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DRY BEAN (*PHASEOLUS VULGARIS*) HARDENING AND THE CONSEQUENCES OF PECTIN METHYLESTERASE ACTIVITY IN STORAGE

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ABSTRACT

Pectin methylesterase (PME) activities (degree of pectin esterification) and hardness (Kramer shear force) of decorticated Malawi bean varieties (Phaseolus vulgaris) *were evaluated at 4 and 8 months storage at 0.55 a_w and 0.85 a_w, and 16°C and 35°C. PME activities were significantly higher at 4 and 8 months than in controls (2°C, 0.30 a_w) and at 8 versus 4 months, irrespective of a_w. Hardening increased with storage time, temperature and a_w. Varieties showed significant hardness differences. PME activity influence on hardness was not clearly revealed during the 8 month storage period in the current study.*

INTRODUCTION

Failure of stored common dry beans (*Phaseolus vulgaris*) to soften sufficiently during normal atmospheric cooking, even after imbibing water (Vindiola *et al.* 1986), is referred to as the hard-to-cook defect. Factors that have been reported to promote the development of the hard-to-cook defect include high temperature ($\geq$ 21°C) high (0.70) water activity (a_w) and phytase activity (Burr *et al.* 1968; Kon 1979; Kon and Sanshuck 1981; Hincks and Stanley 1986; Vindiola *et al.* 1986). The defect is expected to be widely found in Malawi with tropical weather conditions (Bailey 1973) where temperatures and relative humidities range from 13°–28°C and 48%–92%, respectively (Malawi Meteorology Dept. 1986).

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The involvement of pectin methylesterase (PME) in the development of the hard-to-cook legume seed defect has been suggested (Jones and Boulter 1983). PME (Pectin pectylhydrolase, EC 3.1.1.11) catalyses hydrolysis of the methyl group from the pectin molecule to produce methanol and pectinic acid and/or low methoxyl pectin (Delincee and Radola 1979; Versteeg *et al.* 1978). Thus allowing more potential sites for divalent cation cross linking.

Research that has related PME activities to the hard-of-cook defect of legume seeds has been limited and inconclusive. Jones and Boulter (1983) incubated freshly harvested mature dry black beans (*Phaseolus vulgaris* var S-19-N) with 0.03M $CaCl_2$ or 0.03M $CaCl_2$ plus PME at pH 7.0 for 18 h; those incubated with only $CaCl_2$ took 24 min to achieve the 100% cooked state. PME addition further lengthened processing by 4 min as compared to the $CaCl_2$ treated group. This study measured the indirect effect of PME on bean hardness rather than its activity. In a second experiment, Jones and Boulter (1983) stored beans at 34°C and 0.70–0.75 a_w; after six months, the degree of esterification (DE) of pectin had decreased from 51% to 15%, implying PME activity. Bean hardness was not determined in the second investigation.

To clearly define the role of PME in bean hardening during storage, both the enzyme activity and hardness should be assessed in a sample. An understanding of the hard-to-cook mechanism is necessary to alleviate the problems of increased cooking time and energy consumption, and lowered legume seed digestibility (Kon and Sanshuck 1981; Sievwright and Sanshuck 1986). The hard-to-cook defect needs to be addressed to meet the dietary protein needs of populations living in developing societies like Malawi whose resources are generally limited (Bressani *et al.* 1961; Jones and Boulter 1983).

In order to eliminate the hard-shell effect (due to water impermeable seed coats), bean samples used in this study were decorticated. The purpose of the investigation was to evaluate PME activities and hardness changes in decorticated common dry beans (*Phaseolus vulgaris*), and to relate enzyme activity to cooked bean hardness over an 8 month storage period. This project was initiated to evaluate the proposal by Vindiola *et al.* (1986) and Jones and Boulter (1983) that PME may be involved in hardening of beans stored under high temperature ($\geqslant$ 21°C) and high a_w (0.70). The storage conditions were chosen to cover the range where the hard-cook defect has been previously reported in the literature to occur, and also as being relevant and applicable to weather conditions in Malawi. One of the aims of the Malawi Bean/Cowpea Collaborative Research Project (CRSP), which the current research attempted to address, was to examine the genetic variability in culinary aspects of Malawian beans. A survey conducted by the project revealed that Malawian women, who played a major role in bean production, stored many bean lines on their farms to meet agronomic and culinary needs (short cooking time) (Malawi/MSU Bean/Cowpea CRSP, 1989).

The approach followed in the PME assay in the current research was to determine enzyme activity by indirectly measuring the amount of substrate (esterified pectin) remaining in decorticated beans following seed exposure to specific temperatures, a_w and storage time periods (Schwimmer 1981). We hypothesized that decorticated bean seeds stored in a suboptimal environment (35°C; 0.85 a_w) would have increased PME activities as storage time advanced (0–8 month). Elevated PME activities would lead to decreased DE of total pectic substances as compared to the control group (2°C; 0.30 a_w). Cooked bean hardness would be promoted by elevated PME activities which allow for increased free carboxyl site availability on the pectin molecule. Free carboxyl groups would thereafter form cation bridges with available calcium and magnesium ions, thus facilitating pectin desolubilization, a lowering of water soluble pectic substances and bean hardening.

MATERIALS AND METHODS

Environmental Conditions

Controlled environmental cubicles in the Department of Food Science and Human Nutrition at Michigan State University were adjusted to 2°C, 16°C and 35°C ± 2°. Five-gallon high density polyethylene (HDPE) containers with tight-fitting lids (Cole-Palmer Instrument Co., Chicago, IL) were placed in the cubicles. Preliminary studies, over three months, demonstrated that a_w within the HDPE containers held in the 2°C cubicles averaged 0.30 which was in equilibrium with 10% moisture beans.

Saturated solutions of potassium chloride and magnesium nitrate (certified ACS, Fisher Scientific Co., Livonia, MI) were prepared according to the Labuza (1984) method and used to maintain a_w at 0.85 and 0.55 ± 0.05, respectively. A digital hygrometer-thermometer (Fisher Scientific Co., Livonia, MI-model No. 11-661-71) was used to measure a_w and temperature within the containers via an opening resealable with a rubber stopper, weekly for the first month, twice per month the second and third months, and once per month thereafter following initial sample equilibration.

Sample Preparation

Two Malawian bean landraces (varieties), one a small red bean (Acc: 6-5) with a thick seed coat and the other a large white bean (Acc: 2-10) with a thin seed coat were generously provided by the Malawi (Africa) Bean/Cowpea Collaborative Research Project at Michigan State University, East Lansing, MI.

Beans were decorticated to eliminate variation due to seed coat differences. A single layer of beans (500 g) was placed in each of a series of steel wire net

baskets constructed in our laboratory. The baskets were suspended approximately four inches over excess deionized water in dessicators (one basket per dessicator) for 3–4 days, and then sprinkled with ambient temperature deionized water to enhance loosening of the seed coats. The beans were decorticated manually with the aid of a surgical blade, and air dried at room temperature for three days. A preliminary study had demonstrated that previously humidified/decorticated/dried beans were similar in moisture content (9.5%–10%) to untreated decorticated beans. The treated seeds were held in the sealed HDPE containers at 2°C until initiation of the storage study.

Experimental Design

The experiment was a four factor factorial (2^4) model in a completely randomized design where time × temperature × a_w × variety were fixed. All experimental treatments within the experimental design are shown in Table 1. Note that the control was not part of the factorial arrangement. The control was compared to means within the factorial arrangement using the least significant difference (LSD) test (Little and Hills 1978; Steel and Torrie 1980). The nine treatment combinations for each variety were replicated twice.

Thirty-six 400 g decorticated dry bean subsamples (18 red and 18 white) were weighed, put into low density polyethylene (LDPE) Zip-loc™ coded bags (6½ in. × 5½ in., 1.15 mil thick), and randomly assigned to the nine treatment combinations. Samples were then treated with approximately 5 g Captain™[1] dust to control/inhibit mold growth prior to initiation of the storage study. Preliminary work showed no differences between DE of total pectic substances from Captan™ treated decorticated beans and untreated seeds held at 2°C for 3 months. Samples were analyzed for PME activity and cooked bean hardness at zero, four and eight months storage.

Pectin Methylesterase Assay

Chemicals and supplies used for the DE determination of total pectic substances included: calgon and celite (Fisher Scientific Co., Livonia, MI); ground paper pulp (Schleicher and Schueel Co., Keene, NH); macroporus filter paper (pore size: 10-2000 micrometers, Spectrum Medical Industries, Inc., Los Angeles, CA); phenol red indicator (Sigma Chemical Co., St. Louis, MO); HCl and NaCl (ACS certified, Baker Chem. Co., Phillipsburg, NJ); NaOH (1N-Mallinckrodt, Inc., Paris, KY) and ethyl alcohol (absolute-USP, Alcohol and Chemical Co.,

[1]Captain [1,2,3,6 tetrahydro-N-(trichloromethylthio) phthalimidine] is a fungicide with protective and curative action used to treat seeds for control of *Pythium, Phoma,* Rhizoctonia species (Hartley and Kidd 1987).

TABLE 1.
EXPERIMENTAL STORAGE TREATMENT ARRANGEMENT OF DECORTICATED BEANS

Bean Variety (Landrace)	0 Months[1]		4 Months		8 Months	
	Temperature (^{o}C)	Water Activity (A_w)	Temperature (^{o}C)	Water Activity (A_w)	Temperature (^{o}C)	Water Activity (A_w)
White or Red	2	0.30	16	0.55	16	0.55
			16	0.85	16	0.85
			35	0.55	35	0.55
			35	0.85	35	0.85

[1] Control was not part of the factorial arrangement. The control treatment was compared to means in the factorial arrangement using the least significant difference (LSD) test.

Shelbyville, KY). A digital pH meter (Corning model No. 610A, Corning Co., Medfield, MA) was used to measure sample pH.

PME activities were measured indirectly by determining the DE of the total pectic substances extracted from 1 g of flour obtained by grinding a 50 g dry bean sample for five min (80 mesh) using a Braun mill (model No. KSM2, Lynnfield, MA). Total pectic substance extraction and pectic acid titration for the DE determination were carried out according to the method of Owen *et al.* (1952).

Bean Processing/Hardness Determination

Since decorticated beans were used in this investigation, conventional canning procedures could not be employed as processing conditions were greatly lowered. The processing method developed was designed to achieve the 100% cooked state of control white beans (2°C, 0.30 a_w) (base line), since these beans were considered soft. The processing specifications (time, temperature, psig) were arrived at through five trials during preliminary work. A bean was considered cooked upon yielding to slight pressure when individually squeezed between the forefinger and thumb (Jones and Boulter 1983). Pyrex glass canning jars (264 mL) containing 100 g beans and 140 mL of deionized water (Voisey and Larmond 1971) were placed in a boiling water bath for 30 min, drained, rinsed, another 140 mL deionized water added and exhausted for 5 min. The jars were then sealed and the samples were processed at 5 psig (109°C) for five min in a pressure cooker (6 qt.). Cooked samples were allowed to equilibrate in moisture content at 4°C for two weeks, rinsed, and then hardness of the cooked beans (100 g) was measured using a Kramer shear press (Food Technology Corp., Reston, VA, model no. T-2100-C) with a standard shear-compression cell (model no. CS-1) at 1/3 range setting and 3000 lb. transducer force. The force to shear 100 g cooked beans was calculated using the following formula:

$$\text{Shear Force} = \text{Transducer Force} \times \frac{\text{Range}}{100} \times \frac{\text{Peak Height}}{100}$$

(Binder and Rockland 1964). The higher the shear force the harder the bean sample.

Statistical Analyses

The effects of time, temperature, humidity and variety upon PME activities (the DE of total pectic substances) and cooked bean hardness were analyzed statistically using analysis of variance (ANOVA) via the MSTAT statistical software package (Freed *et al.* 1985). The Least Significance Difference (LDS)

test was used for multiple comparisons among means (Little and Hills 1978, Steel and Torrie 1980). When significance is indicated, the $P \leq 0.05$ level is meant unless otherwise stated. Linear correlation coefficients were calculated to determine the relationship between hardness and PME activities (Little and Hills 1978; Steel and Torrie 1980).

RESULTS AND DISCUSSION

Pectin Methylesterase Activity

ANOVA (Table 2) for the DE data of total pectic substances from stored decorticated beans revealed that the DE was highly ($P \leq 0.01$) influenced by storage time. In addition, there was a significant time X a_w interaction which means that the effect of storage time depended on the level of a_w. These results imply that the storage conditions (time and a_w combined) had some effect on the level of PME activities in the legume seed tissue. Data for the legume seed moisture content in equilibrium with a_w for the difference treatments are presented in Tables 2 and 3. Higher enzyme activity levels (by DE) would be expected as storage time increases, since conventionally one unit of PME activity is defined as the amount of enzyme that would liberate one micromole of carboxyl groups per minute (Dahodwala *et al.* 1974; Versteeg *et al.* 1978; Kohn *et al.* 1983; Markovic *et al.* 1983). However, changes in PME activity data of the current research would be interpreted considering both time and a_w, because of the significant time x a_w interaction (Steel and Torrie 1980).

Table 4 illustrates that at four months storage, PME activities were significantly greater at 0.55 a_w than at 0.85 a_w. The reverse was expected. Most enzymatic and biological reactions increase with a_w (Reed 1975). The trend for eight months storage, though not significant was as expected.

PME activities increased significantly over time (zero versus four and eight months; four versus eight months), irrespective of a_w. This is in agreement with Jones and Boulter (1983) who reported a decrease in DE of pectin from 51 to 15% in black beans stored at 34°C, 0.70–0.75 a_w from 6 months. Unless inhibited prior to initiation of the storage period, PME remains active in beans stored at >0.55 a_w for 4 months or longer when storage temperatures are $>$ 16°C.

Cooked Bean Hardness

Hardness of cooked beans was highly ($P \leq 0.01$) influenced by storage time, temperature, a_w and variety (Table 2). In addition, the following interactions were significant: time $\times$ temperature, time $\times$ a_w ($P \leq 0.01$), temperature $\times$ a_w ($P \leq 0.01$) and time $\times$ temperature $\times$ a_w ($P \leq 0.01$). These interactions imply that the effects of storage time on hardness are dependent on levels of temperature

TABLE 2.
ANOVA[1] FOR TOTAL PECTIC SUBSTANCES DE (%), HARDNESS (G FORCE/100 G), PHYTIC ACID/CALCIUM RATIOS, WATER SOLUBLE PECTIC SUBSTANCES (MG/ML) AND MOISTURE CONTENT (%) OF STORED DECORTICATED BEANS FOR TIME, TEMPERATURE, A_w AND VARIETY PARAMETERS

Source	Degrees of Freedom	Mean Square				
		Degree of Esterification	Hardness	Phytic Acid/ Calcium Ratio	Water Soluble Pectic Substances	Moisture Content
Storage Time	1	929.883**	1229704.0**	153320.0	0.011*	3.125**
Temperature	1	8.611	8426539.0**	1679486.0**	0.016*	15.540**
Time X Temperature	1	25.561	529163.0*	77717.0	0.000	0.878
A_w	1	16.103	1735850.0**	664416.0*	0.002	61.328**
Time X A_w	1	58.590*	905522.0**	34388.0	0.000	1.575*
Temperature X A_w	1	2.645	2628351.0**	22525.0	0.002	18.911**
Time X Temperature X A_w	1	0.845	923780.0**	53057.0	0.000	0.151
Variety	1	25.205	12732320.0**	1075678.0*	0.024**	0.551
Time X Variety	1	20.480	50007.0	1668.0	0.000	0.001
Temperature X Variety	1	32.603	91271.0	352170.0	0.005	0.015
Time X Temperature X Variety	1	17.553	16517.0	146205.0	0.000	0.228
A_w X Variety	1	12.005	111510.0	5645.0	0.000	0.070
Time X A_w X Variety	1	3.380	6992.0	77520.0	0.000	0.000
Temperature X A_w X Variety	1	26.633	675.0	6527.0	0.000	0.005
Time X Temperature X A_w X Variety	1	18.758	8224.0	47201.0	0.000	0.061
Error	16	10.610	78012.0	151420.0	0.002	0.237

[1] Factor Factorial ANOVA with four factors at two levels (2^4).

* Significant at 95% confidence level

** Significant at 99% confidence level.

TABLE 3.
INFLUENCE OF BEAN TYPE, STORAGE TIME, TEMPERATURE AND A_w ON MOISTURE CONTENT

Bean Type	Storage Time (Months)	Storage Temperature (^oC)	A_w	Moisture Content (%)
White	0	2	0.30	10.5
	4	16	0.55	10.5
	4	16	0.85	11.6
	4	35	0.55	10.4
	4	35	0.85	14.1
	8	16	0.55	10.7
	8	16	0.85	12.3
	8	35	0.55	10.5
	8	35	0.85	15.6
Red	0	2	0.30	9.5
	4	16	0.55	10.6
	4	16	0.85	11.3
	4	35	0.55	11.0
	4	35	0.85	13.7
	8	16	0.55	10.3
	8	16	0.85	11.8
	8	35	0.55	10.6
	8	35	0.85	15.4

TABLE 4.
INFLUENCE OF BEAN TYPE, STORAGE TIME, TEMPERATURE AND A_w ON DE OF TOTAL PECTIC SUBSTANCES

Bean Type	Storage Time (Months)	Storage Temperature (^oC)	A_w	DE (%)
White	0	2	0.30	56.0
	4	16	0.55	7.5
	4	16	0.85	16.5
	4	35	0.55	14.8
	4	35	0.85	17.8
	8	16	0.55	6.8
	8	16	0.85	5.4
	8	35	0.55	3.8
	8	35	0.85	3.8
Red	0	2	0.30	93.0
	4	16	0.55	19.5
	4	16	0.85	18.3
	4	35	0.55	13.3
	4	35	0.85	19.0
	8	16	0.55	8.3
	8	16	0.85	5.3
	8	35	0.55	3.8
	8	35	0.85	3.1

and a_w; those of temperature on time and a_w; and those of a_w on time and temperature. When relating storage time, temperature, and a_w to cooked bean hardness no one factor can be taken in isolation, because of the significant interactions. Burr *et al.* (1968) found that pinto bean half cooking times increased with storage time, and that the main impact of storage time (24 month) and high moisture (16%) content was at high temperature (21°). Their results were supported by Kon and Sanchuck (1981).

Table 5 shows the influence of storage time, temperature, a_w and variety on cooked bean hardness. At 4 months white beans were significantly harder at 35°C than at 16°C at both 0.55 and 0.85 a_w. The same trend was observed for the 8 month storage period; however, at 8 months, the effect of temperature was of a considerably higher magnitude between the low (16°C) and high (35°C) groups at 0.85 a_w than at 0.55 a_w.

Results were similar for the red beans (Table 5) suggesting that similar mechanisms produced the hard-to-cook defect in both legume seeds. However, variations in water soluble pectic substance, lignin, moisture and phenolic compound levels, phytic acid/calcium ratios and pectin DE prior to and during storage (Kon and Sanshuck 1981; Jones and Boulter 1983; Srisuma *et al.* 1989) need to be thoroughly examined before a definitive statement can be made regarding the similarities in the mechanisms involved in the development of the hard-to-cook defect in these two bean varieties during storage.

Results from our laboratory (Tables 2 and 6) revealed significantly lower phytic acid/calcium ratios in white beans stored at high temperature (35°C) and high a_w (0.85) than those held at 2°C and 0.30 a_w. In contrast, no significant differences were found between red beans stored at 35°C, 0.85 a_w and those at 2°C, 0.30 a_w, even though a trend for decreasing phytic acid/calcium ratios was apparent.

Dissimilarities between varieties were evident. The red beans had significantly lower phytic acid/calcium ratios than the white beans. These results were likely suggest that phytic acid degradation in the white legume variety (which had high initial phytic acid content) may have greater importance on hardness of legume seeds stored at a higher temperature (35°C) and a high a_w as opposed to those seeds with a low initial phytic acid content.

The findings on the relationship between storage time, temperature and a_w of the present study support those reported by Burr *et al.* (1968) and Kon and Sanshuck (1981) who stated that the greatest impact of high a_w and increased storage time on bean hardness occurred at high temperatures. In fact, Jones and Boulter (1983) reported that there was a synergistic relationship between high moisture and high storage temperature on bean hardness.

When comparing the texture of the white and red bean varieties, the latter legumes were significantly harder than the former. The red bean variety is classified as a hard bean by plant breeders (Vindiola *et al.* 1986), a classification

TABLE 5.
INFLUENCE OF BEAN TYPE, STORAGE TIME, TEMPERATURE AND A_w ON COTYLEDON HARDNESS

Bean Type	Storage Time (Months)	Storage Temperature (^oC)	A_w	Cotyledon Hardness (g force/100 g)
White	0	2	0.30	954
	4	16	0.55	759
	4	16	0.85	826
	4	35	0.55	1379
	4	35	0.85	1867
	8	16	0.55	993
	8	16	0.85	929
	8	35	0.55	1474
	8	35	0.85	3319
Red	0	2	0.30	2545
	4	16	0.55	2332
	4	16	0.85	2057
	4	35	0.55	2783
	4	35	0.85	3021
	8	16	0.55	2375
	8	16	0.85	2217
	8	35	0.55	2634
	8	35	0.85	4220

TABLE 6.
INFLUENCE ON BEAN TYPE, STORAGE TIME, TEMPERATURE AND A_w ON PHYTIC ACID/CALCIUM RATIO

Bean Type	Storage Time (Months)	Storage Temperature (^oC)	A_w	Phytic Acid/Calcium Ratio
White	0	2	0.30	3757
	4	16	0.55	4233
	4	16	0.85	3572
	4	35	0.55	3149
	4	35	0.85	2853
	8	16	0.55	3553
	8	16	0.85	3536
	8	35	0.55	3253
	8	35	0.85	2968
Red	0	2	0.30	3242
	4	16	0.55	3363
	4	16	0.85	3048
	4	35	0.55	3065
	4	35	0.85	2922
	8	16	0.55	3275
	8	16	0.85	2903
	8	35	0.55	2913
	8	35	0.85	2695

based on the seed coat characteristics. However, the results of our study suggest that there are more factors contributing to the hardness of the naturally hard bean than simply the seed coat characteristics as suggested by Vindiola *et al.* (1986), since the legume seeds in the study reported here were decorticated before storage. In support of the above suggestion, Hussein *et al.* (1989) found a significant correlation between phytic acid content of different varieties of faba bean cotyledons with cooking time.

Correlation of Cooked Bean Hardness and Pectin Methylesterase Activity

Pectin methylesterase activity was negatively correlated ($r = -0.64$) and not related ($r = 0.29$) with white and red bean hardness, respectively, during the 4 month storage; it was positively correlated with the 8 month stored white ($r = 0.64$) and red ($r = 0.66$) seeds when time and a_w were considered in combination for the significant interaction. PME activity was not correlated ($r = 0.30$) with bean seed hardness during the 8 month storage when all parameters (time, temperature, a_w, variety) were considered. The contradiction in the correlations may have been due to the fact that PME activity was highly influenced by time ($P \leq 0.01$) and an interaction between time and a_w, while cooked bean hardness was highly ($P \leq 0.01$) influenced by time, temperature, a_w and variety and the interactions of time, temperature and a_w; thus more factors (phytase activity, lignin, phenolic compounds) may be influencing hardness, in addition to PME activity, during the 8 month storage. More research is needed to clearly define the relationship of PME activities with cooked bean hardness using an enzyme assay that directly measures PME activities and monitoring PME over short time intervals covering the 8 month period, to confirm the Jones and Boulter (1983) proposal that pectin desolubilization was facilitated by pectin de-esterification, thus promoting legume seed hardness in storage. Results from another experiment conducted in our laboratory, (Tables 2 and 7) revealed a significant decrease in water soluble pectic substances of stored beans with an increase in storage time and temperature. Jones and Boulter (1983) and Kon and Sanshuck (1981) suggested that the source of the divalent cations was mainly from phytic acid degradation. Our laboratory has also shown a decrease in phytic acid/calcium ratio as storage temperature and a_w increased.

CONCLUSIONS

The legume seed hard-to-cook defect was produced in both the red and white decorticated beans stored in suboptimal high temperature and a_w conditions for 8 months. The contribution of PME activity to the hardening of dry beans in storage was not clearly revealed in the current study. More research is needed to further examine the relationship between PME activity and legume seed hard-

TABLE 7.
INFLUENCE ON BEAN TYPE, STORAGE TIME, TEMPERATURE AND A_w ON WATER SOLUBLE PECTIC SUBSTANCES

Bean Type	Storage Time (Months)	Storage Temperature (^oC)	A_w	Water Soluble Pectic Substances (mg/ml)
White	0	2	0.30	0.433
	4	16	0.55	0.419
	4	16	0.85	0.417
	4	35	0.55	0.385
	4	35	0.85	0.338
	8	16	0.55	0.388
	8	16	0.85	0.404
	8	35	0.55	0.327
	8	35	0.85	0.302
Red	0	2	0.30	0.367
	4	16	0.55	0.353
	4	16	0.85	0.340
	4	35	0.55	0.338
	4	35	0.85	0.316
	8	16	0.55	0.306
	8	16	0.85	0.314
	8	35	0.55	0.303
	8	35	0.85	0.271

ness using direct PME enzyme assay procedures over short time intervals during the 8 month storage period. High water soluble pectic substance levels and phytic acid/calcium ratios prior to storage may be other factors to be considered in the hardness changes, beside PME activities.

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EFFECT OF PASTEURIZATION ON MICROBIOLOGICAL AND SENSORY QUALITY OF WHITE GRAPE JUICE AND WINE

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ABSTRACT

The effect of pasteurization on the microbiological and sensory quality of white grape juice and wine was investigated. Heat treatment of less than 3 Pasteurization Units (PUs = minutes at 60°C or equivalent) was sufficient to reduce 10^6 cells/mL of Saccharomyces cerevisiae *and* Hansenula anomala *added to Chenin blanc juice to an undetectable level. In contrast, heat treatment exceeding 10^5 PUs was required to have a measurable browning effect on juice, as measured by absorbance at 420 nm and by formation of 5-hydroxymethyl-furfural. Furthermore, up to 50,000 PUs did not significantly affect flavor, as determined by duo-trio tests with 18 trained judges. Pasteurized (23 PUs) and nonpasteurized juice fermented at the same rate, although not as rapidly as pasteurized, thiamine-supplemented juice. Tunnel pasteurization (13.7 PUs) reduced 5×10^6 yeast cells/mL added to grape juice to an undetectable level and did not significantly affect juice or wine flavor.*

INTRODUCTION

Pasteurization is often used in the beverage industry to prevent microbial spoilage. Brewers apply between 10 and 20 Pasteurization Units (PUs) to beer to eliminate microbial spoilage in the trade (King *et al.* 1978; Fricker 1984). On the other hand, winemakers, despite the early studies of Pasteur (1873), generally hold the belief that heating wine, or even must, causes detrimental

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and irreversible effects on quality of the final product. Thus, winemakers commonly use sulfur dioxide to control microbial spoilage and to protect grape juice or wine against oxidative browning reactions. Sterile filtration and sorbic acid may also be used to prevent refermentation of a wine that contains residual sugar. Normally, SO_2 additions are made at the time of grape crushing, at the end of the alcoholic fermentation, during wine storage and at bottling (Amerine *et al.* 1980). Sulfur dioxide has some liabilities: some asthmatics may react strongly to SO_2 and when concentrations are too high, flavor is compromised. Whereas the elimination of microbial spoilage from wine by heat has been an option for many years, there has been significant commercial interest only in Germany, Italy, South Africa and Australia. In these countries, pasteurization is an attractive way of preventing malolactic fermentation and eliminating browning activity, and it is technically simpler and perhaps cheaper than sterile filtration (Somers 1978).

Pasteurization is defined as a mild heat treatment used to kill or inhibit the vegetative forms of many bacteria and yeasts in liquid or semiliquid food products. This definition ignores the more heat-resistant forms of microorganisms (bacterial spores) the growth of which is inhibited in grape juice or wine by the low pH (or other mechanisms). One PU is defined as 1 min of heating at 60°C or its equivalent (Del Vecchio *et al.* 1951). Parameters used to characterize the heat resistance of microorganisms are the D_T value (the time (min) required to destroy 90% of the cells at temperature T (°C)) and the z value (the increase in temperature (°C) required to reduce D_T by a factor of 10). Typical values for organisms usually found in must are *Saccharomyces cerevisiae* (D_{51} = 16 min; z = 3.2°C in grape juice), *S. bayanus* (D_{54} = 2.4 min; z = 4.9°C at pH 3.2), *Leuconostoc mesenteroides* (D_{59} = 1.7 min; z = 3.9°C in orange juice) and *Lactobacillus fructivorans* (D_{60} = 1.7 min; z = 6.7°C in wine) (Bidan 1986). These values depend on the pH, SO_2 and sugar content of the juice (Hansen and Riemann 1963; Splittstoesser *et al.* 1975; Deveze and Ribereau-Gayon 1977) and the storage temperature of the juice (Stumbo 1973) because thermoresistance is affected by the temperature of growth of the organism. Untreated grape juice usually contains from 10^3 to 10^7 microorganisms with similar thermal resistance per mL.

To determine whether there is an amount of heat input that is sufficient to destroy microorganisms but which does not trigger chemical change and hence effects on juice and wine quality, we studied the effect of pasteurization on flavor and composition of juice and wine, on fermentation rate of juice, and on microbial stability of juice and wine. Up to three different pasteurization techniques (water bath, heat exchanger and tunnel pasteurizer) were used in this study.

MATERIALS AND METHODS

Materials

The following sterile-filtered grape juices and wine were used:

(1) Chenin blanc juice harvested in 1987, with 50 and 25 mg/L SO_2 added at pressing and racking, respectively, leaving 20 mg/L of free SO_2 at the time of the experiments; the juice had a pH of 3.29, 0.67 g/100 mL titratable acidity (as tartaric acid) and 20.4°Brix.
(2) Thompson Seedless juice harvested in 1988, with 50 mg/L SO_2 added, a pH of 3.75, 0.44 g/100 mL titratable acidity (as tartaric acid) and 23.8°Brix.
(3) French Colombard wine, fermented with *Saccharomyces cerevisiae* #522, sterile filtered and bottled under nitrogen atmosphere. The juice, harvested in 1988, had a pH of 3.49, 0.69 g/100 mL titratable acidity and 21.9°Brix. Pectinase and 50 mg/L SO_2 were added at the press.

The yeast strains used in the microbiology experiments, *Saccharomyces cerevisiae* #522 and *Hansenula anomala* #528, were obtained from the yeast collection of the Department of Viticulture and Enology, University of California, Davis. After two days of growth on agar slants, the strains were transferred successively into 10 mL and 100 mL of sterile grape juice diluted 1:1 with water and grown to a density of 10^8 cells/mL. Cultures of both yeast were prepared in duplicate at room temperature. The most viable replicate, as determined by methylene blue staining, was used for the inoculations. For the fermentation experiment, we used an active dry preparation of *Saccharomyces cerevisiae* #522 from Red Star (Milwaukee, WI). The inoculum was prepared by resuspending 5 g of yeast into 50 mL of warm (40°C) sterile water.

Methods

Pasteurization Techniques. Three different techniques were used to pasteurize the grape juice or wine:

(1) A water bath (Precision Scientific, model E5 cat. 6567, Chicago, IL), the initial temperature of which was adjusted to 82°C and maintained at 81.5 ± 0.5°C during the experiments. The juice was put in beer bottles (350 mL) prior to pasteurization and its temperature was adjusted to 20°C. After being pasteurized, bottles were quickly removed from the water bath, cooled, and stored in a cold chamber maintained at 2–4°C. Temperature was recorded at 1-min time intervals during the experiments. The rates

of temperature increase and decrease were measured and factored in the PU calculations.

(2) A heat exchanger (APV, Junior Paraflow Heat Exchanger, Buffalo, NY) used without homogenizer to minimize oxidation, operated at 74.4–76.1°C, 54.21–52.47 mL/s, with a juice holding time of 1.214–1.255 s. These conditions effected between 15.4 and 34.7 PUs, with an average of 23 PUs, calculated according to a model of continuous flow heating systems given by Toledo (1980).

(3) A commerical tunnel pasteurizer set up each day for continuous treatment of beer bottles. Heat input was automatically and continuously recorded. During 51 min of residence in the tunnel, the samples received 13.67 PUs and reached a maximum temperature of 61.4°C.

Sensory Evaluation. Duo-trio tests were conducted using a panel of trained judges to determine if a difference could be detected between pasteurized and unpasteurized samples of juice or wine. In a first series of tests, Chenin blanc juice which had received between 450 and 10^6 PUs in the water bath was compared to unpasteurized juice. The panel consisted of 18 judges who each evaluated one set of duo-trios. For the second series of tests, Thompson Seedless juice and French Colombard wine were pasteurized (13.67 PUs) through the tunnel pasteurizer, and compared, respectively, to unpasteurized juice and wine. The panel consisted of 19 judges who each evaluated twice the set of duo-trios in two separate sessions.

The tests were conducted in individual booths. Samples (25 mL) were served at room temperature in plastic cups. Judges were asked to expectorate the samples and to rinse with water between samples.

Physical and Chemical Analyses. Twenty-six tubes containing 10 mL of Chenin blanc juice were heated in the water bath. Two tubes were used to monitor the temperature of the juice during the heating process. At regular time intervals, duplicate tubes were removed from the bath and rapidly cooled on ice. After cooling, the juice was filtered (0.22 μm) and stored at 4°C. Absorbance was read at 420 nm on a Spectronic 1001 spectrophotometer (Milton Roy, Rochester, NJ) and recorded as browning index (Meydav *et al.* 1977). 5-Hydroxymethylfurfural was measured by HPLC, using a C_{18} Microsorb Rainin column and a 1090M Hewlett Packard detector with diode array monitor set at 284 nm. The mobile phase was 7% water in acetonitrile and the flow rate was 1 mL/min.

Microbiology. First, the survival rate (heat resistance) of *Saccharomyces cerevisiae* and *Hansenula anomala* was determined *vs.* pasteurization units. Two hours before pasteurization, bottles of sterile Chenin blanc juice at 20°C were inoculated with 10^4 to 5×10^7 yeast cells/mL. After pasteurization in the water

bath and cooling, dilutions of the various samples were plated on YM agar supplemented with thiamine for counting.

Second, the effect of tunnel pasteurization (13.7 PUs) on cell survival was measured. French Colombard juice was inoculated as above with the same strains and the number of cells surviving pasteurization was estimated in the same way.

Fermentations. Samples of Chenin blanc unpasteurized juice, juice pasteurized through the heat exchanger (23 PUs), and the same pasteurized juice supplemented with thiamine (0.2 mg/L) were fermented in duplicate at 19°C in 1-gal glass fermenters with *Saccharomyces cerevisiae* #522 (1 g cell dry weight/gal). The fermentations were monitored several times daily for degree Brix, ethanol, pH and cell density.

RESULTS AND DISCUSSION

Sensory Evaluation

No significant difference was detected between pasteurized and unpasteurized Chenin blanc juice when up to 50,000 PUs were applied to the juice in a water bath (Table 1). There was a significant difference between unpasteurized juice and juice pasteurized with 2.05×10^5 PUs ($p<0.05$) and 1.11×10^6 PUs ($p<0.001$).

Similarly, pasteurization (13.67 PUs) of Thompson Seedless juice and French Colombard wine in a tunnel pasteurizer did not significantly affect their sensory quality. The number of correct responses in the duo-trio tests were 19/38 for juice and 15/38 for wine, indicating that the panel was not able to detect a difference between pasteurized and unpasteurized samples.

These results indicate that a surprisingly high level of PUs (up to 50,000) may be applied to juice using a high temperature (81.5°C) for a short time without affecting its flavor. Similarly, juice or wine may be pasteurized for a long time (51 min) at a lower temperature (61°C) without any adverse effect on their flavor.

TABLE 1.
SENSORY EVALUATION OF PASTEURIZED GRAPE JUICE (DUO-TRIO TEST; PANEL OF 18 TRAINED JUDGES)

SUM OF PUs	450	2500	1.24×10^4	5×10^4	2.05×10^5	1.11×10^6
CORRECT ANSWERS	10/18	11/18	11/18	10/18	14/18	17/18
SIGNIFICANCE	NS	NS	NS	NS	$p<0.05$	$p<0.001$

NS: not significant

Physical and Chemical Analyses

Up to 7 × 10^5 PUs were applied to Chenin blanc juice without significantly affecting its browning index. Virtually no 5-hydroxymethylfurfural was found in the juice until more than 10^5 PUs were applied. Above that level, the concentration of hydroxymethylfurfural increased linearly with PUs (Fig. 1). The application of 7 × 10^5 PUs (the maximum used in this study) resulted in the formation of only 5 mg/L of hydroxymethylfurfural. This level is well below the detection threshold in wine (100 mg/L) reported by Amerine and Ough (1983).

Microbiology

Less than 3 PUs were required to eliminate 10^7 yeast cells/mL of juice for both *Saccharomyces cerevisiae* and *Hansenula anomala* (Fig. 2). This is in agreement with Bidan (1986) and implies that pasteurization of grape juice with its natural flora should easily be achieved with about 10 PUs. We choose to apply here a safety factor of 4 (2.5 PUs × 4 = 10 PUs) because the juice used in this study contained a small amount of SO_2. Interestingly, heat resistance did not differ significantly between *S. cerevisiae* (D_{60} = 0.303 min) and *H. anomala*

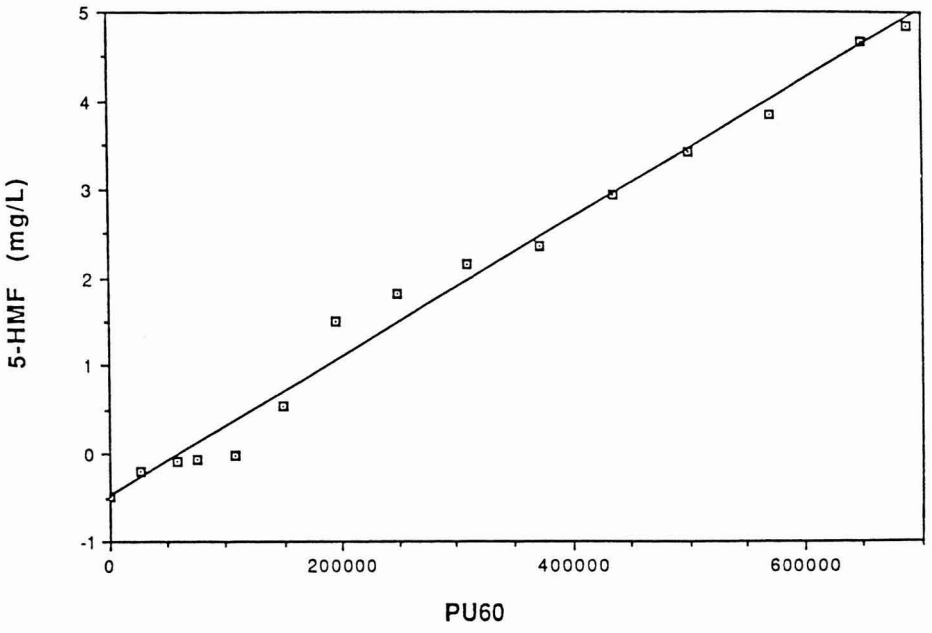

FIG. 1. FORMATION OF 5-HYDROXYMETHYLFURFURAL AS A FUNCTION OF HEATING

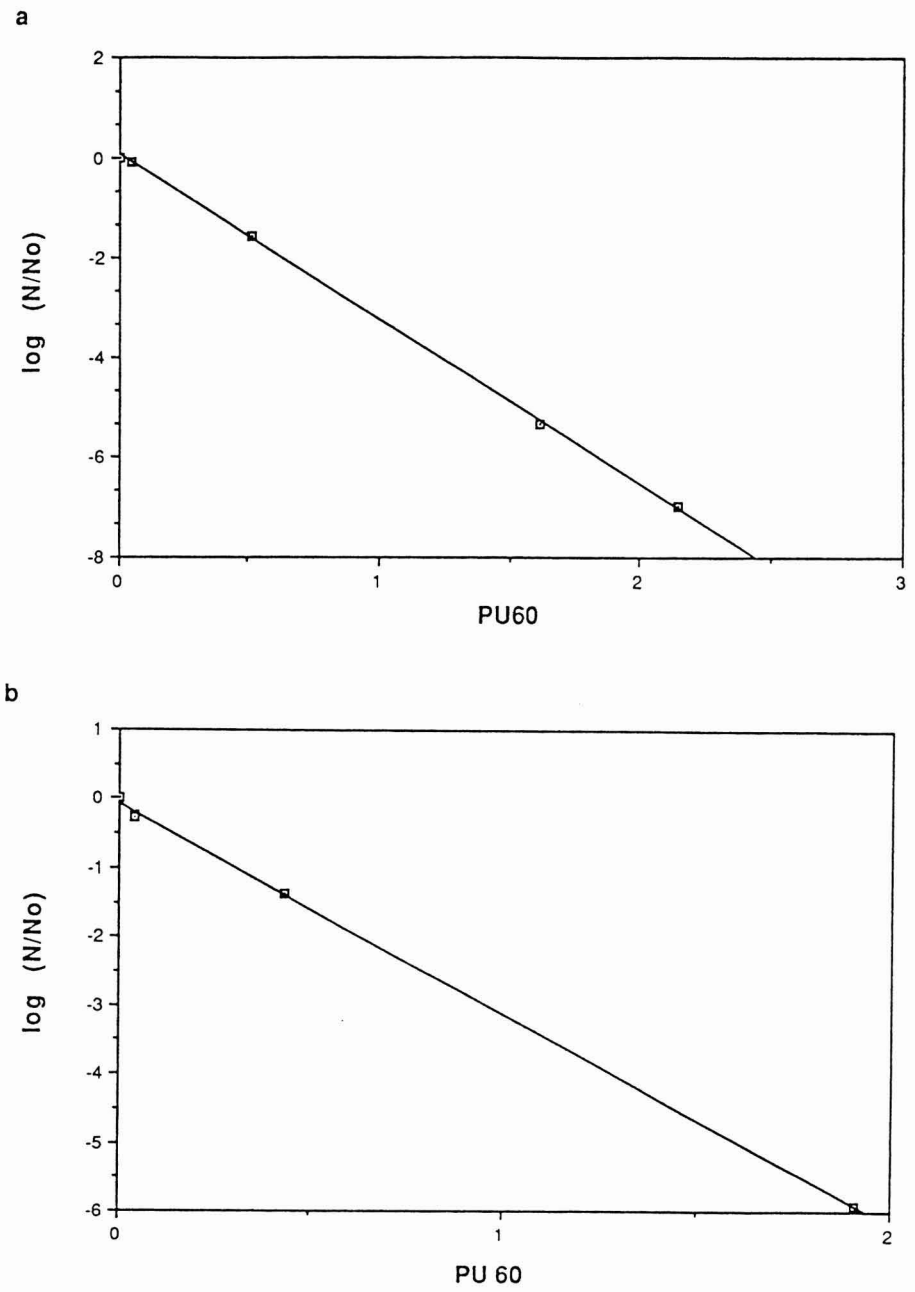

FIG. 2. HEAT RESISTANCE OF (a) *SACCHAROMYCES CEREVISIAE* (#522) AND (b) *HANSENULA ANOMALA* (#128) IN GRAPE JUICE
N_0 and N are the cell densities before and after heat treatment.

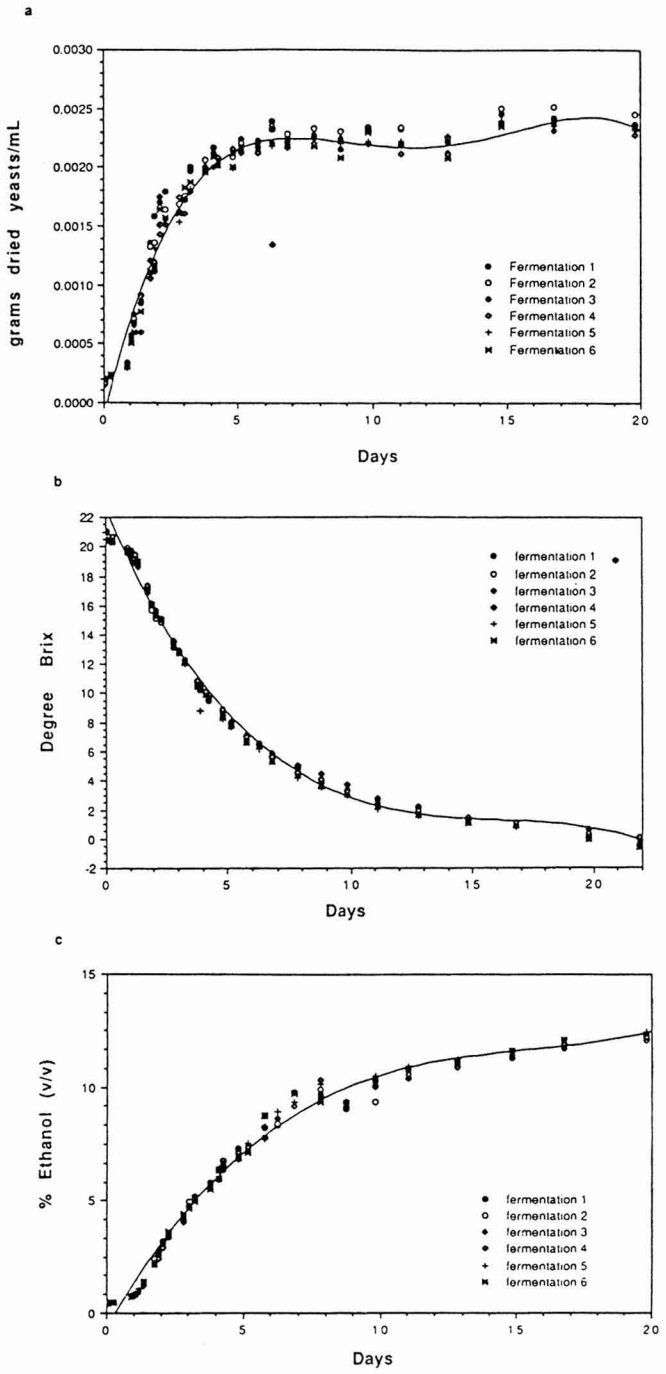

FIG. 3. EVOLUTION OF (a) CELL DENSITY, (b) DEGREE BRIX, AND (c) ETHANOL CONCENTRATION DURING FERMENTATION: comparison among unpasteurized juice (1, 2), pasteurized juice (3, 4) and pasteurized juice supplemented with thiamine (5, 6).

(D_{60} = 0.327 min). These values, measured in grape juice, are somewhat less than those measured by Bidan (1986) in model buffered solutions.

Pasteurization (13.67 PUs) of Thompson Seedless juice in a tunnel pasteurizer reduced 10^7 yeast cells/mL of juice to zero (undetectable level). This result is in agreement with the survival curves described above.

Whether the juice was pasteurized for a short time at high temperature (water bath at 81.5°C) or for a long time at low temperature (tunnel pasteurizer at 61°C), less than 15 PUs were always sufficient to reduce 10^7 cells/mL to zero.

Yeast ascospores are considerably more heat resistant than vegetative cells (Put and De Jong 1982; Splittstoesser *et al.* 1986) and further studies are indicated to determine the level of PUs required for their destruction.

Fermentations

The evolution of cell density, degree Brix and ethanol concentration during fermentation is shown in Fig. 3. Virtually no difference was found between the fermentation behaviors of pasteurized and unpasteurized juices as measured by analysis of variance (Table 2). For all four parameters examined, degree Brix, ethanol, pH and cell density, time was a significant source of variation ($p<0.001$). This was expected since these parameters vary during fermentation. The nature of the treatment received by the juice prior to fermentation was a significant source of variation for cell density, degree Brix and pH. However, examination of Fisher's least significant differences (LSDs) (Table 3) to compare the means obtained for each treatment shows that decrease in degree Brix was unaffected by pasteurization but was faster in the juice supplemented with thiamine. Cell density was significantly higher in unpasteurized juice than in pasteurized juice, even when the latter was supplemented with thiamine. There was no significant difference between replications except for cell density ($p<0.05$)

TABLE 2.
ANALYSIS OF VARIANCE OF FERMENTATION PARAMETERS.
DEGREES OF FREEDOM (df) AND F-RATIOS.

	Degree Brix		Ethanol		pH		Cell Density	
Variables	df	F-ratio	df	F-ratio	df	F-ratio	df	F-ratio
Time (Ti)	31	9020.13***	28	1264.70***	23	36.70***	28	744.47***
Treatment (Tr)	2	42.54***	2	1.25	2	3.42*	2	28.85***
Replication (R)	1	0.2	1	0	1	0	1	4.38*
Ti X Tr	62	1.37	56	0.97	46	1.03	56	1.1
Ti X R	31	1.11	28	0.54	23	0.54	28	0.72
Tr X R	2	7.47**	2	0.49	2	5.44**	2	2.36

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

TABLE 3.
MEANS AND FISHER'S LEAST SIGNIFICANT DIFFERENCES (LSDs) FOR THE FERMENTATION PARAMETERS

	Means			LSDs ($p<0.05$)
	Unpasteurized	Pasteurized	Pasteurized + Thiamine	
Degree Brix	10.487[a]	10.543[a]	10.270[b]	0.062
pH	3.225[b]	3.243[a]	3.239[ab]	0.014
Cell Density (mg/ml)	1.746[a]	1.666[b]	1.668[b]	0.024

Means sharing superscripts are not significantly different at $p<0.05$

indicating that for each treatment, the two fermenting musts behaved almost identically. The fact that degree Brix and ethanol did not differ between pasteurized and unpasteurized juice during fermentation allows us to conclude that pasteurization (23 PUs) does not affect the fermentability of grape juice.

CONCLUSION

Pasteurization of grape juice prior to fermentation in combination with small SO_2 additions is a way to prevent spoilage without affecting juice flavor, composition or fermentation performance. Between 10 and 20 PUs, as is customary with pasteurization of beer, should be amply sufficient to eliminate the native yeast population of grape juice. Further work is indicated to determine whether this amount of PUs would be adequate for control of bacteria.

Pasteurization of wine is an alternative to sterile filtration that could be explored and developed further. It would eliminate the need for SO_2 addition at the time of bottling. We found no difference in sensory quality between pasteurized (13.67 PUs) and untreated wine using 350-mL bottles. At this time, no claims are being made as to the efficacy of tunnel pasteurization with regard to microbial stabilization or the allowance of lowered sulfur dioxide additions in production with standard size (750 mL) bottles. However, we feel this procedure should be especially attractive for those with an eye to marketing in smaller sized containers, which are notoriously more difficult to fill at high speed under sterile conditions.

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EFFECT OF SOY FRACTIONS ON SOME FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF MAIZE-BANANA MIXTURES

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ABSTRACT

Dehydrated maize-banana mix was prepared by drum drying a slurry containing maize grits, banana and soy fraction (soy bean flour, soy milk, or soy residue). A sample containing skim milk in place of the soy was used as a control. Moisture, protein, fat and ash contents of the products were 5.60–9.18%, 17.89–22.80%, 0.46–7.13% and 0.75–2.46%, respectively. The control sample containing skim milk gave the highest value of 0.46 g/mL for its bulk density while the range for other formulations was 0.30–0.41 g/mL. Rheological data showed that all the products were pseudo-plastic, however, the power law parameters were found to depend on the formulation.

Comparative sensory evaluation showed that the most acceptable soy-maize-banana product contained soy milk.

INTRODUCTION

Cereal-based foods are formulated with protein sources such as milk or legume flour to improve protein content and quality (Akinrele and Edwards 1971; Bookwalter *et al.* 1978; Cheryan *et al.* 1979; Milner 1969; and Adeyemi *et al.* 1989). In developing countries, the most widely used source of protein is soybean having been considered as one of the least expensive when compared with egg, beef, milk or cowpeas (Oyenuga 1968). Soy-protein could be introduced into processed foods either as full fat or defatted flour, soymilk or as the 'soy residue' which is obtained after soymilk extraction (Milner 1969; Kapoor and Gupta 1981; and

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Bannde and Gupta 1982). Each of these fractions will exert different effects on the chemical properties and quality of the product.

Processed cereal-based foods are usually characterised by chemical composition and nutritional quality parameters (Milner 1969; Eka 1978; and Fashakin *et al.* 1986) while information appears scanty on the rheological behavior of such food products (Figoni and Shoemaker 1981). In this study physico-chemical properties are complemented with rheological parameters to investigate effect of soy fraction on a drum-dried maize-banana product.

MATERIALS AND METHODS

The batch of maize grits was purchased from Adegbemile Food Industries, Oye-Ekiti, Ondo State; Canco skim milk (99% fat free), and banana (ripe) fruits were obtained at Ile-Ife.

Soybeans were obtained from the Institute of Agricultural Research and Training, Ibadan whilst Cerelac and Nutrend, the two commercially produced weaning foods (as controls) were collected from Food Specialities (Nigeria) Limited, Agbara Factory, Nigeria.

Preparation of Soy-Fractions and Banana Pulp

The soy-fractions prepared were whole and defatted flours, soymilk and 'soy residue'. The whole soy flour was prepared as described by Adeyemi *et al.* (1989); while partially defatted soybean flour was prepared by heating whole soy flour to 90°C and pressing on a hydraulic press (Grezang, Amsterdam) at 3.5×10^7 N/m^2 to express the oil. The partially defatted soyflour was dried in a cabinet dryer at 45°C, milled and sieved on a 300 μm sieve. Soymilk and 'soy residue' were prepared as shown in Fig. 1. Banana pulp was prepared by sulphiting (70 ppm) the peeled, sliced cored banana prior to blending in a Kenwood blender.

Product Formulation and Preparation

The product was formulated from combinations, on dry basis, of maize grits, banana pulp and skim milk powder or soy fraction as presented in Table 1. The banana level was fixed at 10% since preliminary observation showed that dehydrated products from formulations containing more than 10% banana was dark in color. For soybeans, a level of 20% was within the range of 20–30% reported earlier to give a desirable level of protein content and, a relatively stable and acceptable product (Plahar and Leung 1985 and Adeyemi *et al.* 1989).

A slurry of 26% solid was prepared for each of the formulations. For the formulation containing soy milk, total solids of the milk was determined and

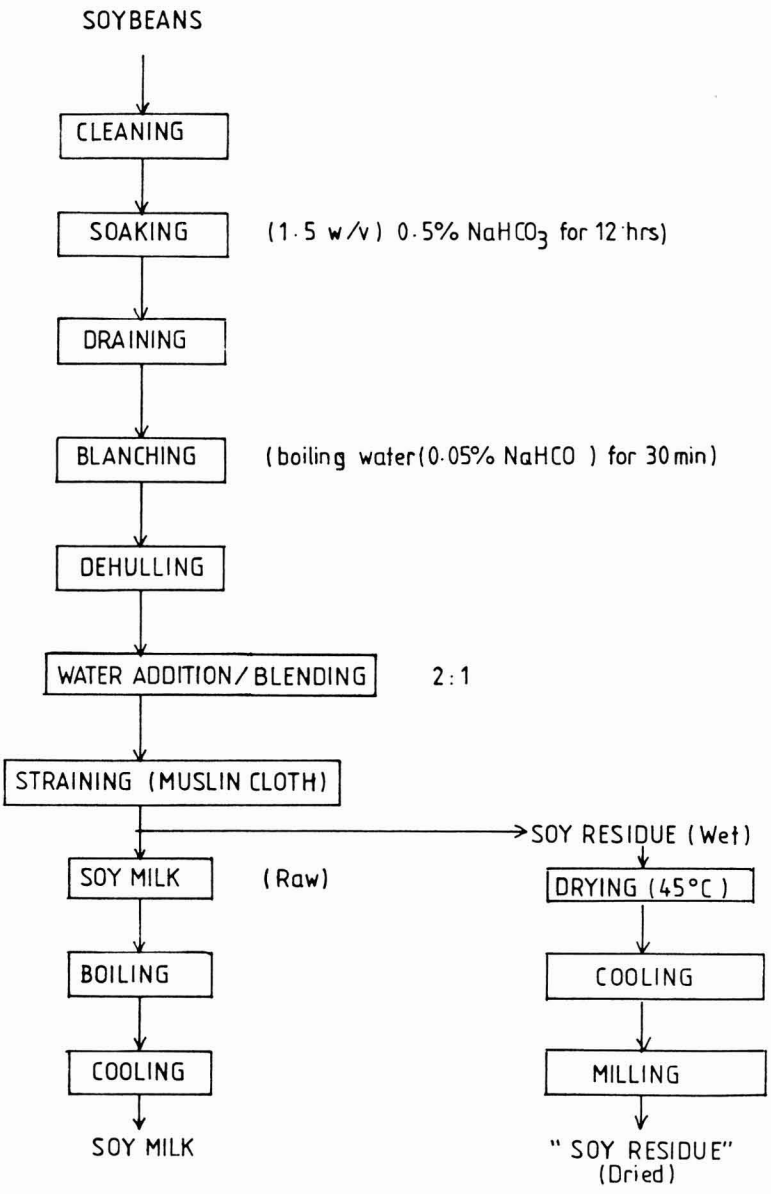

FIG. 1. PREPARATION OF SOYMILK AND SOY RESIDUE

the volume of soy milk corresponding to 20% solid (Table 1) was used in preparing the slurry and then adjusted to the solid content (26%) by adding distilled water. Each slurry was dried on a drum dryer (Goudsche Machinefabrick B. V. Goud, Holland) at a speed of 6.6 r.p.m. and 482.6 kN/m^2 pressure.

TABLE 1.
FORMULATION OF MAIZE-BANANA MIXTURES

INGREDIENTS	A	B	C	D	E	H	M	F*	G*
Maize grits	70	70	70	70	70	70	70		
Banana	10	10	10	10	10	--	10		
Skim milk powder	20	-	-	-	-	-	-		
Whole soy flour	-	20	-	-	-	-	-		
Defatted soy flour	-	-	20	-	-	-	-		
Soy milk	-	-	-	20	-	-	-		
Soy residue	-	-	-	-	20	-	-		

*F = NUTREND
*G = CERELAC

Proximate Analysis and Physico-Chemical Determinations

Analyses were carried out for moisture, protein, fat and ash contents (AOAC 1975). Apparent bulk density, expressed as mass/volume, was determined by obtaining the weight of materials contained in a measuring cylinder tapped to 10 mL mark while water hydration capacity was determined as described by Quinn and Paton (1979). For consistency measurement, a 500 g slurry was prepared with water (60°C) at 15% solid material (dry basis), except for Cerelac prepared at 20%, by blending in a Kenwood blender 5 min. Consistency of the slurry was evaluated on an Adams consistometer as described by Adeyemi *et al.* (1987). Each analysis was in triplicate and the means were separated by Duncan Multiple Range Test (Duncan 1955).

Viscosity Measurement

A slurry of flour fraction <300 μm aperture sieve size was prepared at 12% (w/v) solids with water at 30°, 35°, 40° and 45°C and viscosity measurements were carried out in a Haake MV3 Rotoviscometer (Gebruder HAAKE, Berlin, Federal Republic of Germany). The co-axial cylinder viscometer is equipped with 50/500 Nm^{-1} head; and water from a temperature-controlled water bath (K4R Electronic Messgerate—Work Lauda, West Germany) was circulated through the jacket to maintain the temperature of the slurry at 30°, 35°, 40°, and 45°C. The product was sheared over a wide range of shear rate at increasing

rate of shear up to 914.5 s^{-1} (Urbanski *et al.* 1983) to obtain the rheological behavior of the material using a simple power law model:

$$\eta = K \gamma^{n-1} \quad \ldots\ldots (1)$$

where: η = apparent viscosity (Pa.s)
γ = shear rate (sec^{-1})
n = flow behavior index
K = consistency index ($Pa.s^n$)

To stimulate the influence of mechanical action, especially during pumping of the slurry through pipeline systems, the stress on the sample at a steady shear rate of 832.6 s^{-1} (Urbanski *et al.* 1983) was measured to characterize the materials. The relaxation time, λ, was determined to investigate time dependent nature of the products using the equation:

$$\ln \frac{T - T_{eq}}{T_{max} - T_{eq}} = \frac{-t}{\lambda} \quad \ldots\ldots (2)$$

where T = shear stress at any time, t
T_{max} = maximum shear stress
T_{eq} = shear stress at equilibrium
t = time

Storage Stability Determination and Taste Panel Evaluation

Storage stability was evaluated by determining flour acidity (Adeyemi 1980) on fresh samples and those stored for 6 months at ambient temperature (26 ± 2°C). Sensory evaluation was carried out on the drum-dried and commercial samples by a Panel of 10 experienced and prospective mothers. Using a 9-point hedonic scale, the panelists were asked to score for color, odor, ease of reconstitution of the powder, consistency, smoothness/mouthfeel and taste. The scores were subjected to statistical analysis (Kramer and Twigg 1966) and the means were separated using the least significant difference (LSD) to establish the difference existing within the samples in respect of each of the quality attributes.

RESULTS AND DISCUSSION

Proximate Composition and Physico-Chemical Properties

The range obtained for the protein content of the formulated products was 17.89 to 22.81% while commercial samples had values of 15.98 and 16.32%, respectively, (Table 2). Of all the samples containing soy fraction, that containing 'soy residue' had the least value of 4.06% for its fat content; while ash contents

TABLE 2.
PROXIMATE COMPOSITION OF MAIZE-BANANA-SOY MIX*

Sample Code	Moisture (%)	Protein (%)	Fat (%)	Ash	(CHO %) by difference
A	5.93a	17.89a	0.46a	2.46a	73.59a
B	10.11b	20.71b	8.56b	0.75b	61.80b
C	8.74c	22.81c	5.15c	1.51c	60.51b
D	9.37c	20.51b	4.59c	1.31c	65.02c
E	9.00c	19.11b	4.06c	1.10c	67.47d
F	4.62ad	15.98d	3.06d	2.95a	73.59a
G	3.63d	16.32ad	3.20d	2.87a	74.11a

*Data expressed on dry basis
+ Means with same letters are not significantly different (P = 5%)

varied between 0.75 and 1.51%. Fortification with mineral elements partly explains the relatively higher ash contents of commercial samples. The range obtained for the proximate composition were within the expected values for weaning foods (Milner 1969).

Density values of the formulated products (0.30–0.46 g/mL) were lower than those of the commercial samples (Table 3) probably indicating differences in the nature of the components of the products. Bulk density would be of importance in packaging. The product containing 'soy residue' had the highest water holding capacity (WHC) of 3.96 mL/g while products containing soy flour (whole or defatted) or 'soy residue' had higher consistency value (12.50–13.75) than that containing skim milk (10.00) or the commercial samples with values of 1.75 and 2.00, respectively. This would appear to reflect the nature of the constituent materials or the extent of heat treatment of the samples (Adeyemi *et al.* 1989). The WHC, however, correlated positively with Adam's consistometer values at 0.96 correlation coeffiecient.

Rheological Properties

The data of shear stress and shear rate were fed into programmable calculator (Texas Instrument TI-66) using linear form of Eq. (1) to obtain the rheological parameters presented in Table 4. It is apparent from the data that the ingredients in the formulation affect the rheological parameters of the products. Though

TABLE 3.
PHYSICO-CHEMICAL CHARACTERISTICS OF MAIZE-BANANA-SOY MIX+

Samples	Density (g/ml)	Water holding capacity (ml/g)	Adam Consistometer meter value
A	0.46a	3.40a	10.00a
B	0.33b	3.77a	13.00b
C	0.41a	3.73a	12.50b
D	0.35b	3.86a	13.00b
E	0.30b	3.96a	13.75b
F	0.56c	2.92b	1.75c
G	0.49a	3.16a	2.00c
Correlation Coefficient (r)		0.96	

+ Means with same letters are not significantly different (P = 5%).

there seems not to be a pattern for the products, both the flow behavior index (n) and consistency index (k) values for slurry containing soy flour (whole soy flour, defatted soy flour, or soy residue) are apparently higher than those containing skim milk and the two commercial samples. These correlate favorably with the physico-chemical data of Table 3. Furthermore, addition of banana to grits lowered the flow behavior index (n) and consistency index (k) at 40°C. The values of n obtained for all the products were less than unity (Table 4) thereby indicating the pseudoplasticity of the materials.

The maximum apparent viscosity, η, (Pa.s) was calculated to be 0.202 for the base ingredient containing grits alone (formula H). Values of 0.195, 0.167, 0.167 and 0.131 were obtained for products containing whole soy flour, soy milk, soy residue, and partially defatted soy flour, respectively. However, the value for grit plus banana (formula M) had 0.084 Pa.s while Nutrend and Cerelac had values of 0.058 and 0.020, respectively.

The formulations presented in this work could have a built-in-mechanical and thermal properties against the stresses on the products during subsequent use which would affect the functional and rheological properties of the products. To

TABLE 4.
POWER LAW PARAMETERS OF 12% (W/V) SLURRY OF FORMULATED PRODUCTS AT 30–45°C*

Formulation	30°C		35°C		40°C		45°C	
	n	k	n	k	n	k	n	k
A	0.716	0.214	0.469	0.447	0.398	0.918	0.210	0.550
B	0.471	1.030	0.657	0.512	0.519	1.305	0.481	1.705
C	0.421	1.320	0.682	0.412	0.458	0.961	0.485	0.804
D	0.510	1.490	0.560	1.040	0.650	0.460	0.580	1.050
E	0.380	1.460	0.480	1.050	0.680	0.420	0.650	0.650
Nutrend	0.260	0.300	0.266	0.200	0.239	0.459	0.359	0.024
Cerelac	0.263	0.162	0.348	0.246	0.620	0.270	0.140	0.340
H	-	-	-	-	0.47	1.95	-	-
M	-	-	-	-	0.35	1.55		

*n = flow behavior index; k = consistency index ($N.S_m^{n-2}$).
The n values are less than unity hence they are pseudoplastic.

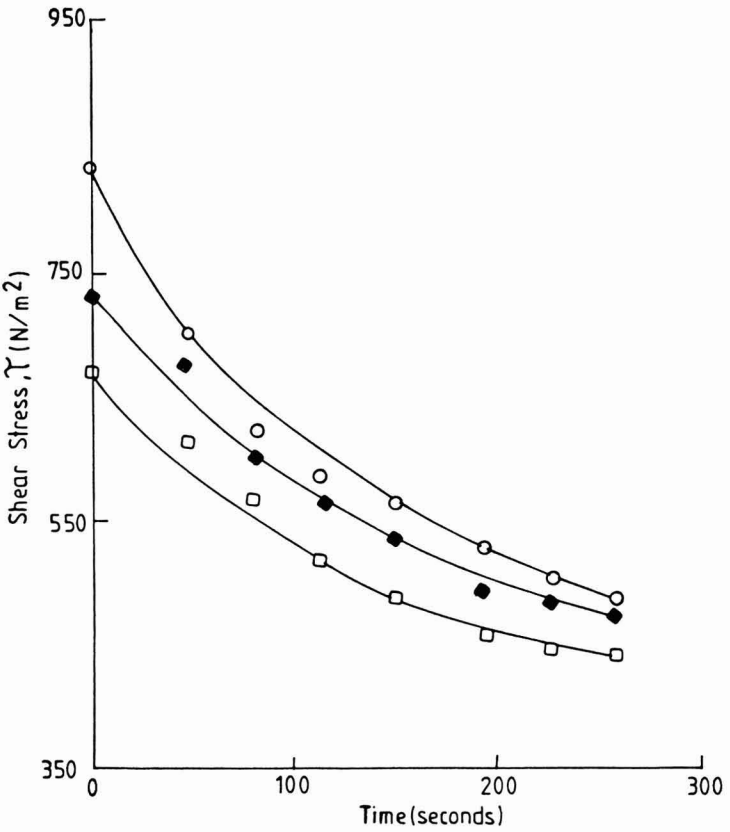

FIG. 2. STRESS DECAY AT A CONSTANT SHEAR RATE 832.65^{-1} AND 12% W/V CONCENTRATION (♦, 30°C; □, 35°C; ○, 45°C)

investigate this, measurement of stress (or viscosity) at a content shear rate (832.6 s^{-1}) was made. This showed that the sample were structurally deformable (Fig. 2). The shear stress relaxes to a constant value after a period of time and the data, in conjunction with Eq. 2 were used to calculate the relaxation time (Table 5).

Storage Stability and Sensory Evaluation

The most stable of all the products containing soy fraction was that with soymilk in its formulation (Table 6). The major source of deterioration is through oxidative rancidity since heat treatment (138°C) during drum-drying would have denatured lipoxidase enzymes (Baker and Mustakes 1973). Sensory evaluation scores showed that there were differences between samples and panelists for the

TABLE 5.
RELAXATION TIME (S^{-1}) OF 12% (W/V) SLURRY OF MAIZE-BANANA-SOY MIX SHEARED AT 832.6 S^{-1}

Formulation	30°C	35°C	40°C	45°C
A	63.8	60.6	91.8	81.7
B	71.3	42.9	60.8	63.1
C	52.9	50.5	55.2	51.6
D	47.2	40.5	53.4	31.6
E	45.1	46.5	61.2	29.7
F	45.3	48.0	43.6	34.8
G	105.5	76.7	8.3	64.0

quality attributes (Table 7). The color of samples B and C was found significantly inferior and unacceptable while none of the samples containing soybean fraction was acceptable on the basis of odor. If a score of 6 were used as the average minimum acceptable score, it would appear that sample D containing soymilk was the most acceptable out of products (B–E) containing soybean fraction. The

TABLE 6.
STORAGE STABILITY OF MAIZE-BANANA-SOY MIX

Samples	Flour acidity		Increase in acidity %
	0 day	6 months	
A	4.13	4.73	14.53
B	2.16	10.02	363.89
C	5.72	9.99	74.65
D	3.35	4.88	33.73
E	2.74	4.86	77.37
F	7.18	7.37	2.65
G	3.65	3.83	4.93

TABLE 7.
MEAN SENSORY SCORES AND LEAST SIGNIFICANT DIFFERENT (LSD) OF MAIZE-BANANA-SOY MIX

Sample Codes	QUALITY ATTRIBUTES*							
	Colour (Powder)	Odour (Powder)	Ease of Reconstitution	Colour (gruel)	Odour (gruel)	Mouth-feel	Sweetness	Consistency (gruel)
A	7.9^{a}	7.6^{a}	8.1^{ab}	7.9^{a}	7.5^{a}	8.1^{a}	6.8^{bc}	7.9^{a}
B	5.2^{c}	4.9^{bc}	5.5^{c}	4.9^{c}	4.4^{c}	4.1^{d}	4.1^{e}	4.6^{d}
C	5.1^{c}	4.4^{c}	7.2^{b}	4.7^{c}	4.3^{c}	5.9^{c}	4.2^{e}	6.2^{c}
D	6.4^{b}	5.7^{b}	7.3^{ab}	6.2^{c}	6.1^{b}	6.4^{bc}	5.7^{cd}	5.8^{cd}
E	6.5^{b}	5.5^{bc}	5.5^{c}	5.4^{bc}	5.5^{bc}	5.7^{c}	4.9^{de}	5.9^{c}
F	8.5^{a}	8.5^{a}	8.3^{a}	8.4^{a}	8.5^{a}	7.7^{ab}	8.6^{a}	7.7^{ab}
G	8.3^{a}	7.8^{a}	7.7^{ab}	7.8^{a}	7.6^{a}	6.1^{c}	7.4^{ab}	6.5^{bc}
$LSD_{(0.05)}$	1.00	1.27	1.09	1.17	1.26	1.37	1.43	1.26

*Data in columns with same letters are not significantly different (at 5% level)

least two acceptable products were those containing whole and partially defatted soy flour, probably due to the distinctive beany flavor characteristic of the products. This is a further confirmation of the adverse effect of beany flavor in the acceptability of soy containing processed products (Cheryan *et al.* 1979; Plahar and Leung 1985).

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USING THE L NUMBER TO PREDICT THE EFFICACY OF MOISTURE BARRIER PROPERTIES OF EDIBLE FOOD COATING MATERIALS

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ABSTRACT

The L number, a dimensionless number that describes the ratio of the moisture permeance of a food substance to that of a coating material, was used to predict the effectiveness of available edible coating materials to prevent moisture migration from or into coated foods. A computer model was developed using the finite difference method to simulate moisture migration through coated food materials. The results indicated that when the L number is smaller than 0.04, the main resistance to moisture transfer for a coated food material is the diffusion of moisture within the food material itself. On the other hand, if the L number is larger than 4, the main resistance to moisture transfer is the permeance of the coating material. The application of this approach to predict the effectiveness of different coating materials for various dried foods is presented.

INTRODUCTION

One of the major problems in multicomponent food systems with components at different water activities, such as dry fruits mixed with cereal or nuts, is moisture migration between the different components. The moisture content of the food components strongly influences texture, the stability of the food because of chemical reactions, and the susceptiblity of the ingredient to microbial spoilage (Labuza 1970). The kinetics of moisture migration between food components was studied by Hong *et al.* (1986) and Gencturk *et al.* (1986) using the finite element method. Hong *et al.* (1986) showed that the limiting factor in moisture migration is the moisture diffusion through the component with the lowest ratio

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of diffusivity to the square of the characteristic length (D_{eff}/L_0^2) in a multicomponent food system.

One approach to block moisture migration in a multicomponent food system with components at different water activities is through the use of an edible moisture barrier on the surface of one of the components, generally the one with the least total area. An ideal moisture barrier will minimize the moisture migration between the food components and should not influence the sensory properties of the product. It should also be cost effective and easy to apply.

Recently there has been much research on the development of edible moisture barriers (Biquet and Labuza 1988; Greener and Fennema 1989a, 1989b; Kamper and Fennema 1984a, 1984b, 1985; Kester and Fennema 1989; Krochta *et al.* 1989; Guilbert 1986.) However, prediction of the efficacy of these barriers in preventing moisture migration has not been addressed. The desirable permeance for a coating material to slow down moisture migration in a food system is not only dependent on the properties of the food material but also influenced by the desired length of shelf life and the environmental conditions, such as temperature and relative humidity. For example, to prevent moisture migration between food materials with two different shelf lives, the food material with the shorter shelf live will require more protection than the other with the longer shelf live. A similar situation can occur for food materials stored at two different environmental conditions such as at different relative humidities. A food material will require more protection when stored at a higher water activity gradient than a lower water activity gradient.

In order to evaluate the effectiveness of an edible coating, one approach is to evaluate the L number (L#) of the coated food system. The L number is a dimensionless number which describes the ratio of the moisture permeance of the food to that of the coating material. It was developed in a previous study for packaged food systems by Taoukis *et al.* (1988). The objectives of this study were to use a computer simulation to study the effectiveness of the L# in predicting moisture transfer through a coated food material and to develop an application scheme for using the L# to evaluate the effectiveness of a coated food system.

MODEL DEVELOPMENT

The Concept of The L Number

The concept of the L number was previously described by Taoukis *et al.* (1988) for the study of moisture transfer as related to the shelf life of packaged foods. Moisture migration in a coated food is controlled by two factors: (1) the permeance of the food itself, and (2) the permeance of the coating material. If the permeance of the food is much greater than that of the coating material, the

main resistance to moisture migration will be the permeance of the coating material. The analytical equation governing this type of moisture transfer was first developed by Karel and Labuza (1969) for packaged food systems and can be written as:

$$ln\ \Gamma = \Phi_{ext}\ t \tag{1}$$

where:

$$\Gamma = \frac{M_e - M_i}{M_e - M_{avg}}$$

$$\Phi_{ext} = \left(\frac{k}{x}\right)\left(\frac{A}{W_s}\right)\left(\frac{P_o}{b}\right)$$

where M_{avg} is the overall average moisture content of the food (dry basis) at any time, t, M_i is the initial moisture content of the food, M_e is the equilibrium moisture content corresponding to the external relative humidity from the linear isotherm of the food, Γ is the inverse of the unaccomplished moisture fraction, Φ_{ext} is the overall external permeance, k is the permeability of the packaging or coating material, x is the thickness of this packaging or coating, k/x is recognized as the permeance of this packaging or coating material, A is the area of transfer, W_s is the weight of the dry solids, P_o is the saturated water vapor pressure at the temperature of food and environment, which are assumed to be in equilibrium, and b is the slope of the assumed linear isotherm of the coated material over the expected moisture range. Equation (1) shows that Φ_{ext} depends on the moisture permeance of the coating, the A/W_s ratio, the environmental conditions, and the isotherm properties of the food. For a semi-infinite slab shape of the food material, the A/W_s ratio can be replaced by the reciprocal of the product of the bulk solids density and the characteristic half thickness, $(\rho_s * L_o)^{-1}$.

If the permeance of the food is much smaller than that of the coating material, the main resistance to moisture migration will be diffusion within the food material. In this case, one can write an approximate analytical solution for diffusion of moisture (Crank 1975) as follows:

$$ln\ \Gamma = \Phi_{int} t + ln\ \frac{\pi^2}{8} \tag{2}$$

where:

$$\Phi_{int} = \frac{D_{eff}\pi^2}{4L_o^2}$$

where Φ_{int} is the overall internal permeance of the food system, and D_{eff} is the effective diffusivity. For a long time period (i.e., $\Phi_{int}\ t >> ln\ \pi^2/8$), using the

assumed linear isotherm over the expected moisture range and the diffusion equation, Taoukis *et al.* (1988) developed a relationship between the food permeability, β_D, and the effective food diffusivity, D_{eff}, based on the principles developed by Krischer and Kroell (1963). This is shown in Eq. (3).

$$D_{eff} = \frac{\beta_D}{\rho_s}\left(\frac{P_o}{b}\right) \tag{3}$$

From this, the food permeance, which is similar to k/x for a coating material, at a given half thickness, L_o, is:

$$\frac{\beta_D}{L_o} = \frac{D_{eff}\,\rho_s}{L_o}\left(\frac{b}{P_o}\right) \tag{4}$$

One can assess the relative importance of the two mechanisms of moisture transfer by calculating the ratio of the overall internal permeance to the overall external permeance where:

$$\frac{\Phi_{int}}{\Phi_{ext}} \cong 2.5\left(\frac{\beta_D / L_o}{k / x}\right) \tag{5}$$

The dimensionless number, L#, can thus be defined as:

$$L_\# = \left(\frac{\beta_D / L_o}{k / x}\right) \tag{6}$$

Moisture Migration through a Coated Food System (Simulation Model)

A one dimensional moisture migration model was used. The unsteady state diffusion equation for moisture transfer in a food material is governed by Fick's second law of diffusion where:

$$\frac{\partial M}{\partial t} = D_{eff}\frac{\partial^2 M}{\partial X^2} \tag{7}$$

with the initial and boundary conditions of:

$$M(X,t) = M_i \qquad \text{at } t = 0\,;\ 0 \le X \le L_o \tag{8}$$

at the center:

$$\frac{\partial M}{\partial X} = 0 \qquad \text{at } X = 0 \tag{9}$$

and at the surface just below the coating:

$$\rho_s(-D_{eff}\frac{\partial M}{\partial X}) = \frac{k}{x}(P_i - P_e) \qquad \text{at } x = L_0 \tag{10}$$

where X is the space dimension, P_i is the water vapor pressure at the interface of the coating and the food, and P_e is the water vapor pressure of the environment. Equation (10) expresses the mass balance of water transport. The amount of water transfer out of the food surface is equal to the amount of water transfer out of the coating material, assuming the edible coating does not gain or lose moisture. As implied earlier, one can assume that over a small moisture range, the linear moisture sorption isotherm can be applied to the food. Thus Eq. (10) can be rewritten as follows:

$$\rho_s(-D_{eff}\frac{\partial M}{\partial X}) = \left(\frac{k}{x}\right)\left(\frac{P_o}{b}\right)(M_{interface} - M_e) \tag{11}$$

A computer program, SIM, was written to simulate the moisture content of the coated food material as a function of time and location. SIM was written in VAX/FORTRAN using the Crank-Nicolson finite difference method to solve the governing Eq. (7) with the boundary conditions shown in Eq. (9) and (11). The time step used was 0.05 h and the space dimension, L_o, was divided into 20 nodes. The volume average moisture contents were reported at one day intervals.

RESULTS AND DISCUSSION

Simulation Studies

Dried banana chips were used as the model system in this study because of their one dimensional moisture transfer characteristics. The parameters used for the computer simulation, including effective diffusivity, thickness, and isotherm data, were adopted from a previous study (Hong *et al.* 1986). These properties and the permeance calculated from these data are listed in Table 1.

The finite difference simulation, in which both food diffusion and coating permeance are controlling moisture migration, for banana chips with various

TABLE 1.
CHARACTERISTIC PROPERTIES AND EXTERNAL ENVIRONMENT CONDITIONS FOR DRIED BANANA CHIPS

Effective diffusivity (D_{eff})	2.515	E-7	$m^2\ day^{-1}$
Bulk dry density (ρ_S)	0.859	E+6	g solid m^{-3}
Half thickness (L_0)	1.76	E-3	m
Initial moisture content (M_i)	1.70		g/100 g solid
Final moisture content (M_e)	4.60		g/100 g solid
Critical moisture content (M_c)	4.22		g/100 g solid
Slope of linear isotherm (b)	7.10		g/100 g solid
Saturated water vapor pressure (P_0) at 25°C	23.76		mmHg
Calculated food permeance (β_D/L_0)	0.367		$g\ m^{-2}day^{-1}mmHg^{-1}$

L#s as the result of different types of coating material permeance, k/x, is illustrated in Fig. 1. The moisture transfer rate decreases with increasing L# as expected. When the L# increased, i.e., the permeance of the coating materials decreased, the coating materials became more effective as moisture barriers. For comparison purposes, one can assume that banana chips become unacceptably

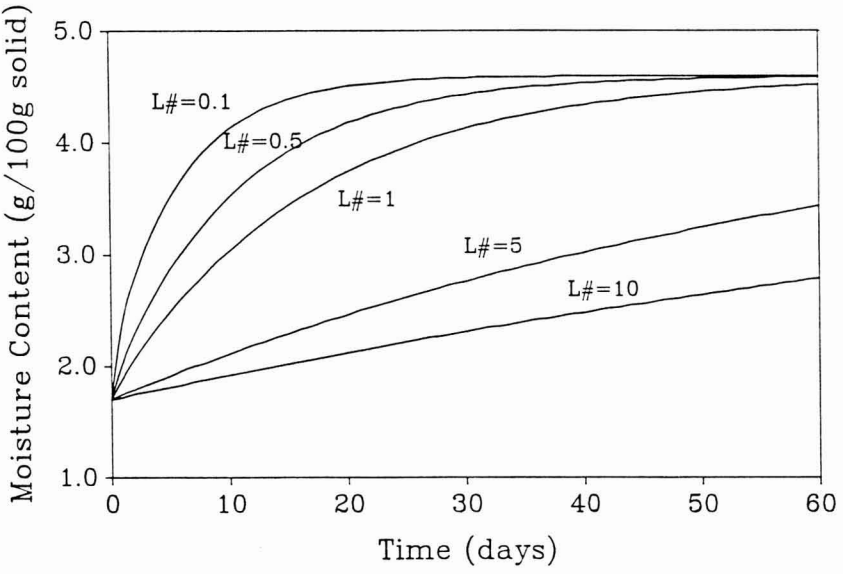

FIG. 1. SIMULATION RESULTS OF MOISTURE MIGRATION IN BANANA CHIPS WITH VARIOUS L# VALUES BY VARYING THE COATING PERMEANCE, USING THE FINITE DIFFERENCE MODEL IN WHICH BOTH INTERNAL DIFFUSION AND COATING PERMEANCE ARE CONTROLLING T MOISTURE TRANSFER

soft at the critical moisture content of 4.22 g/100 g (or a_w = 0.55). As calculated by SIM, the time to reach this critical moisture content for systems with L#s of 0.1, 0.5, 1, and 5 are 12, 22, 34, and 133 days, respectively.

Figure 2 demonstrates the moisture migration into banana chips for a system with an L# of 0.04 based on three different controlling mechanisms respectively, the food diffusion model (Eq. 2), the coating permeance model (Eq. 1) and the finite difference model (Eq. 7–11). Since the finite difference model simulates moisture migration controlled by both internal diffusion and the coating permeance, from the theoretical standpoint, the finite difference model should be the most realistic model as compared to the other two models. In Fig. 2, the finite difference simulated moisture transfer rate is closer to that predicted by the diffusion model than that predicted by the coating permeance model. This result suggests that the major resistance to moisture migration is the diffusion of moisture through the food substance rather than the permeance of the coating. Figure 2 also illustrates that one should not use the linear isotherm model (Eq. 1) to predict the moisture transfer for a food system at L#s of 0.04 or less since the internal moisture diffusion becomes the controlling factor.

When plotting *ln* Γ vs. time, one can get a straight line. The slope of this line can be used as an indication of moisture transfer rate. Table 2 summarizes

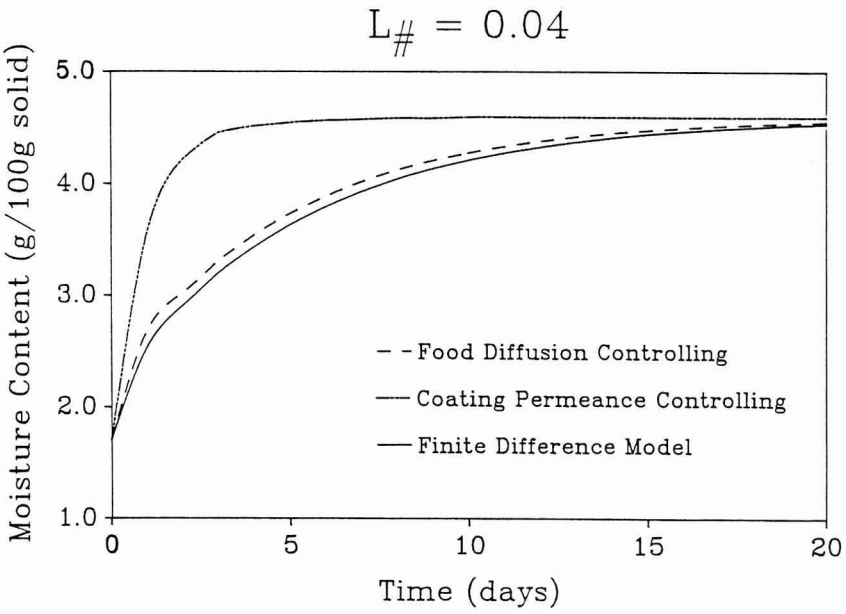

FIG. 2. SIMULATION RESULTS OF MOISTURE MIGRATION IN A BANANA CHIP SYSTEM AT AN L# OF 0.04

TABLE 2.
COMPARISON OF SLOPES OF *ln* Γ VS TIME FOR DIFFERENT L#S

	Slope of *ln* Γ vs time (day^{-1})		% Error*1	
L#	Finite Difference Model	coating Model	Diffusion*2 Model	Coating Model
0.01	0.1968	n.d.*3	2.98	n.d.
0.02	0.1930	n.d.	5.02	n.d.
0.03	0.1893	n.d.	7.07	n.d.
0.04	0.1857	n.d.	9.14	n.d.
0.05	0.1821	n.d.	11.23	n.d.
0.06	0.1788	n.d.	13.33	n.d.
0.07	0.1755	n.d.	15.45	n.d.
0.08	0.1723	n.d.	17.58	n.d.
0.09	0.1692	n.d.	19.73	n.d.
0.1	0.1668	0.8119	21.89	> 100
0.2	0.1412	0.4059	43.49	> 100
0.3	0.1218	0.2706	66.42	> 100
0.4	0.1068	0.2029	89.70	90.02
0.5	0.0949	0.1623	> 100	71.07
0.6	0.0855	0.1353	> 100	58.26
0.7	0.0776	0.1159		49.50
0.8	0.0710	0.1014		42.85
0.9	0.0655	0.0902		37.79
1.0	0.0606	0.0811		33.94
2.0	0.0350	0.0405		16.15
3.0	0.0244	0.0270		10.95
4.0	0.0187	0.0202		8.18
5.0	0.0152	0.0162		6.51
6.0	0.0128	0.0135		5.39
7.0	0.0111	0.0115		4.60
8.0	0.0097	0.0101		4.03
9.0	0.0087	0.0090		3.54
10.0	0.0079	0.0081		3.10

1 % Error = 100%($Slope_{finite\ difference}$ - $Slope_{model}$) / ($Slope_{finite\ difference}$)
*2 Slope of diffusion model is 0.2026 (day^{-1})
*3 n.d. = not determined

the percentage of error of the slope from the diffusion and coating permeance models as compared with the finite difference model at various L#. When the L# increased, the errors from the diffusion model also increased. Less than 10% error for moisture transfer rates between diffusion and finite difference models were observed when the L# is less than 0.04.

From these simulations, it can be shown that a food system with an L# of 0.4 results in a condition where the overall internal permeance and overall external permeance are equally important. This is shown in Fig. 3. In this case, similar results were predicted by the coating and the diffusion models. However,

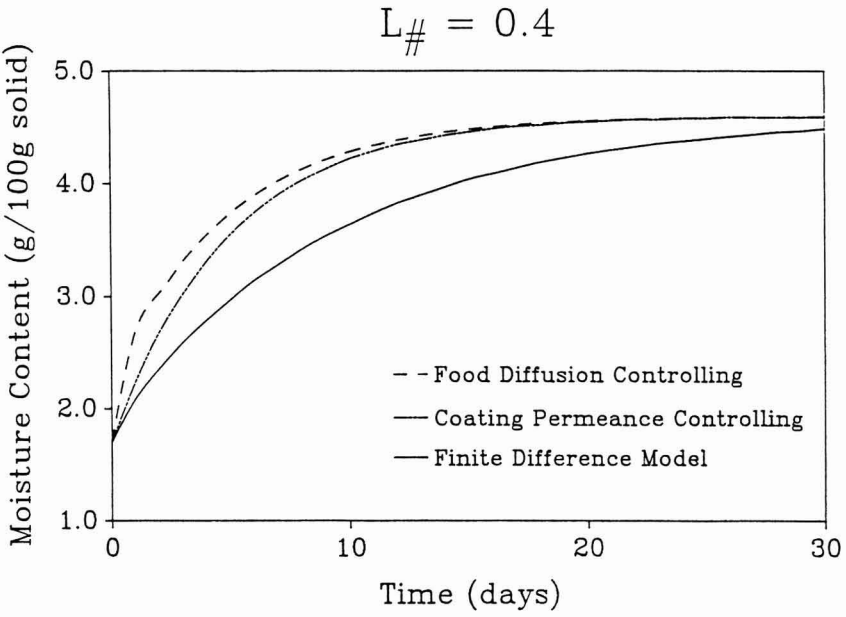

FIG. 3. SIMULATION RESULTS OF MOISTURE MIGRATION IN A BANANA CHIP SYSTEM AT AN L# OF 0.4

both models alone over predict the moisture migration through this system when compared to that predicted by the finite difference model which takes both mechanisms into account and shows a slower moisture gain.

With an increase in the L# for constant internal diffusion properties, the permeance of the coating material decreases thereby becoming the controlling resistance. For a system with an L# of 4 or greater, the rate of moisture transfer is significantly reduced compared to those of systems with L#s of 0.04 or less (as shown in Fig. 1). The main resistance to moisture migration in a system with an L# of 4 is the permeance of the coating rather than the diffusion of moisture through the food material. Thus the linear coating model will effectively predict moisture migration of the system as shown in Fig. 4. Table 2 shows that when the L# increased, the errors from the coating permeance model decreased. Less than 10% error for moisture transfer rates between coating permeance and finite difference models were observed when the L# is greater than 4.

Application Scheme of L# Concept for the Evaluation of the Effectiveness of a Coated Food System

In the literature, many types of edible moisture barriers with different permeabilities have been evaluated. However, a simple method which could be used

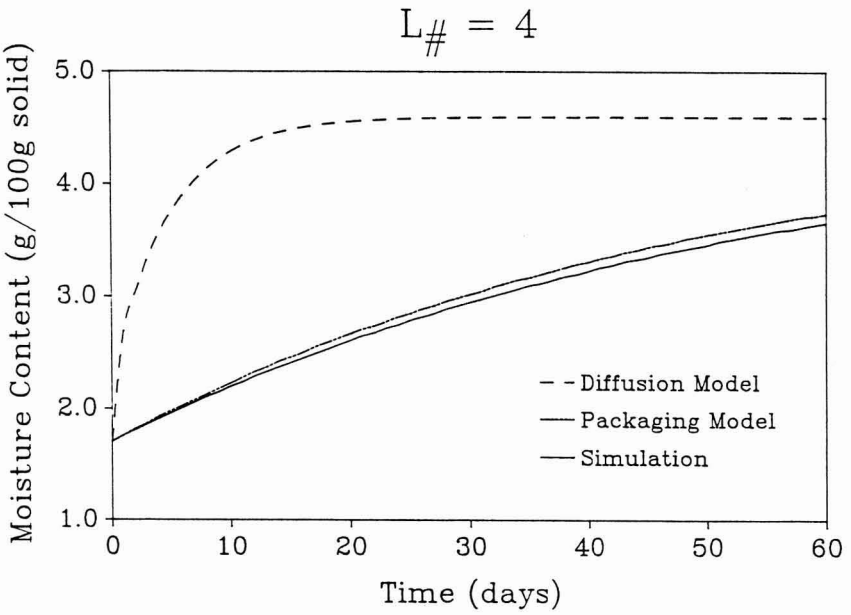

FIG. 4. SIMULATION RESULTS OF MOISTURE MIGRATION IN A BANANA CHIP SYSTEM AT AN L# OF 4

to mathematically calculate the desired moisture permeance of an edible barrier for a particular food system has not been previously addressed. One approach to evaluate the effectiveness of an edible barrier is through the use of the L# concept.

The application scheme for the prediction of the effectiveness of a coated food system using the L# approach is shown in Fig. 5. The properties of the uncoated food materials which include, thickness, the moisture diffusivity of the food, the bulk dry solids density, and the slope of the linear moisture sorption need to be determined. The overall internal permeance of the food can then be acquired using Eq. (4). Next, the critical moisture content, M_c, of the food needs to be known. Above or below this critical moisture content, the food material will change its sensory properties, or increase the rate of quality loss. The external conditions of the environment, including relative humidity and temperature, for which the coated food will be subjected to, need to be known. Then, the desired shelf life, θs, of the coated food product needs to be decided. Generally, dried food systems require a shelf life between 3 months and 1 year.

After determining the properties and conditions, one can calculate the required permeance of the coating material by Eq. (12), assuming the permeance of the

Application Scheme to use the L#

1. Acquire properties of food materials (i.e., Lo, D_{eff}, ρ_s, and b).
2. Determine the critical moisture content, M_c.
3. Define the external conditions that the food material is subjected to (i.e., %RH and T).
4. Define the desired shelf life, θ_s.
5. Calculate the required permeance of the coating, assuming that the coating is controlling moisture migration , using:

$$\left(\frac{k}{x}\right)_{req} = \left(\ln\frac{M_e - M_i}{M_e - M_c}\right)\left(\frac{W_s b}{A P_o \theta_s}\right)$$

$$or \quad = \frac{\ln \Gamma_c L_o \rho_s b}{P_o \theta_s}$$

6. Calculate the $L_{\#}$ and define the controlling mechanism for moisture transfer.
7. Look in the literature for a coating film with the desired k/x.

FIG. 5. THE APPLICATION SCHEME FOR USING THE L# CONCEPT TO EVALUATE THE EFFECTIVENESS OF EDIBLE BARRIER COATED FOOD SYSTEM

coating material is the major resistance. Equation (12) is the reorganized form of Eq. (1).

$$\left(\frac{k}{x}\right)_{req} = \left(\ln\frac{M_e - M_i}{M_e - M_c}\right)\left(\frac{W_s b}{A P_o \theta_s}\right) \tag{12}$$

$$or \quad = \frac{\ln \Gamma_c L_o \rho_s b}{P_o \theta_s}$$

where: $\Gamma_c = (M_e - M_i)/(M_e - M_c)$

The L# for the required permeance can then be calculated using Eq. (6). If the calculated L# is 4 or greater, then the assumption that the permeance of the

coating material is the major resistance is valid. Thus the calculated $(k/x)_{req}$ should be used as the targeted permeance for the edible coating material. One can then look in the literature for a coating with the required permeance. If the calculated L# is between 4 and 0.04, the assumption is not correct and the permeances of both the food and the coating material are controlling moisture migration. However, the coating material with the calculated $(k/x)_{req}$ or a permeance slightly larger than the calculated value can be used with some safety margin. If the calculated L# is smaller than 0.04, then internal diffusion is the major resistance to moisture migration; thus either no coating or a coating material with a permeance much greater than $(k/x)_{req}$ can be used. To assist in screening edible coating materials, Table 3 lists the permeance values of selected edible films in descending order taken from published literature. The published data were obtained by a variety of experimental methods, and values were converted into similar units for ease of comparison.

Table 4 shows some examples of using the L# concept to select the correct coating materials for dried banana chips with different desired shelf lives. The value of the required $(k/x)_{req}$ as calculated from Eq. (12) ranged from 0.025 to 9.175 g m^{-2} $day^{-1}mmHg^{-1}$ for banana chips with desired shelf lives of 1 year to 1 day, respectively. The edible coating material for banana chips with a required one year shelf life leads to a required permeance of 0.025 g $m^{-2}day^{-1}mmHg^{-1}$ which gives an L# of 14.6. Since the L# is greater than 4, one can conclude that the moisture migration in this food system will be controlled by the coating permeance. Therefore, the selection of an edible barrier with a permeance close to the $(k/x)_{req}$ is essential. However, as shown in Table 3, no edible moisture barriers with such low permeance values are currently available. To control moisture migration in this system, other means (such as lowering the external relative humidity or temperature) can be applied. For a shorter shelf life, a higher $(k/x)_{req}$ will sufficiently reduce moisture migration from the banana chips. For banana chips with 5 months shelf life, a coating material with a permeance of 0.061 g $m^{-2}day^{-1}mmHg^{-1}$ is required with an L# of 6.0. From Table 3, one can find that the paraffin film or chocolate liquor film will be suitable for this application. For banana chips with only 1 month shelf life required, a coating material with a permeance of 0.306 g $m^{-2}day^{-1}mmHg^{-1}$ and an L# of 1.2 are calculated using Eq. (12) and (6). Since the L# is less than 4, one can conclude that both internal diffusion and coating permeance are controlling factors. However, if a coating with the calculated $(k/x)_{req}$ or slightly higher is selected, it will still give more than the desired shelf life. From Table 3, one can find that the cocoa butter/sucrose/cocoa solids film and the stearic/palmitic film were applicable to this situation. For comparison purposes, if banana chips require only 1 days of shelf life, the calculated $(k/x)_{req}$ of edible moisture barrier is 9.175 g $m^{-2}day^{-1}mmHg^{-1}$ with an L# of 0.04. In this case, the

TABLE 3.
WATER VAPOR TRANSMISSION, PERMEABILITY, AND PERMEANCE VALUES OF EDIBLE COATINGS LISTED IN ORDER OF INCREASING PERMEANCE

barrier	WVTR	permeability	permeance	temp.	R H	thickness	reference
	$g m^{-2} day^{-1}$	g mil $m^{-2} day^{-1} mmHg^{-1}$	g m-2 day -1 mmHg-1	°C	% gradient	mils	
- parraffin	0.73	0.14 - 0.21	0.036	25	0 to 85	3.9 - 5.7	Kamper and Fennema, 1984a
- chocolate liquor	0.47	2.72	0.041	27	0 to 43	66.4	Landman et al., 1960
- chocolate liquor	1.13	3.81	0.057	27	0 to 74	66.9	Landman et al., 1960
- beeswax	1.40	0.27 - 0.29	0.069	25	0 to 85	3.9 - 5.7	Kamper and Fennema, 1984a
- sweet milk chocolate	1.00	5.02	0.072	27	22 to 74	69.7	Landman et al., 1960
- stearic acid (0-24 hrs study)	2.20	0.14 - 0.19	0.109	25	0 to 85	1.3 - 1.7	Kamper and Fennema, 1984a
- stearic/palmitic acid laminated to beeswax	2.09	0.30	0.140	25	0 to 97	2.2	Kester and Fennema, 1989
- cocoa butter sucrose/cocoa solids	1.36	3.37	0.143	20	0 to 54	23.6	Biquet and Labuza, 1988
- stearic/palmitic acid (0–24 hr study)	3.10	0.20 - 0.26	0.154	25	0 to 85	1.3 - 1.7	Kamper and Fennema, 1984a
- cocoa butter	4.75	11.60	0.183	27	0 to 97	63.4	Landman et al., 1960
- stearic/palmitic acid (0–24 hr study)	4.20	0.27 - 0.33	0.196	25	0 to 90	1.3 - 1.7	Kamper and Fennema, 1984b
- stearic/palmitic acid (0–24 hr study)	4.00	0.28 - 0.37	0.202	25	0 to 85	1.4 - 1.8	Kamper and Fennema, 1984b
- cocoa butter/ sucrose/cocoa solids	1.20	4.84	0.207	20	0 to 33	23.4	Biquet and Labuza, 1988
- cocoa butter/ sucrose/cocoa solids	3.21	5.45	0.226	20	0 to 81	24.1	Biquet and Labuza, 1988
- cocoa butter/ sucrose/cocoa solids	2.74	5.85	0.244	20	0 to 64	23.6	Biquet and Labuza, 1988
- methyl cellulose/ beeswax	5.94	0.50	0.250	25	0 to 100	2.0	Greener and Fennema, 1989b
- stearic/palmitic acid (0–24 hr study)	5.13 - 6.59	0.47	0.265 - 0.334	25	0 to 83	1.4 - 1.8	Kamper and Fennema, 1984a
- stearic/palmitic acid/ beeswax	2.75	0.80	0.360	25	65 to 97	2.2	Kester and Fennema, 1989
- beeswax/paper filter (W50)	8.83	1.67	0.37	25	0 to 100	4.5	Kester and Fennema, 1989

Table 3. (continued)

barrier	WVTR	permeability	permeance	temp.	R H	thickness	reference
	$g\,m^{-2}day^{-1}$	g mil $m^{-2}day^{-1}mmHg^{-1}$	g m-2 day -1 mmHg-1	°C	% gradient	mils	
- stearic/palmitic aicd (0–24 hr study)	2.91	0.69	0.383 - 0.490	25	33 to 65	1.4 - 1.8	Kamper and Fennema, 1984a
- cocoa butter / sucrose/ cocoa solids	2.12	10.53	0.448	20	54 to 81	23.4	Biquet and Labuza, 1988
- stearic/palmitic acid/ beeswax	22.36	1.60	0.940	25	0 to 100	1.7	Greener and Fennema, 1989a
- stearic/palmitic acid (0–24 hr study)	8.00	1.43 - 1.79	1.052	25	43 to 75	1.4 - 1.7	Kamper and Fennema, 1984a
- stearic/palmitic acid (0–24 hr study)	25.00	1.45 - 1.90	1.12	25	0 to 97	1.3 - 1.7	Kamper and Fennema, 1984a
- stearic/palmitic acid/ beeswax	10.87	7.30	1.429	25	65 to 97	1.7	Greener and Fennema, 1989a
- stearic/palmitic acid (0–24 hr study)	11.00	2.026 - 2.460	1.447	25	53 to 85	1.4 - 1.7	Kamper and Fennema, 1984a
- hydrogenated soy oil/rapeseed oil/paper filter (W50)	48.62	9.21	2.050	25	0 to 100	4.5	Kester and Fennema, 1989
- stearyl alcohol/paper filter (W50)	63.36	12.00	2.670	25	0 to 100	4.5	Kester and Fennema, 1989
- hydrogenated soy/ palm oil/mono and diacylglycerols (0–4 hr study)	55.00	10.62 - 16.07	2.724	25	0 to 85	3.9 - 5.9	Kamper and Fennema, 1984a
- chocolate liquor	75.84	86.97	2.924	27	0 to 97	63.8	Landman et al., 1960
- hydrogenated soy/ palm oil/mono and diacylglycerols (20–24 hr study)	80.00	15.45 - 23.37	3.962	25	0 to 85	3.9 - 5.9	Kamper and Fennema, 1984a
- sweet milk chocolate	136.92	27.97	5.279	27	0 to 97	5.3	Landman et al., 1960
- stearic/palmitic acid (0–24 hr study)	50.00	8.55 - 11.18	6.577	25	65 to 97	1.3 - 1.7	Kamper and Fennema, 1984a
- acetyl monogylcerides / paper filter(W50)	195.00	36.94	8.200	25	0 to 100	4.5	Kester and Fennema, 1989

Table 3. (continued)

barrier	WVTR	permeability	permeance	temp.	R H	thickness	reference
	$gm^{-2}day^{-1}$	g mil $m^{-2}day^{-1}mmHg^{-1}$	g m-2 day -1 mmHg-1	°C	% gradient	mils	
- oleic (0 - 7 hr study)	190.00	12.23 - 16.60	9.409	25	0 to 85	1.3 - 1.7	Kamper and Fennema, 1984a
- hextriacontane/paper filter (W50)	244.70	46.37	10.300	25	0 to 100	4.5	Kester and Fennema, 1989
- oleic (0 - 10 hr study)	260.00	16.74 - 21.89	12.876	25	0 to 85	1.3 - 1.7	Kamper and Fennema, 1984a
- tristearin/paper filter (W50)	320.04	60.62	13.47	25	0 to 100	4.5	Kester and Fennema, 1989
- hydrogenated cottonseed/soy oil	290.00	71.66 - 84.74	14.362	25	0 to 85	5.0 - 5.9	Kamper and Fennema, 1984a
- polyunsaturated cornoil/sorbitanmono oleate	340.00	65.65 - 99.34	16.838	25	0 to 85	3.9 - 5.9	Kamper and Fennema, 1984a
- 10% alanate / 20% 10 Myvacet 5-07	363.26	134.22	16.990	25	0 to 90	7.9	Krochta et al, 1989
- stearic acid/paper filter (W50)	468.10	88.67	19.700	25	0 to 100	4.5	Kester and Fennema, 1989c
- 10% alanate/ 10% paraffin/calcium/water	696.27	140.03	32.560	25	0 to 90	4.3	Krochta et al., 1989
- 10%alanate/ 10% beeswax/calcium/ water	696.27	192.13	32.560	25	0 to 90	5.9	Krochta et al., 1989

TABLE 4.
EXAMPLES OF APPLICATION OF THE L# FOR BANANA CHIPS WITH DIFFERENT DESIRED SHELF LIVES

θ_S (day)	$(k/x)_{req}$* ($g\ m^{-2} day^{-1} mmHg^{-1}$)	L#	Potential Edible Coating
365	0.025	14.6	Not available
150	0.061	6.0	Paraffin, Chocolate liquor
30	0.306	1.2	Cocoa butter/sucrose/cocoa solids Stearic/palmitic film
1	9.175	0.04	No film required

*$(k/x)_{req}$ values were calculated from Eq. (12) using food properties in Table 1.

internal diffusion is the controlling factor for moisture migration. Therefore, no moisture barrier will be required for banana chips which require only 1 day of shelf life.

Moisture permeance (k/x) was used in this paper in order to select a proper coating material, especially since many of the films are composed of layers of different materials and in coating a food, it will be very difficult to determine the actual thickness of the coating, which will vary across the surface. In addition for hydrophyllic substances, the actual thickness will vary with the moisture gradient, since these material will swell. In any case for either a composite or a homogeneous coating film, i.e., the same material throughout the whole thickness, one can reduce the overall moisture transfer by increasing the thickness. In this case to determine the limiting factor, one can still follow the same procedure as discussed above, with the addition of determining the maximum critical thickness of each edible coating. Above this critical thickness, an undesirable sensory or coating property might be noted. One can then find the desired permeability. Table 3 also lists the permeability and thickness of the edible coatings found in the literature, for this purpose. One the other hand, especially for composite films, the permeance may not be linearly reduced with increasing thickness. Even for homogeneous films, as was shown by Swarbrick and Amann (1968) for plastic polymers and edible films as shown by Biquet and Labuza (1988) for chocolate, the k/x can vary between a power of -0.8 to 1.2 of the thickness, whereas theoretically it should be -1, i.e., inversely proportional. Thus doubling the thickness will not reduce the overall moisture transfer rate by a factor of 2, so one must determine the composite (k/x) of the finished material at the actual film thickness.

CONCLUSIONS

A computer simulation was developed to study the effectiveness of the L number, a dimensionless number describing the ratio of the moisture permeance of a food substance and an edible coating material, for the prediction of moisture transfer through coated food materials, such as semi-moist foods, dry nuts, cereals or other foods. The results indicated that when the L# is smaller than 0.04, the main resistance to moisture transfer for a coated food material is the diffusion of the moisture through the uncoated food material itself. If the L# is larger than 4, the main resistance of moisture transfer is the permeance of the coating material. The L# concept can be applied to edible as well as synthetic, inedible packaging materials to predict the water vapor barrier (permeance) required to protect a variety of food products from moisture migration. This predictive approach can save time and research dollars in the search for an efficient edible barrier for a food or pharmaceutical product.

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COMPOSITIONAL, NUTRITIONAL AND FUNCTIONAL EVALUATION OF INTERMEDIATE WHEATGRASS (*THINOPYRUM INTERMEDIUM*)

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ABSTRACT

'Oahe' Intermediate Wheatgrass (IWG) seed was found to have higher levels of protein (20.8%), fat (3.21%), and ash (2.64%) than wheat. The IWG protein is nutritionally limiting in lysine, as is wheat, but has higher levels of all other essential amino acids than wheat. A flour beetle larvae bioassay and chemical trypsin inhibitor and hemagglutinin tests demonstrated the absence of significant amounts of antinutrients. IWG kernels were milled with stone, impact, and roller mills. Stone milling resulted in a flour with farinograph characteristics more similar to those of whole wheat flour than did impact and roller milling. No gluten was found in IWG. Bread, muffins, cookies and cake containing various levels of IWG flour were evaluated by a sensory panel and judged to have very favourable appearance, texture, flavor and overall characteristics.

INTRODUCTION

The development of perennial grains as environmentally sound alternative crops for marginal lands has received recent attention amongst agronomists, plant breeders and environmentalists (Jackson 1985; Wagoner 1991). Such crops should form a good soil holding sod to minimize erosion and maintain soil fertility, have favourable grain producing qualities, and provide an acceptable yield. The grain should also be economically viable with establishment and production costs balanced by a break-even price competitive with conventional crops (Watt 1989). In 1983, the Rodale Research Center began testing a large

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number of perennial grains. Compositional and nutritional analyses contributed to the eventual selection of 'Oahe' intermediate wheatgrass (IWG) (*Thinopyron (Agropyron) intermedium)* as one of the apparent best candidates (Becker *et al.* 1986; Wagoner and Schauer 1989; Schauer 1990).

This report describes continuing efforts to evaluate the compositional and nutritional characteristics of 'Oahe' IWG grain and its suitability for use in food products for the marketplace.

MATERIALS AND METHODS

Seed Samples

'Oahe' Intermediate Wheatgrass (IWG) seed samples were obtained from production experiments performed by the Rodale Research Center (RRC). Crops grown in Eastern Pennsylvania in 1985 through 1988 and in Colorado in 1987 were tested. 'Chief' and 'Greenar' varieties of IWG and two varieties of pubescent wheatgrass, 'Greenleaf' and 'Mandan 759' were also evaluated. The seeds were harvested and field cleaned before final cleaning with a grain dockage tester. Visual inspection verified that this cleaning process removed virtually all contaminating plant debris and seeds of other species, as well as under- and over-sized sample seeds.

Wheat seed used for comparison stone milling tests was obtained from the California Wheat Commission; commercial whole wheat flour used in baking tests was purchased in local markets.

Composition

Representative samples of whole grain seed flour and mill fractions were milled to 100 mesh flour in a Udy mill (Udy Corp., Fort Collins, CO) prior to proximate compositional (AOAC 1980) and amino acid analysis. Amino acids in whole grain IWG were determined with a Durrum amino acid analyzer ion exchange method (Spackman *et al.* 1958), with the sulfur amino acids determined after perchloric acid oxidation (Moore 1963). Nitrogen to protein conversion factors were calculated as in Tkachuk (1969).

Starch was isolated (Knight and Olsen 1984) and the melting point determined by differential scanning calorimetry (DuPont Model 990) using excess water. Trypsin inhibitor activity was measured as described by Kakade *et al.* (1974), with one trypsin inhibitor unit (TIU) being defined as the amount of sample causing a 10% inhibition of porcine pancreatic trypsin and N-benzoyl-DL-arginine p-nitroanalide (BAPNA) (Sigma Chemical Co). Hemagglutinin activity was assayed with the method of Beeley (1985) using human erythrocytes, with one unit of activity being the minimum amount of flour causing agglutination in one hour.

Milling

Seed was milled to flour using three different mills. A fine flour (100 mesh) was prepared by milling with a Udy impact mill. Stone ground flour was prepared with a Morehouse Model 530 stone mill (Morehouse Industries, Fullerton CA) equipped with 5 in. stones set at a gap of 0.005 in. Endosperm flour, bran and high ash bran were obtained from a Brabender Quadrumat Jr roller mill (Brabender Instruments, South Hackensack, NJ). Bran fractions from the roller mill were further milled to a 100 mesh flour with the Udy mill.

Nutritional Evaluation

The *in vivo* nutritional value of IWG was compared to wheat using the larval method of Vorha *et al.* (1979). Samples of IWG or whole wheat flour were mixed with water and sampled raw, baked for 30 or 45 min at 177°C, or autoclaved at 110°C for 10, 20 or 30 min. The cooked and uncooked material was lyophilized and fed as 90% of the test diet, the remaining 10% being whole wheat flour. *Tribolium castaneum* eggs were collected, grown for 6 days on a basal diet, and acclimatized for 2 days on the test diet. Three replicates of 10 larvae each were weighed and fed the test diet for 6 days, after which they were reweighed and the mean weight gain per larva calculated for each diet. The results were compared to the weight gain of larva fed unbleached flour diet. See the cited method (Vohra *et al.* 1979) for additional details.

Product Formulation and Evaluation

The farinograph mixing characteristics of IWG whole grain flours and mill fractions were compared to a commercial whole grain flour using AACC methods (AACC 1976; Farinograph Handbook 1972). Absorption, Dough Development Time, Mixing Tolerance Index, Stability and Twenty Minute Drop were calculated as in AACC methods (1976). The IWG whole grain flours and mill fractions were tested for gluten using both the AACC hand washing and AACC machine washing methods.

Baked products were prepared using stone ground IWG flour in modified commercial recipes. Bread was made with 85 parts wheat flour, 15 parts IWG flour, and 5 parts vital wheat gluten. The only flour in muffins was stone ground IWG flour. Cookies were made with IWG flour, oatmeal, butter, sugar, chocolate chips and walnuts. Cake contained 1 part (w/w) IWG flour, 1 part whole wheat flour, 2 parts bananas and proportioned amounts of walnuts and other ingredients.

To evaluate the baked products, a 57 member sensory panel was assembled from laboratory staff volunteers. The judging experience of the panel members ranged from expert to novice; however, most participants had served on multiple panels throughout the year. The panel was supervised by expert staff using

established techniques. The products were hedonically scored for appearance, texture, flavor and overall characteristics, using a scale of 1 (very poor) to 9 (excellent). Results were tabulated and summarized graphically.

Cooking characteristics of blends of 10 and 25% whole grain IWG in brown rice were determined using criteria developed in this laboratory for evaluating wild rice. The rice/IWG mixtures were boiled for 50 minutes and sampled as indicated. Flavor, mouth feel, and appearance were evaluated by 5 experienced judges.

RESULTS AND DISCUSSION

Composition

The proximate composition of multiple lots of Oahe IWG is shown in Table 1. The protein content of IWG is higher than common cereal grains, but near results observed in other perennial grains (Becker *et al.* 1986). The carbohydrate content was determined by difference, so it reflects the protein and fiber observations. The fat and ash are within expected ranges.

The amino acid composition of IWG seed proteins is shown in Table 2. The essential amino acids for wheat (FAO, 1970) and the FAO Standard (1973) are shown for comparison. Lysine is the limiting amino acid in both IWG and wheat, resulting in a chemical score of 54. All other essential amino acids are substantially higher in IWG than in wheat.

The levels of trypsin inhibitor and hemagglutinin activity in IWG are probably not nutritionally significant. Uncooked IWG flour contained 0.01 TIU/mg N and a hemagglutinin titer of 0.0125. For comparison, wheat has 0.5–1.0 TIU/mg N and triticale has 4–7 TIU/mg N (19). Antinutrient levels in cooked IWG were not determined.

TABLE 1.
PROXIMATE COMPOSITION OF INTERMEDIATE WHEATGRASS

Grain	Protein[1] % ± sd n = 14	Carbohydrate[2] % ± sd n = 6	Fat % ± sd n = 6	Crude Fiber % ± sd n = 6	Ash % ± sd n = 3
Intermediate Wheatgrass	20.8 ± 1.8	61.7 ± 2.8	3.21 ± 0.44	1.69 ± 0.41	2.64 ± 0.21
Wheat[3]	14.3	68.6	1.9	3.4	1.8

1. Protein = Nitrogen X 6.25
2. Carbohydrate by difference.
3. NAS/NRC. 1964.

TABLE 2.
PROTEIN AMINO ACID COMPOSITION (g/16 g N) OF INTERMEDIATE WHEATGRASS, WHOLE WHEAT FLOUR, AND THE FAO STANDARD

Amino Acid[1]	Intermediate Wheatgrass[2]	Wheat Flour[3]	FAO Standard[4]
Lysine	2.9 ± 0.2	2.9	5.4
Histidine	2.4 ± 0.2		
Threonine	3.5 ± 0.3	2.9	4.0
Cystine	3.4 ± 0.3	2.5	
Methionine	2.2 ± 0.3	1.5	
Cys + Met	5.6 ± 0.6	4.0	{3.5}
Valine	5.3 ± 0.5	4.4	5.0
Isoleucine	4.0 ± 0.3	3.3	4.0
Leucine	7.2 ± 0.5	6.7	7.0
Tyrosine	3.5 ± 0.3	2.9	
Phenylalanine	4.9 ± 0.4	4.5	{9.6}
Serine	5.4 ± 0.4		
Glycine	4.3 ± 0.2		
Arginine	5.2 ± 0.4		
Alanine	3.8 ± 0.3		
Aspartic Acid	5.1 ± 0.3		
Glutamic Acid	29.6 ± 2.2		
Proline	10.4 ± 0.9		
Chemical Score	54	54	100
Protein Conversion Factor[5]	6.75 ± 0.05	5.85	
Limiting Amino Acid	Lys	Lys	

1. N = 12.
2. Mean ± standard deviation.
3. FAO (1970).
4. FAO (1973).
5. N = 9.
Bracketed values are sums of related amino acids, Cys + Met and Tyr + Phe.

Larvae Bioassay

The results of the *Tribolium castaneum* bioassay are shown in Fig. 1 and 2. This assay has been successfully used to evaluate the nutritional characteristics of wheat, triticale, rice, corn, soy (Shariff *et al.* 1981), sorghum (Banda-Nyirenda *et al.* 1987), and eastern gamagrass (Bargman *et al.* 1989). With this assay, the nutritional value of uncooked IWG is identical to uncooked wheat flour (Fig. 1 and 2). Baking at 177°C for 30 min raised the nutritional value of both IWG

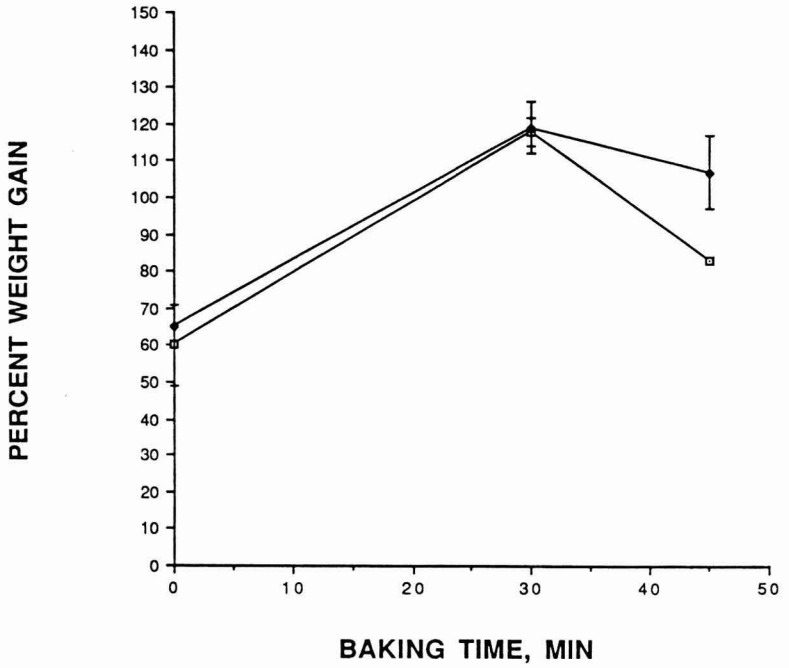

FIG. 1. WEIGHT GAIN OF LARVAE FED BAKED INTERMEDIATE WHEATGRASS (SOLID BOXES) AND WHEAT (OPEN BOXES)

and wheat grains compared to unbaked samples at 0 min (Fig 1). This effect is probably due to the nutritionally beneficial changes associated with cooking, such as cellular disruption, protein denaturation, and inactivation of low levels of antinutrients. Baking for 45 min diminished the nutritional value of both IWG and wheat from levels observed at 30 min, perhaps due to formation of less digestible protein-carbohydrate complexes.

When autoclaved diets were fed, the nutritional value was initially increased, then was diminished by overcooking (Fig 2). Wheat required more autoclaving to attain maximum nutritional values (20 min vs 10 min for IWG), and IWG lost more nutritional value when overcooked.

These results demonstrate that IWG has nutritional characteristics which are similar to wheat, and overcooking is deliterious to both grains.

Milling

Both impact and stone milled whole grain IWG flour had textural characteristics comparable to similarly processed whole wheat flour. In neither case were processing difficulties encountered, other than mill adjustments for the smaller

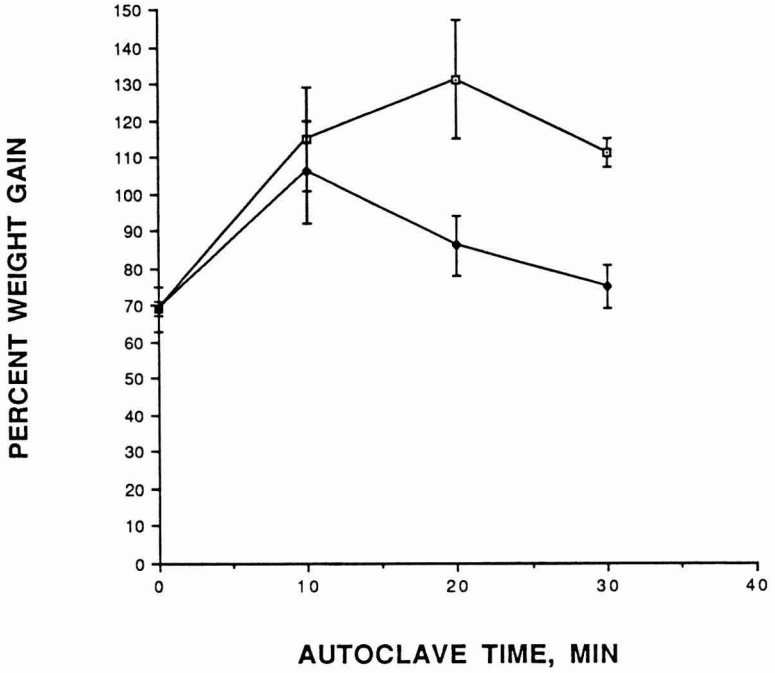

FIG. 2. WEIGHT GAIN OF LARVAE FED AUTOCLAVED INTERMEDIATE WHEATGRASS (SOLID BOXES) AND WHEAT (OPEN BOXES)

kernel size of the IWG. Roller milled endosperm flour from IWG seed had a substantially lower yield (Table 3) and grayer color than other comparable cereal grains. The grayer color is probably due to the larger amounts of bran, as associated with the smaller seed size of the IWG. For example, varieties of IWG seed other than 'Oahe' had a mean kernel weight of 4.7 mg in the 1988 and 4.4 mg in 1989; 'Oahe' samples averaged 5.1 mg and 4.9 mg kernel weights for those years (Wagoner and Schauer 1989; Schauer 1990; Hanners *et al.* 1988), compared to kernel weights of 20–32 mg for commercial hard wheat. Untempered IWG seed shattered and gave very small amounts of pure endosperm flour. Tempering the seed to 15% moisture improved typical roller mill endosperm flour yields to about 38%. Stone milling tempered seeds gave more uniform particles (data not shown).

Flour Characteristics

Stone milling of perennial grains produced flours with high absorptions, long Dough Development Times (DDT), small Mixing Tolerance Indices (MTI), high stability, and small Twenty Minute Declines (TMD) (Table 4); all characteristics

TABLE 3.
ROLLER MILL FRACTION YIELDS FROM PERENNIAL GRAINS, %

Grain	Endosperm Flour	High Ash Flour	Bran
Intermediate Wheatgrass			
Oahe 1988	38.7	5.3	56.0
Oahe 1987	44.5	6.9	48.6
Chief	44.4	4.6	51.0
Greenar	46.1	5.2	47.8
Pubescent Wheatgrass			
Greenleaf	48.1	5.2	53.0
Mandan 759	38.1	4.2	57.8
Hard Red Spring Wheat			
Yecora Rojo	76.4	6.8	16.8

of a thick, viscous dough. It is known that particle size, which is larger in stone milled flours as opposed to impact or roller milled flours, may effect absorption, but has much less impact on the other characteristics. These thicker doughs form very dense breads, even when used as a minor ingredient in composite flours. Such stone milled flours make good ingredients in flours used for muffins or pancakes, where denseness may be an attribute.

Flours made by impact or roller milling do not have these farinograph characteristics and have thinner, pastier doughs and form more dense, compact products.

None of the perennial grains tested contained gluten forming protein. Although the literature is sparse, the genus is reportedly nonuniform (Shibaev 1937), with gluten being reported in some *Agropyron intermedium* samples and not in others.

Since the perennial grain kernels are smaller than wheat kernels, they have a greater surface area per gram of seed and consequently more bran and more dietary fiber. Components of dietary fiber are probably responsible for the viscosity characteristics of the whole grain flour doughs, as evidenced by the farinograms of the weaker doughs from endosperm flours. The chemically determined crude fiber as reported here is for comparison with literature values and has no correlation with dietary fiber.

TABLE 4.
FARINOGRAPH CHARACTERISTICS OF SEVERAL INTERMEDIATE AND PUBESCENT WHEATGRASS GRAINS MILLED WITH DIFFERENT TYPES OF MILLS

Mill Type	Absorption %	DDT (min)	MTI (BU)	Stability (min)	TMD (BU)
INTERMEDIATE WHEATGRASS					
Oahe, 1988 crop					
Stone	63.4	4.6	90	4.7	60
Impact	64.0	1.6	190	0.7	228
Roller	63.8	2.4	140	1.2	170
Oahe, 1987 crop, Pennsylvania grown					
Stone	60.5	3.0	40	6.5	65
Roller	70.0	2.1	195	0.8	240
Oahe, 1987 crop, Colorado grown					
Stone	63.2	2.5	130	1.6	125
Oahe, 1987 crop, Endosperm flour					
	57.7	1.1	280	0.7	375
Oahe, 1988 crop, Endosperm flour					
	58.5	1.2	195	0.7	240
Chief, 1988 crop					
Stone	64.0	7.0	80	8.9	45
Impact	64.8	2.5	80	1.7	90
Roller	66.8	3.3	90	2.4	120
Greenar, 1988 crop					
Stone	64.0	5.0	40	4.4	50
Impact	65.6	2.4	123	1.3	145
Roller	67.7	3.2	115	1.4	145
PUBESCENT WHEATGRASS					
Greenleaf, 1988 crop					
Stone	63.9	5.0	10	12.2	10
Impact	65.6	1.8	120	1.0	135
Roller	68.3	2.4	150	1.5	180
Mandan 759, 1987 crop					
Stone	63.0	2.8	90	1.7	110
Impact	65.2	1.4	180	0.8	205
Roller	70.6	2.0	180	1.0	220
HRS Wheat, Yecora Rojo					
Stone	67.2	6.3	30	6.3	30

Absorption (%) = 2(x+y-50) where x=mls added and y=gms flour
DDT (Dough Development Time) = time of first peak
MTI (Mixing Tolerance Index) = Top of curve at first peak minus top of curve 5 minutes later
Stability = Difference in time between when top of curve reaches the 500 BU line and when it leaves the line.
TMD (Twenty Minute Drop) = Difference between the center of the curve at peak and center of curve 20 minutes later.

The differences in the farinographs of the endosperm flours from 'Oahe' IWG seed grown in two crop years are not significant. These doughs are weak, with little tendency to become viscous. The IWG starch has a gelatinization temperature of 62–68°C, midpoint 65.5°C as compared to wheat which ranges from

58–64°C, midpoint 61°C. Since the endosperm flours are predominantly starch and low in fiber, they would be expected to make composite breads that are lighter than those made from corresponding whole grain flours.

In a separate experiment, seeds from a single lot were grown in both Pennsylvania and Colorado (Wagoner and Schauer 1989). The resultant seeds appeared physically different. The Pennsylvania seeds were typical of those harvested from other lots and crop years while the Colorado grown seeds were about 20% smaller and appeared less developed. The farinograms of flours made from stone ground grain from Colorado were similar to farinograms of impact or roller milled flours in that they had shorter dough development times, larger mixing tolerance indices, lower stabilities and larger twenty minute declines. Not enough compositional data (dietary fiber and starch determinations) are available to fully define the differences, but climatic effects are obviously important. Proximate and amino acid analyses were similar (data not shown).

Baked Products and Their Sensory Evaluation

IWG performed well in the four baked products tested (Table 5). When used as 15% of the flour in bread, IWG imparted a distinct, nutty-grain taste. The loaf had a color and crumb appearance typical of whole grain breads; added gluten was required to produce loaf volumes comparable to whole grain breads. Most panel members rated the overall characteristics between fair and good (5–8), Appearance between good and excellent (7–9), Texture between fair and good (5–8), and Flavor between fair and good (5–8) (Fig. 3A). The bread had a rather coarse texture, which may have influenced judges preferring lighter breads.

The muffins made from 100% IWG flour were judged to have a good to excellent appearance (7–9) by nearly 50% of the judges (Fig. 3B); this is an impressive score considering the diversity of preferences expressed for other products. The muffins had good volume and a plump inviting appearance; they scored good to excellent for appearance (7–9) and overall (6–8), fair to excellent in texture (5–8), and fair to excellent in flavor (5–8).

Cookies made with IWG as the only flour ingredient were judged good to excellent (7–9) in appearance, good in texture and flavor (7–8), and good to excellent (6–9) overall (Fig. 3C). The cookies had a soft texture, broke easily without crumbling and a rather sweet taste.

The banana bread, which was 50% IWG-wheat, was judged good to excellent (7–9) for appearance, flavor and overall; texture scored only slightly lower (6–9) (Fig. 3D). The primary flavor component was banana; IWG and wheat moderated the banana flavor somewhat to produce a mildly banana-nutty flavor. The bread was moist without being soggy and had good crumb and color.

TABLE 5.
RECIPES FOR BAKED PRODUCTS USING INTERMEDIATE WHEATGRASS

Ingredient	gms
BREAD	
Whole wheat flour, Gold Medal	80
Intermediate Wheatgrass	15
Vital wheat gluten	5
Brown sugar	60
Shortening	40
Salt	20
Fresh yeast	25
Water	730
Vitamin C	50 ppm
Straight dough method	
1. Basic fermentation 2 1/2 hours	
2. Floor time 20 min	
3. Proof to height 45-55 min at 38°C.	
4. Bake temperature 405°F for 25 min.	
COOKIES	
Intermediate Wheatgrass flour	100
Oatmeal	90
Butter	106
Granulated Sugar	90
Brown Sugar	70
Whole eggs	50
Vanilla	T
Baking Powder	2
Baking Soda	2
Salt	100
Chocolate Chip	100
Walnut	50
MUFFINS	
Intermediate Wheatgrass	100
Brown Sugar	35
Non-Fat Milk	80
Egg White	28
Salad Oil	25
Raisins	40
Baking Powder	3.5
Salt	1.5
Cinnamon	0.6
WHEATGRASS BANANA CAKE	
Intermediate Wheatgrass	50
Whole Wheat Flour	50
Banana	100
Sugar	85
Salt	0.6
Shortening	40
Eggs	45
Walnut	30
Baking Powder	1.5
Baking Soda	1.5

There were no obvious shelf-life differences between IWG and their all-wheat counterparts when products were held under similar conditions at room temperature for 7–10 days (data not shown).

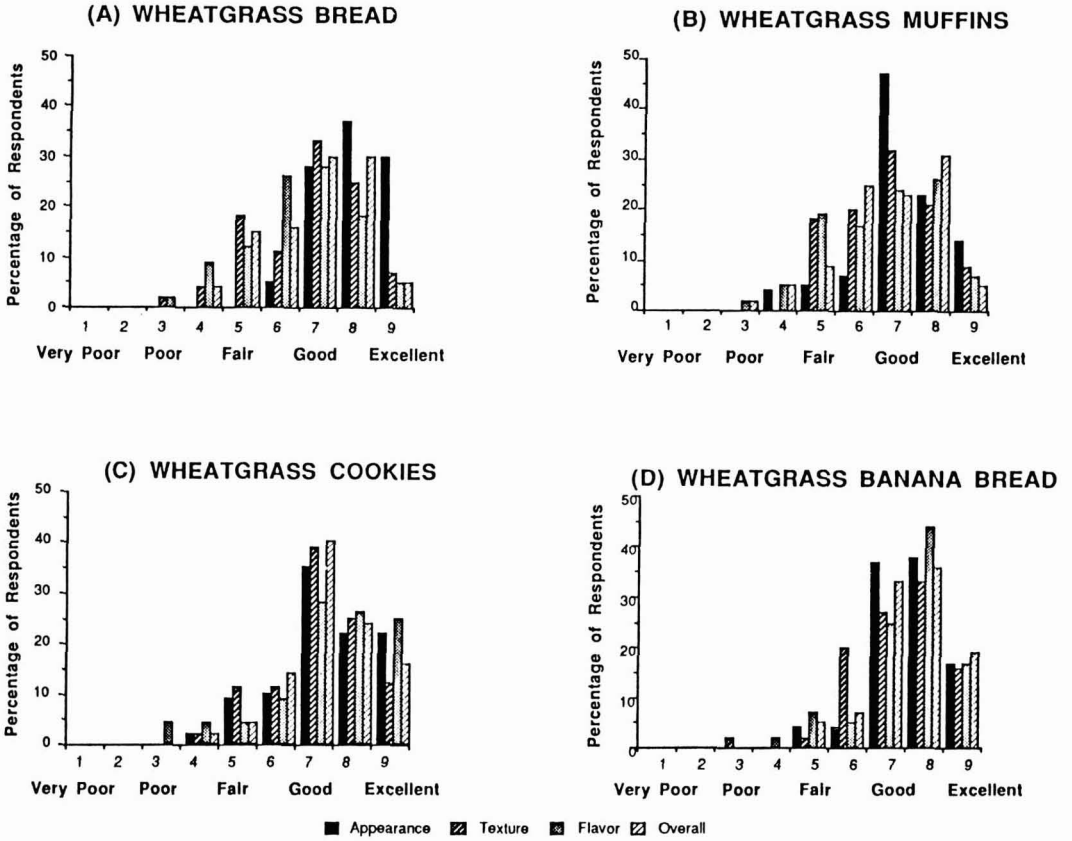

FIG. 3. SENSORY PANEL APPRAISAL SCORES OF BACKED PRODUCTS MADE WITH WHOLE GRAIN INTERMEDIATE WHEATGRASS FLOUR
(A) Bread, (B) Muffins, (C) Cookies, (D) Banana Bread.
■ Appearance □ Texture □ Flavor □ Overall

Cooked Kernel Characteristics

Whole kernel IWG were cooked in boiling water and sampled every 5 min during cooking. Minimum cooking time was about 20 min; the kernels were plump, soft and had a nutty flavor and clean bite with only about 10–15% split. Longer cooking reduced the bran flavor but resulted in more split kernels. Adding 10 or 25% IWG kernels to brown rice was judged to add a mild nutty flavor to the rice dish, similar to but more pronounced than wild rice.

CONCLUSIONS

The quantity of protein present in IWG and the amounts of amino acids in the proteins is superior to the cereal grains now commonly grown, the larvae bioassay indicates IWG is at least nutritionally comparable to wheat, and it has been shown that baked products can be made that are potentially very consumer attractive. All of these factors are supportive of continued consideration of IWG as an alternative perennial grain crop.

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BOOK REVIEW

Nitrite Cured Meat: A Food Safety Issue in Perspective. Robert G. Cassens. 1990. Food and Nutrition Press, Trumbull, Conn. 06611. 169 pp. $65.00.

This book presents an important story that should be read by everyone involved with the food industry. The story is important not so much because it describes the nitrite problem which was critical to the meat industry, but because it also includes a broader, more philosophical discussion on mixing science with politics. The book is valuable in both respects; it serves as an historical record of the major events surrounding the nitrite issue, and it stimulates considerable thought regarding the role that forces outside of science may have on the resolution of food safety issues. The book is written in an easy-to-read style and manages to hold the reader's interest even while presenting the sometimes-mundane recitation of important historical developments.

The author provides an introduction which explains clearly the organization and objectives of the book and which gives the reader an excellent concept of what is to follow. Cassens also attempts to explain the role of major sociological developments in creating the consumer attitudes that existed in the 1970's at the peak of the nitrite controversy. It becomes clear that many seemingly unrelated events probably contributed to the nitrite problem and the magnitude it reached. Further, Cassens is careful to include commentary on the role of politics at each stage of the game. It is almost graphically demonstrated that politics became increasingly important as time went on. The point is well made that scientists who become involved with major issues of public interest should be prepared to consider the impact of political influences on their work. The roles of consumer activists and of the news media are also well described during the developing controversy.

There is a great deal of good technical information in this book as well. To provide background, there is a very good discussion of the work which developed meat curing as a science and of the events leading to discovery of nitrosamines as carcinogens. There is also description of several other significant scientific discoveries in the meat industry; events used to demonstrate that the industry was making rapid advances through basic research prior to the nitrite problem.

After providing the necessary background, Cassens presents the nitrite problem largely in the chronological order of events as they occurred. This effectively demonstrates the manner in which the problem increased in intensity. There is rather detailed coverage of the USDA/FDA Study Group activities and of the USDA Expert Panel meetings. In all cases, however, the author includes interpretive comments which give perspective to the summaries of the group discussions. There is also considerable discussion of the major high points in the controversy, namely the particular problems with bacon, the issue of color "fixation," the Newberne study and the prior sanction issue for cured poultry products.

Cassens also discusses several important scientific accomplishments made during all the activity generated by the nitrite problem. A tremendous amount of time, effort and money was expended on this problem and several scientific advancements were indeed achieved.

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There are a few points in the book where the discussion gets bogged down with details or experimental procedures or analytical techniques; fortunately, these are few and are not distracting. A rather convenient organization of references is used where references are categorized at the end of each chapter according to the subheading within that chapter. Consequently, references can be easily and quickly located.

The book is generally well-written and may be recommended to any food industry professional who is interested in the potential impact of consumer activism and politics on food safety issues.

JOSEPH G. SEBRANEK

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PUBLICATIONS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF MUSCLE FOODS, N.G. Marriott and G.J. Flick, Jr.
JOURNAL OF SENSORY STUDIES, M.C. Gacula, Jr.
JOURNAL OF FOOD SERVICE SYSTEMS, O.P. Snyder, Jr.
JOURNAL OF FOOD BIOCHEMISTRY, J.R. Whitaker, N.F. Haard and H. Swaisgood
JOURNAL OF FOOD PROCESS ENGINEERING, D.R. Heldman and R.P. Singh
JOURNAL OF FOOD PROCESSING AND PRESERVATION, D.B. Lund
JOURNAL OF FOOD QUALITY, R.L. Shewfelt
JOURNAL OF FOOD SAFETY, T.J. Montville and A.J. Miller
JOURNAL OF TEXTURE STUDIES, M.C. Bourne and P. Sherman

Books

NITRITE-CURED MEAT, R.G. Cassens
THE POTENTIAL FOR NUTRITIONAL MODULATION OF THE AGING PROCESSES, D.K. Ingram *et al.*
CONTROLLED/MODIFIED ATMOSPHERE/VACUUM PACKAGING OF FOODS, A.L. Brody
NUTRITIONAL STATUS ASSESSMENT OF THE INDIVIDUAL, G.E. Livingston
QUALITY ASSURANCE OF FOODS, J.E. Stauffer
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ROLE OF CHEMISTRY IN THE QUALITY OF PROCESSED FOODS, O.R. Fennema, W.H. Chang and C.Y. Lii
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Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

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

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

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

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

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

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

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

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

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

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

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

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