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CONTENTS

EFFECT OF HONEY AS AN ANTIBROWNING AGENT IN LIGHT RAISIN PROCESSING

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ABSTRACT

Various process methods were evaluated for the production the light Thompson Seedless raisins. Treatments included: control, commercial (sulfur gas treated), 20% honey solution pressure dip, 10% honey solution pressure dip, 5000 ppm sulfite solution pressure dip, 5000 ppm sulfite solution nonpressure dip, Erthorbic Acid/Citric Acid/$CaCl_2$ solution pressure dip, blanch treatment and a Natick solution. Measured variables included Hunter Colorimeter tristimulus characteristics, visual assessment and rank comparison using Friedman Rank Analysis. The Hunter L, a, b results indicated the honey solution treatments produced lighter and yellower raisins than the commercial and pressure infused sulfite solution treatments. The Erthorbic Acid/Citric Acid/$CaCl_2$ pressure dip treatment resulted in the darkest product. Based on results of the Friedman Rank Analysis, raisins produced using honey with no added SO_2 were ranked highest by each of the 18 untrained panelists.

INTRODUCTION

Current production methods used to produce light Thompson Seedless raisins require the addition of sulfite in order to retard browning and produce the desired light tan colored product. With growing concerns over the use of sulfites, many processors are interested in identifying alternative process methods that exclude the use of these chemicals. A number of processing options have been investigated.

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Grncarevic (1963) developed a variation of the Greek "cold dip" method of dropping grapes into solutions of water, olive oil and potash. This process consisted of dipping grape clusters into an aqueous alkaline emulsion containing 2.5–5% K_2CO_3 and 2% olive oil. Using this treatment, the grapes dried twice as fast as untreated grapes, producing a lighter colored raisin. Also, the skin of the grape was not broken in this treatment as it would be if a caustic solution was used.

Cold dipping was further studied by Radler (1964). He also used a potassium carbonate solution combined with various other materials. He found that certain oleates inhibited browning quite well; however, those oleates tested also increased drying time dramatically. Petrucci *et al.* (1973), found that a cold methyl oleate dip shortened dehydration time when compared to a sodium hydroxide dip and produced a lighter raisin without the use of sulfites.

Lazar *et al.* (1963) inactivated polyphenol oxidase (PPO) by blanching grapes prior to drying. The raisins retained their yellow color but slowly darkened at room temperature. This was suspected to be due to nonenzymatic browning.

Gee (1980) found that mechanically dried grapes resulted in a much lighter raisin than sun dried grapes. As noted by Aguilera (1987), a minimum of three weeks in the field are needed to sun dry. A long dehydration period will permit more time for the enzyme PPO, which is concentrated in the skin of the grape, to darken the raisin.

Gee (1980) noted that lowering the levels of water activity in the grape skin will slow the rate of browning. Halving the grapes before drying allowed the skin to maintain a lower level of water activity, thus producing lighter colored raisins whose color remained stable after a year's storage at 20C.

Aguilera *et al.* (1987) found a correlation between the percentage PPO inactivation and the measured Hunter L value of the raisins. Reduced PPO activity results in higher L (lightness) and +b (yellowness) values. They used a hot water dip followed by drying. Grapes treated with a hot water dip dried to a yellow color only slightly darker than sulfured grapes.

New approaches in the prevention of oxidation using honey have been developed in other areas, such as wine-making. These techniques may be effective in the production of light golden raisins as well. Kime (1982) used honey to clarify and remove hazes from fruit juices and further discovered that honey could be used as an antioxidant in wines instead of SO_2 (Lee *et al.* 1990).

Oszmianski and Lee (1990) found that the addition of honey in apple slices, grape juice and model systems inhibits PPO activity, which has been shown to produce a lighter and yellower raisin (Aguilera *et al.* 1987).

The objective of this study was to compare potential grape treatments that may yield nonsulfited, light-colored golden raisins. These treatments may be considered as alternatives to the current commercial practices.

MATERIALS AND METHODS

Thompson Seedless grapes (~10 kg) were purchased for use in this study from a local fresh fruit market. A large commercial sample of Light Thompson Seedless Raisins was obtained from the Victor Packing Company (Madera, CA) for use as the commercial sample. In the commercial process, grapes were held in 1% solution of caustic soda and then put into metal sulfur gas houses for 6 h. After gassing, grapes were transferred on wooden trays into natural gas drying tunnels for 24 h or until 14% moisture was reached. A subsample was removed and shipped to our laboratories for inclusion in this study.

The locally obtained grapes were subdivided into seven treatment lots of 1 kg each. Analysis was done of 3 replicate sublots for each colorimetry analysis. One lot was set aside with no treatments, to be used as a control sample. Six lots were dipped in a 0.5% NaOH solution for 10 s at ambient temperature, removed and washed with water. Grapes were held under the surface with stainless steel mesh wire. Following this procedure, each sample received a different treatment. The following is a list of all treatment combinations used in the study:

(1) Control. Grapes were untreated and held until ready for dehydration.

(2) 20% honey solution pressurized dip. Grapes were submerged in a 20% honey solution and held for 15 min with 25 psi air over pressure in a sealed, 31.75 cm × 68.58 cm stainless steel pressurization chamber and held 18 h at 0C in sealed plastic bags.

(3) 10% honey solution pressurized dip. Grapes were submerged in a 10% honey solution and held for 15 min with 25 psi air over pressure in a sealed, 31.75 cm × 68.58 cm stainless steel pressurization chamber and held 18 h at 0C in sealed plastic bags.

(4) Sulfite pressurized dip. Grapes were dipped for 5 min in 5000 ppm SO_2 solution using $NaHSO_3$ at 25 psi air over pressure in a sealed, 31.75 cm × 68.58 cm stainless steel pressurization chamber, drained, and held 18 h at 0C in sealed plastic bags.

(5) Sulfite dip and hold. Grapes were dipped for 5 min in 5000 ppm SO_2 solution using $NaHSO_3$, drained, and held 18 h at 0C in sealed plastic bags.

(6) Steam Blanch. Grapes were steam blanched for 3 min at 100C and immediately cooled to room temperature in cold running water bath. They were then held 18 h at 0C in sealed plastic bags.

(7) "Natick 631" solution. Grapes were submerged in combined solution of citric acid, calcium chloride and erthorbic acid as described previously (Anon. 1986). The treatment was held for 15 min at 25 psi air over pressure in a sealed stainless steel chamber. They were then removed and held 18 h at 0C in sealed plastic bags.

After each treatment, samples were drained in a stainless steel basket, put on stainless steel screens and individually placed into a hot air cabinet drier at 63C for 10 h, reaching an approximate moisture content of 14% as determined by an electronic moisture balance (Ohaus, Model MB300-01, Florham Park, NJ).

Upon completion of dehydration, all samples were individually vacuum packaged into plastic bags at 28 in. vacuum. Packages were boxed and stored for 8 months at 21C with 42% relative humidity in order to simulate commercial warehouse storage conditions.

At the end of the treatment period, tristimulus color components L, a and b were measured using a Hunter colorimeter (HunterLab D25 - PC2Δ Hunter Associates Laboratory Inc., Reston, VA) standardized against calibrated instrument tiles. Values of hue and chroma were subsequently calculated (Little 1975).

Due to previous research concerning the application of honey as an active food ingredient (Oszmianski and Lee 1990), a ranked preference test was conducted between the 10% honey treatments and the commercial sample using a Friedman Rank Analysis method and analysis (O'Mahoney 1986; McLellan *et al.* 1987). Overall quality of the two treatment combinations was assessed by 18 untrained taste panelists in one sitting. Additionally, a categorical rating of sample quality was made using a 3 point scale: poor/ okay /good. The samples were randomly coded and presented in cups in a random sequence. Panelists were encouraged to note any comments they wished to share about the products. Panelists were encouraged to use all of their senses in the evaluation process as well. Sample evaluation was carried out in individual booths in a sensory room fitted with northern sky daylight bulbs (MacBeth, Newberg, NY).

Analysis of Hunter colorimetry data was by analysis of variance on triplicate samples from the processed samples using the computer program Statistica (Anon. 1991). Of the sensory data, only the ranking data was analyzed using standardized tests (O'Mahoney 1986). All other sensory results are reported without statistically based conclusions.

RESULTS AND DISCUSSION

In Table 1, the results of an analysis of variance is presented. All five visual characteristic measurements were significant at or beyond the 99% level. Respective p-values are included in the table. Table 2 shows visual measurements for each treatment combination. The samples listed are in descending order from the lightest to darkest according to Hunter L values. A minimum significant difference was calculated using Tukey's Honestly Significant

Difference (HSD) test and is noted in the last line of the table (Gill 1978). This value can be utilized to compare means as reported for each variable. The two honey solutions were effective treatments to maintain lighter raisins as indicated by the Hunter L value. For both honey treatments, the Hunter L results indicated a significantly lighter product than the other treatments. The results also indicated that the honey pretreatment produced a golden raisin that was more saturated yellow (Hunter b+ values) than any of the other treatments.

TABLE 1.
RESULTS OF ANOVA ON HUNTER COLORIMETER MEASUREMENTS, L, a AND b AS WELL AS DERIVED MEASUREMENTS OF HUE AND CHROMA
Sums of squares, mean square error, F-value and probability levels are shown for each variable.

Depend. Variable	Mean Sqr Effect	Mean Sqr Error	f(df1,2) 7,16	p-Level
Hunter_L	31.6018	.477917	66.12418	.0000000
Hunter_a	1.3743	.168333	8.16407	.0002714
Hunter_b	20.1426	.318750	63.19253	.0000000
Hue	257.0782	8.376790	30.68934	.0000000
Chroma	16.0267	.278444	57.55803	.0000000

A polar plot (Fig. 1) of the first quadrant (0–90°) in the Hunter L, a, b color space illustrated the hue and chroma results as calculated for these treatments. Hue was plotted as the angle from zero degrees (red) and chroma (color saturation) was plotted as the radius. Of note is the apparent grouping of the results into two sample categories. The browner, less saturated group consists of the control, sulfite dip and "Natick 631" solution treatments. The plot indicates that this group was darker and less yellow than the remaining samples consisting of the blanch, sulfite pressure treatment, commercial control, and two honey treatments.

The use of a pressure treated sulfite solution appeared to be equally effective as the commercial control. This might indicate the potential for significant process time savings when comparing the commercial 6 h sulfur gas treatment to the 5 min pressurized solution treatment.

Sensory quality tests of the 10% honey treated raisins and commercial raisins showed a significant difference in rank according to the Friedman Rank Analysis. The 10% honey treated raisins with a rank total of 18 was ranked superior to the commercial control with a rank total of 36 using a Friedman rank comparison test statistic of 2.398 ($P = 0.001$). On the 3 point rating scale, using poor, okay or good, 94% of the panelists ranked the 10% honey treatment as good and 6% ranked it as okay. For the commercial control treatment, 44% of

TABLE 2.
VISUAL MEASUREMENTS USING THE HUNTER COLORIMETER AND PANELISTS' COMMENTS FOR VARIOUS GRAPE TREATMENTS USED IN THE PRODUCTION OF LIGHT THOMPSON SEEDLESS RAISINS
Means ± standard deviations and Tukey's HSD minimum significant differences are shown.

Trial	**Hunter Colorimetry Measurements**					**Panelists**
Variable	**L**	**a**	**b**	**Hue**	**Chroma**	**Comments:**
Honey - 20% Sol.	25.2 ± 0.06	4.1 ± 0.38	10.9 ± 0.29	69.5 ± 2.08	11.6 ± 0.22	Yellow, bright
Honey - 10% Sol.	25.0 ± 0.75	4.6 ± 0.87	10.6 ± 0.45	66.6 ± 4.67	11.6 ± 0.23	Yellow, bright
Sulfite - Press. TRT	23.5 ± 0.12	4.2 ± 0.23	9.1 ± 0.15	65.5 ± 0.97	10.0 ± 0.22	Yell-Lt. Green
Blanch	23.5 ± 1.06	3.4 ± 0.17	8.3 ± 0.26	67.7 ± 1.53	9.0 ± 0.21	Lt. Br.-Gr. w/Gray
Commercial Sample	23.0 ± 1.16	4.8 ± 0.25	9.3 ± 0.64	62.5 ± 1.98	10.4 ± 0.58	Yell-Orange, dull
Control	19.3 ± 0.47	5.4 ± 0.17	6.0 ± 0.38	48.2 ± 1.46	8.1 ± 0.36	Medium Brown
Sulfite - Dip/Hold	17.9 ± 0.70	4.2 ± 0.50	5.3 ± 1.20	51.7 ± 4.28	6.7 ± 1.22	Med.-Dark Brown
Natick Sol.	17.2 ± 0.25	3.5 ± 0.15	4.4 ± 0.41	51.9 ± 3.62	5.6 ± 0.26	Dark Brown
Min. Significant Difference	1.892	1.123	1.545	7.922	1.444	

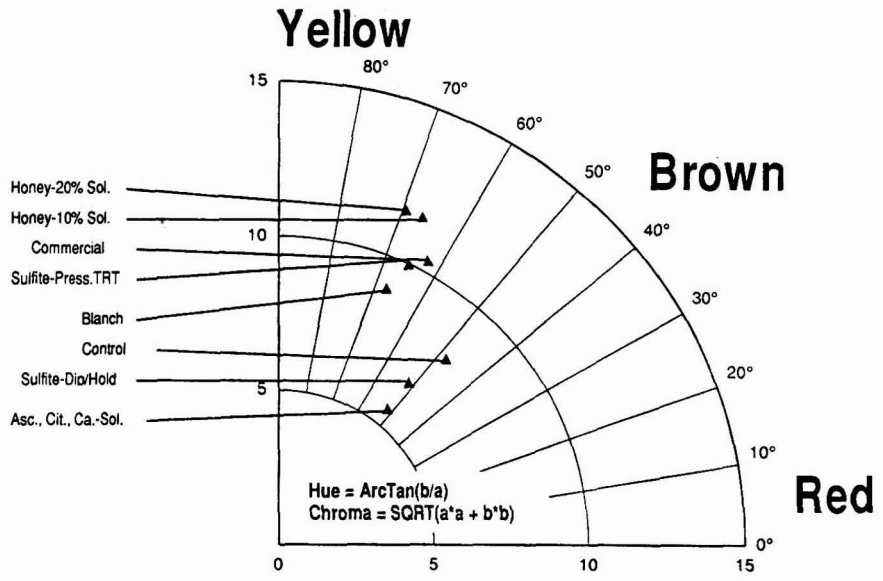

FIG. 1. A POLAR PLOT OF THE FIRST QUADRANT (0° TO 90°) IN THE HUNTER L, a, b COLOR SPACE WITHOUT THE THIRD DIMENSION (L VALUE) PLOTTED
Points are labeled according to treatment.

the panelists ranked it as okay and 50% of the panelists ranked it as poor. Panelists' comments indicated that they found the honey treated sample had a more fruity flavor and a softer, chewier texture.

The results of this study indicate that alternatives to sulfur exist for the pretreatment of grapes planned for dehydration. Based on this study either a blanch treatment or a honey treatment would be effective alternatives. Optimally, our results indicate that a honey treatment will yield the highest quality product as compared to all treatments studied. Significant effects were determined based on a preference result in which 18 out of 18 panelists preferred the honey treated sample.

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ANALYSIS OF FACTORS INFLUENCING LIPID OXIDATION IN HAZELNUTS (*CORYLUS* SPP.)

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ABSTRACT

Significant genotypic variability was found in total fat, fatty acid and α-tocopherol contents and lipoxygenase (LOX) activity of kernels of Corylus avellana *cultivars Barcelona, Ennis, Tonda Gentile Delle Longe (TGDL) and a wild hazelnut,* Corylus cornuta. *Relationships existed between shelf-lives for kernels/nuts of these cultivars and polyunsaturated fat, α-tocopherol and LOX activity levels. Barcelona and Ennis, previously shown to be less resistant to oxidative deterioration (shorter shelf-lives), were higher in polyunsaturated fat and LOX activity. Conversely, the antioxidant α-tocopherol was present in higher concentrations in the more stable TGDL than in the other cultivars. There was no apparent relationship between shelf-life and mineral or protein content.*

INTRODUCTION

From 1985 to 1989, U.S. annual hazelnut production averaged about 16,500 metric tons (Hazelnut Marketing Board 1990), far below domestic

[1]Mention of company or trade name does not imply endorsement by the United States Department of Agriculture over others not named.
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demand. Efforts to increase hazelnut production have been coupled with efforts to improve kernel quality, a characteristic dependent on flavor and shelf-life (Thompson 1969; Lagerstedt 1984).

In a previous report (Pershern 1989), peroxide values (PV) and hexanal production were monitored to assess the storage stability of three cultivars of *C. avellana*, Barcelona, Ennis, and Tonda Gentile Delle Longe (TGDL). Rate constants, activation energies, and shelf-life plots showed the superiority of the cultivar TGDL and to a lesser extent Ennis over Barcelona.

Lipid oxidation, the principal mode of flavor deterioration and shortened shelf-life, is influenced by lipid composition and the presence of antioxidants, enzymes, and trace metals. These factors are not well characterized for American grown hazelnuts. The fat content of hazelnuts is reported to range between 48 and 72% (Thompson and Richardson 1978; Radicati *et al.* 1979; Gargano *et al.* 1981, 1982; Piskornik and Korfel 1983; Rivella 1984). The degree of unsaturation in the total fat influences nut stability. The major fatty acids in hazelnuts are oleic (18:1) at 70–85% of the total (Gargano *et al.* 1981 1982; Piskornik and Korfel 1983), linoleic (18:2) at 10–24%, and linolenic (18:3) at less than 1% (Thompson and Richardson 1978; Piskornik and Korfel 1983). Relative oxidation rates for oleic, linoleic, and linolenic are approximately 1:10:20 (Nawar 1985); hence, the relative concentrations of these three fatty acids will greatly influence oxidation rates, flavor deterioration, and shelf-life.

Lipoxygenase (LOX) enzymes are important because they likely shorten shelf-life by catalyzing the conjugation and hydroperoxidation of the *cis, cis*-1, 4 pentadiene structures within fatty acids such as linoleic or linolenic acid (Vick and Zimmerman 1987). Subsequent reactions of linoleic and linolenic hydroperoxide products have been linked with hexanal production and off-flavors in peanuts (Pattee and Singleton 1981). Though off-flavor and shelf-life problems are similar in hazelnuts, no research has been conducted to determine if genotypically related LOX activity levels are linked to shelf-life. Since LOX mediated hexanal production contributes to off-flavors in many other food products (Gardner 1985), it would be useful to know if the same is true for hazelnuts. Results will be useful in variety development programs for improving the shelf-life and market quality of hazelnuts and the many products containing them.

Tocopherols occur naturally in vegetable oils (Carpenter 1979). They retard hexanal production and off-flavor development by neutralizing free radicals produced by oxidation of unsaturated lipids. The α-tocopherol content in hazelnuts may play an important role in controlling off-flavor, especially since it has been reported to be present at about 20 mg per 100 g hazelnuts (Lambertsen *et al.* 1962; Diem and Lentner 1975).

Our research objective was to determine (1) the relationship of total lipid content, (2) fatty acid profiles, (3) tocopherol content, (4) the content of

minerals potentially catalyzing nonenzymatic lipid peroxidation, and (5) lipoxygenase activity to shelf-life differences observed among the three domestic hazelnut cultivars with established shelf-life properties (Pershern 1989). Results from our investigations on these domestic cultivars and on a limited collection of wild hazelnuts *(C. cornuta)* will be useful in shelf-life and market quality improvement programs.

MATERIALS AND METHODS

Hazelnuts

Three cultivars of *Corylus avellana*, Barcelona, Ennis, and Tonda Gentile Delle Longe (TGDL), were harvested at Oregon State University, Corvallis, OR, in fall, 1986 and 1987, and held in common storage until shipment to our laboratory in January, 1987 and 1988, respectively. Samples were stored unshelled in sealed plastic bags at 4C until the kernels were removed from the shells and analyzed for moisture, fat, protein, minerals, and LOX activity. Kernels of Barcelona, Ennis and TGDL were also analyzed for residual α-tocopherol.

Wild beaked hazelnuts, *Corylus cornuta*, were gathered in northern St. Louis county, Minnesota, in late August, 1986 and 1987, and stored unshelled in sealed plastic bags at 4C until analyzed.

Unless indicated otherwise, each cultivar was sampled and analyzed in triplicate and the results averaged for each analytical determination.

Fat and Total Protein Analysis

The total fat content of the nuts was determined by the hexane extraction method of Christen-Marchal (1986). Fatty acid methyl esters (FAME) were prepared by treating the extracted fat with methanolic NaOH followed by boron trifluoride (AOAC 1984, 28.056–28.059). The FAME and standards (Supelco, Bellefante, PA) were separated using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector, DB-225 (30 m $\times$ 0.25 mm i.d.) capillary column and autosampler. Column temperature was programmed for 3 min at 100C followed by a rise of 10C/min to 220C with a 15 min hold. The helium flow rate was 1.0 ml/min with a 1:20 split. Injection port and detector temperatures were held at 200 and 275C, respectively. Total protein was determined by the semimicro Kjeldahl procedure using a nitrogen to protein conversion factor of 6.25 (Schulte *et al.* 1987).

Lipoxygenase Analysis

Kernels from both the 1986 and 1987 crops were used for analysis within 8 months of harvest. The high oil content of the kernels necessitated the use of acetone powders in enzyme assays.

Aluminum weighing dishes and petri dish bottoms, each of the latter containing a piece of Whatman 41 ashless paper, were preweighed. Acetone and diethyl ether were stored in a solvent safe storage vessel at −18C prior to use and, during the preparation of acetone powders, were held in crushed ice. For extraction, two 95-ml portions each of acetone and diethyl ether were required.

About 14 g of kernels was weighed (accuracy: 0.001 g) and placed in a 100-ml Virtis homogenizer canister to which was added 30 to 35 ml ice-cold acetone and a small piece of dry ice. The canister was assembled on a Virtis "45" homogenizer and run for 45 s at medium speed. A second piece of dry ice was added and the mixture was run again for 45 s at medium speed. This cryogenic milling ensured stability of lipoxygenase. The mixture was poured into a Buchner vacuum funnel containing the preweighed filter paper from the petri dish. The canister was rinsed with the remaining acetone from the first 95-ml portion and this was poured into the Buchner funnel for vacuum augmented filtration. Part of the second 95-ml portion of acetone was used to completely rinse the canister and the remainder was poured over the wet powder in the funnel. The two 95-ml portions of diethyl ether were then poured over the powder one at a time with a gentle breaking of the formed crust between ether washings. A thin spatula was used to loosen the powder around the edges. The paper and powder were carefully placed in a weighed petri dish, which was put into a vacuum desiccator and dried overnight. During the first 1.5 h, the vacuum was released with N_2 three times. The petri dishes with paper and dried powder were weighed. Acetone powders (each about 5.000 g) were stored in plastic tubes with a desiccant and placed in a freezer until the enzyme was extracted for assay.

The enzyme was extracted from the acetone powder by placing 0.5000 g of powder into 5.00 ml of cold 0.05 M potassium phosphate buffer, pH 6.5, in a test tube. Any chunks present were carefully broken up with a thin spatula and the mixture stirred. The preparation was extracted on ice for 45 min, then centrifuged at 4C at 15,000 × g for 20 min. The supernatant was forced through a Millipore filter (0.5 μm cellulose) and the volume of the enzyme extract was measured (about 3.0 ml). The extract was then placed in a heavy-walled glass vial with a Teflon-lined cap and stored in crushed ice until assayed. The linoleic acid (Nu Chek Prep, Elysian, MN) substrate solution was prepared according to the method of Surrey (1964). Hazelnut LOX activity was measured spectrophotometrically at 25C as outlined by Zimmerman and Vick (1970); the constant rate of the production of conjugated diene was measured by

recording the change in absorbance at 234 nm with a Hitachi Perkin Elmer 124 or Beckman Model 35 spectrophotometer. The enzyme assay reaction mixtures routinely consisted of 2.96 ml of 0.05 M acetate buffer (pH 4.0), 0.02 ml of enzyme solution, and 0.02 ml of linoleic acid substrate solution (8.05 mM). In those assays employing more or less than 0.02 ml of the enzyme solution, the buffer volume was adjusted to maintain a total reaction volume of 3.0 ml. The buffer systems employed to develop the pH curve were all 0.05M and consisted of potassium acetate, pH 3.00–5.50; potassium phosphate, pH 6.00–7.50; and sodium borate, pH 8.00–9.00.

One unit of LOX activity in the hazelnuts, on a fresh weight (FW) basis, was defined as the formation of 1 μmol of linoleic acid hydroperoxide/min/g FW using a molar absorptivity of 23,300 (Vioque and Holman 1962). All enzyme assays were conducted within the linear range. Soluble protein concentrations were determined by the Coomassie Brilliant Blue G-250 (Bio-Rad Richmond, CA) method using bovine serum albumin (BSA) as a standard. All analyses were conducted in duplicate (1986) or triplicate (1987) and the results averaged, except that *C. cornuta* was analyzed in duplicate both years.

Thermal stability of hazelnut LOX was determined by placing enzyme extract in temperature controlled water baths at 25, 40, 69 and 95C. Aliquots were withdrawn at timed intervals, promptly chilled on ice and assayed for remaining LOX activity to develop time courses for LOX thermo-lability at each temperature.

Tocopherol Analysis

Following harvest but preceding storage, the hazelnut shells were removed and the kernels were ground and extracted with ethanol using the method of Guzman and Murphy (1986). The method of standard additions (Pease 1980) was used to assess recovery efficiency. Tocopherol standards were a gift from Distillation Products Industries Div., Eastman Kodak Co., Rochester, NY. The chromatographic system consisted of a Waters C-18, 8 mm i.d. $\times$ 10 cm column with a 10 μm particle size packing; a Waters Model 6000 HPLC with M45 solvent delivery system and WISP 710B autosampler (Waters Associates Millipore Corp., Milford, MA); and Hewlett-Packard Photo Diode Array Detector (Hewlett-Packard Co., Palo Alto, CA). The mobile phase was 10% water in methanol at a flow rate of 2.5 ml/min. Twenty-microliter aliquots were injected with the autosampler and monitored at 220 nm.

Storage Effect on Tocopherol Content

Hazelnut kernels were stored for 36 weeks at 25, 35 or 55C and 0.2 or 0.6 water activity (A_w) (Ennis), 25 or 35C and 0.2 A_w (Barcelona) and 25C and 0.2

A_w (TGDL). The kernels were analyzed in triplicate for α-tocopherol content upon shelling and grinding (zero-time) and after 36 weeks of storage. The average percentages of α-tocopherol remaining for each cultivar was then calculated based on its contents at zero-time and after 36 weeks of storage.

Minerals

Kernel samples were analyzed for P, K, Ca, Mg, S, B, Mn, Zn, Cu, Fe, Al, and Na by inductively-coupled plasma (ICP) emission spectrophotometry (Schulte *et al.* 1987). Each individual sample was analyzed using the procedure as outlined by Schulte *et al.* (1987).

Data Analysis

Analysis of variance (SPSS one-way) was used to test for differences among cultivars. Differences ($p \leq 0.05$) between means were analyzed by the Student Newman Kuels test.

RESULTS AND DISCUSSION

Fat Analysis and Total Protein

The fat contents of the nuts used in this study ranged from 43.6–66.1% (Table 1). Since Ennis is an orchard "sport" or mutation of Barcelona, it was not surprising that their fat contents were similar. A total fat level of under 63% has been suggested as contributing to storage stability in hazelnuts (Rivella 1984). However, TGDL, with 66.1%, had the highest level and was found to be the most stable cultivar in earlier shelf-life studies (Pershern 1989).

Results of the FAME analysis are given in Table 2. The ratio of unsaturated to saturated fatty acids was about twice as high for *C. cornuta* as for the *C. avellana* cultivars. The high degree of unsaturation in *C. cornuta* was probably because of its colder habitat. The 18:2 and 18:3 fatty acids would be most susceptible to lipid oxidation and would contribute to poor shelf-life. Among the *C. avellana* cultivars, Barcelona had the highest degree of unsaturation. The degree of monounsaturation in all the nuts is noteworthy when considering current dietary recommendations. Fatty acid content in oilseeds is specific for species, and often for cultivars (Roughan and Slack 1982). The three *C.*

avellana cultivars were grown in Oregon where mean minimum temperatures are considerably higher than in northern Minnesota where the *C. cornuta* nuts were grown. Sunflower seeds were found to have a negative correlation ($r = -0.83$) between minimum temperatures and linoleic acid content (Robertson *et al.* 1979). We also found a relationship between temperature and linoleic acid concentration in our hazelnut data; *C. cornuta* was substantially higher in linoleic acid (13.2%) than the *C. avellana* cultivars (9.4–11.2%).

TABLE 1.
TOTAL FAT AND PROTEIN CONTENT IN HAZELNUT KERNELS

Nuts	Total Fat (%)	Total Protein (%)
Barcelona	60.5[a]	14.5[a]
Ennis	60.4[a]	13.0[a]
TGDL[x]	66.1[b]	14.6[a]
C. cornuta	43.6[c]	18.1[a]

Mean values in the same column followed by different letters are significantly different, $p \leq 0.05$
[x]TGDL = Tonda Gentile Delle Longe

The wild hazelnut, *C. cornuta*, with its smaller kernel/seed was higher in total protein content (18.1%) than the domesticated genotypes (13.0–14.6%). Values of 9.6% and 13.5% (Klein and Gunther 1985) and 12.7% (Diem and Lentner 1975) were reported previously for *C. avellana*. Combined, total protein and total fat comprised a very large part of the kernel, ranging from 73.4 – 80.7% for Barcelona, Ennis and TGDL and 61.7% for *C. cornuta*. However, total protein content alone did not appear to influence LOX activity or shelf-life.

Lipoxygenase Activity

Figure 1 shows the pH response curve for LOX using linoleic acid substrate. Maximum activity occurred in a relatively narrow range around pH xxxxxx

TABLE 2.
MEAN CONTENT ± SD OF FATTY ACIDS IN HAZELNUT KERNELS (% OF TOTAL)

	C. avellana cultivars			
Fatty Acids	Barcelona	Ennis	TGDL[x]	*C. cornuta*
4:0	0.15 ± 0.01	1.08 ± 0.10	1.06 ± 0.21	ND
6:0	0.13 ± 0.00	0.11 ± 0.03	0.11 ± 0.00	ND
8:0	ND	ND	Tr	ND
10:0	ND	0.16 ± 0.00	Tr	ND
12:0	Tr	Tr	Tr	Tr
14:0	Tr	Tr	Tr	ND
16:0	5.22 ± 0.26	6.38 ± 0.15	5.87 ± 0.69	3.46 ± 0.02
16:1	0.17 ± 0.01	0.26 ± 0.15	0.24 ± 0.05	0.14 ± 0.01
17:0	Tr	Tr	Tr	Tr
17:1	Tr	0.09 ± 0.00	0.09 ± 0.04	Tr
18:0	2.43 ± 0.38	2.29 ± 0.28	2.38 ± 0.07	1.48 ± 0.00
18:1	79.85 ± 0.85	79.46 ± 0.41	81.27 ± 0.37	81.12 ± 0.02
18:2	11.18 ± 1.01	10.65 ± 0.50	9.36 ± 0.48	13.18 ± 0.02
18:3	0.12 ± 0.02	0.10 ± 0.10	0.12 ± 0.02	0.22 ± 0.00
20:0	0.15 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.25 ± 0.02
20:1	0.31 ± 0.03	0.18 ± 0.04	0.17 ± 0.04	0.24 ± 0.01
18:1+18:2	91.03	90.29	90.63	94.30
18:2+18:3	11.30	10.75	9.48	13.40
Ratio Uns/Sat	11.34	8.95	9.56	18.29

ND = None detected
[x] TGDL = Tonda Gentile Delle Longe

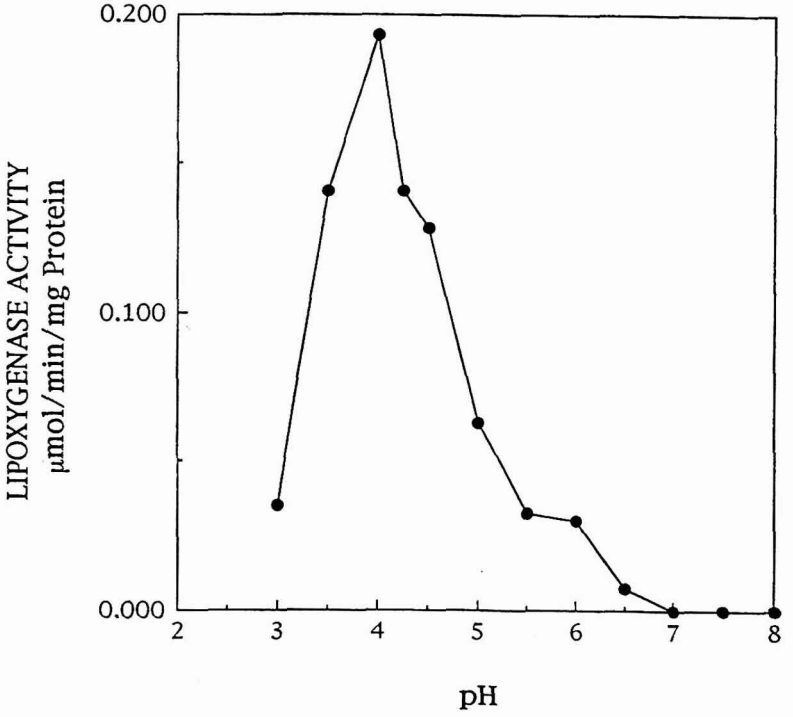

FIG. 1. EFFECT OF pH ON ACTIVITY OF CRUDE BARCELONA HAZELNUT LIPOXYGENASE EXTRACT WITH LINOLEIC ACID SUBSTRATE AT 25C

4.0. It is well known that pH extremes result in denaturation of enzymes. Indeed, little activity was seen above pH 6.0, and none at pH 7.0, for hazelnut LOX. Few other lipoxygenases show as low a pH optimum as 4.0. Those in legumes have optima around pH 6.5 (Chang and McCurdy 1985). However, LOX in tomato with an optimum at pH 4.5 (Daood and Biacs 1988) and in watermelon cotyledons with optima at pH 4.4 and 5.5 (Vick and Zimmerman 1976) are also lower than the majority of LOX studied.

The acetone powders were very stable if kept frozen and dry. However, after extraction of the enzyme into buffered solutions, stability decreased. When kept at 4C, the crude extract showed a 10–15% loss of enzyme activity in 24 h. At 25C, activity was completely lost in 24 h. Therefore, it was essential during all analyses using the crude extract that it be held at 0C and that new extractions be made daily.

Lipoxygenase was less stable as temperature increased, losing all activity in 20 min at 95C while at 69C about 30% of original activity remained (Fig. 2).

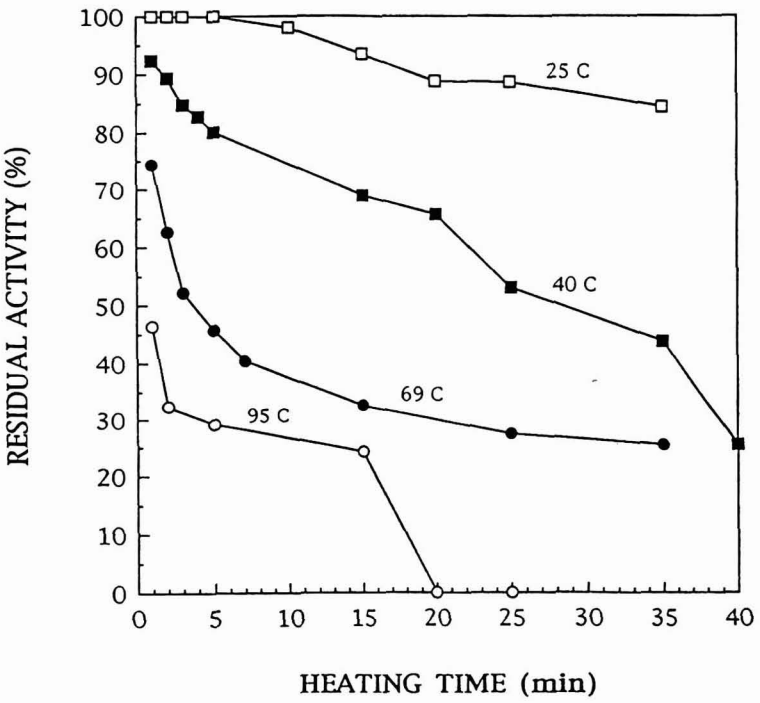

FIG. 2. INFLUENCE OF HEATING TIME AND TEMPERATURE ON HAZELNUT (CV BARCELONA) LIPOXYGENASE ACTIVITY

Thus, the enzyme was moderately resistant to the heat treatments in this experiment and much more resistant than tomato LOX (Daood and Biacs 1988) and sunflower LOX (Leoni *et al.* 1985). Hazelnut LOX was also somewhat more heat stable than pinto bean LOX (McCurdy *et al.* 1983). At 65C, pinto bean LOX lost 93% of activity in 10 min and hazelnut LOX lost only 60% of activity in 10 min at 69C in our study.

Lipoxygenase inactivation in soybeans has been shown to follow a first order rate of degradation (Brown *et al.* 1982). The activation energy for inactivation of the crude LOX enzyme was calculated from Arrhenius plot data to be 20.9 Kcal/mol in pH 6.5 K_3PO_4 buffer. Brown *et al.* (1982) reported activation energies for the inactivation of LOX in soybean cotyledons to be 127.4 and 103.3 Kcal/mol in pH 9.8 and 10.8 buffers, respectively.

A comparison of maximum LOX activity in the three cultivars and in *C. cornuta* revealed that all activities were relatively low (Table 3). Barcelona displayed the highest activity, 0.347 μmol/min/g FW, compared with TGDL

TABLE 3.
LOX ACTIVITY EXTRACTED FROM ACETONE POWDER OF HAZELNUT KERNELS

Nut and Year of Crop	LOX Activity	
	Specific Activity (μmol/min/mg Protein) ± SD	Activity/gFW[x] (μmol/min) ± SD
Barcelona		
1986	0.215 ± 0.021	0.347 ± 0.000
1987	0.227 ± 0.003	0.351 ± 0.004
Ennis		
1986	0.110 ± 0.003	0.227 ± 0.003
1987	0.128 ± 0.010	0.296 ± 0.045
TGDL[y]		
1986	0.119 ± 0.005	0.211 ± 0.001
1987	0.120 ± 0.001	0.252 ± 0.006
C. cornuta		
1986	0.107 ± 0.011	0.164 ± 0.014
1987	0.189 ± 0.002	0.302 ± 0.003

x gFW = Grams fresh weight
y TGDL = Tonda Gentile Delle Longe

at 0.213 μmol/min/g FW for samples from the 1986 crop. Nuts from the 1987 crop showed values of 0.353 and 0.248 μmol/min/g FW for Barcelona and TGDL, respectively.

A summary of LOX activity in terms of specific activity and activity per gram fresh weight of nut is also shown in Table 3. Interestingly, LOX activity on a fresh weight basis varied considerably between years in *C. cornuta*. It is common to see wide biological variation in wild tissue. In addition, there was a clearly observed difference in the size and quality of the *C. cornuta* nuts between the two years. The 1986 crop was very sparse and most clones, in the northern Minnesota location where the wild nuts were gathered, did not set nuts. Nuts were very small with an exceptionally large amount of pellicle compared to solid nut tissue. It may be that the environmental stress that reduced nut production and size also reduced LOX activity for that year.

TABLE 4.
α-TOCOPHEROL AND POLYUNSATURATED FATTY ACID CONTENTS (18:2+18:3) OF HAZELNUT KERNELS

Cultivar	α-tocopherol mg/100 g±SD	PUFA (%)
Barcelona	11.63±1.88	11.30
Ennis	19.53±1.00	10.75
TGDL[x]	21.24±1.06	9.48
C. cornuta	8.16±1.25	13.40

[x] TGDL = Tonda Gentile Delle Longe

TABLE 5.
α-TOCOPHEROL REMAINING IN HAZELNUT KERNELS AFTER 36 WEEKS OF STORAGE AT 0.2 OR 0.6 A_w AND 25, 35 OR 55C

Cultivar	A_w	Storage Temperature (C)	α-tocopherol (mg/100)	% Remaining
Barcelona	0.2	25	5.74	49.4
Ennis	0.2	25	11.46	58.7
Ennis	0.6	25	13.93	71.4
TGDL[x]	0.2	25	13.63	64.2
Barcelona	0.2	35	4.17	35.9
Ennis	0.2	35	9.09	46.6
Ennis	0.6	35	11.78	60.4
Ennis	0.2	55	3.54	18.1
Ennis	0.6	55	7.21	36.9

[x] TGDL = Tonda Gentile Delle Longe

Tocopherol

There were significant differences ($p \leq 0.05$) in α-tocopherol levels among the three cultivars and *C. cornuta* (Table 4). Only *C. cornuta* contained γ-tocopherol at a detectable level; it contained 8.55 mg/100 g. The γ-tocopherol in the wild nut may be an important factor because it is a better antioxidant in

TABLE 6.
MINERAL CONTENT OF HAZELNUTS, FRESH WEIGHT BASIS

Nut	Minerals mg/100 g FW										
	Cu	Fe	Mn	Ca	K	Mg	P	S	Zn	Al	Na
Barcelona	2.2	6.2	1.8	135	960	125	333	120	243	<6.16	<6.10
Ennis	2.0	6.1	1.3	135	890	160	323	150	245	<3.50	<6.10
TGDL[y]	2.0	5.6	1.5	120	660	165	346	150	205	<3.50	<6.10
C. cornuta	2.2	5.7	2.1	360	730	230	405	210	253	<3.50	<6.10
Diem and Lentner (1975)	1.4	4.5	4.2	250	618	150	320	198	x	x	3

x Data not given
[y] TGDL = Tonda Gentile Delle Longe

model systems than α-tocopherol (Dougherty 1988). The α-tocopherol content in plant foods generally correlates positively with polyunsaturated fatty acid (PUFA) content (Lehmann *et al.* 1986), but the reverse was true for the hazelnuts analyzed in this study (Table 4).

The α-tocopherol remaining in kernels after 36 weeks of storage is given in Table 5. At 25 and 35C, where Barcelona can be compared with Ennis at the same A_w (0.2), the former underwent the greater loss in α-tocopherol, perhaps because more was consumed in its antioxidant role. At 25C and 0.2 A_w, TGDL underwent the lowest percentage loss in α-tocopherol. For Ennis, which was stored at 0.2 and 0.6 A_w and 25, 35 and 55C, percentage loss of α-tocopherol varied inversely with A_w and directly with temperature.

Minerals

The analysis of minerals involved in lipid oxidation, Cu, Fe, and Mn, showed little variation among cultivars (Table 6). Ca, Mg, S, P, and Zn were higher in *C. cornuta* than in the domesticated cultivars. These results differed somewhat from previously reported values (Diem and Lentner 1975).

The transition metals such as cobalt, copper, iron, and manganese are major prooxidants in lipid systems (Labuza 1971; Tannenbaum *et al.* 1985). Copper and iron, particularly, catalyze the oxidation of unsaturated lipids, thereby decreasing the induction period and increasing the rate of oxidation. In addition, a single, tightly bound iron atom is required for lipoxygenase and the iron status of plants can be studied by measuring lipoxygenase activity (Boyer and Vander Ploeg 1986).

CONCLUSIONS

We believe this is the first report that relates lipoxygenase activity and endogenous antioxidant levels to previously reported shelf-life differences in three domestic hazelnut cultivars. LOX activity levels among the domesticated cultivars reflected shelf-life differences observed previously (Pershern 1989) where TGDL was the most stable, followed by Ennis and Barcelona. Consequently, it is important to note that LOX activity levels in these cultivars varied inversely with shelf-life. Both TGDL and Ennis were lower in lipoxygenase activity than Barcelona.

The (α-tocopherol levels in American grown hazelnuts have not been previously reported. We found that higher α-tocopherol levels (TGDL > Ennis > Barcelona) in hazelnuts improved shelf stability, thus reflecting its antioxidant function.

Contrary to the results obtained by Rivella (1984) (which indicated that hazelnuts containing more than 63% fat are likely to exhibit poor shelf stability) we found that TGDL, which contained 66% fat, was the most stable of the three *C. avellana* cultivars. Hence, it would appear that total fat content is not a good predictor of shelf-life. The degree of unsaturation of the fat was a better predictor within the genotypes that we tested. Barcelona, with the highest unsaturation/saturation ratio (11.34) had the poorest shelf stability. Ennis had a slightly lower unsaturation/saturation ratio (8.95) than TGDL (9.56); yet, Ennis was less stable. This difference in stability may have been due to the higher level of α-tocopherol and lower total polyunsaturated fatty acid content, i.e., 18:2 and 18:3 acids, in TGDL.

Assessment of lipoxygenase activity, tocopherol content, fatty acid profile, fat unsaturation/saturation ratio and other factors that might affect lipid oxidation, e.g., mineral content, could help hazelnut breeders to develop high quality cultivars with good shelf stability. The information reported here on the wild beaked hazelnut, *C. cornuta*, could also be useful to variety developers seeking to introduce cold tolerance into commercial cultivars, thereby expanding domestic production areas and decreasing dependence on imports.

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PHYTIC ACID INHIBITS THE PRODUCTION OF AFLATOXIN B_1

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ABSTRACT

Phytic acid inhibition of Aspergillus flavus *aflatoxin B_1 production is well observed. Although this fungus grew well in Czapak-Dox medium, mycotoxin production was eliminated by adding a small amount of phytic acid. Possible reasons are discussed, and the importance of some metallic ions is observed. Results suggested that phytate may be an effective anti-AFB_1 agent for preventing the contamination of the fungus.*

INTRODUCTION

Phytic acid, myo-inositol hexaphosphoric acid, is widely present in crops, oil seeds, nuts and peanuts, especially in their shells and buddings. The function of phytic acid is believed to provide cations (Milliams 1970), phosphorus (Hall and Hodges 1966), or high-energy phosphoric groups (Biswas *et al.* 1978) necessary for the early development and growth of plants. In addition, it is suggested that there must be other unknown effects for the development and well-being of plants.

Aflatoxin B_1 (AFB_1) produced by *Aspergillus flavus* as well as other fungi is an intermediate metabolite of glucose aerobic oxidation, and an important role is played in this process by enzymes which need metal ions as their coenzyme or activator for their activity. It is the homoenzyme that in some way inhibits the production of AFB_1. There is evidence that AFB_1 causes cancer and has the potential for other neoplasms in animals. According to Hiscocks (1965),

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growth of fungi results in loss of damaged foods and industrial materials amounting to $200 billion every year. AFB[1] is known to play a role in oncogenesis (Busby *et al.* 1984), and its production is inhibited by phytic acid. A preliminary assay was done to confirm this and the results are reported.

MATERIALS AND METHODS

Materials

AFB_1 standard and AF standard strain were provided by the Chinese Institute of Preventive Medicine, phytic acid by Tianwei Company, Sichuan, and Silica gel (type 60) by E. Merck.

All other reagents are analytical reagent grade.

Methods

Czapak-Dox median (pH 6.0) was prepared as follows:

(1) Add 9.0 ml distilled water to 1.0 ml of phytic acid and adjust its pH to 6.0 with 1.0 mol/L NaOH solution.

(2) The AF standard strain was cultured on slants of rose bengal agar and kept at 27C for 10 days, and 20 ml of aseptic saline was added to make AF a suspension.

(3) The 70 g of corn powder were weighed into a 1000 ml beaker, 500 ml water added and the mixture brought to a boil. After filtering the mixture into another 1000 ml beaker, 30 g of sucrose, 3 g of sodium nitrate, 1 g of dipotassium hydrogen phosphate, 0.5 g of potassium chloride, 0.5 g of magnesium phosphate, and 0.01 g of ferrous sulphate were added. One ml of 0.5% cupric sulphate solution, and 1 ml of 1% zinc sulphate solution were added. The resulting solution was diluted to 1000 ml with distilled water and portioned into 100 ml flasks, which were then stoppered and sterilized (121C for 20 min).

Effect of phytic acid concentration on AFB_1 production was tested by adding 1.0 ml of AF suspension into each flask containing 100 ml of Czapak-Dox medium with a specified amount of phytic acid solution (see Table 1) and determining the AFB_1 produced after 10 and 25 days incubation at 25C on a rotating shaker.

AFB_1 was measured as follows: From each incubated flask, pipette 20 ml of the culture into a series of 250 ml flasks with stoppers. Add 30 ml of n-hexane and 100 ml of 55% methanol, and stopper tightly to avoid leakage.

TABLE 1.
EFFECT OF CONCENTRATION OF PHYTIC ACID ON PRODUCTION OF AFB_1, BY AF GROWING IN CZAPAK-DOX CULTURE

Items	Days of Incubation (20 C)	Culture Number 1	2	3	4	5	6	7
phytic acid (mg/100ml)		0.00	0.01	0.02	0.04	0.06	0.12	0.24
AFB_1	10	10.5	10.8	10.0	8.9	5.3	no	no
(μg/100ml)	25	12.4	12.0	11.5	8.2	3.4	no	no

After shaking for 30 min and a short time standing, filter through paper into a 125 ml separating funnel for layering. Collect the methanol-water portion in a flask, and pipette 20 ml into another 125 ml separating funnel. Add 20 ml of chloroform, and after shaking 2 min let it stand for layering. Collect the chloroform portion and filter it through about 10 g of anhydrous sodium sulfate on filter paper into a 50 ml evaporating dish, followed by three washings with chloroform, each being collected into the dish. Evaporate the chloroform extract to dryness at room temperature. Add 1.0 ml of a mixture of benzene and acetonitride (98:2 by volume), and when the residue dissolves transfer it into a 2 ml test tube, which is subsequently stoppered.

TLC plate preparation was: Mix 10 g of silica gel G with 2 ml of water, heat to 65C, and spread on a clean glass plate (7 × 14 cm) about 3 mm thick. Allow to dry in air. The plates were activated by heating in an oven at 100C for 2 h. The final gel film was about 0.25 mm thick.

TLC is performed in a developing chamber (25 × 10 × 8cm) at room temperature (20C).

(1) A 50 μl portion of sample to be tested was applied on the point (origin) 2 cm apart from the lower edge of the TLC film, and the TLC plate was placed in the first developing chamber containing absolute diethyl ether as the mobile phase. When the moving edge of the ether reached 12 cm from the origin, the process was stopped, the plate removed from the chamber and allowed to dry in air. After drying, the plate was placed in the second chamber containing a mixture of acetone and chloroform (8:92 by volume) as the mobile phase. When the moving edge of the mixture reached 12 cm beyond the origin, the process was stopped and the plate was removed from the chamber.

(2) Fluorescence intensity of AFB_1 spots was measured at 365 nm using thin-layer chrometo-scanner (model CS-930 Shimadzu, Japan). The RF value of the standard AFB_1 was 0.70.

The effect of different metals on production of AFB_1 by AF was assessed by culturing with Czapak-Dox media with 0.04% of phytic acid and, individually, absent of $FeSO_4$, $MgSO_4$, $ZnSO_4$, or $CuSO_4$.

RESULTS AND DISCUSSION

Results of this study suggest that phytic acid present at a concentration of 0.12% in the culture can totally inhibit production of AFB_1 by AF, and at a concentration of 0.04% the effect of inhibition will be seen in the 10 and 25 day incubations (Table 1). It appears that the longer the incubation time, the more effective the inhibition suggesting that the inhibition is long-acting. How soon this effect is noticeable requires further study.

Although it was not the objective of this study to investigate the specific mechanisms of phytic acid inhibition, it is interesting to note that in the growth process of fungi (Detroy and Hesseltine 1970), during the late stage of their trophophase, the respiratory activity of fungi is reduced, resulting in reduced growth. As a consequence, there is a relative accumulation of acetyl coenzyme A and intermediates of the tricarboxylic acid cycle. By the inductive effect of these intermediates, the secondary biological synthesis system is activated, leading to the commencement of AFB_1 synthesis by AF (Bu'Lock 1965). The secondary biological synthesis is regulated through enzymatic synthesis just as that of the primary metabolic intermediates synthesis (Purchase 1974). When small amounts of phytic acid are added to the culture, it chelates metals and the activity of enzymes is decreased or lost. As the amount of phytic acid is increased, the production of AFB_1 by AF is gradually inhibited (see results of Table 2).

TABLE 2.
EFFECT OF METAL IONS ON ABILITY OF AF TO PRODUCE AFB_1

Culture Number[a]	1	2	3	4	5	6	7
AFB_1 produced (μg/100 ml)	10.3	8.4	4.6	7.6	0	0	0

[a]1. Czapak-Dox Culture; 2. Czapak-Dox Culture containing 0.04% of phytic acid; 3. Czapak-Dox Culture containing 0.06% of phytic acid; 4. Czapak-Dox Culture containing 0.04% of phytic acid without $CuSO_4$; 5. Czapak-Dox Culture containing 0.04% of phytic acid without $ZnSO_4$; 6. Czapak-Dox Culture containing 0.04% of phytic acid without $FeSO_4$; 7. Czapak-Dox Culture containing 0.04% of phytic acid without $MgSO_4$.

The results of this study show that AF grows in Czapak-Dox culture containing 0.04% phytic acid (i.e., this concentration is not sufficiently viscous to inhibit production of AFB_1, see Table 1) and at this phytic acid concentration metal ions from sources such as water, reagent, etc. are chelated. In the absence of added $ZnSO_4$ or $FeSO_4$ production of AFB_1 was also inhibited (culture numbers 5,6 and 7 in Table 2). The absence of $CuSO_4$ did not show the effect (culture 4 in Table 2), suggesting that ions of Mg^{2+}, Zn^{2+}, Fe^{2+} may be cofactors of the related enzymes, which is in agreement with observations of Marsh *et al.* (1975), or may stabilize the structural component of the enzyme active center. The inhibiting effect of phytic acid on AF to yield AFB_1 is probably due to the interaction of phytic acid with such ions. Further study is necessary to confirm this hypothesis.

Phytic acid is distributed mainly in the buddings and outer shells of plant seeds and evenly covers the surface of seeds like a protective membrane. The function of phytic acid is not only for energy storage and antioxidation of fats of seeds (Graf *et al.* 1987) but also for protecting seed from fungus invasion and hence blocking production of AFB_1 by AF as stated in this study. This explains why there is no growth of fungi in seeds with intact protective membranes. The result of this study suggests that when seeds of plants and tobacco are sprayed or soaked with phytic acid solution of appropriate concentration prior to storage, protection from mold follows, reducing loss of crops or foods. This treatment could also reduce cancer incidence by reducing exposure of people to AFB_1.

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THERMAL PROPERTIES AND MOISTURE SORPTION ISOTHERMS OF SPRAY-DRIED ENCAPSULATED MILKFAT

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ABSTRACT

Stable emulsions containing 40 or 60% anhydrous butter oil and carbohydrate encapsulants (sucrose, modified starch or all-purpose flour) were spray dried to produce free-flowing shelf-stable powders, according to a 2 × 3 factorial design, replicated three times. Differential scanning calorimetry profiles showed well-defined melting ranges that were related to encapsulant used. Butter oil was almost completely encapsulated when fat content was 40% and sucrose was used as the wall material. Moisture sorption isotherms of powders with sucrose showed characteristic breaks caused by sugar crystallization followed by moisture desorption, whereas powders with modified starch or all-purpose flour continuously absorbed moisture with increasing relative humidity. The solvent-extractable fat fraction increased with increasing relative humidity in all cases. Scanning electron microscopy showed that sucrose-containing powder particles partially dissolved and fused together as a result of moisture uptake, whereas powders with modified starch or all-purpose flour maintained particle identity, even at 80% relative humidity.

INTRODUCTION

Spray drying of milkfat with functional encapsulants, such as starch or other carbohydrates, to form free-flowing powders can reduce storage costs and enhance stability by forming microcapsules to protect the milkfat from oxidative deterioration during storage (Young *et al.* 1993; Imagi *et al.* 1992; Gejl-Hansen

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[2]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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and Flink 1977). Onwulata *et al.* (1994a,b) have demonstrated that anhydrous butter oil or cream may be successfully encapsulated in carbohydrate matrices (sucrose, all-purpose flour or modified starch); however, the physical and structural properties varied with the source of milkfat and the type of encapsulant used. Evaluation of some thermal properties of the encapsulated materials is also needed, since they may differ from those of the individual constituents.

Milkfat is considered to be a quality-enhancing ingredient in foods, with the melting characteristics being very significant. Proper and timely melting of milkfat is necessary in developing flavor and enhancing texture. The melting pattern of milkfat has been well-studied in the range of −10 to 40C (Taylor *et al.* 1978; van Beresteyn and Schaap 1971; Patel and Frede 1991). Milkfat oxidizes and decomposes during prolonged heating at high temperatures. Some decomposition products of butter oil have been reported (Tangel *et al.* 1977; Chang *et al.* 1978; Keogh 1989). Encapsulation may modify the melting range of butter oil and reduce the rate and extent of decomposition.

Starch retrogrades upon heating, with gelatinization being influenced by the presence of sugars and emulsifiers. Differential scanning calorimetry has been used to monitor changes in chemical properties of starches as a function of temperature by detecting changes in heat capacity associated with such processes (Kim and Walker 1992; Wootton and Bamunua-Rachchi 1980; Buck and Walker 1988). Processing also may alter the physical state of disaccharides, changing them either to an amorphous or crystalline form. Altering the physical state of carbohydrates in foods affects their properties during processing and storage (Niediek 1988).

The physical state of the encapsulation matrix and its moisture absorption pattern is critical in stopping oxidation and rancidity development during storage (Shimada *et al.* 1991). The effect of imbibed water on the structure of the microcapsule determines its stability when blended into a dry food mix. An amorphous glass entraps flavor compounds and protects encapsulated materials from oxidation due to slow diffusion of, for example, encapsulated oil from the interior to the surface of the capsule and that of oxygen to the interior when the carbohydrate is in the glassy form; however, encapsulant crystallization results in higher diffusion rates or causes complete release of encapsulated compounds through capsule rupture (Roos and Karel 1991; Labrousse *et al.* 1992). In the crystalline state, sugars imbibe little water at low relative humidity. At high water activity (A_w = 0.8–0.85), sugars such as sucrose begin to dissolve. Amorphous sugars absorb substantial amounts of water at low relative humidity, leading to crystallization and sudden moisture desorption (Riganakos *et al.* 1992). An excellent example of such behavior is that of the milk sugar, lactose, in milk or whey powder which, if present in the amorphous state, absorbs water and crystallizes to form the alpha monohydrate, resulting in caking (Mistry *et al.* 1992; Saltmarch and Labuza 1980; Bushill *et al.* 1965) .

The objectives of this study were to investigate the thermal behavior of anhydrous butter oil encapsulated in a variety of carbohydrate matrices and to examine the effect of water imbibition on the structural integrity of the spray dried microcapsules when exposed to high humidity at ambient temperatures.

MATERIALS AND METHODS

Anhydrous butter oil was purchased from a commercial distributor (Land-O-Lakes, Minneapolis, MN). Encapsulants chosen were sucrose (Domino's, Domino Sugar Corp, New York, NY), modified starch (M-starch) Capsul™ (National Starch and Chemical Co., Bridgewater, NJ) and all-purpose flour (N-starch) (ADM Milling Co., Kansas City, MO). An emulsifying agent (mono- and diglycerides) (American Ingredients Co., Kansas City, MO) was also used. The protein source was nonfat dry milk (Maryland and Virginia Milk Producers Association, Inc., Laurel, MD).

Encapsulated powders were formulated to have 40 or 60% milkfat, 5% emulsifier, 5% nonfat dry milk and the remainder disaccharide. Sample preparation was done as follows: The encapsulant was blended with nonfat dry milk solids, dissolved in water, then mixed with a warmed (23.9C) emulsion of anhydrous butter oil and emulsifier, and heated at 23.9C for 5 min with stirring. The constantly stirred slurry (40% total solids) was slowly brought to the final temperature (62.8C), and homogenized at 17.2 MPa with a Manton-Gaulin Model 100 DJF3 855X Triplex homogenizer (APV Gaulin, Inc., Everett, MA). The homogenized emulsion was spray dried in a compact dryer (APV Crepaco Inc., Attleboro Falls, MA). Spray dryer inlet temperature was 180–190C, and an outlet temperature of 80–110C was maintained. The powders were produced in batches, removed from the dryer after 30 min and stored at 4C. A 2×3 factorial design was completed, replicated 3 times. Milk protein content was about 2% in the finished powders. When all-purpose flour was used as the encapsulant, it was necessary to homogenize at a lower pressure and temperature (10.3 MPa and 54.4C) to accommodate pasting properties.

A Perkin-Elmer differential scanning calorimeter, Model DSC-7, equipped with an Intracooler II refrigeration unit was used to measure thermal characteristics (Perkin Elmer Corp., Norwalk, CT). The purge gas was nitrogen, at 20 ml/min. High purity indium was used to calibrate the instrument. A 10 mg ($\pm$ 1 mg) portion of sample was weighed into aluminum pans (Perkin-Elmer) and hermetically sealed. An empty sample pan was used as a reference. Heating was from -25 to 350C at 20C/min after initial cooling to -30 at 20 C/min. The heat of melting, in joules per gram of sample, was determined by dividing the area under the curve by the sample weight. Samples were analyzed in duplicate.

^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (CPMAS) spectra were obtained on a Bruker MSL-300 spectrometer (Bruker, USA, Billerica, MA) operating at 75.5 MHz, using a Doty Scientific, Inc. (Doty Scientific, Inc., Columbia, SC) CPMAS NMR probe. The CPMAS spectra were obtained using a 1 ms contact time and 1.5 s recycle time. The sweep width was 25,000 Hz; 1024 data points were collected. 4096 transients were collected for each spectrum. The high resolution experiments were conducted using magic angle spinning nuclear magnetic resonance (MAS). The spectra were obtained using high powered gated decoupling. The ^{13}C pulse width was 7 μs pulse with a 5 s recycle time. The sweep width was 25,000 Hz and 2048 data points were collected. 512 transients were collected for each spectrum.

Moisture sorption isotherms were obtained at 25C by equilibrating 10-g powder samples with known water vapor pressures provided by the following saturated salt solutions: $CaSO_4$, LiBr, LiCl, K_2CO_3, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, KI, and $(NH_4)_2SO_4$ (Rockland and Nishi 1980) for 72 h. Moisture uptake by encapsulated powders was determined after equilibrating for 200 h over anhydrous K_2CO_3. The weight change was determined after drying under vacuum for 4 h at 102C (AOAC 1984).

Extractable fat (ŸF) was determined by dispersing 10 g powder in 50 ml carbon tetrachloride and shaking for 15 min (Anon. 1978). (Warning: carbon tetrachloride is a known carcinogen and appropriate safety precautions must be taken for its use.) After decanting and filtering the supernatant, the filtrate was evaporated, leaving the extracted milkfat. The extractable fat fraction was expressed as the fat weight recovered from the powder, divided by the original weight (Anon. 1978).

Surface structures of encapsulated powders after equilibration at 20 or 80% relative humidity were evaluated with a scanning electron microscope (JEOL Model 840A, JEOL, Peabody, MA) in the secondary electron imaging mode. Samples of powders were sprinkled on aluminum specimen stubs coated with Spot-O-Glue labels (Avery, Azusa, CA). Excess, nonadherent particles were removed from the surfaces of the sample stubs with a jet of pressurized air. Specimen stubs were coated with a thin layer of gold in a DC cold sputtering module in an E306A vacuum evaporator (Edwards High Vacuum, Inc., Grand Island, NY).

RESULTS AND DISCUSSION

The transition points of the components used for the encapsulation of milkfat are presented in Table 1. The DSC thermogram for butter oil had a melting pattern with numerous peaks from −40 to 40C. Continued heating

TABLE 1.

MELTING PROPERTIES OF MATERIALS USED FOR ENCAPSULATION

Product	Peak (C)	Melting range (C)	ΔH (J/g)
Butter oil	*	-40 - 40	**
Sucrose	190	180 - 196	122.2
M-Starch	99	60 - 150	85.4
N-Starch	118	60 - 201	250.4
Emulsifier	46	37 - 165**	27.6

* Numerous peaks within the melting range

** More than one peak

M-starch = modified starch; N-starch = all-purpose flour; emulsifier = mono- and diglycerides

above this region led to gradual thermal decomposition, without the appearance of additional peaks. Thermal decomposition of butter oil beyond this melting region has been reported (Taylor *et al.* 1978). The melting range for the emulsifier (mono- and diglycerides) used was from 37–165C with peaks at 46 and 150C. The M-starch matrix had melting peaks around 99C, N-starch at 118C, and sucrose at 190C. Identification of the melting peaks of the various components was essential in identifying shifts and complex patterns in the thermograms of the encapsulated powders.

DSC thermograms of the spray dried powders containing 40 or 60% anhydrous butter oil encapsulated in three different carbohydrate matrices are shown in Fig. 1, 2 and 3; the heat of melting and peak temperatures are reported in Table 2. The thermal profiles show two major melting zones for each product containing 40 or 60% encapsulated fat, indicating the melting of the surface or unencapsulated fat and fusion of the wall material. Thermograms of butter oil encapsulated within the N-starch matrix (Fig. 1A and B) show melting of the butter oil from 0 to 40C. The butter oil peaks were more defined for thermograms of powders with 60% fat. The heat of melting of the milkfat was much greater at 60% fat, indicating the presence of more butter oil. Thermograms for the M-Starch/butter oil capsules show one main peak and a curve from 53 to 175C (Fig. 2C and D). Heat of melting again varied with the amount of fat in the M-starch-encapsulated powder. In contrast to the other samples, capsules with sucrose as the encapsulating agent (Fig. 3E and F) had

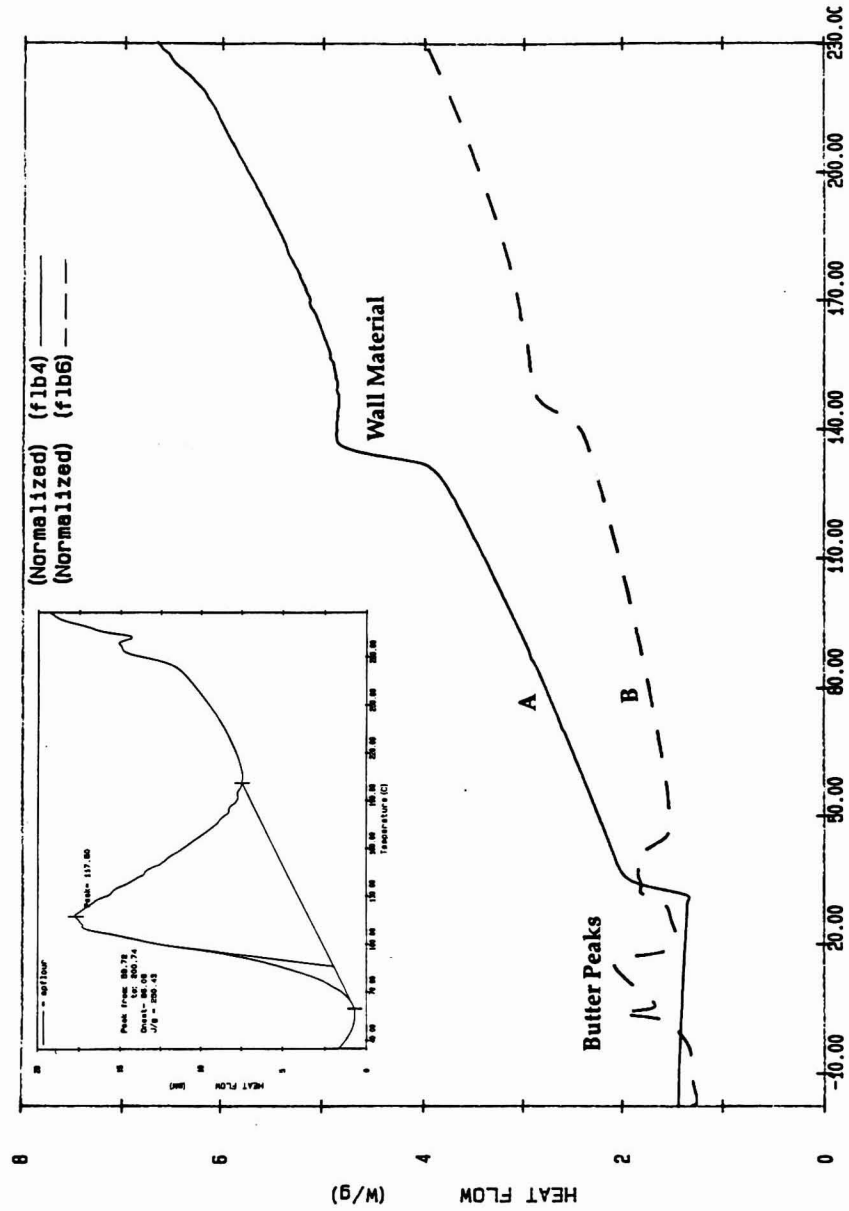

FIG. 1. DSC THERMOGRAMS OF BUTTER OIL ENCAPSULATED IN ALL-PURPOSE FLOUR (N-STARCH)
(A) 40% Fat level; Flb4. (B) 60% fat level; Flb6. Insert: DSC thermogram of all-purpose flour.

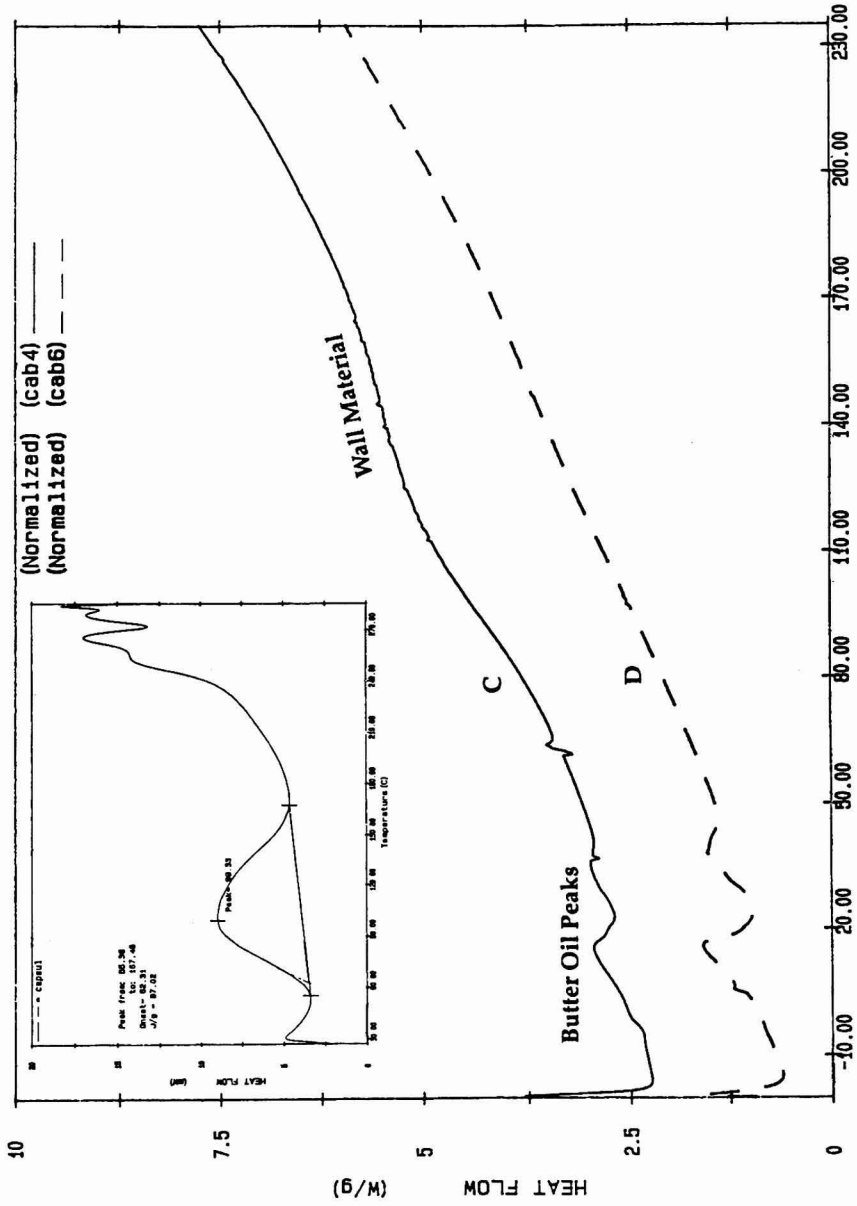

FIG. 2. DSC THERMOGRAMS OF BUTTER OIL ENCAPSULATED IN MODIFIED STARCH (M-STARCH) (C) 40% Fat level; CAB4. (D) 60% Fat level; CAB6. Insert: DSC thermogram of modified starch.

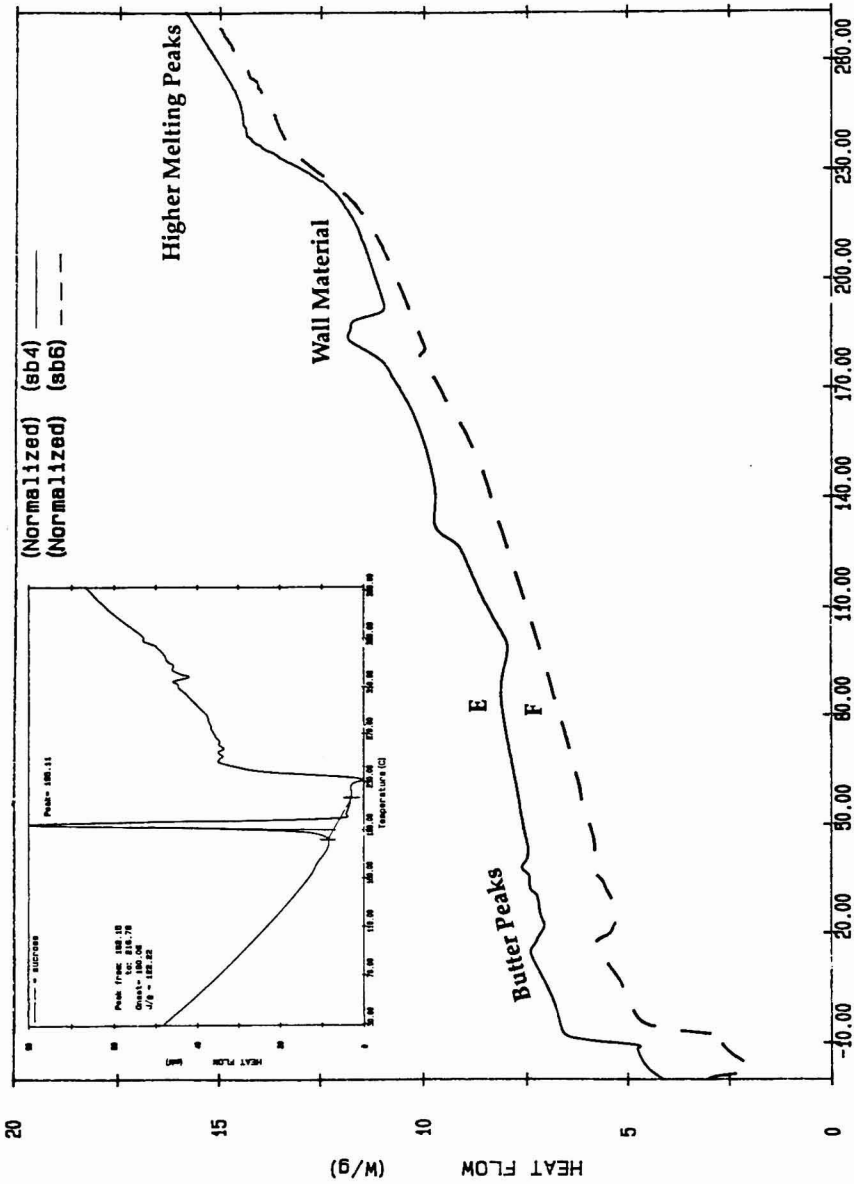

FIG. 3. DSC THERMOGRAMS OF BUTTER OIL ENCAPSULATED IN SUCROSE
(E) 40% Fat level; SB4. (F) 60% Fat level; SB6. Insert: DSC thermogram of sucrose.

three fusion zones: surface fat peaks, 17–38C, sucrose peaks, 178–184C, and high temperature peaks above 220C. The capsules with sucrose and 40% fat show a disassociation product peak at 239C (Fig. 3E and Table 2); the sample with 60% fat disassociates at a maximum of 236C (Table 2). The thermal patterns of these powders are those of true capsules with defined event times for the fusion of the various components comprising the powders.

TABLE 2.
MELTING CHARACTERISTICS OF MILKFAT ENCAPSULATED IN CARBOHYDRATE MATRICES[1]

Product	Surface fat				Encapsulant load			
	Peak	ΔH	Peak	ΔH	Peak	ΔH	Peak	ΔH
	ΔH = J/g				Peak = C			
N-starch40	*	*	36.0	10.6	134.4	27.6	--	--
SD			0.0	2.1	2.6	0.4		
N-starch 60	4.0	25.2	39.1	13.6	148.5	19.6	--	--
SD	0.0	0.0	0.1	0.5	0.5	4.3		
M-starch 40	15.2	15.3	34.1	2.5	112.8	58.4	--	--
SD	0.2	0.2	0.1	0.8	0.2	4.1		
M-starch 60	15.8	24.9	36.1	8.3	96.7	9.6	--	--
SD	0.2	4.0	2.1	0.0	0.3	0.8		
Sucrose 40	17.6	14.5	38.0	4.9	183.8	42.5	239.1	23.9
SD	0.6	0.0	0.0	0.1	0.2	0.0	0.1	1.7
Sucrose 60	16.8	24.3	36.2	3.6	177.8	4.7	235.8	15.0
SD	0.2	2.0	0.2	0.2	0.2	2.4	0.2	1.9

*: Insignificant melting peaks.

--: No thermal products after carbohydrate peak.

N-starch = All-purpose flour and butter oil.

M-starch = Modified starch and butter oil.

40 and 60 = 40% and 60% milkfat.

SD = Standard Deviation

[1]Moisture content of powders ranged from 1 to 3%.

Thermal properties of milkfat polymorphs and their crystallization properties have been studied in detail (van Beresteyn and Schaap 1971; Patel and Frede 1991). Solid fat indices and melting patterns were determined by the triglyceride structure and fatty acid composition. We made no attempt to identify the crystalline forms of the anhydrous milkfat in our powders; melting peaks (Table 2) for the six powders studied all fall within the known melting range for milkfat.

It is known that complexes formed in the presence of saccharides change the melting patterns of the components. Krog *et al.* (1989) reported a disassociation of amylose-lipid complex at 100–120C, with the heat of disassociation increasing in the presence of monoglycerides. Lipid-saccharose complexes appeared as high melting peaks. Starch degradation studies have shown that in the presence of sugar and emulsifiers, peak temperatures are increased (Buck and Walker 1988; Donovan 1977). In our study, the M-starch (modified starch) showed a peak temperature increase of 13C with a 40% fat content, whereas, with 60% fat, there was little change (2C) in peak temperature (Tables 1 and 2). In contrast, the N-starch (all-purpose flour) showed peak temperature increases of 16 and 30C for powders with 40 and 60% fat, respectively (Tables 1 and 2), suggesting that greater changes are occurring in the starch moiety of this encapsulant as a result of the association with fat. When sucrose was used as the encapsulant, maximum peak temperature decreased, with the greatest decrease (13C) observed in the powder with 60% fat (Tables 1 and 2). If encapsulated milkfat is to be used as a shortening in, for example, dry bakery mixes, upon reconstitution and subsequent baking, the capsule must rupture to deliver the shortening load at the appropriate time. Our results suggest that it might be possible to tailor capsule rupture temperature to the baking process by careful choice of encapsulant. In addition, the capsules must be able to withstand the mechanical stress of dry-mixing and blending to be shelf stable; milkfat completely enclosed in a competent capsule should be protected from oxidative deterioration during storage. Storage stability of milkfat encapsulated under our experimental conditions will be the subject of a future study.

To investigate the possibility of complex formation between sucrose and milkfat, as suggested by the appearance of a third high-melting peak in the thermograms of this formulation (Fig. 3E), samples encapsulated in sucrose were analyzed by nuclear magnetic resonance (NMR). NMR studies were not done on samples encapsulated in M-starch or N-starch, since these samples did not contain a high-melting fraction. A ^{13}C CPMAS spectrum of a powder encapsulated with sucrose and 40% butter oil is presented in Fig. 4. The CPMAS spectrum shows a low crystalline order sucrose spectrum (110–50 ppm). The high resolution mass spectrum for butter oil in the same powder (Fig. 4, mobile phase) shows the presence of the fatty acid side chains (35–18 ppm), a glycerol peak (70–60 ppm), monounsaturates (130 ppm) and a carbonyl peak

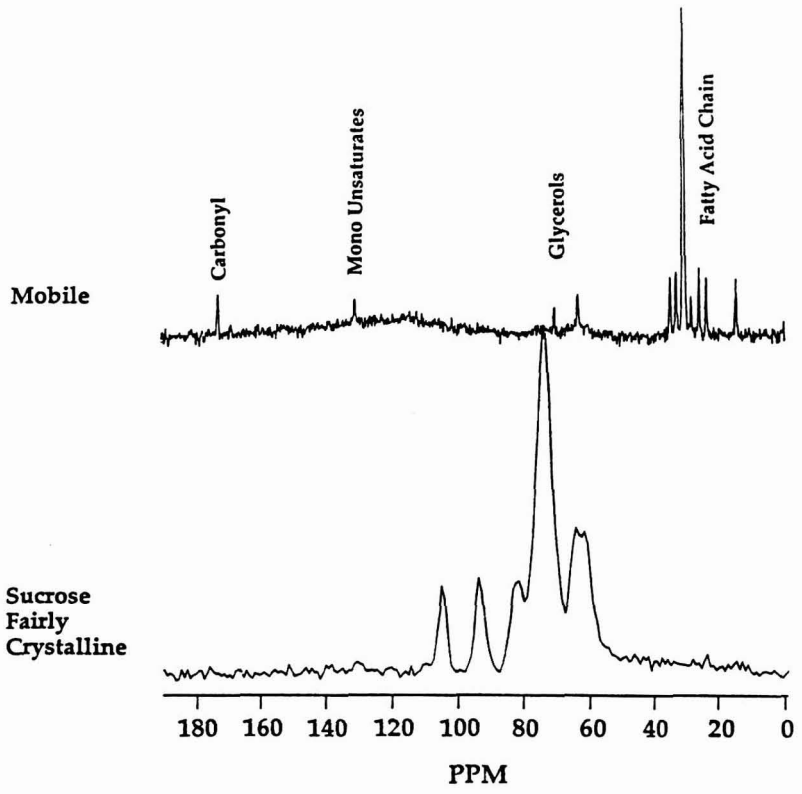

FIG. 4. ^{13}C NMR SPECTRUM OF POWDER WITH 40% BUTTER OIL ENCAPSULATED IN SUCROSE, SHOWING SOME CRYSTALLINE ORDER
Mobile phase shows intact triglyceride structure.

(170 ppm). The ^{13}C spectrum shows no evidence of thermal oxidation of the butter oil caused by the encapsulation process. NMR spectra of sucrose and other polysaccharides have been reported (Seino and Uchibori 1984; Akoh and Swanson 1987). The NMR spectra did not support the formation of sucrose-milkfat complexes such as ester linkages under our experimental conditions; the high melting peak present in the thermograms of sucrose/butter-oil powders still remains unidentified. Encapsulation may be stabilized by other interactions such as formation of fat-protein complexes by homogenization before drying.

Moisture uptake by the powders encapsulated with sucrose or M-starch and containing 40 or 60% anhydrous butter oil after exposure to 40% relative humidity at 25C for more than 200 h is plotted in Fig. 5. This demonstrates that the moisture sorption patterns were dependent on the type of encapsulant used. Sucrose/butter-oil powders showed initial moisture uptake and subsequent

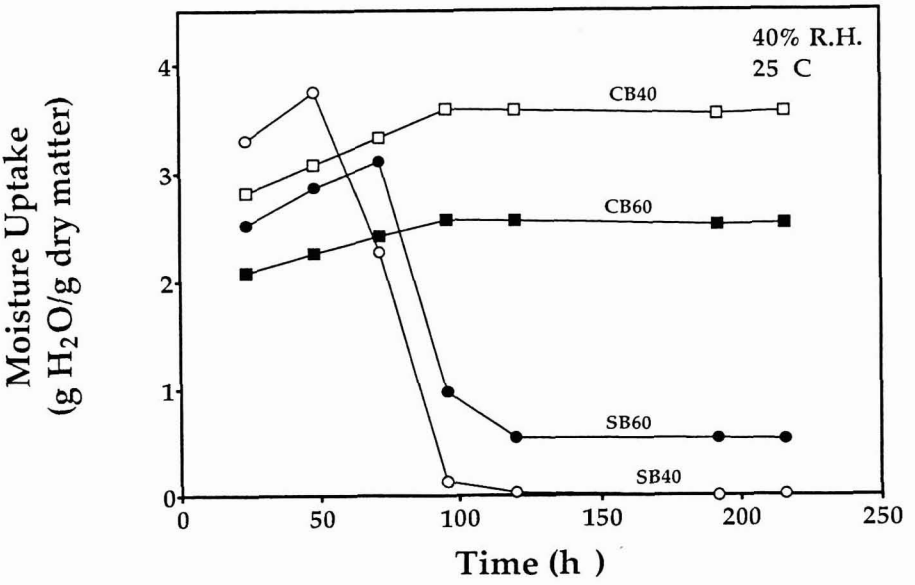

FIG. 5. UPTAKE OF MOISTURE AT 25C AND 40% RELATIVE HUMIDITY FOR SAMPLES WITH 40 OR 60% BUTTER OIL AND WITH SUCROSE OR MODIFIED STARCH (M-STARCH) AS ENCAPSULANT AFTER EQUILIBRATION FOR 200 H OVER ANHYDROUS POTASSIUM CARBONATE
-○- = Sucrose 40 (SB40); -●- = Sucrose 60 (SB60); -□- = M-starch 40 (CB40); -■- = M-Starch 60 (CB60).

moisture loss after 100 h. This pattern of moisture uptake is typical of an amorphous powder which desorbs moisture upon crystallization. Sucrose in the amorphous form in a low humidity environment has been shown to recrystallize and lose moisture (Iglesias and Chirife 1978; Karel 1975). In contrast, the M-starch/butter-oil capsules showed a steady uptake of moisture until equilibrium was reached. There was no desorption of moisture even after 200 h. The moisture uptake patterns were similar to those of food gums that retain absorbed water. N-starch capsules imbibed negligible amounts of water under these conditions (data not shown). In both cases, samples with 60% anhydrous butter oil imbibed less water than did the samples with 40% fat.

Sorption isotherms of powders encapsulated in sucrose, N-starch and M-starch, with 40 or 60% butter oil are presented in Fig. 6. Sucrose/butter-oil powders show characteristic sorption patterns across the range of water activities examined. A similar break in the sorption isotherm between 40–50% relative humidity was reported for spray dried milk powders, attributed to the crystallization of α-lactose monohydrate (Berlin *et al.* 1969; Pisecky 1992). The sorption

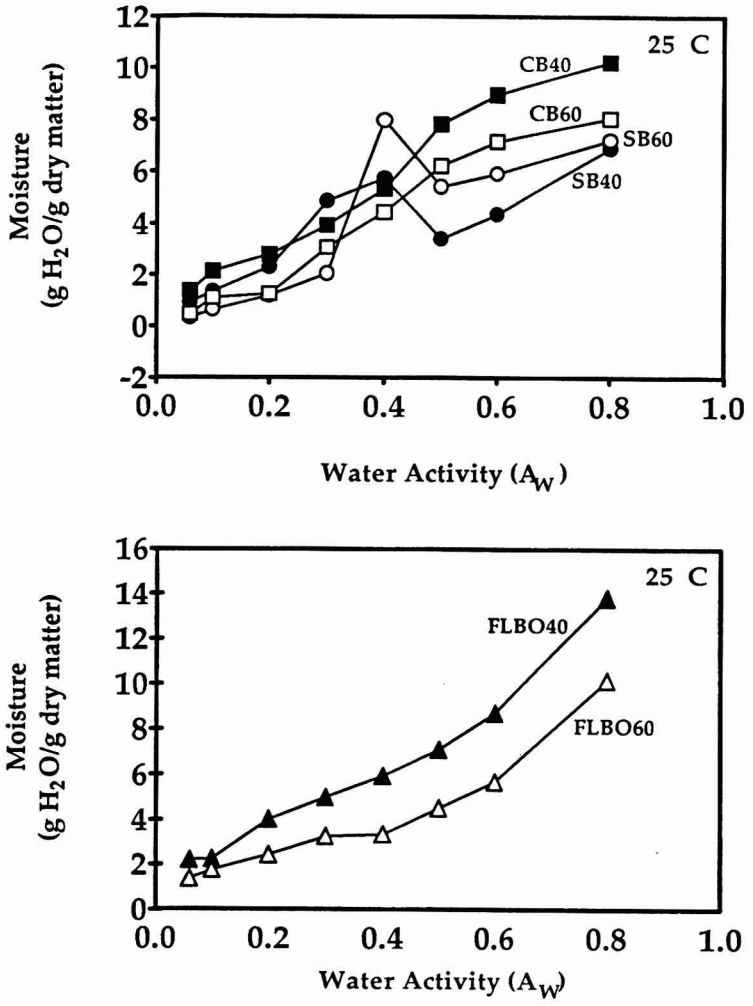

FIG. 6. MOISTURE SORPTION ISOTHERMS OF POWDERS WITH 40 OR 60% BUTTER OIL AND SUCROSE, MODIFIED STARCH (M-STARCH) OR ALL-PURPOSE FLOUR (N-STARCH) AS ENCAPSULANT

-●- = Sucrose 40 (SB40); -○- = Sucrose 60 (SB60); -■- = M-Starch 40 (CB40); -□- = M-Starch 60 (CB60); -▲- = N-Starch 40 (FLBO40); -Δ- = N-Starch 60 (FLBO60).

isotherm for crystalline sucrose differs from that of amorphous sucrose (Niediek 1988; Moreyra and Peleg 1981). Even though a small amount of lactose is present (about 1/20 of the total disaccharide present), we attribute the characteristic breaks in the isotherms of the sucrose/butter oil powders to crystallization of amorphous sucrose. It is highly likely that powders encapsulated with sucrose would crystallize eventually, even in low humidity, suggesting that these

powders must be protected from moisture uptake during storage by low-water-vapor-permeable packaging. M-starch/butter oil and N-starch/butter-oil isotherms showed patterns typical of water absorption for flours or gums, with steady increases in moisture sorption with increasing relative humidity. Oxidative stability may be expected for the sucrose/butter-oil powders between 0.1–0.2 A_w, and for M-starch/butter-oil and N-starch/butter oil powders between 0.2-0.4 A_w, based on the moisture monolayers (Karel 1975); this remains to be confirmed in future studies.

The amount of extractable fat (ŸF) at different relative humidities for each powder was measured, to determine the effect of high humidity on fat retention; results for sucrose/butter-oil and M-starch/butter-oil powders are shown in Fig. 7. ŸF increased with increasing relative humidity at each fat level, for all encapsulated powders, up to the limit of fat encapsulated. This suggests that the uptake of moisture dissolved some of the encapsulating matrix, or ruptured the matrix as a result of particle swelling, exposing the enclosed fat. Previous studies (Onwulata *et al.* 1993a,b) had shown that the least amount of extractable fat (< 55%) was obtained from samples containing 40% butter oil encapsulated in sucrose.

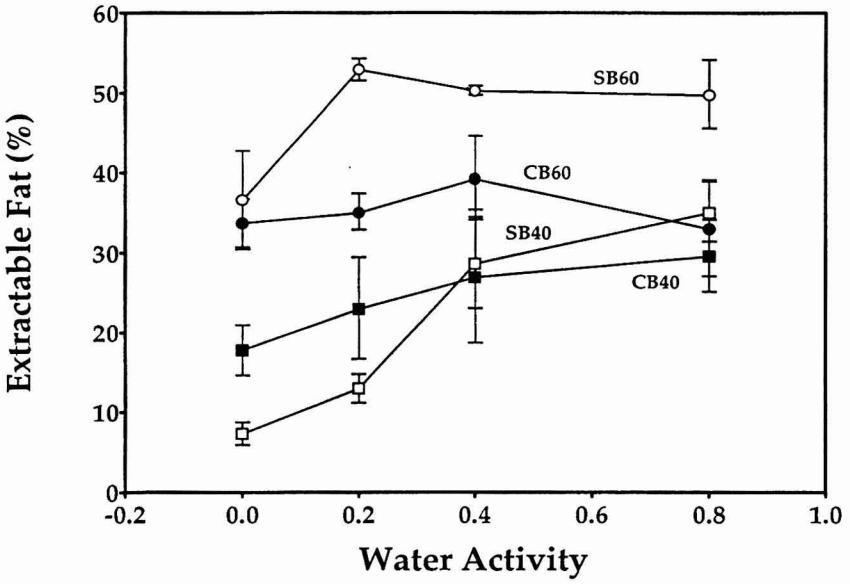

FIG. 7. INCREASE IN EXTRACTABLE FAT WITH INCREASING WATER ACTIVITY (A_w) OF POWDERS WITH 40 OR 60% FAT ENCAPSULATED IN SUCROSE OR MODIFIED STARCH (M-STARCH)
-□- = Sucrose 40 (SB40); -○- = Sucrose 60 (SB60); -■- = M-Starch 40 (CB40); -●- = M-Starch 60 (CB60).

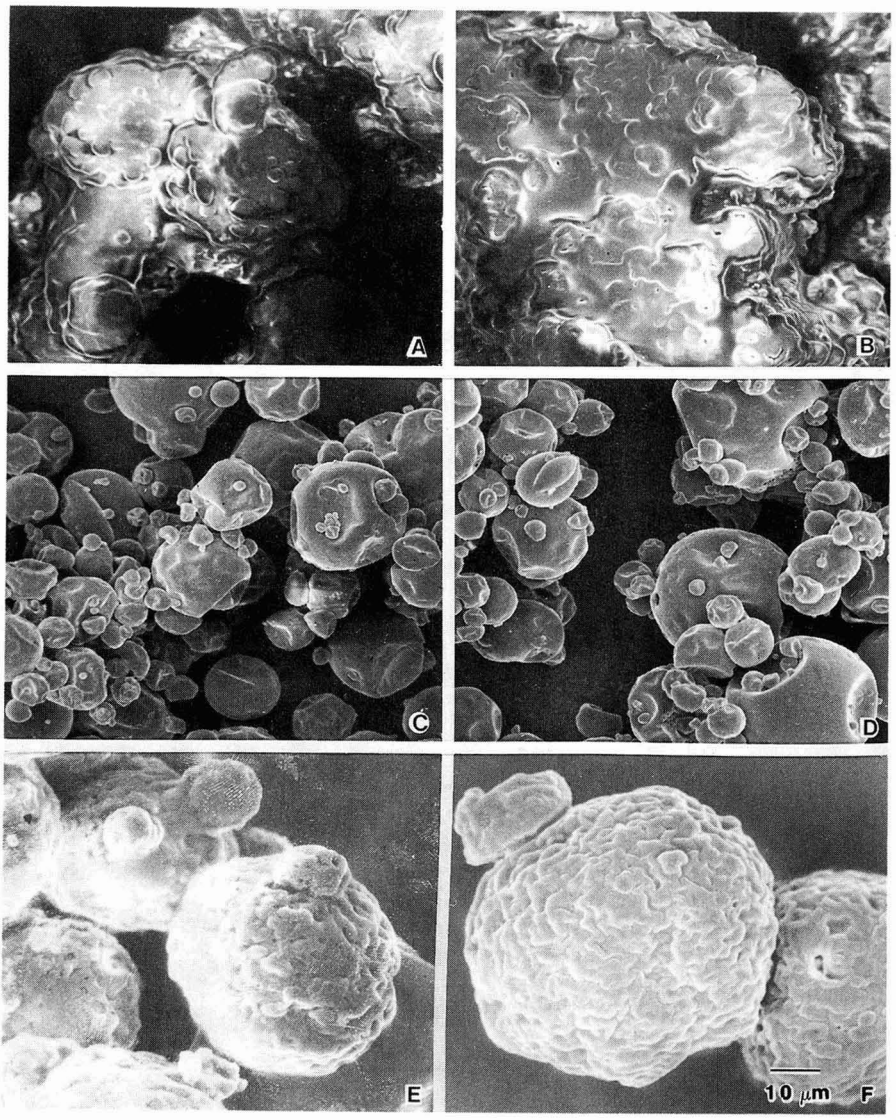

FIG. 8. SCANNING ELECTRON PHOTOMICROGRAPHS OF BUTTER OIL IN CARBOHYDRATE MATRICES (40% FAT)

Sucrose at 20% relative humidity (8A); sucrose at 80% relative humidity (8B); modified starch (M-Starch) at 20% relative humidity (8C); M-Starch at 80% relative humidity (8D); all-purpose flour (N-Starch) at 20% relative humidity (8E); and N-Starch at 80% relative humidity (8F).

Based on ^{13}C CPNMR analysis, the sucrose comprising the microcapsule wall appeared to retain some crystalline order. However, isotherms done at 25C

(Fig. 5 and 6) showed that sucrose was mostly amorphous, only fully crystallizing after exposure to moisture. As shown by the data in Fig. 7, sucrose crystallization had an undesirable effect on fat retention within the capsule.

Scanning electron photomicrographs of the microcapsules containing 40% butter oil at 20 and 80% relative humidity are shown in Fig. 8A-F. Powders with sucrose as the encapsulant (8A) have absorbed sufficient moisture, even at 20% relative humidity to begin to lose their particulate identity. At 80% relative humidity (8B), the particles have fused and caked as a result of the crystallization of the sucrose. In contrast, photomicrographs of M-starch/butter-oil powders show typical spray dried powder particles at 20% relative humidity (8C); upon moisture imbibition, at 80% relative humidity, the particles have swelled slightly but have not lost their structural identity and appear to be intact (8D). N-starch/butter-oil powders show distinct surface ridges at 20% relative humidity (8E), but at 80% relative humidity, the ridges are partially reduced in size and appear smooth (8F). DSC analysis of milkfat extracted from these capsules by treatment with carbon tetrachloride shows partial dissolution of the capsule wall as a result of exposure to high relative humidity through presence of the encapsulant in the thermograms (data not shown). Based on these results, special packaging will be required to ensure structural integrity of the microcapsules if long shelf-life at ambient temperatures is to be attained.

CONCLUSIONS

Spray dried encapsulated milkfat powder shows potential for use as a food ingredient in such products as dry bakery mixes. Well-defined melting ranges are identifiable in DSC profiles; melting temperatures for capsule rupture and release of the fat load are associated with type of encapsulating agent chosen. Low levels of extractable fat show protection of the encapsulated butter oil, when sucrose is the encapsulant, which is essential in maintaining milkfat quality. Moisture uptake and sorption isotherms are also related to encapsulant and demonstrate the need for special packaging to prevent moisture imbibition during storage. Ease of moisture uptake can be advantageous in a batter when dry mixes are reconstituted for baking.

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APPLICABILITY OF A SHEAR INDUCED RATE CONSTANT MODEL TO CONVERSION OF CORN MEAL IN A TWIN-SCREW EXTRUDER

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ABSTRACT

Data obtained from extruding corn meal in a corotating twin-screw extruder was used to test an Arrhenius type shear induced rate constant model, $k_s = k_{s,o} \exp(-E_s/\tau v)$, where k_s was shear induced rate constant, E_s was shear activation energy, τ was shear stress, and v was molar volume. A rheological zone was created by placing a reverse screw 300 mm from the die where the temperature was at 60C, sufficiently low that thermal effect on conversion of the material into gel or melt could be neglected. Throughput was used as an independent variable to generate various levels of shear input, while screw speed and feed moisture content were kept constant. Conversion of the material in the rheological zone was determined using differential scanning calorimetry, and the rate contant k_s was calculated assuming zero order kinetics. A plot of k_s vs. $1/\tau v$ followed a straight line, confirming the applicability of the model to twin-screw extrusion under the experimental conditions. The shear activation energy E_s was 40 cal/mole, comparable to the literature values obtained from single screw extrusion and capillary rheometry.

INTRODUCTION

The quality of extrudate depends on the various physicochemical changes occurring in extrusion. For example, during extrusion of corn meal, gelatiniza-

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tion and melting of starch are two important changes, along with complex interactions among starch, protein and lipid components. The extent of these changes is a function of order of reaction, rate constant, and reaction time. In extrusion, the rate constant depends on both shear and thermal energy inputs.

The order of reaction for starch and starch based products was studied by several investigators. Bhattacharya and Hanna (1987) showed that starch gelatinization in single screw extrusion followed pseudo-zero-order reaction kinetics at moisture contents between 18 and 42% (d.b.). The rate constant was higher for waxy corn than for high amylose corn, presumably due to the differences in structure. Wang *et al.* (1989) also showed that the conversion of amioca, a waxy corn starch, measured with DSC followed pseudo-zero-order. Burros *et al.* (1987) studied the kinetics of degerminated yellow corn meal in the moisture range of 13.4–34.4% using DSC, and they found that the reaction order for gelatinization and melting was between 0.5 and 0.8, the order decreasing with moisture content. In a critical review of starch gelatinization Lund (1984) suggested that gelatinization did not appear to be a first-order reaction, although it could be modeled as such over limited extents of gelatinization. At moisture contents above 90%, several authors showed that for starch based products such as rice and potato starches (Kubota 1979), parboiled rice (Bakshi and Singh 1980), and potato (Shiotsubu 1983), gelatinization followed first-order kinetics. Cai and Diosady (1993) suggested the use of two cooking zones to describe the kinetics of gelatinization of wheat starch in twin-screw extrusion: the first cooking zone followed pseudo-second-order and the second cooking zone followed pseudo-first-order. However, our experiments on shear conversion of starch in corn meal in a twin-screw extruder, showed zero-order kinetics in the transition zone and in the rheological zone created by reverse screw elements. In summary, the above findings suggest that starch gelatinization is not an elementary reaction, and the order of reaction is affected by material composition, structure, temperature-shear history, equipment design, operating conditions, etc.

Researchers have used various kinetic models to account for the shear effect in extrusion process. For example, Davidson *et al.* (1984) and Diosady *et al.* (1985) correlated undegraded starch remaining in the material (X) to the mean shear stress (τ) and mean residence time (t) of wheat starch in the active volume of a single screw extruder according to the equation

$$\ln X = -k'(\tau t) + b \qquad (1)$$

where k′ was modified rate constant and b was constant. The active volume was defined as the barrel volume between the beginning of the transition zone and the barrel end, where the shear effect was high. In their experiments, temperatures ranged from 80 to 120C and moisture contents from 20 to 30%. Cai and

Diosady (1993) represented the rate constant for gelatinization of wheat starch k_g in twin-screw extrusion as

$$k_g = k_o \exp[-(\Delta E_o - \beta \tau)/RT] \quad (2)$$

where k_o was preexponential component, ΔE_o was bond energy, β was active volume, τ was shear stress, R was the gas constant, and T was temperature. Equation (2) suggests that k_g decreases with temperature, if $(\Delta E_o - \beta\tau)$ is positive. This equation gives a fairly good fit to gelatinization data obtained from extruding wheat starch with barrel temperature from 100 to 160C.

Another approach to modeling the rate constant of starch conversion was discussed by Wang *et al.* (1992) using an 'energy equivalent concept'. Assuming thermal and shear energy are thermodynamically additive and interactive, the authors proposed that the rate constant could be described as

$$k = k_T + k_s + (k_T.k_s)^{0.5} \quad (3)$$

where k_T was thermally induced rate constant, and k_s was shear induced rate constant. This decoupling of the rate constant seemed to provide more insight into the physicochemical changes which took place due to the individual effects of thermal and shear energy in an extruder. An Arrhenius type of relationship was used to represent these rate constants as

$$k_T = k_{T,o} \exp(-E_T/RT) \quad (4)$$

and

$$k_s = k_{s,o} \exp(-E_s/\tau\upsilon) \quad (5)$$

where E_T was thermal activation energy, E_s was shear activation energy, τ was shear stress, υ was molar volume of anhydroglucose, and $k_{T,o}$ and $k_{s,o}$ were preexponential coefficients. The term $\tau\upsilon$ was interpreted as a characteristic energy, and the uniqueness of this approach was its attempt to separate the thermal and the shear effects. At low temperatures (below 75C) when thermal effect was negligible (Xiaoge 1992), Eq. (3) was reduced to

$$k \approx k_s \quad (6)$$

It has been argued that shear effect is a major cause for conversion in extrusion at low temperatures. The activation energy required for shear induced reactions was relatively low (Heinicke 1984; Xiaoge 1992). During cold extrusion of corn starch with single screw, shear activation energies were shown to be about 3 orders of magnitude smaller than thermal activation energies

(Wang *et al.* 1992). Shear energy alone could cause conversion of corn meal at low temperatures (Yam *et al.* 1993).

The thermally induced rate constant model Eq. (4) is commonly accepted for a wide range of temperatures in many processes. However, the shear induced rate constant model (Eq. 5) has been proven only in the following cases. Wang *et al.* (1992) showed that it was applicable to single screw extrusion at 70 – 100C and capillary rheometry at 21–50C. Basedow *et al.* (1979) also showed that a similar model was applicable to shear degradation of polyacrylamide in a high-shear rotational viscometer at 40C. Since the applicability of Eq. (5) in a twin-screw extrusion remained unknown, this work was aimed at testing its general applicability of in twin-screw extrusion of corn meal, which is widely used in the manufacture of breakfast cereals.

MATERIALS AND METHODS

A Werner & Pfleiderer ZSK-30 corotating twin-screw extruder with L/D ratio of 28.6 and screw length of 878 mm was used in this study. The screw configuration was assembled with segmental forwarding screw elements, two mild mixing elements, and a reverse screw element. The feed material was degerminated yellow corn meal (supplied by Lauhoff Grain Co., Danville, IL) consisting of 80% starch, 8% protein, 10.5% moisture, and the balance 1.5% being lipids, fiber and ash.

Yam *et al.* (1993) have recently demonstrated that placing reverse screws in an extruder could create rheological zones where significant conversion of corn meal occurred. In the present study, a 10 mm long half-pitch reverse screw was placed at 300 mm from the die to create a rheological zone or reaction zone as shown in Fig. 1. Directly above the reverse screw was a removable 160 mm × 60 mm vent port, and a temperature-pressure sensor that was connected to a data acquisition system.

Throughput or mass flow rate, ranging from 150 to 200 g/min, was used as an independent variable to generate various levels of shear rate. To keep temperature low, most experiments were conducted without external heat input and with cooling water circulating through the barrel. Under these conditions the temperature in the rheological zone could be controlled at about 60C, which is sufficiently low that thermal effect on starch conversion was negligible (Wang *et al.* 1992). However, slight adjustments were sometimes required: the steady state temperatures in the rheological zone at throughputs of 150 and 170 g/min were slightly below 60C, and it was necessary to raise the barrel temperature by 1C to achieve the target temperature of 60C. The steady temperatures at 180 and 200 g/min were close to 60C, and no adjustment of barrel temperature was needed.

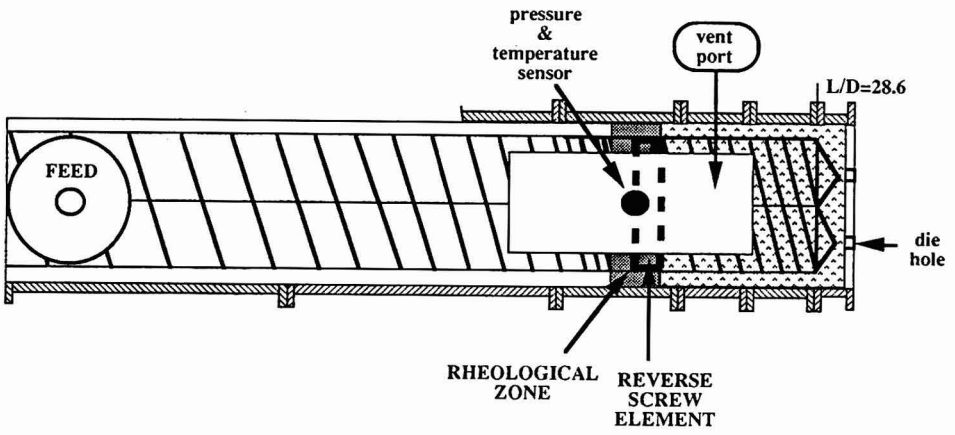

FIG. 1. SET UP OF EXTRUDER SHOWING LOCATION OF VENT PORT ABOVE REVERSE SCREW ELEMENT

After the rheological zone reached 60C for at least 3 min, the feeder and the extruder were dead stopped, and cold water was sprayed immediately over the barrel. The vent port was removed within 3 min. Samples in the rheological zone were taken through the vent port for conversion measurement using differential scanning calorimetry. The degree of conversion C in corn meal was defined as (Yam *et al.* 1993):

$$C = 1 - \frac{\Delta H}{\Delta H_o} \tag{7}$$

where ΔH was enthalpy of extrudate and ΔH_o was enthalpy of raw material. The heating rate was 5C/min, and the scan range was from 40 to 100C.

RESULTS AND DISCUSSIONS

Material Phases (Zones) Created Exclusively by Reverse Screw Element

Figure 2 shows the four distinct zones that were observed inside the extruder after the removal of the vent port. These zones were identified by

visual examination (based on degree of fill, color, connected/powdery state), by physical examination (piercing the sample with a sharp knife), and by measurement of degree of starch conversion. Zone 1 was partially filled and consisted of unconverted powdery material. Zone 2 was also partially filled and consisted of semi-connected (about 10–20%) material with yellowish glaze, quite different from the dull yellow of raw corn meal. This zone was identified as a transition zone where low degree of conversion occurred. Zone 3 was completely filled and consisted of a hard, yellow solidified shiny mass. This zone was identified as a rheological zone where significant conversion occurred. Zone 4 was partially filled and consisted of patches of connected material.

Estimation of k_T

To justify the assumption that thermal effect was negligible, the following empirical correlation of Wang *et al.* (1989) was used to estimate the thermally induced rate constant for starch conversion at 60C

$$k_T = \frac{x}{74.97x^2 - 167.21x + 93.38} \tag{8}$$

where x was defined as T/T_p, T was operating temperature, and T_p was peak temperature measured in DSC endotherm. Equation (9) was derived from kinetic data of waxy corn starch (Wang *et al.* 1989), rice starch (Lund *et al.* 1984) and potato starch (Pravisani *et al.* 1985), with the assumption of zero-order kinetics. The equation was valid for $0.63 < T/T_p < 1.06$. For raw corn meal at 20% moisture content, the measured value for T_p was 151.6C, and thus the equation was applicable for temperatures between $95C < T < 160C$. The estimated k_T from the equation at 95C was 0.035 per min; the k_T at 60C was expected to be smaller. Conversion at 60C in the rheological zone was estimated to be less than 0.5%, sufficiently small to be neglected.

Model Testing

The validity of Eq. (5) in the rheological zone was determined by plotting $\ln k_s$ vs. $1/\tau v$. k_s was obtained, based on zero-order kinetics, using the equation

$$k_s = \frac{C_r - C_f}{t_r} \tag{9}$$

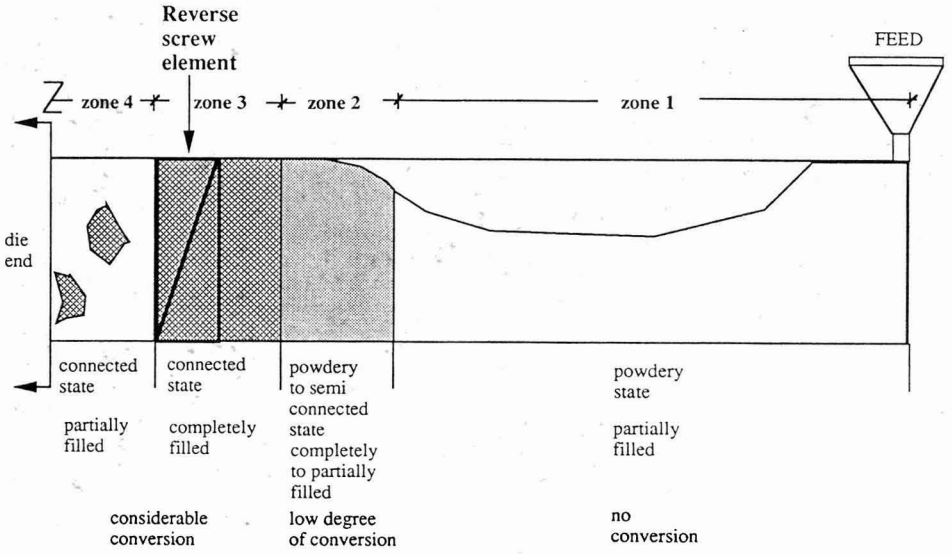

FIG. 2. FUNCTIONAL ZONES ACROSS REVERSE SCREW ELEMENTS DURING TWIN-SCREW EXTRUSION

where C_r was average degree of conversion at the middle of the reverse screw, and C_f was average degree of conversion at the middle of the adjacent forwarding screw (see Zone 3 in Fig. 2). t_r was average reaction time estimated using

$$t_r = V/Q \tag{10}$$

where V was reaction volume, and Q was volumetric flow rate. Although only two points were used to estimate k_s in Eq. (9), duplicate or triplicate runs were conducted at each throughput.

The shear stress τ in the rheological zone was estimated using the equation of Yaku (1984)

$$\dot{\gamma} = \frac{Q\ D\ (1 - m_1 B\ G/\pi D h)}{h^4\ \tan\theta} \tag{11}$$

where D was screw diameter, m_1 was number of cross channels drilled in the reverse screw, B was width of drilled cross channel, G was depth of drilled

cross channel, h was screw channel depth, and θ was helix angle of reverse screw. Since the reverse screw used in the study had no drilled slot, Eq. (11) was simplified as

$$\dot{\gamma} = \frac{Q\ D}{h^4\ \tan\theta} \tag{12}$$

The viscosity was estimated using the equation of Kokini *et al.* (1989)

$$\eta = 0.399\dot{\gamma}^{-0.6872}\ \exp(5152.8/T)\exp(-0.1464M) \tag{13}$$

where η was viscosity, T was absolute temperature, and M was moisture content. In the rheological zone where temperature was at 333 K (60C) and moisture content was at 20%, Eq. (13) became

$$\eta = 112093\dot{\gamma}^{-0.6872} \tag{14}$$

The molar volume of starch (Xiaoge 1992) in corn meal was expressed as

$$\nu = \frac{162}{1.4A_s + A_w} \tag{15}$$

where A_s and A_w were percentage of starch and water, respectively. ν was 123 cc/mole for corn meal with 80% starch and 20% moisture content. The shear stress was obtained according to

$$\tau = \eta\ \dot{\gamma} \tag{16}$$

The results obtained from the above equations are presented in Table 1. Pressure, average shear rate, average shear stress, and k_s in the rheological zone increased with throughput. Conversely, viscosity, degree of conversion, and reaction time decreased with increasing throughput. Figure 3 is a plot of ln k vs. $1/\tau\nu$; the coefficient of determination r^2 is 0.91. The linearity of the plot supports the proposal that the shear induced rate constant model is applicable to twin-screw extrusion. The preexponential rate constant $k_{s,o}$ was estimated to be 17.3 min^{-1}. The shear activation energy E_s was estimated to 40 cal/mole, which was comparable to the values obtained from single screw extrusion (10–100 cal/mole) and capillary rheometry (60–290 cal/mole) reported by Xiagoe (1992).

TABLE 1.
SUMMARY DATA ON SHEAR INDUCED KINETIC STUDIES

Through put (g/min)	Pressure over RSE (psi)	Average shear rate (s^{-1})	Viscosity (poise)	Av. Shear Stress (dynes/cm^2 x 10^7)	Conversion in rheological zone	time in rheological zone (min)	ks (min^{-1})
150	250	541	1483	0.080	0.09	0.054	1.68
150	285	541	1483	0.080	0.09	0.054	1.68
170	300	614	1361	0.084	0.11	0.048	2.32
180	300	650	1301	0.085	0.19	0.045	2.72
180	350	650	1301	0.085	0.21	0.045	4.94
200	340	722	1217	0.089	0.21	0.040	5.25
200	350	722	1216.8	0.089	0.32	0.040	8.00
200	370	722	1217	0.089	0.32	0.040	8.00

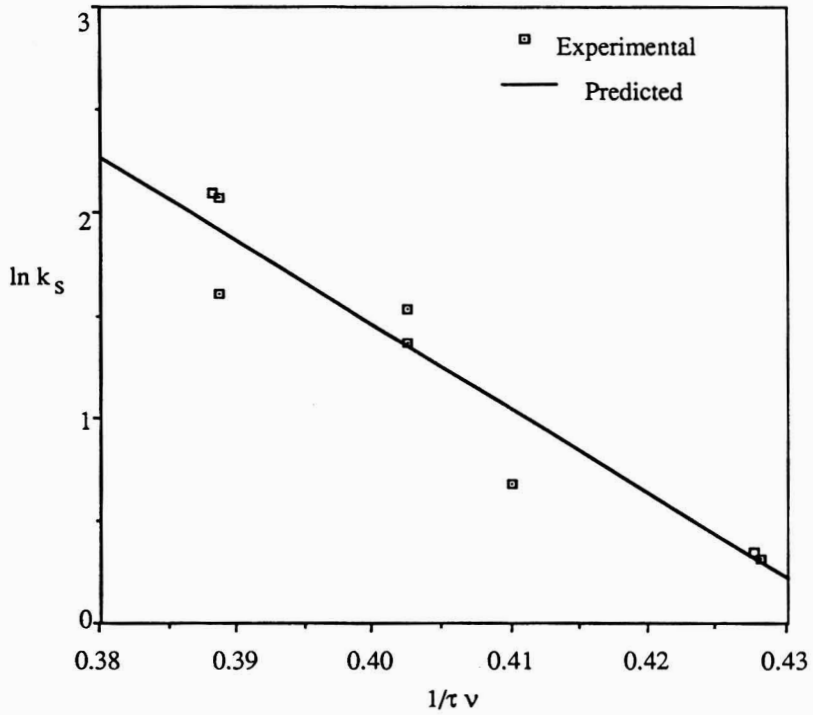

FIG. 3. PREDICTED VS. EXPERIMENTAL SHEAR INDUCED RATE CONSTANTS IN RHEOLOGICAL ZONE DUE TO A REVERSE SCREW ELEMENT

CONCLUSION

The results of this study support the proposal that the shear induced rate constant model, $k_s = k_{s,o} \exp(-E_s/\tau v)$, is applicable to twin-screw extrusion. Thus the model could be used as a design equation to relate the shear induced rate constant with shear stress. Restrictions in an extruder, such as reverse screws, could be used as design tools to impart various levels of shear inputs.

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EFFECT OF PROCESSING CONDITIONS ON THE PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF STANLEY PLUM PASTE

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ABSTRACT

Stanley plums were processed into pastes by heat concentration to 25 and 30°Brix. The soluble solids of these two pastes were increased to 40 and 45°Brix, respectively, by sugar addition. The influence of processing techniques on the physicochemical and sensory characteristics of pastes was evaluated. Heat concentration resulted in a significant decrease in titratable acidity, total anthocyanins and total pectin. Pastes showed pseudoplastic behavior within the shear rate range of 20 to 100 rpm. Sugar addition had darkening effect on color, but no noticeable effect on rheological properties of the pastes. It was not feasible to concentrate plum pastes beyond 40°Brix. Paste pH was not affected by processing conditions. Sensory evaluation indicated that preference could be adequately predicted by flavor and color under the condition that °Brix/acid ratio was suitable.

INTRODUCTION

Plums can be an important fruit crop in Michigan, but in order to increase the economic significance of this commodity there is a need for development of new products from plums. Stanley plums are the most abundant plum cultivar in Michigan and its importance to the industry is derived from the fact that, (1) timing of harvest falls between two major fruit crops, cherries and apples, thus optimizing available labor and equipment, and (2) the Stanley cultivar is especially suited to Michigan climate (Elliott 1983). The development of a strong market for Michigan plums is particularly relevant for the state's fruit

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growers because it is one of their most important minor crops. A considerable amount of research has already been done in our group to develop plum juice from Stanley, as well as from several other plum cultivars (Arnold *et al.* 1992; Siddiq *et al.* 1992, 1994; Chang *et al.* 1994). In addition to juice, the apparent success of several recently developed new products in the U.S. which use fruit pastes indicates that plum paste might find very good acceptance in the marketplace. Such a product could utilize fairly large quantities of fruit and give a "value added" product, which would increase use of processed plums. Therefore, the objectives of this study were to (1) develop a procedure to produce pastes from Stanley plums and, (2) determine the effect of processing conditions on the chemical, physical and sensory characteristics of plum pastes.

MATERIALS AND METHODS

Stanley plums, grown in northern Michigan, were frozen to −20C immediately after harvest and kept at this temperature until further processing was required.

Plum Paste Production

Frozen plums were allowed to thaw overnight at 4C. After washing with tap water plums were heated to 95C and macerated for 10 min in double jacketed stainless steel kettles. The macerate was then slightly cooled and the pits removed by passing through a stainless steel screen. The pitted macerate was passed through a finisher (Dodge, Mishawaka, IN) equipped with 0.060 in. screen. The plum puree was stored at −20C until further processing. For paste production, puree was concentrated to 25° or 30°Brix in a steam jacketed kettle at 85–90C with thorough stirring. One batch of each °Brix was filled hot (85C) into 12-oz jars. The other batch had sucrose added to give a 15°Brix increase. The paste with added sugar was mixed well, heated, and hot filled into glass jars. The jars were sealed immediately, cooled to room temperature, and then stored at 4C in dark.

Quality Evaluation of Plum Paste

The plum pastes were evaluated for various quality indices. All measurements were made in duplicate unless stated otherwise.

Soluble Solids and Total Solids

Percent soluble solids of plum paste were measured using an Abbe-3L (Bausch & Lomb Optical Co., Rochester, NY) refractometer. The results are expressed as °Brix at 20C. For total solids, 6 g of paste were weighed into aluminum weighing dishes which had been dried in a vacuum oven (Hotpack, Philadelphia, PA) for 1 h at 100C under a pressure of 27" Hg. Drying of samples was done in the vacuum oven for 8 h at 100C under 27" Hg. The dried samples were allowed to cool in a desiccator for 30 min and then weighed. The percent total solids were calculated using the formula: % Total Solids = (dried sample weight/fresh sample weight) × 100.

Moisture Content and Water Activity

The moisture content of plum paste was calculated from percent total solids using the formula: % moisture content = 100 − % total solids. Water activity of paste (2 g) was measured using a water activity system (Decagon Devices, Inc., Pullman, WA).

pH and Titratable Acidity (TA)

Paste samples, 5g each, were diluted with 45 ml distilled water and pH measured with a pH meter (Model 601A, Corning Glass Works, Medfield, MA). For titratable acidity, 5 g of paste were mixed with 95 ml distilled water and titrated to pH 8.1 with 0. lN NaOH. Results are expressed as percent malic acid by weight.

Total Pectin and Protopectin

Extraction of pectin was done according to the method of McCready and McComb (1952). The colorimetric measurement of galacturonic acid was done using the method of Kintner and Van Buren (1982). The concentration of pectin was calculated from the standard curve of galacturonic acid. Procedure of Kanujoso and Luh (1967) was used to determine protopectin concentration.

Total Anthocyanins

Total anthocyanins were extracted according to the method of Skalski and Sistrunk (1973) and Cash *et al.* (1976) with a slight modification based on the

method of Lees and Francis (1971). A 10-g portion of paste was mixed with 20 ml of 0.1N HCl buffer. The mixture was blended in a Waring Blendor for 1 min with 100 ml of acidified ethanol solution (95% ethanol: 1.5N HCl = 85: 15, v/v). The extraction solution was made up to 200 ml and kept in the dark for 2 h. The mixture was centrifuged under refrigeration at 9000 × g for 10 min and absorbance was read at 535 nm using a spectrophotometer (Milton Roy Spectronic-70, Rochester, NY). Total anthocyanins were calculated using the formula: Total Anthocyanin (mg/100g) = [(absorbance × dilution factor)/E] × (100g/10g). The factor E (extinction coefficient) of 98.2 was used for unidentified anthocyanins in acidified ethanol (Fuleki and Francis 1968).

Polyphenol Oxidase (PPO) Activity

The method of Cash *et al.* (1976) modified by Siddiq *et al.* (1992) was used for extraction and assay of PPO. All extraction materials were maintained at low temperature (2–5C) to reduce enzymatic activity during extraction and assay.

Hunter Color

Color of triplicate samples of paste was measured by Hunter Color Difference Meter (D25 DP-9000 system, Hunter Associates Laboratory, Reston, VA). About 200 g of paste were placed in a standard optical cell for the measurement after standardization with a pink tile (L* = 73.49; a* = 17.34; b* = 10.28).

Rheological Properties

Rheological properties (apparent viscosity, flow behavior index and consistency index) were determined according to the method of Castell-Perez (1990). A Brookfield HBTD viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) connected with a data acquisition system (Dianachart PC-Acquisition Model PCA-14, Dianachart Inc., Rockaway, NJ) was used to measure the rheological properties of plum pastes.

Paste samples were loaded into a cylindrical cup, with flat bottom, made of stainless steel with fluid jackets for temperature control. The sample temperature was maintained at 30C. Inside diameter (D) was 2.54 cm (d/D = 0.59) and cup height was 4.0 cm. Fluid level was kept at 1.2D and impeller depth (distance from the bottom of impeller to the bottom of the cup) set at 0.5d

to allow complete immersion of the flag impeller (Fig. 1). After equilibration of temperature at 30C, the flag impeller was immersed and readings at a selected rotational speed were recorded after steady state was reached. For any given run, the rotational speed varied (a stepwise increase) from 20 to 100 rpm.

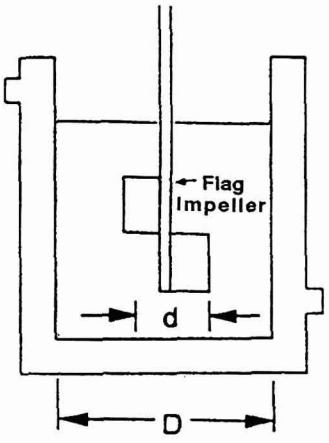

FIG 1. MIXER VISCOMETER APPARATUS WITH FLAG TYPE IMPELLER

Sensory Analysis

The sensory analysis of plum paste was carried out using the descriptive test with unstructured scaling (Poste *et al.* 1991). Samples were tested by a panel of 60 judges (including faculty, staff, and students in the Department of Food Science and Human Nutrition) for color, acidity, sweetness, consistency, flavor, and overall acceptance. All sensory tests were held in the sensory evaluation laboratory of the Department of Food Science and Human Nutrition at Michigan State University under cool white fluorescent lighting. Panelists were asked to evaluate the first 5 descriptors by intensity only and the last one by personal preference. A 15-cm horizontal line was used for each sensory attribute to be evaluated. Panelists were asked to record each evaluation by marking the horizontal line at the point that best reflected their perception of the magnitude of that property. At each sensory test about 15 g paste samples were evaluated at room temperature.

Microbial Content

Samples were tested for total (standard) plate count (SPC), coliform counts, and yeast and mold counts using the methods of Pestka (1993).

Statistical Analysis

The experiment was designed as a three factor (replication × heat concentration × sugar addition) randomized model with balanced data. Mean, standard deviation, standard errors, ANOVA tables, and correlations were determined using the Super ANOVA software (Abacus Concepts, Inc., Berkeley, CA), Lotus software, and SAS software. LSD test for multiple comparisons was applied to determine significantly different treatment effects.

RESULTS AND DISCUSSION

Paste Production

The yield of puree from plums was 71.25% by weight. The yields of pastes were 59.62% for 25°Brix and 50.23% for 30°Brix, based on puree weight. The corresponding yields of pastes were 42.47% and 35.78%, based on fruit weight. Our preliminary experiment showed that it was not feasible to concentrate plum pastes beyond 40°Brix. However, Wani *et al.* (1990) reported that the pulp from 3 plum varieties could not be concentrated even beyond 26°Brix. It was found that plum paste with 25–30°Brix had most attractive color and best consistency. Thus, 25 and 30°Brix pastes became the standards for all subsequent work. Due to the high acidity of paste, it was necessary to add sweetener to ameliorate the flavor. Pastes sweetened with sugar addition to increase 5, 10, 15 and 20°Brix were evaluated. Preliminary sensory tests showed that sweetened pastes with sugar addition to increase 15°Brix were best accepted. Therefore, unsweetened paste and pastes sweetened to give a 15°Brix increase were used to study the effect of sugar addition on paste characteristics.

Soluble Solids and Total Solids

During the manufacturing process, it is important to know the degree of concentration of puree or paste for the quality control (Goose and Binsted 1964). Soluble solids and percent total solids were increased by heat evaporation, which removed water from puree and sugar addition, which directly increased the soluble solids (Table 1). The total solids increased significantly ($p \leq 0.05$) with an increase in soluble solids. Time required for heat concentration of puree to 25 and 30°Brix, at 100C, was approximately 30 and 45 min, respectively.

Moisture Content and Water Activity (A_w)

Moisture content and A_w loss were inversely ($p \leq 0.05$) related to the increase in soluble solids due to heat evaporation and sugar addition (Table 1). After heat evaporation, plum pastes still had relatively high A_w, 0.978 and 0.973 for 25 and 30°Brix pastes. The addition of sugar to food products has been applied to enhance taste and prevent microbial growth by reducing water activity (Jay 1992). Plum pastes with added sugar had an A_w of 0.949 and 0.931 for 40 and 45°Brix pastes, respectively. The A_w decreased by an average of 0.35 due to sugar addition. Although in the A_w range of 0.978–0.931 some spoilage bacteria can grow, the low pH of plum pastes (3.45–3.50) was considerably below the minima for most food spoilage and all food poisoning bacteria (Jay 1992).

pH and Titratable Acidity (TA)

The pH values of pastes were similar regardless of the process employed for production (Table 1), although minor changes in pH during concentration process have been reported in literature (Wani *et al.* 1990; Exama and Lacroix 1989). Bash *et al.* (1984) reported that sugar addition had little effect on pH of tomato paste. TA of sweetened pastes decreased significantly ($p \leq 0.05$) due to dilution effect. Similar results have been reported for tomato paste (Bash *et al.* 1984). The decrease in TA appeared to be a function of heating time.

Total Pectin and Protopectin Content

Heat process resulted in a significant degradation ($p \leq 0.05$) of total pectin (Table 1). Luh *et al.* (1954) stated that marked losses in pectic substances occurred when tomato pastes were processed. Sawayame and Kawabata (1989) studied the effect of pH and heat on the physicochemical properties of pectic substances and reported that heating induced acid-hydrolysis of pectin in the pH range of 2–5 results in lower molecular size distribution of pectin. No protopectin was detected in either paste. It is possible that heating caused depolymerization to smaller segments and then decomposition.

Total Anthocyanins (T ACYs)

Heat concentrated 30°Brix paste had lower T ACYs as compared to 25°Brix paste (Table 1). This was most likely due to the heat sensitivity of the

anthocyanin pigments, as Siddiq *et al.* (1994) reported that plum juice lost 16% of T ACYs after heat treatment at 65C for 70 min. Significant decrease ($p \leq 0.05$) in T ACYs of samples with sugar added was due to the dilution effect of sugar. For plum pastes at pH 3.45–3.50, the anthocyanins were in the form of AH+ and represent red color (Brouillard and Delaporte 1977).

TABLE 1.
EFFECT OF PROCESSING CONDITIONS ON SOME PHYSICOCHEMICAL PROPERTIES OF PLUM PASTES

Treatment / Characteristics	Puree	Heat concentrated to 25°Brix	Heat concentrated to 30°Brix	25°Brix Sugar added to 40°Brix	30°Brix Sugar added to 45°Brix
Soluble solids (°B)	16.42[a1]	24.50[b]	29.69[b]	39.55[c]	44.95[c]
Total solids (%)	18.08[a]	25.73[b]	30.69[b]	40.70[c]	46.06[c]
Moisture content (%)	81.92[c]	74.27[b]	69.31[b]	59.25[a]	53.94[a]
Water activity (a_w)	0.998[c]	0.978[b]	0.973[b]	0.949[a]	0.931[a]
pH	3.45[a]	3.45[a]	3.45[a]	3.45[a]	3.45[a]
Titratable acidity[2]					
(Wet basis)	1.357[a]	1.873[c]	2.230[c]	1.621[b]	1.837[b]
(Dry basis)	7.506[c]	7.278[b]	7.266[b]	3.979[a]	3.989[a]
Total Pectin[3]					
Wet basis	0.722[a]	0.982[c]	1.111[c]	0.811[b]	0.838[b]
Dry basis	3.996[c]	3.816[b]	3.622[b]	1.990[a]	1.820[a]
Protopectin[3]					
Wet basis	0.317	nd[4]	nd	nd	nd
Dry basis	1.754	nd	nd	nd	nd
Total anthocyanins[5]					
Wet basis	18.46[b]	20.12[c]	17.60[b]	15.35[a]	14.13[a]
Dry basis	102.11[c]	78.21[c]	57.35[b]	37.68[a]	30.69[a]

[1] Values with the same letters in the same soluble solids (°Brix) in horizontal rows are not significantly different at 5% level of significance
[2] Calculated as % malic acid
[3] g/100g
[4] Not detected
[5] mg/100g

Polyphenol Oxidase (PPO) Activity

No PPO activity was detected in any of the paste samples, which indicated that the possibility of enzymatic browning in these products was negligible. However, Wesche-Ebeling and Montgomery (1990) indicated that even when enzyme activity is inhibited, the quinones and intermediate oxidation products formed before the enzyme was inactivated could be sufficient to initiate polymerization reactions and degradation of anthocyanins. The enzymatic reaction could occur during thawing of the frozen fruit, as well as during preheating before the inactivation temperature was reached. Enzymes derived from mold contamination (Pilando *et al.* 1985) can also be as detrimental as native fruit enzymes. Minimizing mold contamination, enzyme inactivation, rapid thawing and preheating procedures are recommended to reduce browning reaction.

Hunter Color

Hunter L* values were significantly ($p \leq 0.05$) decreased by heat concentration for all pastes (Table 2). Browning as a result of heat processing has been observed previously (Friedman and Molnar-Perl 1990; Velisek *et al.* 1989). The addition of sugar also darkened the color of pastes significantly ($p \leq 0.05$). According to Leszkowiat *et al.* (1990), sucrose contributed to the nonenzymatic browning by thermal hydrolysis to yield glucose and fructose, which are the main reactants in the Maillard reaction. All paste samples had positive Hunter a* values which were decreased significantly ($p \leq 0.05$) by heat concentration and sugar addition (Table 2). Hunter b* values, which indicate yellow when positive and blue when negative, were significantly decreased ($p \leq 0.05$) by processing, as shown in Table 2. The positive values for both Hunter a* and b* indicated that color of plum puree and pastes were red-yellow.

Hue angles of all paste samples increased slightly due to heat concentration or sugar addition (Table 2) but the change was not significant ($p \leq 0.05$). Hue angle is the attribute of color perception by which an object is judged to be red, yellow, green, blue, etc. (Anon. 1987). The smaller the hue angle, the more red than yellow the object is. Chroma (saturation index) determines how far the color is from the gray toward the pure hue (Anon. 1987). Significant decrease ($p \leq 0.05$) of chroma due to heat concentration and sugar addition was detected in all paste samples.

Rheological Properties

The response of decreasing shear stress to time at constant shear rate indicated that plum paste exhibited short time-dependent thixotropic behavior

TABLE 2.
EFFECT OF PROCESSING CONDITIONS ON HUNTER CDM[1] VALUES OF PLUM PASTES

Treatment / Characteristics	Puree	Heat concentrated to 25°Brix	Heat concentrated to 30°Brix	25°Brix Sugar added to 40°Brix	30°Brix Sugar added to 45°Brix
Hunter L*	21.92[c2]	17.24[b]	16.36[b]	14.46[a]	13.49[a]
Δ L*	--	-4.68[b]	-5.56[b]	-2.78[a]	-2.87[a]
Hunter a*	39.23[c]	27.73[b]	24.05[b]	22.91[a]	19.40[a]
Hunter b*	12.61[c]	7.99[b]	7.03[b]	6.63[a]	5.70[a]
Hue angle[3]	0.311[b]	0.281[a]	0.284[a]	0.282[a]	0.286[a]
Chroma[4]	41.21[c]	28.86[b]	25.06[b]	23.85[a]	20.22[a]

1 Color difference measurement
2 Values with the same letters in the same soluble solids (°Brix) in horizontal rows are not significantly different at 5% level of significance
3 Calculated as $\tan^{-1}(b/a)$
4 Calculated as $(a^2+b^2)^{1/2}$

(Fig. 2). However, after about 5 min plum paste exhibited time-independent behavior. Rao and Anantheswaran (1982) showed that the time span related to this time-dependent behavior was relatively short, and because of various mechanical operations in a processing line, the behavior will not persist for long. Shear stresses required to induce a given rate of shear on pastes are shown in Fig. 3. Shear rates in the order of 20 to 100 rpm with increasing heat concentration generally resulted in increased resistance to flow, but sugar addition resulted in a slightly lower resistance flow. The apparent viscosity (i.e., shear stress/shear rate) at different rpm showed similar tendency (Fig. 4), therefore, the apparent viscosity at 50 rpm was used to illustrate the viscosity of paste.

The apparent viscosity (n_a), consistency index (K) of plum paste increased significantly ($p \leq 0.05$) with an increase in heat concentration (Table 3). Sugar addition showed little effect on rheological behavior of the paste. These results are similar to those reported previously (Canellas *et al.* 1993; Exama and Lacroix 1989). Values obtained for the flow behavior index (n) of the plum pastes ranged from 0.367 to 0.406, confirming the pseudoplastic nature of plum pastes.

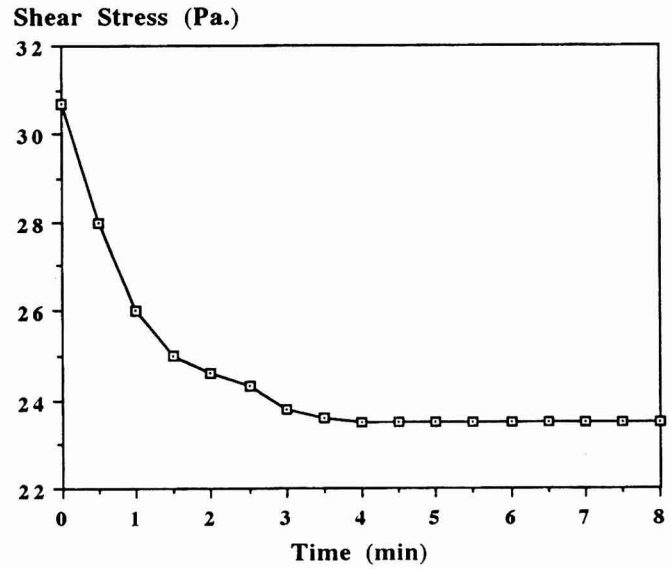

FIG. 2. RHEOGRAM OF PLUM PASTE AT CONSTANT SHEAR RATE (20 RPM) AT 30C

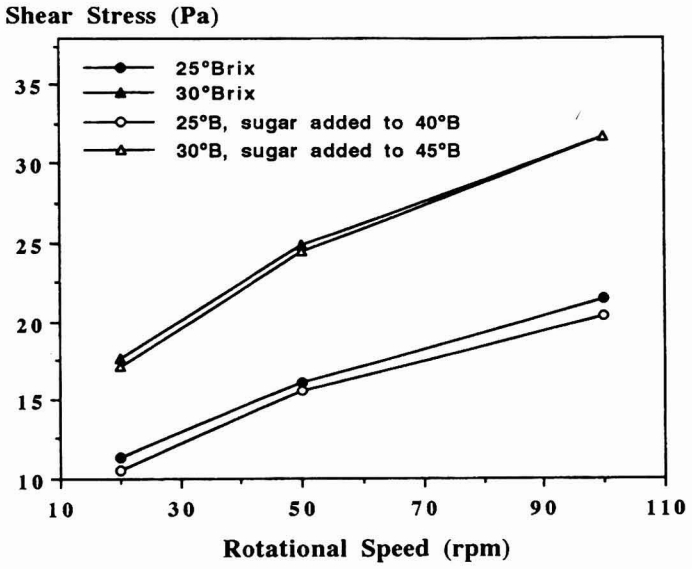

FIG. 3. SHEAR STRESS OF PLUM PASTE AS A FUNCTION OF ROTATIONAL SPEED AT 30C
(Rotational speed directly proportional to shear rate.)

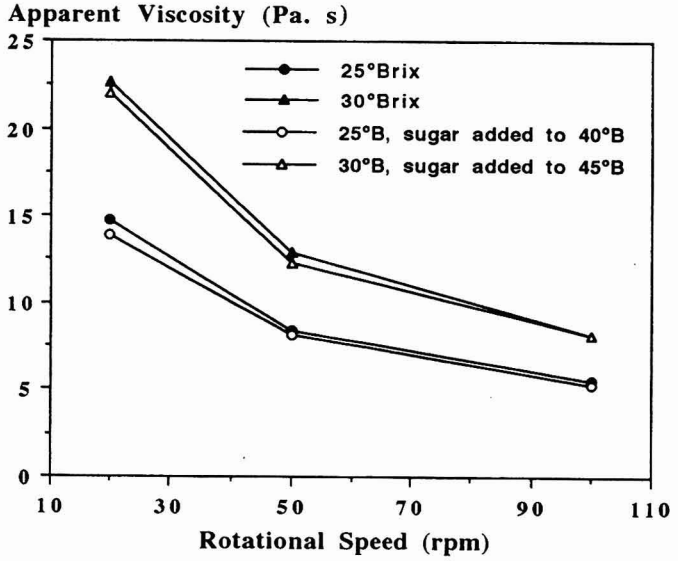

FIG. 4. APPARENT VISCOSITY OF PLUM PASTE AT DIFFERENT SHEAR RATES AT 30C
(Rotational speed directly proportional to shear rate)

TABLE 3.
EFFECT OF PROCESSING CONDITIONS ON RHEOLOGICAL PROPERTIES OF PLUM PASTES

Treatment / Characteristics	Puree	Heat concentrated to 25°Brix	Heat concentrated to 30°Brix	25°Brix Sugar added to 40°Brix	30°Brix Sugar added to 45°Brix
Apparent viscosity (n_a)[1]	4.05[a2]	8.36[b]	12.86[b]	8.08[b]	12.64[b]
Consistency index (K)[3]	20.87[a]	34.66[b]	56.46[b]	32.29[b]	54.03[b]
Flow behavior index (n)	0.306[a]	0.397[b]	0.367[b]	0.406[b]	0.381[b]

1 Unit is Pa. s
2 Values with the same letters in the same soluble solids (°Brix) in horizontal rows are not significantly different at 5% level of significance
3 Unit is N. s^n/m^2

Sensory Evaluation

Mean values of the sensory scores for color, acidity, sweetness, consistency, flavor and overall acceptance are shown in Table 4, and P values of analysis of

variance are shown in Table 5. Heat processed 30°Brix paste was darker than 25°Brix paste as perceived by the sensory panelists who also found that sugar addition darkened the paste. Acidity was significantly affected by the addition of sugar. Sugar addition decreased acidity rating and increased sweet perception of pastes. This may be due to the increased °Brix/acid ratio of plum pastes by sugar addition. Heat concentration showed a significant effect ($p \leq 0.01$) on consistency as compared to that of sugar addition ($P = 0.0497$). Sugar addition gave only a slight increase in consistency of plum pastes. Both heat concentration and sugar addition showed little influence on flavor of plum pastes. Sugar addition showed significant influence on overall acceptance ($p \leq 0.01$).

The increase in mean score by an average of 4.24 due to sugar addition indicated that people accepted sweetened plum pastes better than the unsweetened one. Panelists commented that unsweetened plum pastes were too sour to accept. The correlation coefficient between overall acceptance and other sensory attributes (Table 6) confirmed this perception. For unsweetened pastes, the overall acceptance was significantly correlated ($\sim p < 0.01$) negatively to acidity and positively to sweetness. But for sweetened plum pastes, panelists graded the overall acceptance based on not only sourness and sweetness but also on flavor, color, and thickness, in order of significance. These results indicated that plum pastes could be characterized by acidity, sweetness, thickness sensations, but the preference could be adequately predicted by flavor and color under suitable °Brix/acid ratio.

TABLE 4.
MEAN VALUES OF SENSORY ATTRIBUTES FOR PLUM PASTES

Treatment / Characteristics	Heat concentrated to 25°Brix	Heat concentrated to 30°Brix	25°Brix Sugar added to 40°Brix	30°Brix Sugar added to 45°Brix
Color	8.16	10.53	10.60	12.02
Acidity	9.60	10.35	5.38	5.23
Sweetness	4.34	4.34	8.76	8.74
Consistency	7.98	10.34	8.85	10.63
Flavor	8.35	9.11	9.26	9.10
Overall acceptance	5.98	5.58	10.24	9.10

TABLE 5.
P VALUES OF ANALYSIS OF VARIANCE FOR SENSORY ATTRIBUTES OF PLUM PASTES

	Source		
Sensory Attributes	A	B	AxB
Color	0.0001	0.0001	0.0887
Acidity	0.4070	0.0001	0.2147
Sweetness	0.9784	0.0001	0.9849
Consistency	0.0001	0.0497	0.3332
Flavor	0.4564	0.2679	0.2553
Overall acceptance	0.2097	0.0001	0.9652

A - Heat concentration
B - Sugar addition

TABLE 6.
CORRELATION COEFFICIENTS OF OVERALL ACCEPTANCE WITH COLOR, ACIDITY, SWEETNESS, CONSISTENCY AND FLAVOR OF PLUM PASTES

Treatment / Characteristics	Heat concentrated to 25°Brix	Heat concentrated to 30°Brix	25°Brix Sugar added to 40°Brix	30°Brix Sugar added to 45°Brix
Color	-0.058	-0.056	0.326**	0.335**
Acidity	-0.347**	-0.482**	-0.393**	-0.091
Sweetness	0.508**	0.435**	0.447**	0.349**
Consistency	0.106	-0.078	0.293*	0.245
Flavor	0.090	0.105	0.439**	0.682**

* Significance at 5% level
** Significance at 1% level

Microbial Analysis

No microorganisms were detected in any of the paste samples. Although plum puree was contaminated with yeasts and molds, pastes made from it were

free from microbial contamination. Heat treatment necessary for concentration of puree to paste was sufficient to destroy yeasts and molds.

CONCLUSIONS

Heat concentration resulted in component degradation, such as titratable acidity, total anthocyanins and pectin content, as well as concentration of these components. On the other hand, sugar addition showed dilution effect on paste composition. Both heat concentration and sugar addition resulted in decreased lightness of color. Rheological properties were significantly affected by heat concentration but not by sugar addition. Sensory evaluation indicated that plum paste could be characterized by acidity, sweetness, and consistency sensation, but the preference could be adequately predicted by flavor and color under suitable Brix/acid ratio. Future studies may focus on concentration of pastes under vacuum to shorten processing time and lower processing temperatures. This can help minimize quality loss by heat, such as browning and degradation of anthocyanins and pectin.

ACKNOWLEDGMENT

Acknowledgment is made to Michigan Agricultural Experiment Station, the Food and Crop Bioprocessing Center, Michigan State University and Michigan Plum Advisory Board for their support of this research.

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BOOK REVIEWS

ENZYMES IN FOOD PROCESSING, 3RD EDITION. Edited by Tilak Nagodawithana and Gerald Reed, Academic Press, Inc., 1250 Sixth Ave., San Diego, CA 92101-4311. 1993. 480 pages. $110.00.

When Gerald Reed wrote the first edition of *Enzymes in Food Processing* in 1966, it would have been difficult to imagine just how radically enzyme technology would evolve and grow during the ensuing 28 years. To accommodate the many new technologies and applications, the third edition represents a complete rewrite of the previous two. The strategy adopted by the editors was to omit topics that have not substantially changed during the past but rather to focus on newly discovered applications and experimental tools. The end product of their endeavor is a well-rounded, easily digestible volume that should appeal to a broad range of readers.

For those wishing a refresher course in basic enzymology, the opening three chapters provide a succinct and well-written review of enzyme structure, nomenclature and catalytic properties. With special emphasis on the effects of pH, water and temperature, including freezing, on enzyme activity, interests of the food scientist have clearly been kept in mind. The flow then turns to enzyme technology with chapters on enzyme design using protein engineering and enzyme immobilization. The middle portion of the book focuses on specific enzyme systems with chapters on carbohydrases, proteases, lipases and oxidoreductases. Finally, the remaining chapters cover specific industrial applications including enzymes in baking, starch and syrup production, dairy products, uses of pectic enzymes, enzymes associated with flavor enhancement, winemaking, brewing and fish processing.

In many books of this nature, oftentimes the forest is obscured by the trees, but not here. This book successfully avoids the common pitfall of overdosing on small details. To obtain highly specialized information, most of the chapters contain extensive citation lists directing readers to appropriate core journals. This book should serve as a valuable addition for enzymologists and non-enzymologists alike. The editors are to be applauded for this truly first class effort.

BRUCE P. WASSERMAN

Journal of Food Processing and Preservation **19** (1995) 83–87.

CARBOHYDRATE POLYESTERS AS FAT SUBSTITUTES. Casimir C. Akoh and Barry G. Swanson. Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016. 1994. 269 pages. $125.00.

This book is a useful review of the subject through 1991. It represents a well-written collection of papers dealing with fat substitutes, particularly the carbohydrate polyesters. While several authors are involved, the editors have managed to generate a text that flows well. This is a well-written, concise book on an important contemporary subject. It should be a particularly useful reference for nutritionists, food scientists and dietitians.

The first five chapters deal with a history of carbohydrate polyesters, their synthesis and analysis and finally a chapter covering patent literature. This section should provide an excellent overview of the general subject. The chapter on patent literature is particularly valuable and is an area often overlooked in texts of this nature. It is a credit to the editors (particularly the editor who wrote this chapter!), that this important topic was included. Chapter 4 deals with the analysis of carbohydrate polyesters using supercritical fluid extraction and chromatography. This is particularly timely, as these techniques are proving to be extremely valuable tools in lipid chemistry.

The next two chapters deal with the physical, chemical properties of sucrose polyesters and their use in food systems as emulsifiers and as fat replacers. Chapters 6 and 7 should be of interest to food scientists and ought to be required reading for anyone contemplating the use of carbohydrate polyesters.

Chapter 8 represents a valuable inclusion for it deals with a property of sucrose polyesters that is often overlooked, namely, their antimicrobial properties. Fatty acid esters of sucrose have been shown to have a broad spectrum of antimicrobial effects against bacteria, yeasts and molds. Such properties can be useful but could also present problems in certain food products. By and large the antimicrobial properties of sucrose polyesters do not seem to interfere with yeast leavening or usual food fermentations. Nonetheless, an awareness of this important property is critical for successful food formulation with sucrose polyesters.

Chapters 9 and 10 deal with toxicological and nutritional studies, including the effects of sucrose polyester on cholesterol metabolism. The review of metabolism and nutritional aspects is well done but rather cursory. The chapter dealing with the effects on cholesterol metabolism is quite extensive and well written.

Chapters 11 and 12 cover the "other" fat substitutes. Chapter 11 is a good, readable review of the subject and includes a review of the patent literature. Chapter 12 is an assessment of the potential market for fat substitutes which makes the point that the market could be huge; however, there are many

assumptions that make these estimates rough at best. Nonetheless, this chapter conveys to the reader the possible enormity of this market.

The final chapter is a valuable inclusion that deals with the regulatory environment. This is a consideration generally overlooked by academicians but is one that will be a critical determinant of the commercial success of fat substitutes. It is, therefore, extremely important that any student of this topic have an awareness of the regulatory climate which will surely support the evolution of this potential commercial venture and its possible health impacts.

GILBERT A. LEVEILLE, Ph.D.

NUTRITION IN THE '90s, CURRENT CONTROVERSIES AND ANALYSIS, VOL. 2. Edited by F.N. Kotsonis and M.A. Mackey. Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016. 1994. 170 pages. $35.00.

This edited book reviews some very interesting and timely topics in a lucid and easily understandable manner for both experts and lay readers. The emphasis in the first seven chapters is on the impact of nutrition and physical fitness (exercise) in relation to the major debilitating diseases and disorders afflicting Americans, with major attention to an ever increasing older population.

The first two chapters, written by two eminent physician-researchers, David P.Rose and David J.A. Jenkins, cover state of the art findings on dietary fat, fiber and complex carbohydrates in relation to cancer and heart disease. Rose points out that animal experimentation confirms the epidemiological suggestion that a fat intake of 30% of total calories is probably threshold for lowering the incidence of certain types of cancer. Jenkins states that soluble fiber, by reducing insulin secretion, lowers the key enzyme responsible for cholesterol synthesis.

Roger R. Williams from the University of Texas contributes a most interesting chapter on the genetics of heart disease and high blood pressure. He has the good fortune of access to the Mormon genealogical database that proved so useful recently in isolating the gene of a certain type of breast cancer.

An interesting article contributed by David A. Levitsky shows that moderate weight loss in obese individuals may be attained by taking advantage of the imprecise control of food intake when the diet contributes between 20 and 40% fat of total calories.

The first of three chapters on physical activity, by Steven N. Blair, was very cautious. On one hand, we are given to understand that active people have a lower mortality from both heart disease and cancer, but Mr. Blair also points

out that heart attack patients who were told to lose weight and exercise had a 60% higher mortality than those who did not lose weight John O. Holloszy strongly emphasizes the benefits of exercise in the prevention of several chronic diseases associated with aging, an important consideration for our expanding population of senior citizens. In the third chapter on exercise, but also including an excellent discussion of calcium nutrition, Bess Dawson-Hughes stresses the role of exercise in relation to the prevention or worsening of osteoporosis.

Chapter 8 cover the interesting subjects: nonnutritive components of food in the health and well-being of the consumer by Mark L. Wahlquist and the enormous potential for new research approaches using Transgene Technology by John A. Thomas. The latter chapter describes the many new transgenic mouse models that are now available to study such human diseases as AIDS, diabetes and hypertension, among others.

In the final chapter, one of the editors of the book, Maureen A. Mackey (together with Betsy P. Hill), discusses the new FDA regulations concerning food labelling and health claims.

One of the fascinating trends illustrated by this book is the ever-increasing role played by the medical profession in the field of nutrition. Six of the 10 chapters are authored by M.D.s. Considering that nutrition is still not a major subject taught in medical schools this is a puzzling observation. This aside, the editors and authors have done a fine job in assembling a thoroughly readable and useful treatise.

HANS FISHER

PRINCIPLES OF ENZYMOLOGY FOR THE FOOD SCIENCES, 2ND EDITION. John R. Whitaker. Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016. 1994. 625 pages. $185.00.

This book is a revision of Dr. Whitaker's 1972 original with the same title. It deals primarily with enzyme kinetics and the properties of enzymes. It covers the characteristics of proteins (enzymes), enzyme purification, and the nature of the active sites of enzymes. It then describes in detail reaction rates, effects of enzyme and substrate concentrations, inhibition, effects of temperature and pH, and cofactors. There is a chapter on classification and nomenclature of enzymes and then a discussion of several specific hyrolases and oxidoreductases, which are useful as illustrations of the important properties of enzymes and the type of information that can be obtained from these studies.

The emphasis in the book is on kinetics. The book evolved from a course taught over many years by Dr. Whitaker, and it would make an excellent textbook for a course in enzyme kinetics as well as a good reference book.

After each chapter there are a number of questions for self-testing. Answers are not provided so maximal use of this as a textbook may be obtained with some instructional guidance.

The title of the book, *Principles of Enzymology for the Food Sciences* should not be taken to imply that it is limited to researchers in food science. It is a well-written book that takes one through basic enzyme kinetics step by step and does not require an extensive background in mathematics. It would be a useful book for students and researchers in any of the life sciences. The book does not emphasize the role of enzymes in quality of foods during processing and storage. The possible role in food processing of some of the enzymes discussed is only cursorily examined. For example, the potential effect of peroxidases and catalase as antioxidative components of food tissues is not discussed although the kinetics of the enzymes are well-described.

This Second Edition has been updated somewhat, but the changes from the first edition are not extensive. Many of the references suggested for further reading (a strength of the book) are relatively old. This is not a problem since basic kinetics have not changed, but it does reemphasize that the book is about kinetic evaluation of enzymic activities and not an extensive discussion of the role of enzymes in food quality. The quality of the printing and layout of the Second Edition is far superior to that of the First Edition and makes the book easier to read. The book had very few typographical or other errors. One of the few confusing points I found was the referral to 8,11,14-eicosatrienoic acid as linolenic acid rather than dihomogammalinolenic acid.

I have used the First Edition of this book over the years as a reference whenever I needed more information than was available in the typical biochemistry textbook. I have found this one of the two most useful reference books in my work on enzymic catalysis. (The other was William P. Jenck's *Catalysis in Chemistry and Enzymology*, which is now unfortunately out of print.) The price of the book is high. It is probably not worth purchasing this book if you have a copy of the First Edition. If you do not, and have a need to evaluate enzyme kinetics in your work, I can recommend it highly. Even at $185.00 it would be a valuable addition to either your individual or institutional library.

H.O. HULTIN

PUBLICATIONS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD LIPIDS, F. Shahidi
JOURNAL OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY, D.Y.C. Fung and M.C. Goldschmidt
JOURNAL OF MUSCLE FOODS, N.G. Marriott and G.J. Flick, Jr.
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Books

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Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

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 that have already been published elsewhere.

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

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

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

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

RIZVI, S.S.H. 1986. Thermodynamic properties of foods in dehydration. In *Engineering Properties of Foods*, (M.A. Rao and S.S.H. Rizvi, eds.) pp. 133–214, Marcel Dekker, New York.

MICHAELS, S.L. 1989. Crossflow microfilters ins and outs. Chem. Eng. *96*, 84–91.

LABUZA, T.P. 1982. *Shelf-Life Dating of Foods*, pp. 66–120, Food & Nutrition Press, Trumbull, CT.

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

Tables should be numbered consecutively with Arabic numerals. Type tables neatly and correctly as they are considered art and are not typeset. The title of the table should appear as below:

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ACTIVITY OF POTATO ACYL-HYDROLASES ON NEUTRAL LIPIDS, GALACTOLIPIDS AND PHOSPHOLIPIDS

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

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

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

Standard nomenclature as used in the engineering literature should be followed. Avoid laboratory jargon. 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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CONTENTS