATION OF *E. COLI* FOR FOOD PASTEURIZATION BY HIGH-STRENGTH PULSED ELECTRIC FIELDS

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ABSTRACT

Pulsed electric fields of very high field strength and short duration are effective in the inactivation of E. coli. *Nine log reduction in* E. coli *viability was achieved using a stepwise pulsed electric field treatment where* E. coli *suspensions were treated repeatedly in batches. It was demonstrated that high-strength pulsed electric field treatment is adequate for pasteurization of liquid foods.*

A 40,000 volt pulse generator was constructed to supply high voltage electric pulses to a treatment chamber with two parallel plate stainless steel electrodes where fluid food was contained. The gap between electrodes was 0.51 cm and the chamber volume was 14 ml. Pulse electric field strength ranged from 35 to 70 kV/cm. Pulse width was selected at 2 μs. Number of pulses per treatment varied from 1 to 80.

E. coli *were suspended in a simulated milk ultra-filtrate (SMUF) and treated with pulsed electric fields in a batch mode. The suspension fluid was maintained at constant temperatures of 7, 20, or 33C. Maximum temperature change occurring during each pulse was 0.3C measured by a fiber optics temperature probe.* E. coli *viability before and after treatment were assayed by counting colony forming units (cfu).*

INTRODUCTION

Pulsed electric field (PEF) treatment is a potential nonthermal food pasteurization technique (Mertens and Knorr 1992; Castro *et al.* 1993). PEF treatment inactivates microorganisms without adversely affecting the flavor, taste

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and nutrients which occur when using traditional thermal pasteurization (Jayaram *et al.* 1992).

Many researchers reported inactivation of vegetative microbial cells with PEF including Hamilton and Sale (1967); Sale and Hamilton (1967, 1968); Hülsheger *et al.* (1980, 1981, 1983); Jacob *et al.* (1981); Hofmann (1984); and Mizuno and Hori (1988). The lethal effect of PEF is related to the electric field strength and the treatment time (Hülsheger *et al.* 1981, Pothakamury *et al.* 1994, Zhang *et al.* 1994a). The lethal effect is also a function of the temperature of the fluid medium being treated (Dunn and Pearlman 1987; Jayaram *et al.* 1992). However, microbial inactivation achieved by PEF was on the order of 2 to 5 log cycles, primarily due to previous limitation in the maximum electric field. Breakdown strength of air, 25 kV/cm, was the major source of limitation. The application of repetitive pulses with a large amount of energy results in an increase in temperature and the formation of gas bubbles (Zhang *et al.* 1994b). High pulse energy may result in electrolytic reactions, Ohmic heating, and disintegration of food particles (Sale and Hamilton 1967; Hülsheger *et al.* 1980; Dunn and Pearlman 1987; Grahl *et al.* 1992).

From the engineering point of view, minimizing the energy level of PEF treatment results in safer (Zhang *et al.* 1994b) and more economical processes. Electric field strength plays a more important role than pulse duration in the microbial inactivation (Grahl *et al.* 1992). Therefore, increasing the field strength and decreasing the pulse duration may result in increased microbial inactivation without increasing pulse energy.

In this study we investigated the effect of high strength (35–70 kV/cm), short duration (2–3 μs) pulsed electric fields on the viability of *Escherichia coli* suspended in simulated milk ultra-filtrate (SMUF) under controlled temperatures of 7, 20, or 33C.

MATERIAL AND METHODS

Pulse Generator

A high voltage charged transmission line produced a square pulse when connected to a matching load with an ideal switch. The peak output voltage, in this case, is half of the charged voltage. By constructing two transmission lines in series, a 40,000 volt Blumlein pulse generator (Fig. 1) provided high voltage short duration electric pulses with peak output voltage very close to charged voltage. Two spools of high voltage coaxial cables (RG-8/Au), each 152 m long, were used as the transmission lines. A typical electric pulse is illustrated in Fig. 2. Pulsed electric field was calculated as the voltage measurement divided by the electrode gap, 0.51 cm. The pulse was distorted from its square shape due to

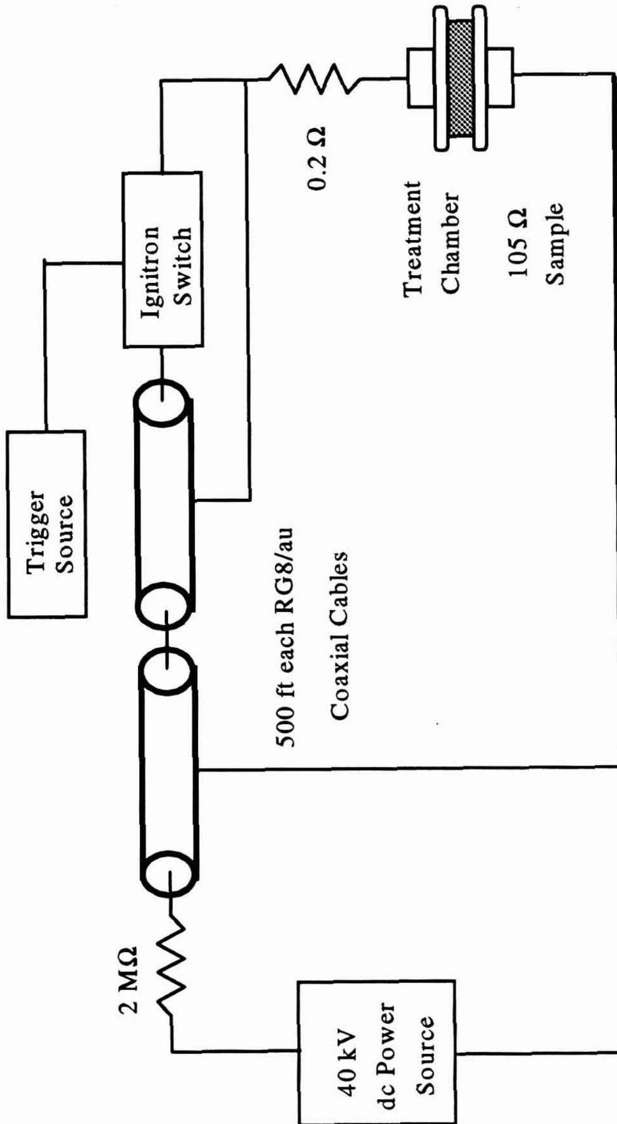

FIG. 1. CIRCUIT DIAGRAM OF BLUMLEIN PULSE GENERATOR USING COAXIAL CABLES

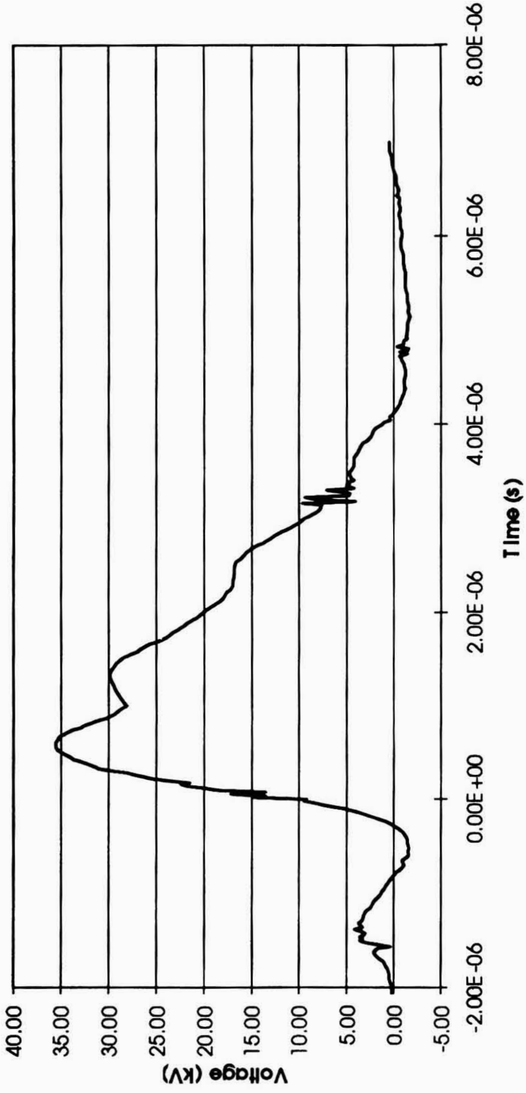

FIG. 2. VOLTAGE MEASUREMENT OF ELECTRIC PULSES GENERATED BY A BLUMLEIN PULSE GENERATOR
Pulse duration was 2 μs, and the peak electric field was 70 kV/cm.

characteristics of the ignition switch. The characteristic impedance of this pulse generator was 100Ω since two 50Ω cables are used in series. A matching load resistance, i.e., resistance of the food in a treatment chamber, was necessary to obtain a meaningful pulse wave form.

Treatment Chamber

A 0.51 cm gap parallel-plate static treatment chamber was used for the tests. Technical data for the static treatment chamber is summarized in Table 1. With the Blumlein pulse generator set at 37 kV charging voltage, pulse strength obtained in the food was 70 kV/cm.

Control and Measurement of the Temperature

The food temperature was maintained by circulating constant temperature water through the cooling jackets built into the stainless steel electrodes. The accuracy of the temperature control is +/− 0.1C. The temperature of the electrode was determined with a high precision thermocouple (Omega T-TT-40, Stamford, CT) attached to one electrode. The temperature of the fluid food in the chamber was monitored with a fiber optics transducer (Model T22-03D, Photonetics, Wakefield, MA) which had a precision of 0.1C and response time of 0.2 s. Electrode temperature during PEF treatment was kept constant, but fluid food temperature demonstrated a transient at each electrical pulse. A typical fluid center temperature measurement is illustrated in Fig. 3 where time 0 indicates the moment an electrical pulse was applied. A temperature pulse at time 0 may be computed from the energy balance since the electric pulse was much shorter in duration (2 μs) than the temperature pulse (20s). The calculated temperature pulse of 0.29C was the same as the measured temperature pulse.

SMUF Composition

The composition of the fluid food, simulated milk ultra-filtrate (SMUF), is listed in Table 2. The apparent load resistance of the original SMUF sample in the treatment chamber was 4Ω at 20C. In order to obtain the matching impedance with the Blumlein pulse generator, the original SMUF was modified by diluting it with water (Table 2). The apparent load resistance of the modified SMUF was 105Ω at 20C.

TABLE 1.
STATIC PULSED ELECTRIC FIELD TREATMENT APPARATUS PARAMETERS

	Description	Operating Parameters
A	Static PEF Treatment Chamber:	
	Electrode arrangement	Parallel disk plates with contoured edge
	Orientation of electrodes	Horizontal
	Volume (ml)	14.0
	Electrode contact area (cm^2)	27.1
	Gap between electrodes (cm)	0.51
	Apparent load resistance (Ω)	105
	Electrode material	Stainless Steel
	Insulation material	Plexiglas
	Electric field uniformity (%)	< 2
	Electrode cooling volume (ml)	14
	Electrode cooling area (cm^2)	74
B	High Voltage Pulse Generator:	
	Charging voltage (kV)	37 (Nom) 40 (Max)
	Maximum electric field (kV/cm)	75
	Effective capacitance (μF)	0.02
	Discharge Switch	Ignitron
C	Auxiliary Cooling Device:	
	Cooling fluid	Water
	Cooling fluid temperature (C)	7, 20, 33
	Cooling flowrate (ml /mim)	1200
D	Voltage and Current Monitor:	
	Voltage monitor	resistor divider ratio = 710
	Current monitor	Pearson current transducer 0.1V : 1A
	Waveform measurement	HP54520A, 500 MHz digital oscilloscope

Microbial Preparation

Escherichia coli (ATCC 11229) was grown in nutrient broth (DIFCO 0003-01-6) continuously agitated in a temperature control shaker (Model MSB-3322A-1, GS Blue Electric, Blue Island, IL) at 37C until reaching an absorbance of 1.8 at 600 nm, corresponding to the end of logarithmic growth phase with a viable count of 80×10^7 cf/ml. The bacteria were chilled on ice for

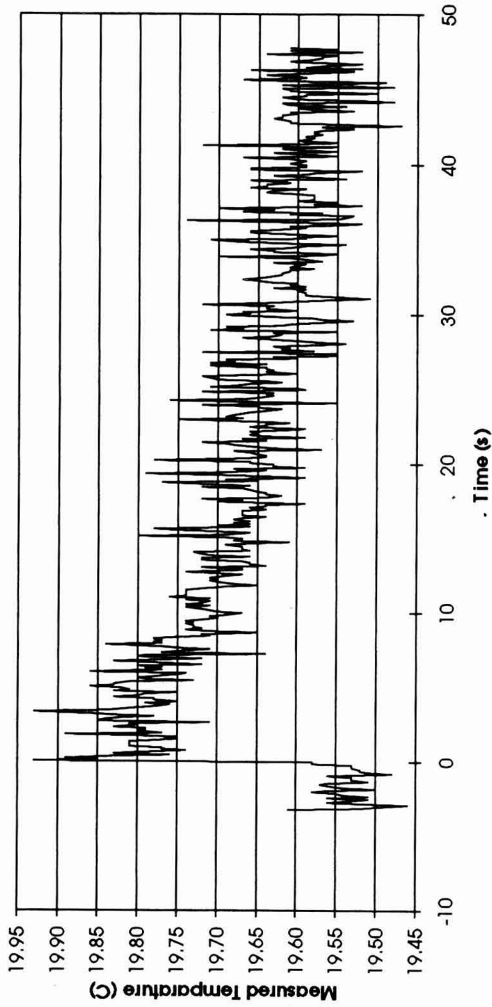

FIG. 3. TYPICAL FLUID FOOD CENTER TEMPERATURE AS DETECTED BY A FIBER OPTIC TRANSDUCER
Pulse electric field strength was 70 kV/cm and pulse energy was 17.2 J.

TABLE 2.
SIMULATED MILK ULTRA FILTRATE COMPOSITION

Components	Original SMUF	Modified SMUF
Lactose	50.0 g	1.389 g
KH_2PO_4	1.58 g	0.044 g
K_3citrate	0.98 g	0.027 g
Na_3citrate•$2H_2O$	1.79 g	0.050 g
K_2SO_4	0.18 g	0.005 g
$CaCl_2 \bullet 2H_2O$	1.32 g	0.037 g
Mg citrate	0.38 g	0.011 g
K_2CO_3	0.30 g	0.008 g
KCl	1.08 g	0.030 g

5 min, transferred to a 50 ml sterile disposable centrifuge tube, centrifuged at 4000g at 3C for 15 min, and the supernatant discarded. The precipitate was resuspended and washed with an equal volume of chilled nutrient broth and centrifuged. Resuspension and centrifugation were repeated twice. One ml of a cryoprotectant (20% glycerol) was added to the pellet obtained. Pellets of *E. coli* were frozen and stored at −70C until further use.

Frozen pellets of *E. coli* were thawed, centrifuged to remove the glycerol and kept in ice until use. Each pellet was hydrated at 20C in 40 ml SMUF, 20 min before treatment. Chilling injury was taken into consideration by assaying microbial viability without PEF treatment, as the control.

The viability of the *E. coli* before and after PEF treatments was assayed by counting colony forming units (cfu). One milliliter SMUF samples were taken, serially diluted with 0.1% sterile peptone solution, and 1 ml of the dilution plated on tryptone soy agar with violet red bile agar overlay and incubated at 37C for 24 h. Plates with cfu in the range between 25 and 250 were selected. Each dilution was replicated in four plates for the purpose of calculating the mean cfu.

PEF Treatment Procedures

The chamber was filled for each treatment with *E. coli*-inoculated SMUF at 20C. Gas bubbles were completely expelled from the chamber before

treatment. Time between pulses (15–30 s interval) was controlled to maintain the electrode temperature within ±0.2C of the selected cooling water temperature. After each treatment, the SMUF was removed from the treatment chamber, the chamber was washed with a 70% sterile alcohol solution and rinsed with sterile distilled water.

Two basic types of PEF treatments were conducted in this study. A one step treatment was conducted by treating a batch of *E. coli*-inoculated SMUF with selected number of PEF pulses. Each data point was obtained using one batch of freshly inoculated SMUF for a one step treatment.

A stepwise treatment was conducted by treating the *E. coli*-inoculated SMUF in batches with 16 pulses. The SMUF was recollected and retreated in batches with 16 pulses. Only the first stepwise treatment was loaded with batches of freshly inoculated SMUF. Consecutive stepwise treatments were loaded with SMUF treated previously. All PEF pulses had a field strength of 70 kV/cm and a pulse duration of 2 μs.

As a certain volume of sample was needed to assay the microbial viability of *E. coli* before and after each stepwise treatment, a sequence of 8, 6, 4, 2 and 1 batches of SMUF was treated in the first, second, third, fourth and fifth stepwise treatments. In the first stepwise treatment, eight batches of freshly inoculated SMUF were each treated with 16 PEF pulses. Six batches of SMUF were collected after the first stepwise treatment and were each treated with 16 additional PEF pulses as the second step. Five treatment steps were conducted for each stepwise test. The means of all the batches in each stepwise treatment are reported.

RESULTS AND DISCUSSION

A nine log reduction in *E. coli* viability was accomplished by pulsed electric field treatment of inoculated SMUF (Fig. 4). Survival fractions before and after each treatment step were assayed and accumulated. The initial inoculation was 80×10^7 cfu/ml, and the final viable *E. coli* concentration was practically zero. The energy required to achieve the nine log reduction in viable of *E. coli* concentrations was 97 kJ/L. Figure 4 reports two replicate stepwise tests with similar inactivation results.

Table 3 summarizes the microbial inactivation accomplished by various researchers using pulsed electric field treatment. Inactivation *E. coli* ranges from 2–5 log cycles. Matsumoto *et al.* (1991) achieved five log reduction using a converged electric field strength of 40 kV/cm. Hülshenger *et al.* (1983) achieved four log reduction using a long treatment time (1,080 μs) with field strength of 20 kV/cm. Grahl *et al.* (1992) obtained three log reduction for *E. coli* in whole

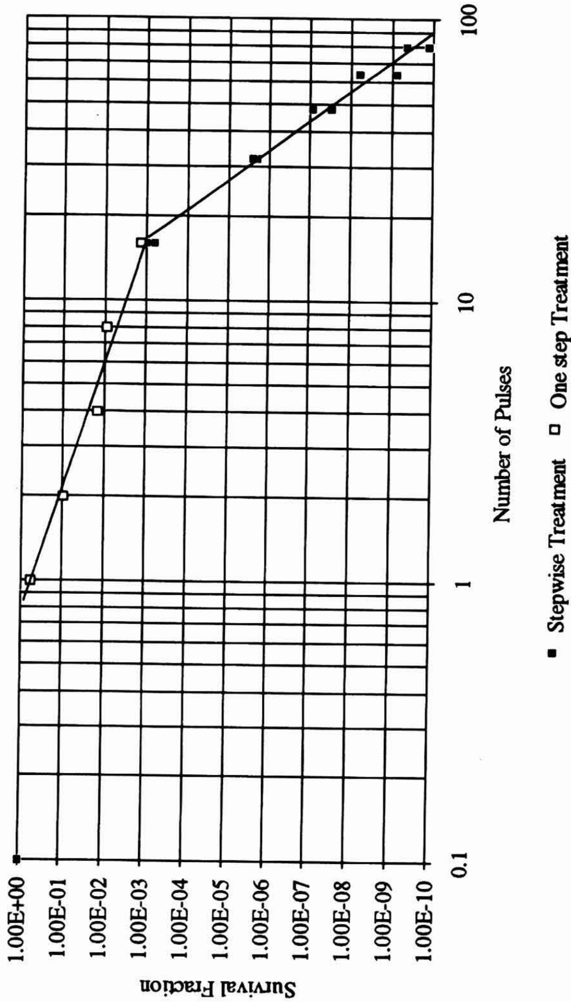

FIG. 4. INACTIVATION OF *E. COLI* WITH HIGH-STRENGTH SHORT-DURATION PULSED ELECTRIC FIELDS
PEF strength was 70 kV/cm. Pulse width was 2 μs. Suspension fluid temperature was 20C.

TABLE 3.
SUMMARY OF MICROBIAL INACTIVATION ACCOMPLISHED BY PULSED ELECTRIC FIELDS

Researchers	Microorganisms and initial inoculation	Suspension media	Log reduction in viability	Temperature C	Peak electric field strength (kV/cm)	Treatment time (μs)	Number of pulse treatment
Jayaram *et al.* 1992	*Lactobacillus brevis* $10^8 \sim 10^9$ cfu/ml	$Na_2HPO_4/NaH_2PO_4 \bullet H_2O$ (0.845/0.186 mM)	6	30	25	15,000	204
Mizuno and Hori 1988	*S. cerevisiae* (10^4 cfu/ml)	NaCl (1%)	3	-	17	-	780
Matsumoto *et al.* 1991	*S. cerevisiae*	phosphate buffer	5	-	30	-	-
Matsumoto *et al.* 1991	*E. coli*	phosphate buffer	5	-	40	-	-
Gupta and Murray 1988	*S. typhimurium* (10^9 cfu/ml)	NaCl	5	-	83	-	20
Gupta and Murray 1988	*P. fragi* (10^7 cfu/ml)	milk	5	-	90	-	5
Hamilton and Sale 1967	*E. coli*	Saline (0.1%)	2	-	19.5	-	-
Hamilton and Sale 1967	*Staph. aureus*	Phosphate buffer (20mM)	2	-	27.5	-	-
Hülsheger *et al.* 1983	*E. coli*	Phosphate buffer	4	20	20	1,080	30
Grahl *et al.* 1993	*E. coli* (10^7)	milk	3		22	200	5
This study	*E. coli* (10^9 cfu/ml)	Modified SMUF	9	20	70	160	80

- Treatment conditions not reported.

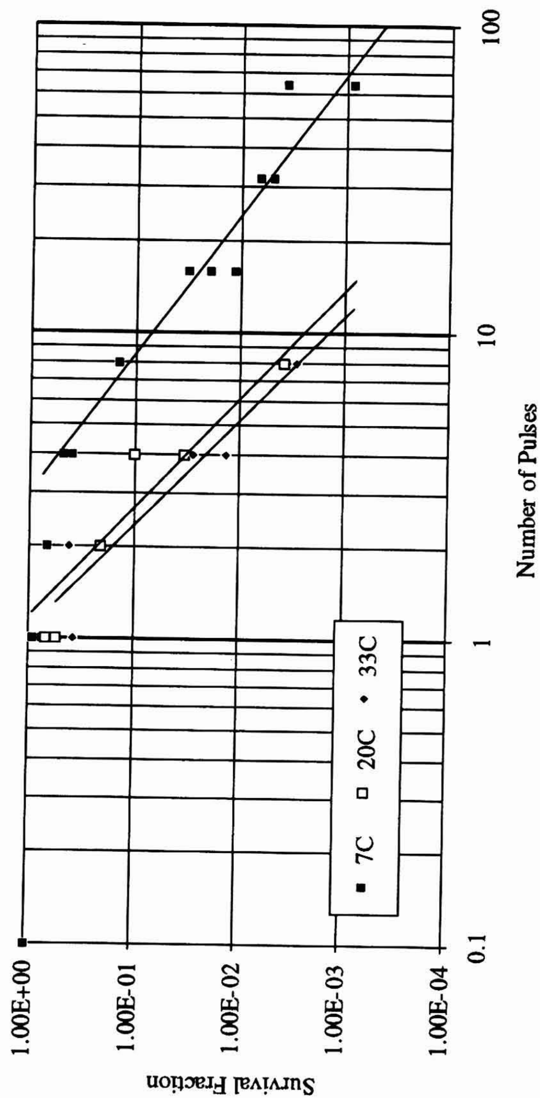

FIG. 5. SURVIVAL FRACTION OF *E. COLI* AFTER PEF TREATMENT AT 7, 20 AND 33C
PEF strength was 36 k V/cm. Pulse duration was 2 μs.

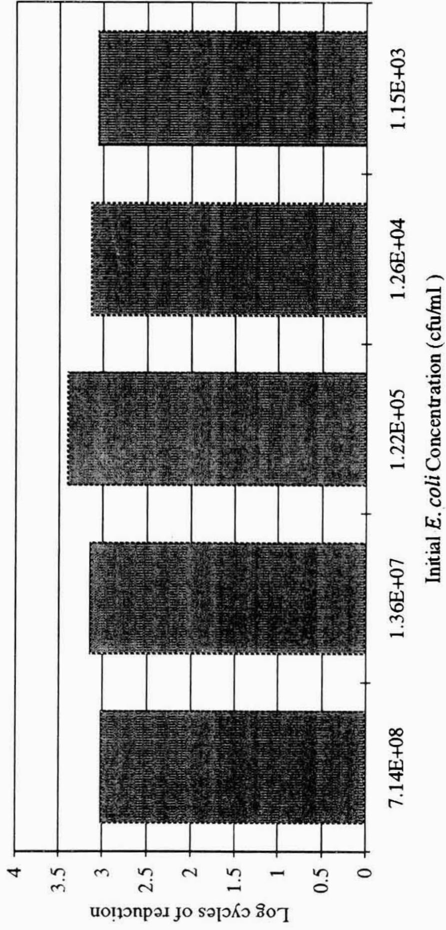

FIG. 6. LOG CYCLES OF INACTIVATION AT SELECTED INITIAL CONCENTRATIONS OF *E. COLI*

PEF strength was 70 kV/cm. Pulse duration was 2 μs. Number of pulses treated was 16.

milk with field strength of 22 kV/cm. Gupta and Murray (1988) are the first researchers to treat microbial suspensions with higher than 25 kV/cm field strength, and they achieved five log reduction for *S. typhimurium* and *P. fragi*. However, previous studies were unable to demonstrate a 6–7 log reduction of *E. coli*, the commonly accepted standard for food pasteurization.

Nine log reduction of *E. coli* viability is first repeated herein, indicating that pulsed electric field treatment can be used for commercial pasteurization of foods. A batch PEF treatment system may have a limiting number of log cycle reduction in microbial viability. A space charge can develop about 10 μm from the electrode surface where electric field strength is much lower than in the bulk (Spencer 1994). These space charge layers serve as protective regions for some microorganisms. A stepwise treatment makes microbial cells receive equal amount of PEF treatment. A high degree of inactivation may be achieved using stepwise treatment.

Media Temperature

Inactivation of *Lactobacillus brevis* by pulsed electric fields is a function of suspension media temperature (Jayaram *et al.* 1992). Figure 5 illustrates inactivation of *E. coli* at three media temperatures, 7, 20 and 33C by one step PEF treatment. Electric field strength and pulse duration were selected to be 35 kV/cm and 2 μs, respectively. Energy input for each pulse was 4.3 Joules. Increasing the temperature from 7 to 20C significantly increased PEF inactivation of *E. coli*. The additional increase in temperature from 20 to 33C did not result in an increase in PEF inactivation of *E. coli*.

Initial Inoculation

Population load of *E. coli* did not significantly change the PEF inactivation as illustrated in Fig. 6. Initial *E. coli* concentration ranged from 1.15×10^3 to $7.14 - 10^8$ cfu/ml. PEF microbial inactivation is not a function of initial concentration of *E. coli* in the range studied.

CONCLUSIONS

Nine log reduction of *E. coli* in a modified SMUF was achieved by a stepwise pulsed electric field treatment, which is adequate for food pasteurization. Ohmic heating during the PEF treatment was negligible. Increasing the suspension temperature of *E. coli* from 7 to 20C promoted microbial inactivation by PEF treatment. Initial concentration of *E. coli* in the range of 1.15×10^3 to $7.14 - 10^8$ cfu/ml did not alter PEF inactivation.

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REFERENCES

CASTRO, A.J., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1993. Microbial inactivation of foods by pulsed electric fields. J. Food Processing Preservation *17*,47–73.

DUNN, J.E. and PEARLMAN, J.S. 1987. Methods and apparatus for extending the shelf life of fluid food products. U.S. Pat. 4,695,472.

GRAHL, T., SITZMANN, W. and MAKL, H. 1992. Killing of microorganisms in fluid media by high-voltage pulses. DECHEMA Biotechnol. Conf. Ser., *5B*, 675–678.

GUPTA, R.P. and MURRAY, W. 1988. Pulsed high electric field sterilization. IEEE Pulsed Power Conf. Record, pp. 58–64.

HAMILTON, W.A. and SALE, A.J.H. 1967. Effects of high electric fields on microorganisms II. Mechanism of action of the lethal effect. Biochim. Biophys. Acta *148*, 789–800.

HOFMANN, G.A. 1984. Microflora reduction in liquids with pulsed electric fields. Internal Tech. Rept., Biotechnologies & Experimental Research, San Diego.

HÜLSHEGER, H. and NIEMANN, E.G. 1980. Lethal effects of high-voltage pulses on *E. coli* K12. *Radiat. Environ. Biophys. 18*, 281–288.

HÜLSHEGER, H., POTEL, J. and NIEMANN, E.G. 1981. Killing of bacteria with electric pulses of high field strength. Radiat. Environ. Biophys. 20, 53–65.

HÜLSHEGER, H., POTEL, J. and NIEMANN, E.G. 1980. Electric field effects on bacteria and yeast cells. Radiat. Environ. Biophys. 22, 149–162.

JACOB, H.E., FORSTER, W. and BERG, H. 1981. Microbiological implications of electric field effects. II. Inactivation of yeast cells and repair of their cell envelope. Z. Allg. Mikrobiol. *21*, 225–233.

JAYARAM, S., CASTLE, G.S.P. and MARGARITIS, A. 1992. Kinetics of sterilization of *Lactobacillus brevis* by the application of high voltage pulses. Biotech. Bioeng. 40(11), 1412–1420.

MATSUMOTO, Y., SATAKE, T., SHIOJI, N. and SAKUMA, A. 1991. Inactivation of microorganisms by pulsed high voltage applications. Conf. Rec. of IEEE Industrial Applications Society Annual Meeting, pp. 652–659.

MERTENS, B. and KNORR, D. 1992. Developments of nonthermal processes for food preservation. Food Technol. *46*(5), 124–133.

MIZUNO, A. and HORI, Y. 1988. Destruction of living cells by pulsed high-voltage applications. Trans. IEEE Ind. Appl. 24(3), 387–394.

POTHAKAMURY, U.R., MONSALVE-GONZÁLEZ, A., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1994. Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric field technology. Food Preservation 2000 Proceedings, Natick, MA.

SALE, A.J.H. and HAMILTON, W.A. 1967. Effects of high electric fields on microorganisms I. Killing of bacteria and yeasts. Biochim. Biophys. Acta 148, 781–788.

SALE, A.J.H. and HAMILTON, W.A. 1968. Effects of high electric fields on microorganisms. III. Lysis of erythrocytes and protoplasts. Biochim. Biophys. Acta. 163, 37–43.

SPENCER, D.B. 1994. Space charge evolution in dilute binary electrolytes exposed to high voltage transients. M.Sc Thesis, Washington State University.

ZHANG, Q., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1994a. Engineering aspects of pulsed electric field pasteurization. J. Food Eng. *25*(2), 261–281.

ZHANG, Q., MONSALVE-GONZÁLEZ, A., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 1994b. Inactivation of *E. coli* and *S. cerevisiae* by pulsed electric fields under controlled temperature conditions. Trans. ASAE 37(2), 581–587.

THE INFLUENCE OF EXTRUSION PROCESSING ON IRON DIALYZABILITY, PHYTATES AND TANNINS IN LEGUMES[1]

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ABSTRACT

Iron dialyzability, chemical forms of iron, tannin content, and phytic acid degradation in boiled, low impact and high impact extruded navy beans, chickpeas, cowpeas and lentils were determined. In boiled legumes, the dialyzable iron was 1.2–2.7% of total iron. Dialyzable, soluble and ionic iron were highest in low impact, extruded legume flours. Lower forms of inositol phosphates (inositol tri-, tetra- and penta-phosphates) increased to 51–71% of total phytate in legumes processed by boiling or extrusion compared to 21–33% in raw legumes. Tannin content was lower in extruded products compared to boiled or raw legumes. The influence of extrusion processing on iron dialyzability varied with processing conditions. Results suggest that the degradation of phytate and changes in tannin content may not be responsible for the increase in iron dialyzability and solubility associated with low impact extrusion processing.

INTRODUCTION

Extrusion processing is being used increasingly to process new cereal and legume products. Extrusion is a high temperature, short time (HTST) cooking process which uses high shear at elevated pressure and temperature. Interactions among food components during extrusion processing may have a positive or negative effect on the bioavailability of nutrients, including bioavailability of iron.

Bioavailability has been defined as the proportion of the total nutrient in a food, meal or diet that is utilized for normal body functions (O'Dell 1984).

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Important chemical factors affecting the bioavailability of iron in foods include the valence, solubility and degree of chelation or complex formation of the iron (Lee and Clydesdale 1979). Measurements of the percentage of soluble or dialyzable iron in a food are commonly used in vitro techniques to assess potential iron bioavailability (Tanner and Whittaker 1989).

Several factors, including phytates and tannins, may contribute to the low bioavailability of iron in legumes which has been reported. Phytate has been identified as an inhibitor of iron bioavailability (Brune *et al.* 1992; Hallberg *et al.* 1987). During food processes involving heat treatment, naturally occurring phytate in plants may be degraded to lower forms of inositol phosphates which have been reported to induce an increase in iron solubility through the formation of small soluble iron complexes (Sandberg *et al.* 1989). Tannins, located mainly in the seed coat or testa of beans, are another factor that has been suggested to be responsible for the low bioavailability of iron in legumes (Torrance *et al.* 1982; Rao and Prabhavathi 1982).

Due to the significance of dietary fiber and complex carbohydrate in nutrition and health, an increased consumption of legumes is being promoted (Morrow 1991). Because legumes also are an important plant source of iron, the purpose of the present research was to assess the influence of extrusion processing on iron bioavailability by determining in vitro iron dialyzability and chemical forms of iron. The effect of extrusion processing on phytate degradation and tannin content of legumes also was determined.

MATERIALS AND METHODS

Legumes

Domestically grown navy beans, chickpeas, cowpeas and lentils were obtained from Morrice Grain and Bean Company, Morrice, MI. All dry bean samples were ground using a Fitzpatrick mill with a 3A 187 mesh screen to obtain legume flours.

Extrusion Processing

A Baker Perkins, MPF-50 (25:1 L/D) corotating twin-screw extruder was used to extrude the legume flours under low and high impact conditions. The major differences in the configuration of low and high impact processes include (a) screw configuration, (b) screw speed, (c) moisture content and (d) barrel zone temperatures. The specific conditions of the two extrusion processes are as follows:

	Low impact	High impact
Screw speed (rpm)	350	400
Moisture (%)	16	10–14
Die temperature (F)	221–243	268–278
Barrel temperature (F)	179–199	214–227

The extruded products (moisture content: 5.8–13.6%) were ground using the Fitzmill with a 0.04 inch mesh screen prior to analyses.

Boiling

The nonextruded legumes were cooked using a conventional home cooking procedure. The legumes were soaked overnight in tap water, heated to boiling point and held at that temperature for 30 min, by which time they were soft. The boiled legumes were homogenized to a smooth consistency. All the water used for soaking or boiling the legumes was used for homogenization. The samples were stored in the refrigerator (for 3 days or less) until they were analyzed.

Total Iron Analysis

Extruded and nonextruded samples were wet-ashed with concentrated nitric acid and 30% hydrogen peroxide. The ash was dissolved in 0.1 N hydrochloric acid and the solution was analyzed for iron using atomic absorption spectroscopy (AAS) (Perkin-Elmer Model 2380).

In Vitro Iron Dialyzability

The iron dialyzability assay developed by Miller *et al.* (1981) was used as a measure of potential iron bioavailability in boiled and extruded legume products. The extruded legume flours were mixed with water in a ratio of 1:3 (flour:water), heated to boiling temperature and held for 15 min. The slurry was cooled to room temperature prior to analysis. Iron content of the dialysate was determined using ferrozine color reagent.

Quantification of the Chemical Forms of Iron

Boiled legumes were analyzed for elemental, total nonelemental, soluble and ionic iron according to the method proposed by Lee and Clydesdale (1979). The extruded legume flours that were prepared for the iron dialyzability assay were also analyzed for the chemical forms of iron. The complexed iron was measured as the difference between the soluble iron (determined by AAS) and ionic iron (determined using the ferrozine color reagent).

Determination of Inositol Phosphates

Phytic acid and its degradation products including inositol hexa-, penta-, tetra-, and tri-phosphates were determined using ion exchange chomatography and high pressure liquid chromatography techniques according to Graf and Dintzis (1982) and Sandberg and Ahderinne (1986) modified as follows.

Sample Preparation. Raw legumes were milled using a Micro-Mill (Chemical Rubber Co., OH) to pass through a sieve equipped with a 60 mesh. Samples of 0.5 g raw, boiled or extruded legume flours were extracted under mechanical agitation with 20 ml 0.5 M HCl for 2 h at 20C. The extract was centrifuged and supernatant decanted, frozen overnight and filtered under pressure. The filtrate was diluted with 10 ml distilled deionized water (DDW) and passed through an ion exchange column containing 0.65 ml resin (AG 1-X8, 200-400 mesh) at 0.4 ml/min followed by 10 ml of 0.025 M HCl. Inositol phosphates were removed from the resin with ten 1 ml portions of 2 M HCl. The eluent was evaporated to dryness and diluted with 1 ml of DDW.

Mobile Phase. The mobile phase consisted of 0.05 M formic acid:methanol (46:54) to which 1.5 ml/100 ml of tetrabutylammonium hydroxide was added. The pH was adjusted to 4.3 by addition of 9 M sulfuric acid. The mobile phase was filtered through a Millipore filter (0.45 μm) under vacuum and degassed.

HPLC Procedure. HPLC analyses were conducted using a reverse phase Supelcosil LC-18 column (Supelco) 5 micron particle size equilibrated with the mobile phase for 1 h and a HPLC pump (miniPump, Bodine Elec. Co., IL). Injections were made with a 20 μl loop. The optimal flow rate was 1 ml/min. Inositol phosphates were detected using a differential refractometer (Waters, Model R401). Retention times and peak areas were measured with a Peak Simple II integrator (SRI Instruments, NV).

Determination of Tannin Content

Tannin content of legumes, expressed as catechin equivalents, was determined using a procedure described by Price *et al.* (1978). Ground sample (200 mg) was mixed with 10 ml of 1% concentrated HCl in methanol and incubated at room temperature for 20 min. The mixture was centrifuged and filtered and the filtrate was analyzed for tannins using the vanillin-HCl reagent.

Statistical Analyses

Student's t test and one-way analysis of variance followed by the test for least significant difference were used to compare means at 95% confidence level.

RESULTS AND DISCUSSION

Iron Dialyzability

Total iron content was significantly higher in extruded legume flours (Table 1). A similar increase in iron content in extruded products was reported by other researchers (Fairweather-Tait *et al.* 1987; Lombardi-Boccia *et al.* 1991; Hazell and Johnson 1989). The wear of certain parts of the extruder was presumed to be the cause for the contamination of iron in the extruded products. However, there was no detectable elemental iron in our extruded products. Thus, the equipment used in extrusion processing may not have been responsible for the increase in iron in the extruded products.

The amount of dialyzable iron in low impact extruded products was higher than in boiled legumes. However, the dialyzable iron content of high impact extruded and boiled legume products was similar except in navy beans. In navy beans, both low and high impact extruded products had higher dialyzable iron than the boiled legumes.

The increase in iron dialyzability in low impact extruded products cannot be fully explained. Speculations have been made that during extrusion some of the high molecular weight compounds (including phytate) are degraded, thus releasing iron (Fairweather-Tait *et al.* 1987). The reason for the differences in the effects of low and high impact extrusion processing on iron dialyzability is not readily apparent. The extent of protein denaturation during the extrusion process and the resulting effect on protein solubility may produce variable effects on iron dialyzability.

TABLE 1.
TOTAL IRON AND DIALYZABLE IRON IN BOILED AND EXTRUDED LEGUME PRODUCTS[1]

Sample preparation	Total iron	Dialyzable iron	
	(μg/g dry wt)	(μg/g dry wt)	(% total)
		NAVY BEANS	
Boiled	61.5 ± 1.25	1.68 ± 0.15	2.74 ± 0.25
Extruded-low[2]	70.8 ± 0.42*	2.85 ± 0.27*	4.03 ± 0.38*
Extruded-high[3]	64.7 ± 1.56*	2.89 ± 0.34*	4.47 ± 0.53*
		CHICK PEAS	
Boiled	49.60 ± 0.92	1.34 ± 0.31	2.70 ± 0.10
Extruded-low[2]	51.86 ± 0.26*	2.16 ± 0.25*	4.16 ± 0.48*
Extruded-high[3]	51.94 ± 0.51*	1.43 ± 0.24	2.76 ± 0.47
		COWPEAS	
Boiled	47.90 ± 1.01	0.65 ± 0.07	1.36 ± 0.17
Extruded-low[2]	53.87 ± 0.77*	1.00 ± 0.08*	1.86 ± 0.14*
Extruded-high[3]	53.13 ± 1.26*	0.87 ± 0.07	1.63 ± 0.14
		LENTILS	
Boiled	57.15 ± 1.52	0.68 ± 0.11	1.18 ± 0.20
Extruded-low[2]	61.82 ± 1.72*	1.60 ± 0.21*	2.59 ± 0.35*
Extruded-high[3]	58.95 ± 2.09	0.70 ± 0.08	1.18 ± 0.14

[1]Each value represents the mean ± standard deviation of samples analyzed in triplicate

[2]Legume flour extruded under low impact conditions

[3]Legume flour extruded under high impact conditions

*Student's t-test at 95% confidence indicates significant difference from values for respective boiled legumes

Chemical Forms of Iron

Nonelemental iron in navy beans, chickpeas and cowpeas was significantly increased in the extruded products compared to their respective boiled legumes (Table 2). In lentils, an increase in nonelemental iron was seen only in the low impact extruded product compared to the boiled legume.

An increase in total soluble iron in low impact extruded products compared to the high impact extruded or boiled legume products was observed (Table 2). Iron solubility may be altered by extrusion processing due to the effects of heat, pressure and shear on protein solubility and distribution. Iron solubility may also be increased due to the degradation of phytate that occurs during extrusion processing. Dialyzable iron (Table 1) in all legume products was lower than the total soluble iron (Table 2) because iron has to be both soluble and in a low molecular weight form to be dialyzable.

Ionic iron was also higher in low impact extruded than high impact extruded or boiled navy beans and chickpeas (Table 2). In cowpeas and lentils, there were no significant differences in ionic iron between low impact extruded and high impact extruded products. However, the values were higher than the ionic iron in boiled legumes.

The results of the analyses of iron dialyzability and chemical forms of iron suggest that low impact extrusion processing increased iron solubility and, as a consequence, iron dialyzability. High impact extrusion, on the other hand, did not change iron solubility compared to boiled legumes; hence no differences were seen in iron dialyzability of the two legume products. Ionic iron did not seem to accurately reflect iron dialyzability.

Degradation of Phytate

The inositol tri-, tetra-, penta-, and hexaphosphate contents in raw, boiled, and extruded legume flours are presented in Table 3. Although no changes in total phytate content were observed, processing (either boiling or extrusion) increased the conversion of inositol hexaphosphate to its lower phosphate forms.

Phytic acid in raw legume flours ranged from 66 to 79% of the total inositol phosphate forms whereas, in boiled or extruded legume products, phytic acid content ranged from 20 to 50% only. Extrusion processing did not result in any consistent pattern of degradation of phytic acid. If iron dialyzability is directly correlated to the extent of phytate degradation, greatest conversion of phytate to lower inositol phosphates would be expected in low impact extruded legume products, since iron dialyzability was highest in these products. However, this pattern was observed only in navy beans and chickpeas and not in the case of cowpeas or lentils.

TABLE 2.
NONELEMENTAL, SOLUBLE AND IONIC IRON IN BOILED AND EXTRUDED LEGUME PRODUCTS[1]

Sample preparation	Nonelemental (μg/g dry wt)	Soluble (% total)	Ionic (% total)
	NAVY BEANS		
Boiled	61.5 ± 1.25	7.29 ± 0.15	6.86 ± 0.26
Extruded-low[2]	70.8 ± 0.42*	13.42 ± 0.51*	11.93 ± 0.18*
Extruded-high[3]	64.7 ± 1.56*	7.33 ± 1.12	5.89 ± 0.33*
	CHICK PEAS		
Boiled	49.60 ± 0.92	5.33 ± 1.12	4.04 ± 0.92
Extruded-low[2]	51.86 ± 0.26*	14.02 ± 1.00*	11.73 ± 1.48*
Extruded-high[3]	51.94 ± 0.51*	5.04 ± 0.54	3.43 ± 0.27*
	COWPEAS		
Boiled	47.90 ± 1.01	6.87 ± 0.11	6.15 ± 0.14
Extruded-low[2]	53.87 ± 0.77*	10.14 ± 0.48*	7.09 ± 0.14*
Extruded-high[3]	53.61 ± 1.20*	7.33 ± 1.80	6.93 ± 0.12*
	LENTILS		
Boiled	56.62 ± 0.52	14.13 ± 2.71	5.14 ± 0.50
Extruded-low[2]	59.14 ± 1.12*	21.32 ± 0.92*	7.58 ± 0.09*
Extruded-high[3]	56.39 ± 0.67	13.80 ± 0.74	7.53 ± 0.19*

[1]Each value represents the mean ± standard deviation of samples analyzed in triplicate

[2]Legume flour extruded under low impact conditions

[3]Legume flour extruded under high impact conditions

*Student's t-test at 95% confidence indicates significant difference from values for respective boiled legumes

TABLE 3.
DEGRADATION PRODUCTS OF PHYTIC ACID IN RAW, BOILED AND EXTRUDED LEGUME PRODUCTS[1]

Sample preparation	IP3	IP4	IP5	IP6	Total phytate (μmole/g dry wt)
	—% total phytate—				
NAVY BEAN					
Raw	0	5.4	15.4	79.3	14.0 ± 1.9
Boiled	8.4	16.5	28.1	47.0	14.0 ± 0.6
Extruded-low[2]	9.5	21.5	37.1	37.4	15.4 ± 0.1
Extruded-high[3]	10.8	13.6	25.9	49.8	14.6 ± 0.2
CHICKPEA					
Raw	0	13.4	20.7	66.0	7.7 ± 0.0
Boiled	25.3	19.0	21.9	31.0	8.7 ± 0.1
Extruded-low[2]	25.2	25.7	25.2	24.6	6.1 ± 0.4
Extruded-high[3]	9.9	14.0	27.5	48.7	8.2 ± 0.1
COWPEA					
Raw	0	7.1	20.8	72.3	11.5 ± 0.3
Boiled	27.1	19.7	22.2	31.0	11.1 ± 2.1
Extruded-low[2]	14.5	24.6	29.4	31.6	9.6 ± 0.1
Extruded-high[3]	21.3	31.9	27.1	19.7	9.8 ± 0.3
LENTIL					
Raw	0	10.8	22.8	66.6	5.2 ± 0.1
Boiled	36.8	14.4	16.9	31.9	4.5 ± 0.4
Extruded-low[2]	16.9	19.7	28.2	35.4	5.2 ± 0.6
Extruded-high[3]	14.7	26.0	30.5	28.8	4.9 ± 0.1

[1]Each value represents the mean or mean ± standard deviation of samples analyzed in triplicate

[2]Legume flour extruded under low impact conditions

[3]Legume flour extruded under high impact conditions

Previously, Sandberg *et al.* (1989) reported that only inositol hexa- and pentaphosphates decreased iron solubility at simulated physiological conditions, and their degradation seemed to significantly reduce the inhibiting effect of phytate on iron availability. Sandberg and Svanberg (1991) reported that complete hydrolysis of inositol hexa- and pentaphosphates through activation of endogenous phytase led to a strong increase in in-vitro iron availability. Our results suggest that although extrusion processing causes degradation of phytate, the presence of different forms of inositol phosphates does not appear to explain the varying effects of low impact and high impact extrusion on iron dialyzability.

TABLE 4.
TANNIN CONTENT (MG CATECHIN EQUIVALENTS/G DRY WEIGHT) OF RAW, BOILED AND EXTRUDED LEGUME PRODUCTS[1]

Legume[2]	Raw	Boiled	Extrusion condition	
			low[3]	high[4]
Chickpea	2.32±0.3	2.64±0.3	1.10±0.3*	1.61±0.1*
Cowpea	2.91±0.1	2.31±0.1*	1.97±0.0*	1.91±0.0*
Lentil	7.65±0.2	7.98±0.8	2.22±0.1*	1.84±0.2*

[1]Each value represents the mean ± standard deviation of samples analyzed in triplicate

[2]Navy beans showed no detectable tannins

[3]Legume flour extruded under low impact conditions

[4]Legume flour extruded under high impact conditions

*ANOVA test at 95% confidence level indicates significant difference from values for respective raw legumes.

Tannin Content

Extrusion processing decreased the tannin content of legume flours by about 31–76% compared to raw legumes (Table 4). The effects of low impact and high impact extrusion processing on iron dialyzability cannot be explained by the decrease in tannin content of extruded legume flours, since the decrease was seen in both low impact and high impact extrusion processing. Boiling decreased tannin content by 20% in cowpeas only. No detectable tannins were seen in raw, boiled or extruded navy bean products.

Boiling and extrusion processing produced different effects on the tannin content of legumes. In the preparation of boiled legumes for analyses, the water used for soaking or boiling the legumes was used for homogenization. Thus, losses of tannins due to leaching during soaking or boiling were avoided. Another speculation is that during extrusion, tannins may complex with other components of legumes, such as proteins or sugar, and may become undetectable by the vanillin assay.

The factors investigated in our study, phytates and tannins, do not seem to fully explain the varying effects of extrusion processing on iron dialyzability. Another component of legumes thought to interfere with iron bioavailability is fiber. The modification of fiber during extrusion processing is not fully elucidated; however, it has been shown that at mild or moderately severe conditions, extrusion does not significantly change dietary fiber content but solubilizes some fiber components. At more severe conditions, the dietary fiber content is seen to increase, mainly due to the formation of enzyme-resistant starch fractions (Asp and Björck 1989; Theander and Westerlund 1987). A redistribution of insoluble to soluble dietary fiber in extruded wheat flour also has been reported (Björck *et al.* 1984). The extent to which the effects of extrusion processing on iron dialyzability in legumes might be attributed to alterations in the distribution or characteristics of fiber components is yet to be determined. The role of other factors such as competing minerals, lignin complexes and the interactions between the various components in determining the effects of extrusion on iron bioavailability needs to be investigated.

REFERENCES

ASP, N.G. and BJÖRCK, I. 1989. Nutritional properties of extruded foods. In *Extrusion Cooking*, (C. Mercier, P. Linko and J.M. Harper, eds.) pp. 399–434, Am. Assoc. Cereal Chem., St. Paul, MN.

BJORCK, I., ASP, N.G., BIRKHED, D. and LUNDQUIST, I. 1984. Effects of processing on availability of starch for digestion in vitro and in vivo. I. Extrusion cooking of wheat flours and starch. J. Cereal Sci. *2*, 91–103.

BRUNE, M., ROSSANDER-HULTEN, L., HALLBERG, L., GLEERUP, A. and SANDBERG, A.S. 1992. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J. Nutr. *122*, 442–449.

FAIRWEATHER-TAIT, S.J., SYMSS, L.L., SMITH, A.C. and JOHNSON, I.T. 1987. The effect of extrusion cooking on iron absorption from maize and potato. J. Sci. Food Agric. *39*, 341–348.

GRAF, E. and DINTZIS, F.R. 1982. Determination of phytic acid in foods by high performance liquid chromatography. J. Agric. Food Chem. *30*, 1094–1097.

HALLBERG, L., ROSSANDER, L. and SKANBERG, A.B. 1987. Phytates and the inhibitory effect of bran on iron absorption in man. Am J. Clin. Nutr. *45*, 988–996.

HAZELL, T. and JOHNSON, I.T. 1989. Influence of food processing on iron availability in vitro from extruded maize-based snack foods. J. Sci. Food Agric. *46*, 365–374.

LEE, K. and CLYDESDALE, F.M. 1979. Quantitative determination of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Food Sci. *44*, 549–554.

LOMBARDI-BOCCIA, G., DILULLO, G. and CARNOVALE, E. 1991. In-vitro dialysability from legumes: influence of phytate and extrusion cooking. J. Sci. Food Agric. *48*, 599–605.

MILLER, D.D., SCHRICKER, B.R., RASMUSSEN, R.R. and CAMPEN, D.V. 1981. An in vitro method for estimation of iron availability from meals. Am. J. Clin. Nutr. *34*, 2248–2256.

MORROW, B. 1991. The rebirth of legumes. Food Technol. *45*, 96, 121.

O'DELL, B.L. 1984. Bioavailability of trace elements. Nutr. Rev. *42*, 301–308.

PRICE, M.L., SCOYOC, S.V. and BUTLER, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. *26*, 1214–1218.

RAO, B.S.N. and PRABHAVATHI, T. 1982. Tannin content of foods commonly consumed in India and its influence on ionisable iron. J. Sci. Food Agric. *33*, 89–96.

SANDBERG, A.S. and AHDERINNE, R. 1986. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. J. Food Sci. *51*, 547–550.

SANDBERG, A.S., CARLSSON, N.G. and SVANBERG, U. 1989. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. J. Food Sci. *54*, 159–161.

SANDBERG, A.S. and SVANBERG, U. 1991. Phytate hydrolysis by phytase in cereals: effects on in vitro estimation of iron availability. J. Food Sci. *56*, 1330–1333.

TANNER, J.T. and WHITTAKER, P. 1989. Comparison of in vitro, animal, and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability. Am. J. Clin. Nutr. *49*, 225–238.

THEANDER, O. and WESTERLUND, E. 1987. Studies on chemical modifications in heat-processed starch and wheat flour. Staerke *39*, 88–93.

TORRANCE, J.D., GILLOY, M., MILLS, W., MAYET, F. and BOTHWELL, T.H. 1982. Vegetable polyphenols and iron absorption. In *The Biochemistry and Physiology of Iron*, (P. Saltman and J. Hegenauer, eds.) pp. 819–820, Elsevier Biomedical, New York.

THE ROLE OF PULP INTERPARTICLE INTERACTION IN DETERMINING TOMATO JUICE VISCOSITY

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ABSTRACT

Pulp interparticle interaction plays a major role in determining the viscosity of tomato juice. Indices of second order and of compounded high order interparticle interaction have been evaluated by diluting tomato juice samples with their own serum, and determining the slope of the reduced viscosity curves at the very dilute and at the original concentration regions, respectively. The second order interaction index, reflecting the behavior of a pair of colliding particles, has practically a similar value for samples having the same process history. On the other hand, tomato juice samples differ markedly in their value of the interaction index describing the effect of high order interparticle interaction on the viscosity at the original juice concentration. The higher that value, the higher the viscosity. The value of this index decreases by concentrating the tomato juice and increases by homogenization. It has a maximum value at pH 4–4.5 and most importantly it is greatly dependent on the mechanical abuse history of the juice. Enzymatic depolymerization of the soluble and especially the insoluble pectin markedly reduces the value of the interaction index, indicating that pectin plays a major role in determining the level of that interparticle interaction.

INTRODUCTION

Much has been published on the relationship between tomato juice viscosity and different compositional characteristics, but few attempts have been made to establish this relationship quantitatively. Tanglertpaibul and Rao (1987a) have found a correlation between the apparent viscosity of the juice, as well as of the concentrate, and the serum viscosity and the pulp content. The relative

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contribution of the pulp content to the juice viscosity was larger than that of the serum viscosity, which in turn was also correlated with the concentration of soluble pectin. Similarly, Marsh *et al.* (1980) have developed an empirical correlation between consistency of the concentrate and of the juice and the ratio between the water insoluble solids (WIS) and the total solids. In both cases, the main focus was the consistency of the concentrate rather than that of the single strength juice.

A different approach was taken by Takada and Nelson (1983a) who established an empirical correlation between the juice viscosity, and the precipitate weight ratio (PPT), which is the weight percent of the pulp obtained by centrifugation at 12880×g for 30 min at 4C. In this study, the pulp content was the only important factor that was responsible for determining the tomato juice viscosity.

Although of different cultivars, the tomato juice and concentrate samples in each of these studies were obtained by more or less the same processing techniques. It is doubtful whether the established empirical correlations are applicable to variations in processing conditions. Moreover, these correlations do not account for the effect of particle size distribution on the apparent viscosity of tomato juice (Tanglertpaibul and Rao 1987b), and they also cannot explain the large drop in viscosity of reconstituted juice (Harper and El Sahrigi 1965; Mannheim and Kopelman 1964).

The present study is an investigation of the role of other primary factors affecting tomato juice viscosity, mainly of pulp interparticle interaction, and how they are influenced by different processing conditions.

MATERIALS AND METHODS

Commercial tomato juice and concentrate samples of cultivar M-82-1-8 were obtained from a local plant (Pardess Citrus Products, Yavneh, Israel) during four consecutive seasons.

Serum of the tomato juice was produced by centrifugation at 2360×g for 30 min. Volumetric pulp content (%v/v) was determined in duplicate, by centrifuging 50 g of juice sample at 1100×g for 20 min and measuring the volume of the wet precipitate. Water insoluble solids (WIS) were determined similarly, except that the precipitate was resuspended in distilled water and reprecipitated by centrifugation until the supernatant was free of soluble solids. The precipitate was then dried in a vacuum oven at 60C until constant weight was achieved.

The viscosity of tomato juice was determined by using an industrial efflux tube viscometer. The analysis was carried out at 20.0±0.05C, or at other

temperatures as indicated, by measuring the flow time in a pipette consisting of an insulated cylinder (40 mm i.d., by 175 mm height) with an outlet capillary (2.2 mm i.d., by 300 mm height). The flow time of the sample was measured by the travel time of the upper liquid meniscus from the top of the cylinder to a mark 95 mm below it. Under these conditions the flow time of water at 20C was 25 s. The results of the juice viscosity determination are expressed in terms of relative viscosity, which is the ratio between the flow time of the juice and that of the water. Serum viscosity relative to water was determined by an Ostwald-Cannon-Fenske capillary viscometer at 20 ± 0.1C.

The interaction indices were evaluated by determining the reduced viscosity (η_{red}) of the tomato juice that was diluted with its own serum. The reduced viscosity was calculated from the following expression:

$$\eta_{red} = \left(\frac{\eta_j}{\eta_s} - 1\right) / WIS \qquad (1)$$

where: η_j is the relative juice viscosity
η_s is the relative serum viscosity
WIS is the concentration of water insoluble solid (%)

Enzymatic treatment of pulp or serum was carried out using Pectinex 3XL (Swiss Ferment) at room temperature. After treatment, the enzyme was thermally inactivated. Different levels of serum viscosity were obtained by mixing enzymatically depectinized serum with an untreated one. The pulp and serum were recombined after treatment and gently mixed to obtain a tailor-made juice sample.

Adjusting of the juice pH was carried out by using 4N HCl or 4N NaOH to change the pH below and above the original value, respectively. In all cases, all samples were also adjusted to contain the same level of added liquid and chloride by adding 4N NaCl.

Laboratory concentration of tomato juice was carried out at 35C under vacuum in a rotary evaporator (Rotavap, Ika-Werk, Germany). The external water bath temperature was 53C. Concentrate samples were withdrawn at different stages of the process and were reconstituted to the same Brix as that of the original juice and tested for viscosity.

Using the same procedure, but at room temperature and with no vacuum, tomato juice samples were subjected only to the same mechanical history, namely the same length of mixing time in the evaporator, before measuring their viscosity.

Concentration of juice was also carried out, with no mechanical effect, by placing the tomato juice, into which 2000 ppm of sodium benzoate was added,

in cellulose dialysis tubing and leaving it at room temperature to evaporate through the film until a concentration of 12 Brix was achieved. This concentrate was then diluted to original concentration for viscosity determination.

Data analysis was carried out using stepwise regression analysis.

RESULTS AND DISCUSSION

Problems in Correlating Viscosity to Composition

The data in Table 1 presents three typical groups of tomato juice samples and indicates the problem of correlating the viscosity to compositional parameters. In the first group (samples 1, 2 and 3) the viscosity is about the same, yet the composition is different. The first and third samples have the same serum viscosity but differ in pulp content. On the other hand, the second and third samples have the same pulp content but different serum viscosity. Moreover, the viscosity of the third sample combines the effect of low pulp content and low serum viscosity, and yet has about the same value as the other two samples.

The second group (samples 4 and 5) shows a typical increase in viscosity by mechanical means (homogenization) with no change in any of the compositional parameters. In a similar way, the third group (samples 6, 7, 8 and 9) shows the well-known phenomenon of a drop in the viscosity of tomato juice that has been concentrated and reconstituted. Here again the large change in viscosity would not be expected on the basis of the negligible changes in composition.

Viscosity-Dilution Curves

A reduced viscosity curve of tomato juice sample, diluted with its own serum, (Fig. 1) indicates a fast increase in the slope at high water insoluble solids (WIS) concentration. Similar curves are obtained when analyzing systems of stable dispersions, which rheological behavior was reviewed for rigid particulates, among others, by Frish and Simha (1956), Russel (1980) and Goodwin (1987). Normally, the reduced viscosity is expressed as a power series of the volume fraction (ϕ) of the dispersed particulates. The typical equation will have the following form:

$$\eta_{red} = [\eta] + k_2\phi^2 + \text{high order terms} \tag{2}$$

TABLE 1.
CHARACTERISTICS OF THREE GROUPS OF TOMATO JUICE SAMPLES

Group	No	Sample Description	Juice relative viscosity	Serum relative viscosity	Volumetric Pulp content (%v/v)	Water insoluble solids (%w/w)	Main Interaction index
I	1	Commercial sample	18.7	1.25	36	1.13	32
	2	"	19.4	2.46	31	1.01	39
	3	"	17.7	1.25	31	0.96	65
II	4	Commercial sample	27.7	2.04	-	0.86	43
	5	Homogenized	36.3	2.04	-	0.87	54
III	6	Commercial sample	25.8	1.36	38	0.96	25
	7	Reconstituted from 9.7 Brix	7.9	1.36	-	-	20
	8	Reconstituted from 14.5 Brix	8.6	1.32	29	0.93	18
	9	Reconstituted from 27.3 Brix	8.1	1.30	26.5	0.96	12

where $[\eta]$ is the intrinsic viscosity, having a value of 2.5 for uncharged spheres (Einstein 1906) and k_2 is a coefficient accounting for the second order interaction, namely between a pair of colliding particles. A number of models have been proposed to evaluate k_2.

The curve in Fig. 1 clearly indicates, from the fast changing slope, that high order interaction plays an important role in determining the viscosity of the tomato juice. No physical model is available to evaluate the parameters of such high order interaction, especially for soft particulates such as tomato pulp.

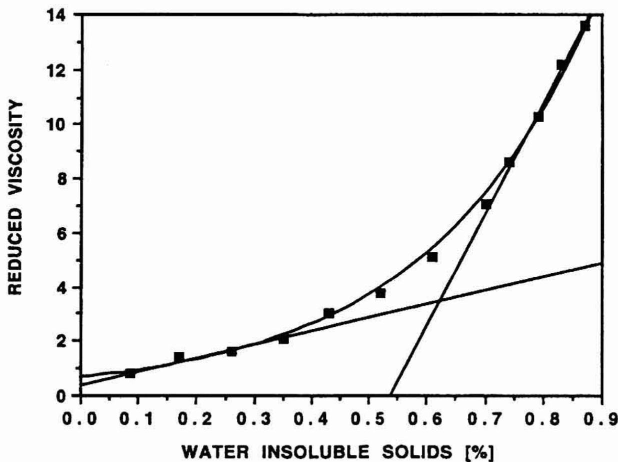

FIG. 1. TYPICAL REDUCED VISCOSITY CURVE OF TOMATO JUICE DILUTED WITH ITS OWN SERUM

Nevertheless, the slope of the reduced viscosity curve of the tomato juice, at normal concentration still provides very valuable information about the compounded effect of such high order interactions. The value of this slope is denoted as the main interaction index (M). In addition, the dilution curve also provides information on the intrinsic viscosity, $[\eta]$, (the intercept) and the second order interaction index (S), namely the initial slope. In this study all these values are given in terms of WIS rather than in volume fraction, which value is changing with the method of its determination. Therefore the interaction indices and intrinsic viscosity values cannot be compared with theoretical models.

The value of the main interaction index, evaluated for the samples in Table 1, apparently accounts for the similarity in the viscosity of the samples of the first group where it seems that interparticle interaction compensates for low serum viscosity or pulp content, as well as for the changes in viscosity in the other two groups. Juice homogenization increases the value of the interaction index, whereas concentration and reconstitution decreases it (Table 1).

It is interesting to note that in the case of the reconstituted juice samples (samples 7, 8 and 9), the reduction in the value of the main interaction index, as compared to the original juice, is also coupled with a decrease in the volumetric pulp content (Table 1). Since there is no change in the concentration of water insoluble solids, the value of the volumetric pulp content has to do with particulates that are capable of forming more voluminous aggregates due to higher interparticle interaction. The volume of the pulp, precipitated by centrifugation, depends therefore, not only on the actual mass of insoluble material but also on the extent of interparticles interaction. This should be true also for the precipitate weight ratio (Takada and Nelson 1983a).

TABLE 2.
VALUES OF SECOND ORDER INTERACTION INDEX AND OF PARTICLES INTRINSIC VISCOSITY FOR ORIGINAL AND RECONSTITUTED TOMATO JUICE SAMPLES

Juice Samples	Number of samples	Second order interaction index (S)	Particle intrinsic viscosity [η]
Commercial	10	5.00 ± 0.04 (S.E.)	0.42 ± 0.04 (S.E.)
Reconstituted	10	3.30 ± 0.04 (S.E.)	0.45 ± 0.04 (S.E.)

Mathematical Model

It is interesting to note that there is a typical second order interaction index (S) of 5.0 and 3.3 for regular commercial and reconstituted juice samples, respectively (Table 2). Moreover, there is no statistically significant difference in the value of particle intrinsic viscosity $[\eta]$ for these two groups.

Based on Fig. 1, the reduced viscosity of the original tomato juice can be expressed with the aid of two lines (Fig. 1), that are drawn to evaluate the two interaction indices, as follows:

$$\eta_{red} = [\eta] + S \times WIS_i + M(WIS_o - WIS_i) \quad (3)$$

where:

WIS_i = water insoluble solids at the intersection of the two lines
WIS_o = original concentration of water insoluble solids

The intersection point (WIS_i) between the two lines can be therefore calculated by:

$$WIS_i = \frac{\eta_{red} - [\eta] - M \times WIS_o}{S - M}$$

Calculated values of WIS_i for 39 samples of tomato juice, including reconstituted samples, were found to correlate well (Correlation coefficient R = 0.896) with WIS_o, resulting in an equation of the form:

$$WIS_i = a \times WIS_o \quad (5)$$

where a is a constant, having a value of 0.72.

Combining Eq. 3 and 5, results in an equation having the following general form:

$$\eta_{red} = [\eta] + C_1 \times S \times WIS_o + C_2 \times M \times WIS_o \quad (6)$$

where C_1 and C_2 are constants.

Using values of $[\eta] = 0.45$, $S = 5.0$ or 3.3 for regular or reconstituted samples (Table 2), respectively, as well as the values of M obtained for each sample, a regression analysis of the 39 different test juice samples resulted in the following empirical equation:

$$\eta_{red} = 0.45 + 1.1 \times S \times WIS_o + 0.224 \times M \times WIS_o = 0.45 + WIS_o \times (1.1\ S + 0.224 \times M) \quad (7)$$

$(R = 0.997)$.

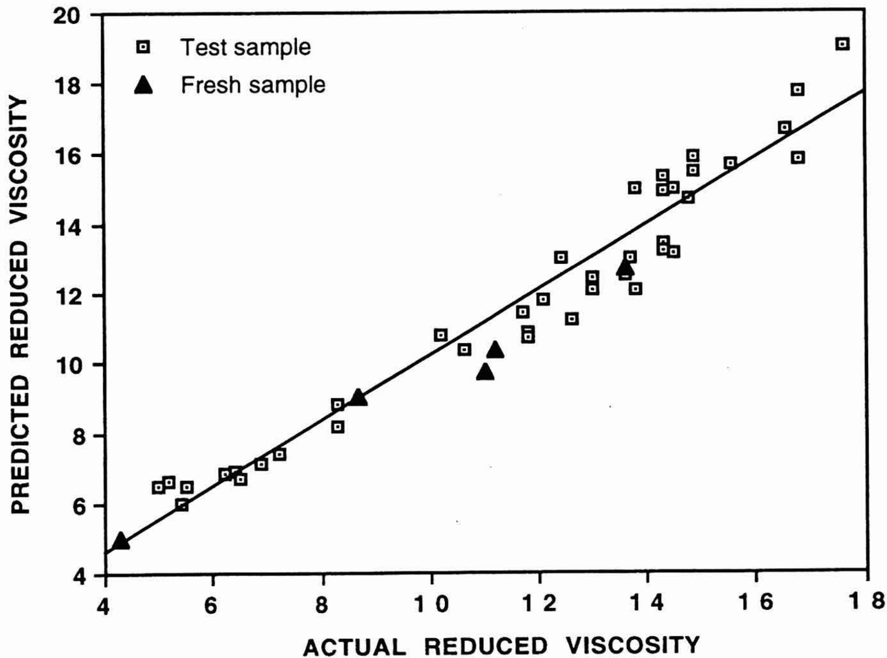

FIG. 2. CALCULATED AND EXPERIMENTAL VALUES OF REDUCED VISCOSITY OF DIFFERENT COMMERCIAL AND RECONSTITUTED TOMATO JUICE SAMPLES

The correlation between the predicted and actual values (Fig. 2) shows a good agreement also with the values of an additional five fresh samples that were not included originally in the regression analysis. These five samples represent a wide range of viscosity.

TABLE 3.
THE EFFECT OF DEPOLYMERIZATION OF THE PECTIN IN THE PULP ON THE MAIN INTERACTION INDEX

Enzyme concentration (p p m)	Enzyme action time (min)	Juice relative viscosity	Serum relative viscosity	Water insoluble solids (% w/w)	Main interaction index
0	0	32.8	2.16	1.05	32
10	1.5	28.6	2.08	1.03	28
100	3.0	20.9	2.00	1.02	19
100	14.40	15.5	1.96	1.03	12

Effect of Pectin on Interparticle Interaction

Enzymatic depolymerization of the pectin in the pulp particulates causes a large reduction in the value of the main interaction index (Table 3) and thus in the viscosity. In this case again, the reduction of viscosity is accomplished with negligible changes in the concentration of water insoluble solids or other commonly used compositional parameters.

TABLE 4.
THE EFFECT OF DEPOLYMERIZATION OF SOLUBLE PECTIN IN THE SERUM ON THE MAIN INTERACTION INDEX

Serum relative viscosity	Water insoluble solids W.I.S. (%w/w)	Juice relative viscosity	Main interaction index
2.28	1.14	39.1	32
1.92	1.16	33.5	30
1.72	1.14	28.1	27
1.52	1.10	21.9	25
1.16	1.10	13.3	18

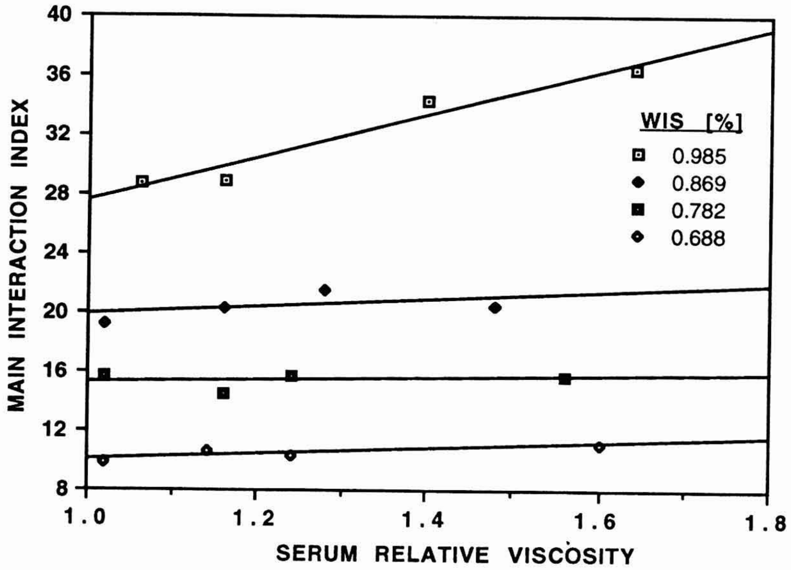

FIG. 3. THE EFFECT OF pH ON THE VALUE OF THE MAIN INTERACTION INDEX

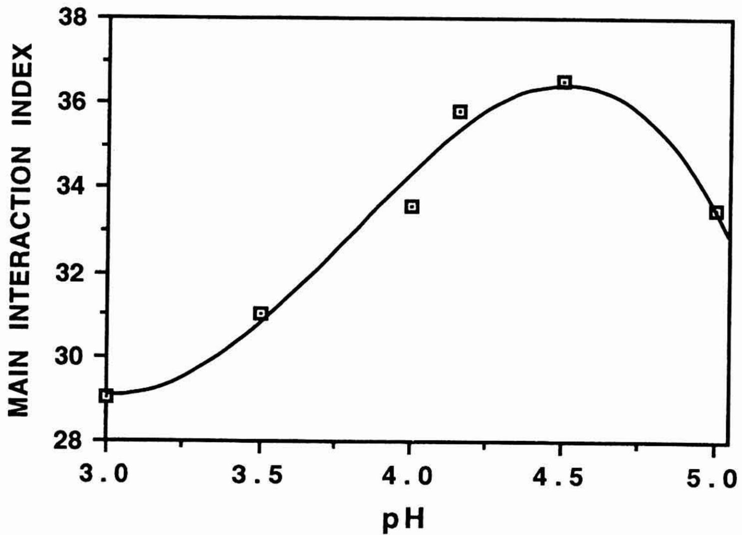

FIG. 4. THE EFFECT OF THE SERUM VISCOSITY AND THE CONTENT OF WATER INSOLUBLE SOLIDS ON THE VALUE OF THE MAIN INTERACTION INDEX

In a similar way to the pulp, enzymatic depolymerization of the soluble pectin in the serum also reduces the value of the main interaction index and thus the juice viscosity (Table 4). However, the soluble pectin affects the interaction index only at high WIS levels (Fig. 3). On the other hand, at a low enough level of WIS, the effect of soluble pectin on the value of the interaction index is practically negligible.

Data in Fig. 4 show a maximum value for the main interaction index at a pH of 4.5. This phenomenon is in accordance with the findings of Takada and Nelson (1983b) who have reported that the maximum juice viscosity is reached between pH 4 and 4.5, a result that was attributed to pectin/protein interaction.

It is clear that pectin, soluble and insoluble, plays an important role in the pulp interparticle interaction, probably through electrostatic attraction between the negatively charged pectin and the positively charged protein, which is present in the particles. However, it is also possible that the pectin exerts its effect via polymer entanglement as well as hydrogen and calcium pectate bonds.

Effect of Juice Concentration on Its Viscosity

One of the puzzling questions in the industry is how the process of concentrating and reconstituting of tomato juice impairs its viscosity. According to the findings discussed above, this question may be translated into why there is a considerable drop in the value of the main interaction index during the concentration process. One possible explanation is the damage caused to the pectin, either soluble or insoluble, by the exposure to elevated temperatures in the evaporator. However, data in Fig. 5 clearly show that a relatively very long exposure time is needed at temperatures of 75C and above to cause appreciable viscosity drop. The experimental time here is much longer than the normal residence time of the juice in a commercial evaporator. Therefore, the time-temperature history of the tomato juice can hardly be the main cause for the considerable drop of the viscosity of reconstituted juice.

However, when subjecting tomato juice only to the same mechanical mixing history as that of the concentrate, even at room temperature without any evaporation, its viscosity dropped to the same extent (Fig. 6). Moreover, when tomato juice, concentrated in a dialysis tube by evaporation through the film with no stirring at all, was reconstituted, the resulting tomato juice was of the same viscosity as that of the original juice. These results clearly indicate that mechanical effects, probably by breaking of aggregates that are formed and held together through the interparticle interaction, may play an important part in determining the viscosity of the tomato juice. The mechanical effect is practically irreversible and the reconstituted juice does not regain its original viscosity.

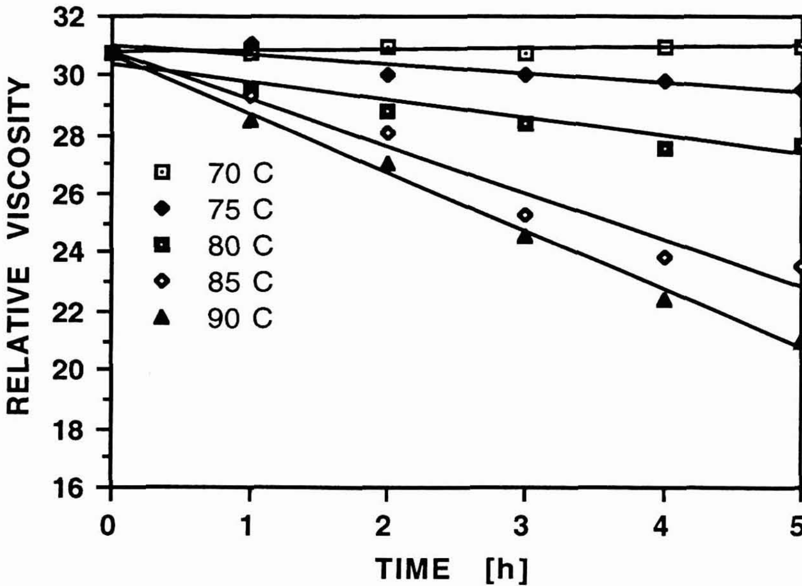

FIG. 5. EFFECT OF TIME-TEMPERATURE HISTORY ON TOMATO JUICE VISCOSITY

Minimizing mechanical abuse of the product should be, therefore, one of the main processing considerations. Producing 22 Brix concentrate by direct concentration or by concentrating first to 31 Brix and cutting back with tomato juice (Table 5) resulted in different viscosities of the reconstituted juice. The fact that the "cut back" 22 Brix concentrate (sample 4) contained a quantity of "unabused" juice resulted in a higher reconstituted juice viscosity.

Mechanical effects play a dual role. As discussed above, they may reduce interparticle interaction, thus also viscosity. On the other hand, a strong enough mechanical treatment such as homogenization, is sometimes used in the industry to increase the viscosity. Therefore, depending on the properties of the tomato juice and on the intensity of the mechanical effect, one may expect either an increase or a decrease in viscosity. This may explain Crandall and Nelson's (1975) findings that anything can be expected in the mechanical treatment of tomato juice, namely increase, decrease or no change in viscosity.

In order to evaluate the apparent juice viscosity in more common units, Eq. 7 can be modified, taking into account the definition of reduced viscosity and the relative serum viscosity (η_s), as follows:

$$\eta = 10^{-3}\eta_s\,[1 + 0.45\ \mathrm{WIS_o} + \mathrm{WIS^2_o}\ (1.1\mathrm{S} + 0.224\ \mathrm{M})] \qquad (8)$$

where η = tomato juice viscosity in Pa.s

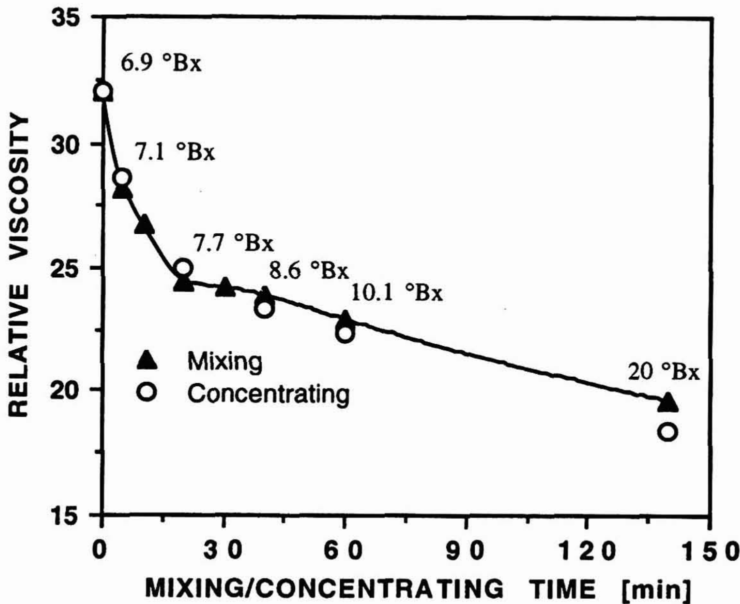

FIG. 6. EFFECT OF MIXING AND CONCENTRATION HISTORY ON THE JUICE VISCOSITY

TABLE 5.
EFFECT OF MODE OF CONCENTRATE PREPARATION ON RECONSTITUTED JUICE VISCOSITY

Sample No	Concentrate description	Juice relative viscosity
1	Original Juice	38.6
2	22 Brix	12.5
3	31Brix	11.5
4	22 Brix from 31 Brix by cut-back with original juice	16.5

This empirical equation is valuable in understanding the factors affecting viscosity. However, it should be noted that this equation may be of limited use for prediction of the tomato juice viscosity in practical applications. The reason is simply due to the fact that the interaction indices that appear in the equation should be determined by analysis of the actual juice sample. The same may be true for determination of serum viscosity and of precipitate weight ratio.

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REFERENCES

CRANDALL, P.G. and NELSON, P.E. 1975. Effects of preparation and milling on consistency of tomato juice and puree. J. Food Sci. *40*, 710–713.

EINSTEIN, A. 1906. *Investigations on Theory of Brownian Movement*, Dover, New York.

FRISCH, H.L. and SIMHA, R. 1956. The viscosity of colloidal suspensions and macromolecular solutions. In *Rheology Theory and Application* (F.R. Eirich, ed.) pp. 525-591, Academic Press, San Diego, CA.

GOODWIN, J.W. 1987. The rheology of colloidal dispersions. In *Solid/Liquid Dispersions*, (Th.F. Tadros, ed.) pp. 219–221, Academic Press, San Diego, CA.

HARPER, J.C. and El SAHRIGI, A.F. 1965. Viscometric behavior of tomato concentrates. J. Food Sci. *301*, 470–476.

MANNHEIM, C.H. and KOPELMAN, I.J. 1964. Evaluation of two methods of juice concentration. II. Product comparison. Food Technol. *18*, 911–914.

MARSH, G.L., BUHLERT, J.E. and LEONARD, S.J. 1980. Effect of composition upon Bostwick consistency of tomato concentrate. J. Food Sci. 45, 703–706.

RUSSEL, W.B. 1980. Review of the role of colloidal forces in the rheology of suspensions. *J. Rheol. 24*(3)287–317.

TAKADA, N. and NELSON, P.E. 1983a. A new consistency method for tomato products: The precipitate weight ratio. J. Food Sci. *48*, 1460–1462.

TAKADA, N. and NELSON, P.E. 1983b. Pectin-protein interaction in tomato products. *J. Food Sci. 48*, 1408–1411.

TANGLERTPAIBUL, T. and RAO, M.A. 1987a. Flow properties of tomato concentrates: effect of serum viscosity and pulp content. J. Food Sci. *52*, 318–321.

TANGLERTPAIBUL, T. and RAO, M.A. 1987b. Rheological properties of tomato concentrates as affected by particle size and methods of concentration. J. Food Sci. *52*, 141–145.

IMPROVING THE FUNCTIONAL AND BREAD MAKING PROPERTIES OF SPROUTED INDIAN WHEAT

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ABSTRACT

The effect of reducing dough pH to 4.2 and addition of 2–4% gluten on rheological and pasting properties of sound and sprouted wheat flours are reported. Baking properties studied using "Medium Time Fermentation" and "Short Time Fermentation" methods revealed that an acceptable bread could be produced from even highly sprouted wheat by lowering the pH and adding 4% vital gluten to the flour, and using the "Short Time Fermentation Method".

INTRODUCTION

Adverse effects of sprouting due to increased enzymatic activity on the bread making properties of wheat are well documented. Reducing or circumventing the adverse effects of increased enzymatic activity has been attempted. Reducing pH of slurry and dough to inactivate the enzyme were attempted by several workers (Meredith 1970; Fuller *et al.* 1970; Dorfer and Koball 1980; Westermarck-Rosendahl *et al.* 1980). Addition of multivalent salts (Ca, Fe) or a diaminocarboxylic acid or their mixture had also been tried (Gerbhardt and Wiesener 1979). Blending and pearling to reduce the amylases activity of sprouted wheat was also studied (Liu *et al.* 1986; Henry *et al.* 1987; Sharma *et al.* 1988; Sekhon *et al.* 1992). Attempts have also been made to modify the baking process for producing acceptable quality baked products from sprouted wheats. A combination of lowering the pH, increasing the salt concentration and adding L-cystine HCl was found to be effective in producing acceptable quality bread from high maltose-value flours (Ranhotra *et al.* 1977). These approaches

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were mainly directed to reduce or inactivate the enzyme activity and proved to be successful to varying degrees. One of the important aspects in sprouted wheats is the damage to gluten caused by the increased proteolytic activity (Singh *et al.* 1987). This damage may have a detrimental effect on medium-protein and weak-gluten Indian wheats (Sekhon *et al.* 1992). This paper describes the combined effect of pH, salt and added vital gluten along with the modification of the baking process on functional and baking properties of sound and sprouted wheat.

MATERIALS AND METHODS

Preparation of Samples

Representative samples of wheat variety WL-1562 were procured from 1990–91 harvest and sprouted as described earlier (Singh *et al.* 1987).

Milling

The cleaned samples were conditioned to 14% moisture and milled in a Buhler Pneumatic Mill (MLU-202, Buhler Brothers, Uzwil, Switzerland) after determining the appropriate conditions of feed rate and roll settings for the mill. The yield of straight grade flour was calculated on recovered product basis.

Chemical Composition

Analytical methods for ash, protein, total sugars and diastatic activity were carried out as per AACC (1976) methods. Color grade values were determined using Kent-Jones and Martin flour color grader (Series III). To study the gluten strength the SDS-sedimentation method of Axford *et al.* (1979) was used. The pH of the dough was determined as described earlier (Harinder and Bains 1988).

Farinographic Characteristics

Constant weight flour method (MCC 1976) was followed for farinographs. Preliminary experiments were carried out to determine the quantity of 0.1 N HCl (2.5 ml/100 g flour) necessary to obtain the desired pH of 4.2 in the dough system. The pH of dough for normal and the two sprouted wheat flour samples was 5.6, 5.5 and 5.4, respectively. Salt (2% on a flour weight basis) was added to study the combined effect of lower pH and salt.

Pasting Properties

Brabender Visco-amylograph was used to study the pasting behavior of the flour. The amounts of acid and salt were added on the system weight basis (flour and water) such as the amounts simulated the concentration of salt in the bread system. Samples (60 g) containing optional ingredients (vital gluten of 2 or 4%) were mixed with 450 ml distilled water. The contents were mixed to smooth dispersion, transferred to the Visco-amylograph bowl and brought to 35C and then heated to 95C at the rate of 1.5C/min and held at 95C for 15 min and cooled at the same rate to 50C. Similarly, the effects of adding vital gluten and change in pH plus salt on falling number values were studied using AACC (1976) methods.

Baking Performance

For pan bread making "Medium Time Fermentation" (Fig. 1) and "Short Time Fermentation" (Fig. 2) methods were used for normal and sprouted wheat flours. The formulas used are given in Table 1. All ingredients were mixed to optimum development in a mixing bowl (National Manufacturing Company, Lincoln, NE). The kneading was finished at 30C into smooth dough by stretching and folding manually and fermented in a fermentation cabinet (National Manufacturing Company, Lincoln, NE) maintained at 30C and 85% RH. The dough was remixed in the same mixer and relaxed in the fermentation cabinet. The dough was sheeted and moulded on a National moulder, after which it was proofed and then baked for 25 min at 235C in a reel-type test baking oven (National Manufacturing Company, Lincoln, NE). Bread volume was measured by rapeseed displacement and the breads were organoleptically evaluated as described earlier (Singh *et al.* 1990).

Statistical Analysis

The data were statistically analyzed using two factors in randomized block design as described by Steel and Torrie (1960) using HP-1000 computer system.

RESULTS AND DISCUSSION

Chemical Composition

Sprouting of wheat resulted in a significant reduction in flour yield, the decrease being 10% in flour milled from 48 h sprouted wheat (Table 2). This decrease in flour yield of sprouted wheat could be attributed to heavy yield of

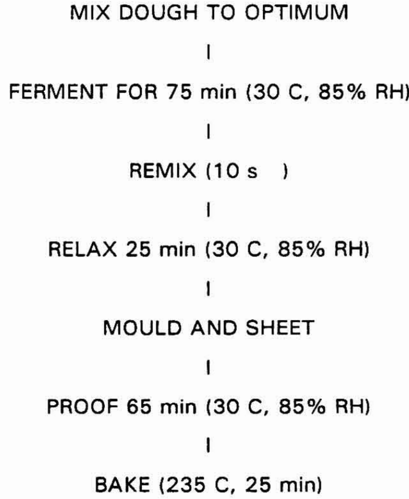

FIG. 1. SCHEME FOR MEDIUM TIME FERMENTATION METHOD BAKING SCHEDULE

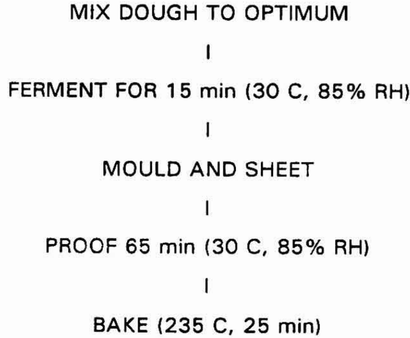

FIG. 2. SCHEME FOR SHORT TIME FERMENTATION METHOD BAKING SCHEDULE

shorts. Similar results have been reported earlier by Sekhon *et al.* (1992). Flour milled from sprouted wheat showed significantly higher protein and ash content. However, the differences were nonsignificant between flours milled from 24 and 48 h sprouted wheat. The higher protein and ash content in flours from sprouted wheat could be due to loss of starch in sprouted grains and the higher proportion

TABLE 1.
INGREDIENTS USED IN "MEDIUM TIME FERMENTATION" AND "SHORT TIME FERMENTATION" METHODS

Ingredients	Baking Method	
	Medium Time	Short Time
Flour (14% mb, g)	100	100
Salt (g)	2	2
Yeast (g)	2.25	2.25
Potassium bromate (ppm)	5	40
Ascorbic Acid (ppm)	5	35
Vital gluten (0, 2, 4%)	Optional	Optional
Water (%)	Optimum	Optimum

of outer branny layers rich in these constituents. Similar results have been reported earlier by Lemar and Swanson (1976); Ranhotra *et al.* (1977); Sekhon *et al.* (1992). SDS-sedimentation values were significantly lower in sprouted wheat flours, thereby indicating poor gluten strength. The results are in general agreement to those reported earlier (Ranhotra *et al.* 1977; Singh *et al.* 1987; Sekhon *et al.* 1992).

The falling number values were significantly lower in the sprouted wheat flour (Table 3). Addition of vital gluten to sound wheat flours significantly increased the falling number values. Decreasing the pH and adding salt significantly lowered the falling number values of sound wheat flour but significantly increased the values in sprouted wheat flours. The addition of vital gluten further increased the falling number values; however, the increase was nonsignificant.

Pasting Properties

Peak viscosity, viscosity at 95C and 50C, and set back decreased significantly with the addition of gluten to sound wheat flours (Table 4). Decreasing the pH of sound wheat flour significantly increased the values of these characteristics except set back. The corresponding values for sprouted wheat flour were significantly lower, thereby showing a profound effect of sprouting on amylographic properties. The effect of addition of gluten and decrease in pH plus salt on these parameters except on set back values for sprouted wheat flours were just reverse to that observed for sound wheat flours, which is understandably a favorable effect. The reduced viscosities in sprouted wheat flours were

TABLE 2.
CHARACTERISTICS OF DIFFERENT FLOUR USED

Characteristics	Sprouting (h)		
	0	24	48
Flour Yield (%)	75.5±0.40[a]	70.7±0.30[b]	65.4±0.50[c]
Protein (% N X 5.7)	8.7±0.12[a]	9.0±0.14[a,b]	9.2±0.20[b]
Ash (%)	0.52±0.01[a]	0.55±0.01[b]	0.57±0.01[b]
Sugar (% Maltose)	1.7±0.16[a]	2.1±0.15[b]	2.9±0.16[c]
Diastatic activity (mg maltose/10g)	250±4.00[a]	430±8.00[b]	585±8.20[c]
SDS-Sedimentation value (ML)	52±2.60[a]	46±2.10[b]	40±1.60[c]

Values with similar superscripts do not differ significantly (P<0.05)

TABLE 3.
EFFECT OF pH 4.2 AND 2% SALT, AND GLUTEN ON THE FALLING NUMBER VALUES OF SOUND AND SPROUTED WHEAT FLOUR

Sprouting time (h)	Gluten (%)					
	0		2		4	
	Control	pH+Salt	Control	pH+Salt	Control	pH+Salt
0	795	700	845	730	885	750
24	196	410	205	445	215	475
48	90	268	100	258	110	280

LSD (0.05) : 20

due to degradation of starch brought about by the increased amylolytic activity. Sekhon *et al.* (1992) also reported similar findings. The degradation of starch also led to reduced retrogradation, which was reflected by the lower set back values obtained. The difference in the viscosity values between hot paste and paste cooled to 50C referred to as the set back value (Beachell and Stansel 1963)

TABLE 4.
EFFECT OF pH 4.2 AND 2% SALT, AND GLUTEN ON THE PASTING PROPERTIES OF SOUND AND SPROUTED WHEAT FLOUR

Type	pH	Salt (%)	Gluten (%)	Characteristics				
				Gelatinization Temp (C)	Peak Viscosity (Bu)	Viscosity at 95 C (Bu)	Viscosity at 50 C (Bu)	Setback
Sound	5.8	-	0	60.0	800	790	1570	770
	5.8	-	2	61.2	690	660	1275	585
	5.8	-	4	62.5	600	575	1135	535
	4.2	2	0	64.2	1060	1050	1750	960
	4.2	2	2	65.0	900	819	1380	480
	4.2	2	4	65.5	800	790	1285	485
Sprouted*	4.2	2	0	67.5	220	215	285	65
(24h)	4.2	2	2	68.0	235	220	325	90
	4.2	2	4	68.5	265	245	360	95
Sprouted*	4.2	2	0	69.5	55	45	65	-15
(48h)	4.2	2	2	68.0	80	70	65	-15
	4.2	2	4	68.5	95	85	80	-15

* At pH 5.8 flour paste viscosity was nil so the data were not reported

and reflects the retrogradation behavior of a starch (Masurs *et al.* 1957; Nishita and Bean 1979). The favorable effect of lowering pH plus salt could be due to their inhibitory effect on the activity of the amylolytic enzymes. Addition of gluten reduced the viscosities of paste in the sound wheat flour due to formation of complexes with starch granule surfaces, preventing the release of extrudate and so lowering peak viscosity (Olkku and Rha 1978). Similar effects of substitution of gluten proteins for an equivalent weight of starch on peak viscosities have been observed by Anker and Geddes (1944). However, it brought about improvement in viscosities in sprouted wheat flours, probably due to its being a macromolecule and the property of forming a viscoelastic system with starch.

Farinographic Characteristics

Farinograph water absorption, dough development time and stability significantly decreased whereas degree of softening significantly increased with the increase in sprouting time thereby showing weakening effect of sprouting on dough strength (Table 5).These observations have been made earlier also (Singh *et al.* 1987 and Sekhon *et al.* 1992). This trend of weakening of dough strength with increasing sprouting time was visible even if the pH of the dough was reduced to 4.2 and gluten was added up to 4%. In sprouted flours the decrease of dough pH plus salt brought about a decrease in water absorption. In contrast, the addition of gluten brought about an increase in water absorption. The favorable effect of gluten addition on water absorption is overtaken by the reduction in pH to the extent that even with the addition of gluten at 4% the water absorption values were less than those obtained at pH 4.2 with addition of 2% salt.

Decreasing pH of the dough in sprouted flours to 4.2 plus salt improved the dough development time, dough stability and decreased the degree of softening. Thus the decrease in pH plus salt had a favorable effect on the strength of dough, which may be due to reduction in activity of proteolytic enzymes (Bean *et al.* 1976; McDonald and Chen 1964). Addition of gluten to the dough at normal and reduced pH also improved the strength of sprouted flours significantly. The effects were more pronounced at 4% addition of gluten. A combination of change of pH plus salt and addition of gluten was found to be more effective in increasing the strength of dough of sprouted wheat flours. The improvement in dough strength of sprouted wheat flours might be due to the inactivation of amylolytic and proteolytic enzymes due to the reduction of pH to 4.2 (Sandsted *et al.* 1937; Miller and Johnson 1948; Meredith 1970; McDonald and Chen 1971) and improvement of gluten content and strength due to added gluten.

TABLE 5.
EFFECT OF pH 4.2 AND 2% SALT, AND GLUTEN ON FARINOGRAPHIC CHARACTERISTICS OF SOUND AND SPROUTED WHEAT FLOUR

Sprouting time (h)	Characteristic	Gluten (%) 0		2		4	
		Control	pH+Salt	Control	pH+Salt	Control	pH+Salt
0	Water absorption (%)	58.4	51.2	58.6	56.4	60.4	54.0
	Dough development time (min)	1.7	1.3	3.5	2.4	2.8	9.0
	Dough stability (Bu)	4.3	4.1	4.6	12.0	5.6	23.8
	Degree of softening (Bu)	90	105	75	50	70	40
24	Water absorption (%)	53.4	51.6	56.2	53.2	56.6	52.2
	Dough development time (min)	1.6	1.4	2.1	2.0	2.4	4.0
	Dough stability (Bu)	1.3	3.6	1.8	5.8	2.5	8.6
	Degree of softening (Bu)	215	155	215	140	200	75
48	Water absorption (%)	53.0	52.0	54.0	52.4	54.8	50.0
	Dough development time (min)	1.2	1.3	1.5	1.6	1.5	1.7
	Dough stability (bu)	1.0	1.5	1.2	1.7	1.9	3.2
	Degree of softening (Bu)	315	215	305	200	290	180

LSD (0.05) for water absorption, 2.0; dough development time, 1.4; dough stability, 3.0; degree of softening, 11.

TABLE 6.
EFFECT OF pH 4.2 AND 2% SALT, AND GLUTEN ON CHARACTERISTICS OF BREAD PREPARED FROM SOUND AND SPROUTED WHEAT FLOUR BY MEDIUM TIME FERMENTATION METHOD

Sprouting time (h)	Characteristic	Gluten (%)					
		0		2		4	
		Control	pH+Salt	Control	pH+Salt	Control	pH+Salt
0	Specific loaf volume (cc/g)	3.54	3.46	3.88	3.76	4.06	3.86
	Total scores (100)	74.2	71.0	81.2	78.1	86.5	84.4
24	Specific loaf volume (cc/g)	4.04	4.23	4.26	4.20	4.34	4.22
	Total scores (100)	55.1	58.8	65.5	69.7	75.0	76.8
48	Specific loaf volume (cc/g)	3.13	3.23	3.25	3.20	3.25	3.10
	Total scores (100)	38.2	44.0	49.1	57.0	57.5	61.6

LSD (0.05) for specific loaf volume, 0.15; total scores, 2.

TABLE 7.
EFFECT OF pH 4.2 AND 2% SALT, AND GLUTEN ON CHARACTERISTICS OF BREAD PREPARED FROM SOUND AND SPROUTED WHEAT FLOUR BY SHORT TIME FERMENTATION METHOD

Sprouting time (h)	Characteristic	Gluten (%)					
		0		2		4	
		Control	pH+Salt	Control	pH+Salt	Control	pH+Salt
0	Specific loaf volume (cc/g)	3.34	3.65	4.02	4.35	4.50	4.70
	Total scores (100)	69.7	67.5	76.4	76.1	85.3	84.0
24	Specific loaf volume (cc/g)	4.20	4.36	4.55	4.68	4.94	4.98
	Total scores (100)	59.2	66.0	71.2	75.6	80.8	83.2
48	Specific loaf volume (cc/g)	3.5	3.28	3.35	3.45	3.40	3.80
	Total scores (100)	43.3	51.3	54.0	62.5	63.0	72.0

LSD (0.05) for specific loaf volume, 0.15; total scores, 2.

Baking Performance

The dough handling properties of sprouted wheat flours were excellent at pH 4.2 compared to those having normal pH in which case the dough was sticky (data not shown). Specific loaf volume of breads prepared by both the methods of baking used were found to be significantly higher for 24 h sprouted wheat flours with respect to the control flour. However, a significant reduction in loaf volume was observed in case of breads prepared from wheat sprouted for 48 h. Thus augmentation of enzymatic activity in the flours up to 24 h sprouting was conducive for bread making. This could be particularly true for Indian wheats, which are normally lower in diastatic activity. The favorable effect of limited sprouting of wheat on the loaf volume and specific volume of breads produced by a long fermentation time method has been reported earlier (Ranhotra *et al.* 1977; Sharma *et al.* 1988; Singh *et al.* 1990).

Change of dough pH as well as the addition of gluten separately or in combination were found to increase the specific volume of breads using either of the two methods of baking adopted. The combination of change in pH and addition of gluten was found to be more effective in this respect. However, of the two methods, the "Short Time Fermentation" method gave better response with the combination of these changes than that by the "Medium Time Fermentation" method. The bread evaluation scores prepared by this method were also higher than those with the latter method. The reason for this superior response was that the "Short Time Fermentation" method did not allow much time for the enzymes to act (Table 6 and 7).

The present study showed that the reduction of pH to 4.2 and addition of gluten improves the rheological properties of dough produced from sprouted wheat. Acceptable quality bread could be produced from flours having diastatic activity up to 585 mg maltose/10 g flour by addition of 4% gluten along with change of dough pH to 4.2 and adopting the "Short Time Fermentation" method.

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REFERENCES

AACC. 1976. *Cereal Laboratory Methods*, Am. Assoc. of Cereal Chemists, St. Paul, MN.

ANKER, C.A. and GEDDES, W.F. 1944. Gelatinization studies upon wheat and other starches with amylograph. Cereal Chem. *21*, 335.

AXFORD, D.W.E., McDERMOTT, E.E. and REDMAN, D.G. 1976. Note on the sodium dodecyl sulphate test of bread making quality: Comparison with Palshenke and Zeleny test. Cereal Chem. *56*, 582.

BEACHELL, H.M. and STANSEL, J.W. 1963. Selecting rice for specific cooking characteristics in a breeding programme. Intern. Rice Comm. Newsl. (special issue), 25.

BEAN, M.M., KEAGY, P.M., FULLINGTON, J.G., JONES, F.T. and MECHAM D.K. 1976. Dried Japanese noodles. 1. Properties of laboratory prepared noodle doughs from sound and damaged wheat flours. Cereal Chem. *5*, 416.

DORFER, J. and KOBALL, G. 1980. Inhibition of alpha-amylase by selected organic and inorganic substances. Baker Konditor. *28*, 58.

FULLER, R., HUTCHINSON, J.B., McDERMOTT, E.E. and STEWART, B.A. 1970. Inactivation of alpha-amylase in wheat and flour with acid. J. Sci. Food. Agr. *21*, 27.

GERBHARDT, E. and WIESENER, B. 1979. Treatment of enzyme-rich flour for baking purposes. Chem. Abstr. *91*, 522.

HARINDER, K. and BAINS, G.S. 1988. Studies on baking of high alpha-amylase flour: Effect of pH, salt and L-cysteine HCl in dough. Nahrung *32*, 481.

HENRY, R.J., MARTIN, D.J. and BLACKENEY, A.B. 1987. Reduction of the alpha-amylase content of sprouted wheat by pearling and milling. J. Cereal Sci. *5*, 155.

LEMAR, L.E. and SWANSON, B.G. 1976. Nutritive value of sprouted wheat. J. Food Sci *41*, 719.

LIU, R., LIANG, Z., POSNER, E.S. and PONTE, J.G. 1986. A technique to improve functionality of flour from sprouted wheat. Cereal Foods World *31*, 471.

LORENZ, K. 1980. Cereal sprouts: composition, nutritive value, food applications. CRC Crit. Rev. Food Sci. Nutr. *11*, 353.

MASURS, E.G., SCHOCH, T.J. and KITE, F.E. 1957. Graphical analysis of the Brabender viscosity curves of various starches. Cereal Chem. *34*, 141.

McDONALD, K.E. and CHEN, L.L. 1964. Properties of wheat flour proteases. Cereal Chem. *41*, 443.

MEREDITH, P.1970. Inactivation of cereal alpha-amylase by brief acidification. The pasting strength of wheat flours. Cereal Chem. *47*, 492.

MILLER, B. and JOHNSON, J.A. 1948. High levels of alpha-amylase in balking. II. Proteolysis in straight and sponge doughs. Cereal Chem. *25*, 178.

NISHITA, K.D. and BEAN, M.M. 1979. Physico-chemical properties of rice in relation to rice bread. Cereal Chem. *56*, 185.

OLKKU, T. and RHA, C. 1978. Gelatinization of starch and wheat flour starch: A review. Food Chem. *3*, 293.

RANHOTRA, G.S., LEOWE, R.J. and LEHMANN, T.A. 1977. Bread making quality and nutritive value of sprouted wheat. J. Food Sci. *49*, 1373.

SANDSTEDT, R.M., BLISH, M.J., MECHAM, D.K. and BODE, C.E. 1937. Identification and measurement of factors governing diastasis in wheat flour. Cereal Chem. *14*, 17.

SEKHON, K.S., SINGH, N. and NAGI, H.P.S.1992. Effect of pearling and blending on the bread-making properties of sprout-damaged wheat. Cereal Foods World. *37*, 715.

SHARMA, S., NAGI, H.P.S. and SEKHON, K.S. 1988. Effect of blending laboratory sprouted grains on milling and baking properties of wheat. J. Food Sci. Technol. *25*, 330.

SINGH, N., SEKHON, K.S. KAUR, A., SINGH, R.P. and NAGI, H.P.S. 1990. Hailstorms damage to wheat: Grain quality and functional properties. Cereal Foods World. *55*, 258.

SINGH, N., SEKHON, K.S. and NAGI, H.P.S. 1987. Laboratory sprouted damage and effect of heat treatment on milling and baking properties of Indian wheat. J. Food Sci. *52*, 176.

STEEL, R.G.D. and TORRIE, J.H. 1960. *Principles and Procedures of Statistics,* McGraw-Hill, New York.

WESTERMARCK-ROSENDAHL, C., JUNNLA, L. and KOIVISTOINEN, P. 1980. Efforts to improve the baking properties of sprout-damaged wheat by reagents reducing alpha-amylase activity. III. Effects on technological properties of flour. Lebensm. Wiss. Technol. *13*, 193.

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