Stabilizing Brown Rice to Lipolytic Hydrolysis by Ethanol Vapors

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ABSTRACT

Brown rice can be stabilized to lipolytic hydrolysis by exposure to vapors from boiling aqueous ethanol (EtOH). During six months of storage at 36°C, free fatty acids increased little or none in brown rice kernels treated with EtOH vapors for 3–10 min. Flours produced from treated kernels had low residual lipase activity. Treated kernels and flours prepared from them were more susceptible to oxidative deterioration than untreated kernels and flours, as indicated by increases in conjugated diene hydroperoxide content during storage. EtOH vapor treatment lowered the moisture content of the 12.8%-moisture brown rice kernels approximately 1.5%; loss of kernel oil was less than 3%. The water content of 8%-moisture kernels was not changed, and no oil was extracted by the EtOH vapor treatment. Thiamin and tocopherols were not lost in EtOH vapor-treated kernels. Thermal curves of treated and untreated kernels obtained by differential scanning calorimetry indicated no starch gelatinization in the treated kernels. EtOH vapor treatment of brown rice kernels reduced microbial populations to very low levels.

Brown rice has a short shelf life (about three to six months) because of hydrolytic and oxidative deterioration of bran lipids. Brown rice lipids are readily hydrolyzed by lipases, both natural to the bran and of microbial origin, that release free fatty acids (FFAs). FFAs are precursors of off-flavors and off-odors associated with lipid degradation products generated in subsequent oxidation reactions.

Three approaches have been taken to stabilize brown rice to lipolytic hydrolysis. In the first approach, lipase is inactivated by heating raw or brown rice with moist or dry heat (Van Atta et al 1952, Bardet and Glease 1961, Tadahiko Hirokawa et al 1986) or by parboiling or precooking it (Miller 1963, McCabe 1976, Sowbhagya and Bhattacharya 1976). In the second approach, kernel oil, which serves as a substrate for lipase, is removed by organic solvent extraction (Kester 1951, Wayne 1966). In the third approach, bran lipases and lipase-producing bacteria and mold are denatured and inactivated by liquid ethanol (EtOH) extraction (Champagne et al 1991).

This article describes a process for stabilizing brown rice to lipolytic hydrolysis by exposing it to EtOH vapors. We report the effects of EtOH vapor treatment on the appearance, composition, and bacterial and mold populations of brown rice kernels and on the storage stability of brown rice kernels and their flours.

MATERIALS AND METHODS

Rice Samples

Rough rice samples of Tebonnet (1989 crop) were obtained from the Louisiana State University Rice Experiment Station at Crowley, LA. Samples were dehulled in a McGill sheller (H. T. McGill, Houston, TX).

EtOH Vapor Treatment

A 40-g sample of freshly dehulled brown rice was placed in a jacketed glass butt tube (3 cm in diameter, 12 cm high) fitted with a wire mesh sample-retaining screen (Fig. 1). Water from a bath set at 83°C was circulated through the jacket. When the temperature of the sample reached 78°C, which required 20 min, the glass butt tube was inserted into the neck of a 500-ml round-bottom flask containing boiling aqueous EtOH (95% v/v; bp 78°C). Samples were exposed to EtOH vapors for 3, 5, and 10 min. After treatment, the samples were transferred to shallow stainless steel pans and allowed to cool in room-temperature (24°C) air.

Brown rice samples at 12.8% (the moisture level of control kernels) and 8.0% moisture were treated with EtOH vapors. Samples at 8.0% moisture were obtained by drying 12.8%-moisture kernels for 2.5 hr in a 65°C oven. Untreated brown rice kernels and flours prepared from them served as controls. Moisture levels were determined by drying ground material in an oven at 130°C for 1 hr, as described by the Agricultural Marketing Service (AMS 1959).

To evaluate the effect of heat on kernel stability, we placed

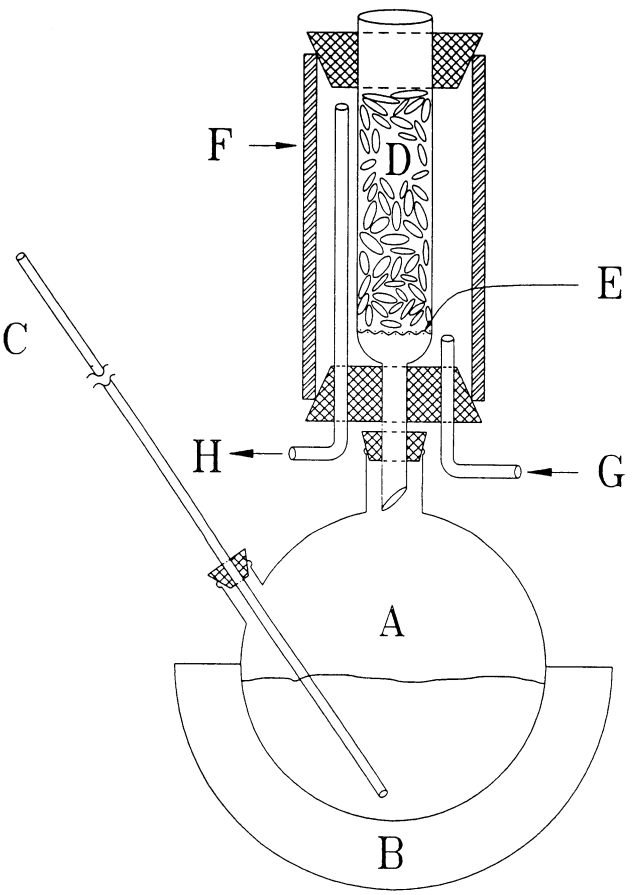

Fig. 1. Apparatus used to treat brown rice kernels with ethanol vapors: A, 500-ml round-bottom flask with a side arm; B, heating mantle; C, glass tubing for venting; D, glass butt tube (3 cm in diameter, 12 cm high) holding 40 g of brown rice; E, wire mesh screen to retain the sample; F, Plexiglas jacket; G and H, inlet from (G) and outlet to (H) circulating water bath.

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brown rice kernels in an 83°C jacketed glass butt tube for 30 min (20 min to reach 78°C and 10 min at 78°C). To compare the action of aqueous EtOH with that of the common, commercial vegetable oil extractive solvent hexane, we also treated brown rice kernels with vapors from boiling hexane (bp 68°C).

Flours were prepared by grinding kernels to a powder in a Udy cyclone mill (Udy Corp., Fort Collins, CO) with a 20-mesh sieve screen. Brown rice kernels and flour samples were stored in capped half-pint glass jars with air headspace at 36°C. Two batches each of vapor-treated and control kernel and flour samples were analyzed to determine thiamin and tocopherol content, bacterial and mold populations, and storage stability.

FFA Content

To measure the extent of lipolytic hydrolysis of brown rice kernels and flours during storage, we determined the FFA content of treated and control samples the day after vapor treatment and periodically thereafter by a micromethod (Hoffpauir et al 1947). Metacresol purple was substituted for the phenolphthalein indicator. FFA content was calculated as oleic acid and expressed as percentage of oil.

Conjugated Diene Hydroperoxides

To measure oxidative deterioration of unsaturated lipids in brown rice kernels and flours during storage, we used the method of St. Angelo et al (1972) to determine the content of conjugated diene hydroperoxides (CDHPs) in samples on the day following treatment and during storage. Samples were ground in a Udy cyclone mill to pass through a 20-mesh screen. Samples (0.5 g) were shaken with 25 ml of high-performance liquid chromatography (HPLC) grade hexane for 30 min and then filtered through 0.45-μm Millex-HV Millipore filters. Absorbancy of the filtrates at 234.0 nm was determined, using hexane as a reference. A molar absorptivity (A_s) of 24,500 $mol \cdot L^{-1} \cdot cm^{-1}$ was used to calculate the concentration of CDHP in micromoles per gram of brown rice (dry basis). To compare changes (Δ) in CDHP from the day following treatment through six months of storage, initial CDHP values were subtracted from values obtained during storage.

Microbiological Assays

Rice samples (10 g) were weighed, transferred aseptically into sterile blender jars, and blended with 90 ml of sterile, phosphate-buffered distilled water (pH 7.2). Serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were prepared with sterile, phosphate-buffered distilled water (pH 7.2). Duplicate nutrient agar pour plates for total plate counts and triplicate potato-dextrose agar plates for total molds were inoculated with the appropriate dilutions and incubated as described by DeLucca et al (1978).

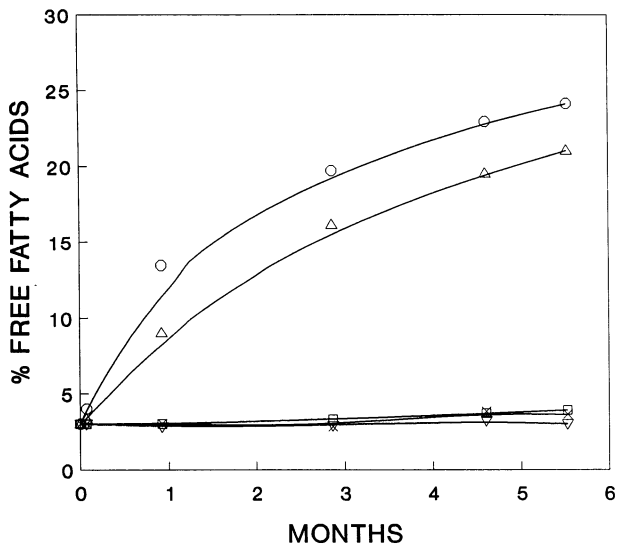

Fig. 2. Effects of storage at 36°C on free fatty acid formation in ethanol vapor-treated (□, 3 min; X, 5 min; ▽, 10 min), heat-treated (△), and control (○) brown rice kernels. Values plotted are means of analyses on two batches of rice.

Thiamin and Tocopherol Content

Thiamin content of duplicate EtOH vapor-treated and control rice samples was determined by AOAC method 43.165 (AOAC 1985). Tocopherol content was determined by an HPLC method developed by General Mills Inc. (Medallion Laboratories, Minneapolis, MN).

Microscopy

We examined the surfaces of 10 kernels randomly chosen from each batch of EtOH vapor-treated and control kernels with an Olympus BH-2 compound microscope (Olympus Corp., Lake Success, NY) at ×40 magnification.

Differential Scanning Calorimetry

To determine the effect of EtOH vapor treatment on starch gelatinization, we compared thermal curves of treated and untreated brown rice kernels obtained by differential scanning calorimetry (DSC). DSC was conducted with a thermal analyzer (model 7701, Hart Scientific, Provo, UT) equipped with a Hart Scientific 707 cell base designed for a system that includes a reference and one to three samples. The procedure of Normand and Marshall (1989) was followed to prepare and run samples on the differential scanning calorimeter. Thermal curves were obtained from 20 to 110°C, with a heating rate of 1.0°C/min.

RESULTS

Lipolytic Hydrolysis of Brown Rice During Storage

Figure 2 shows the effect of storage at 36°C on the accumulation of FFAs in brown rice kernels (12.8% moisture) treated with EtOH vapors or heat or not treated (control). Samples were stored at 36°C to accelerate the rate of lipolytic hydrolysis. FFA levels in kernels treated with EtOH vapors for 3 or 5 min increased from 3.0% to 3.9% and 3.6%, respectively, after six months of storage. FFA content of kernels treated with vapors for 10 min did not change, whereas that of control kernels increased from 3% to 24%. The increase in FFA content of heat-treated kernels during storage was approximately 15% lower than that of the control kernels, indicating some deactivation of lipase by heat-denaturation.

Vapors from boiling aqueous EtOH extracted surface water from the kernels and condensed on the kernels during treatment. The vapor treatment lowered the moisture content of the kernels approximately 1.5%; loss of kernel oil was less than 3%. To determine whether the action of EtOH vapors in stabilizing brown rice kernels to FFA formation depended on the vapors condensing on the kernels, we also treated 8%-moisture kernels with EtOH vapors. The EtOH vapors did not condense on the 8%-moisture kernels; the water content of the kernels did not change, and no oil was extracted. The FFA level of 8%-moisture kernels treated with EtOH vapors for 10 min did not increase during storage at 36°C. These results show that vapors from boiling aqueous EtOH are effective in stabilizing brown rice kernels to FFA formation and that stabilization does not depend on the vapors condensing to a liquid.

We also determined the effect of storage at 36°C on FFA levels in flours prepared from 12.8%-moisture brown rice kernels treated with EtOH vapors (Fig. 3). After five months in storage, flours prepared from kernels treated with vapors for 3, 5, or 10 min showed increases in FFA levels from 3% to 9, 7, and 6%, respectively. In contrast, FFA levels in flours prepared from control and heat-treated kernels rose from 3% to 80% and 46%, respectively. The low increases in FFA content in flours prepared from vapor-treated kernels indicated a low level of residual lipase activity in the flours.

Hexane Vapor Treatment

Vapors from boiling hexane were not effective in preventing FFA formation in brown rice kernels. After one month of storage at 36°C, the FFA levels in kernels treated with hexane vapors for 3, 5, or 10 min increased from 3% to 18, 17, and 12%, respectively; the FFA levels in control and heat-treated kernels increased to 17 and 14%, respectively.

Oxidative Deterioration of Unsaturated Lipids

CDHP levels increased rapidly in kernels treated with EtOH vapors (Fig. 4) and in their flours (Fig. 5). Only a slight increase in CDHPs was observed for control and heat-treated kernels during storage. The larger increases in CDHPs in EtOH vapor-treated kernels and their flours relative to the controls indicate that lipids in the former are more susceptible to oxidative deterioration.

Effect of Treatment on Microorganisms

Table I shows the effect of EtOH vapor treatment on the bacterial and mold populations of brown rice kernels. Total plate counts and mold counts were very low in EtOH vapor-treated kernels. The vapors from boiling aqueous EtOH killed the microorganisms.

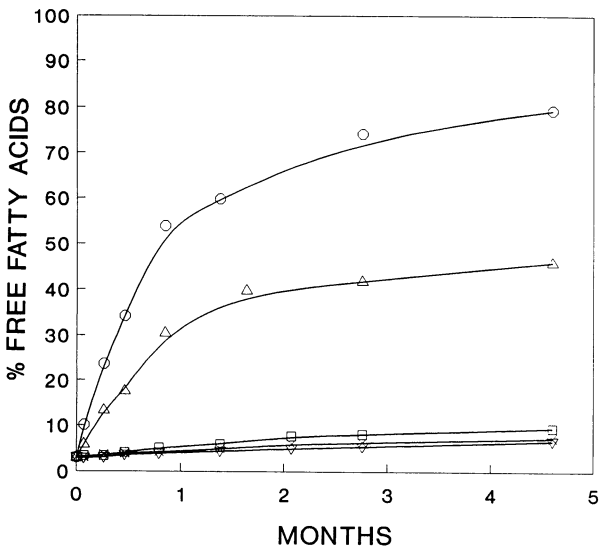

Fig. 3. Effects of storage at 36°C on free fatty acid formation in flours prepared from ethanol vapor-treated (□, 3 min; X, 5 min; ▽, 10 min), heat-treated (△), and control (○) brown rice kernels. Values plotted are means of analyses on two batches of flour.

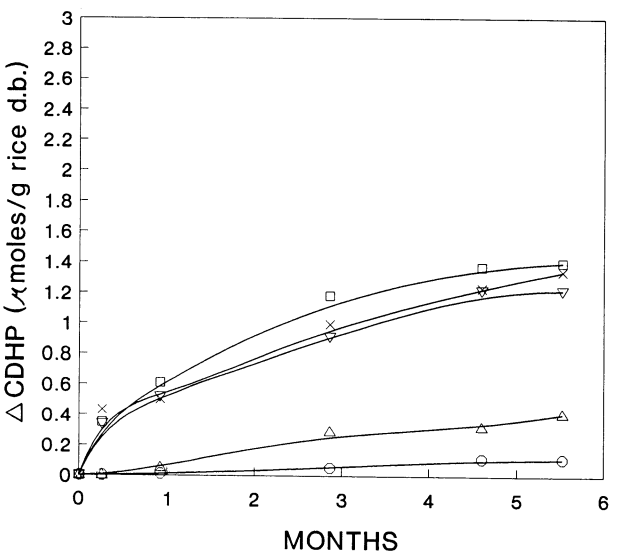

Fig. 4. Change in conjugated diene hydroperoxide content (ΔCDHP) in ethanol vapor-treated (□, 3 min; X, 5 min; ▽, 10 min), heat-treated (△), and control (○) brown rice kernels during storage at 36°C. Values plotted are differences of means calculated from analyses on two batches of rice.

Thiamin and Tocopherol Retention

Brown rice is rich in B vitamins. Thiamin was chosen as an indicator of the degree of retention of B vitamins in kernels treated with EtOH vapors. Thiamin was not lost in EtOH vapor-treated kernels (Table II). Tocopherols, known for their antioxidant properties, were also retained in EtOH vapor-treated kernels (Table II).

Kernel Surface Appearance

There were no visible differences between brown rice kernels treated with EtOH vapors and control kernels. Narrow, superficial transverse fissures were observed at ×40 magnification on the surfaces of kernels treated with EtOH vapors for 5 or 10 min. Kernels treated for 5 min had zero to two fissures. Kernels treated for 10 min had more extensive fissuring; some had a few transverse fissures, while others were covered with a web of fissures. No transverse fissures were seen on the surfaces of control kernels or those treated with EtOH vapors for 3 min.

Starch Gelatinization

Thermal curves for EtOH vapor-treated and control rice kernels (Fig. 6) were each characterized by a broad starch gelatinization endotherm (Normand and Marshall 1989). The calculated peak gelatinization temperature (T_p) of kernels treated with EtOH vapors for 10 min (98.5°C) was 2°C lower than that for control kernels (100.5°C). The enthalpy associated with starch gelatini-

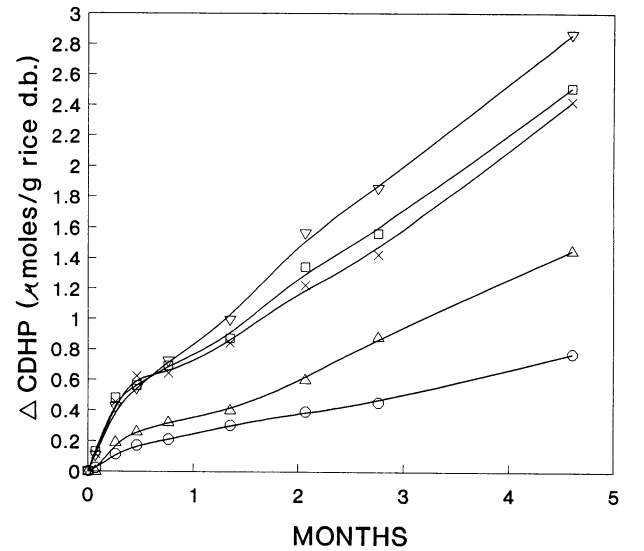

Fig. 5. Change in conjugated diene hydroperoxide content (ΔCDHP) in flours prepared from ethanol vapor-treated (□, 3 min; X, 5 min; ▽, 10 min), heat-treated (△), and control (○) brown rice kernels during storage at 36°C. Values plotted are differences of means calculated from analyses on two batches of flour.

TABLE I
Effect of Ethanol (EtOH) Vapor Treatment on Microbial Population of Brown Rice[a]

Treatment	Total Plate Count (no./g)	Mold Count (no./g)
Control	14,000	10
Heat-treated	250	<10
3-min EtOH vapors	10	<10
5-min EtOH vapors	10	<10
10-min EtOH vapors	<10	<10

[a] Counts were determined one week after kernels were dehulled. Initial moisture content was 12.8%.

zation (ΔH) (area under the endotherm) could not be calculated because no final baseline was observed, due to temperature limitations of the instrument. However, there appears to be no difference in peak size for the curves for EtOH vapor-treated and control kernels. This indicates that there was no difference in the thermal energy required to gelatinize starch in EtOH vapor-treated and control kernels, which in turn implies that the EtOH vapor treatment did not gelatinize kernel starch. The presence of a small low-temperature shoulder on the starch gelatinization endotherm for EtOH vapor-treated kernels indicates that the treatment increased the porosity of the kernel surface (Normand and Marshall 1989, Champagne et al 1990).

DISCUSSION

A stable, full-fat product with ungelatinized starch can be produced by treating brown rice with vapors from boiling aqueous EtOH. Brown rice kernels treated with EtOH vapors are stable to lipolytic hydrolysis, as indicated by minimal or no increases in FFAs during storage.

EtOH vapors act by denaturing lipases endogenous to the brown rice kernel, with concomitant deactivation. The longer the treatment time, the more effective EtOH vapors were in denaturing endogenous lipases. Flours produced from kernels treated with EtOH vapors for 3-10 min had low residual lipase activity. Within the intact rice kernel, lipases are localized in the testa-cross layer region of the caryopsis coat, while oil is localized in the aleurone and germ (Shastry and Raghavendra Rao 1971). Damage to kernels during shelling disrupts these regions, allowing lipases to make contact with oil and lipolysis to proceed. Denaturation by EtOH vapors is plausible because endogenous lipase is so close to the kernel surface.

The action of EtOH vapors can also be attributed to ethanolic denaturation of bacteria and mold on kernel surfaces, which kills the organisms. DeLucca et al (1978) determined that about 10% of the total bacterial population on rough rice and all of the isolated molds showed lipolytic action. Bacterial and mold lipases may contribute more to FFA formation in brown rice than endogenous lipases (DeLucca and Ory 1987). Loeb and Mayne (1952) found a relationship among moisture content of rice, microflora, and FFA formation in rice bran.

Heat-treated brown rice kernels and those treated with vapors from boiling hexane were not stable to lipolytic hydrolysis. The temperatures of the heat (78°C) and hexane vapor (68°C) treatments were too low to substantially denature and inactivate lipases. Unlike ethanol, hexane does not appreciably denature proteins (or enzymes) (Belter and Smith 1952).

EtOH vapor-treated kernels were more susceptible to oxidative deterioration than untreated kernels. This can be attributed in part to ethanolic and heat denaturation and inactivation of the hemoproteins catalase and peroxidase found in brown rice kernels. No catalase activity was observed in vapor-treated kernels, and peroxidase activity was reduced by 52, 63, and 86% in kernels treated for 3, 5, and 10 min, respectively (*data not shown*). Eriksson et al (1969) demonstrated that the rate of linoleic acid oxidation in the presence of heat-denatured catalase and peroxidase was as much as 11 times higher than that for unheated controls. They attributed this result to unfolding of the enzyme, which increased exposure of the heme groups to the substrate. St. Angelo et al (1972) demonstrated that the nonenzymatic oxidation of peanut lipids by heat-denatured hemoproteins proceeded more rapidly than the enzymatic oxidation of these lipids with active lipoxygenase and hemoproteins.

Another possible explanation for the increased susceptibility of EtOH vapor-treated kernels to oxidative deterioration is disruption of the caryopsis coat by EtOH vapors. Microscopy and DSC results indicated that EtOH vapor treatments resulted in kernel fissuring, which increased kernel porosity, thus rendering lipids more susceptible to oxidation.

Oxidative deterioration in brown rice is inhibited by naturally occurring antioxidants such as tocopherols and their derivatives (Sowbhagya and Bhattacharya 1976). Heat processing may destroy these antioxidants (Herting and Drury 1969). Tocopherol loss was minimal in kernels treated with EtOH vapors; about 10% of α-tocopherol and less than 5% of γ-tocopherol were lost. Therefore, the heightened susceptibility of EtOH vapor-treated kernels to oxidative deterioration cannot be attributed to the destruction of tocopherols during processing.

Although CDHP analyses indicated an increased susceptibility to oxidative deterioration in EtOH vapor-treated kernels and their flours, they do not allow the prediction of differences in vapor-treated and untreated kernels in the development of off-flavors or off-odors from hydroperoxide secondary oxidation reactions. Sensory analyses and gas chromatography studies of volatile oxidation products are underway to evaluate the development of off-flavors and off-odors in vapor-treated and untreated brown rice during storage.

TABLE II
Effect of Ethanol (EtOH) Vapor Treatment on Thiamin and Tocopherol Content (mg/100 g, db) of Brown Rice at 12.8 and 8.0% Moisture

Treatment	Thiamin	Tocopherol	
		α	γ
12.8%-Moisture kernels			
Control	0.72	1.63	0.80
3-min EtOH vapors	0.72	1.51	0.79
5-min EtOH vapors	0.72	1.48	0.81
10-min EtOH vapors	0.73	1.49	0.78
8.0% Moisture kernels			
Control	0.72	1.58	0.80
10-min EtOH vapors	0.71	1.43	0.77

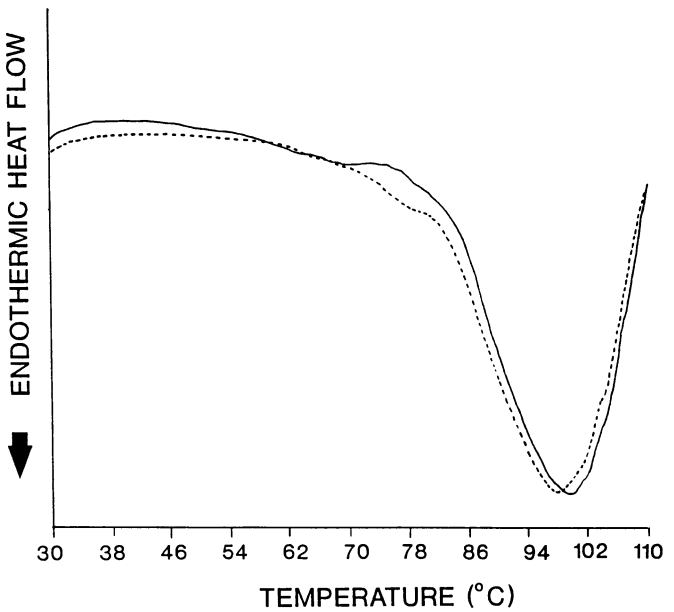

Fig. 6. Thermal curves for brown rice kernels treated for 10 min with ethanol vapors (dashed curve) and control kernels (solid curve).

ACKNOWLEDGMENTS

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