

1989

# Properties of soybean-corn mixtures processed by low-cost extrusion

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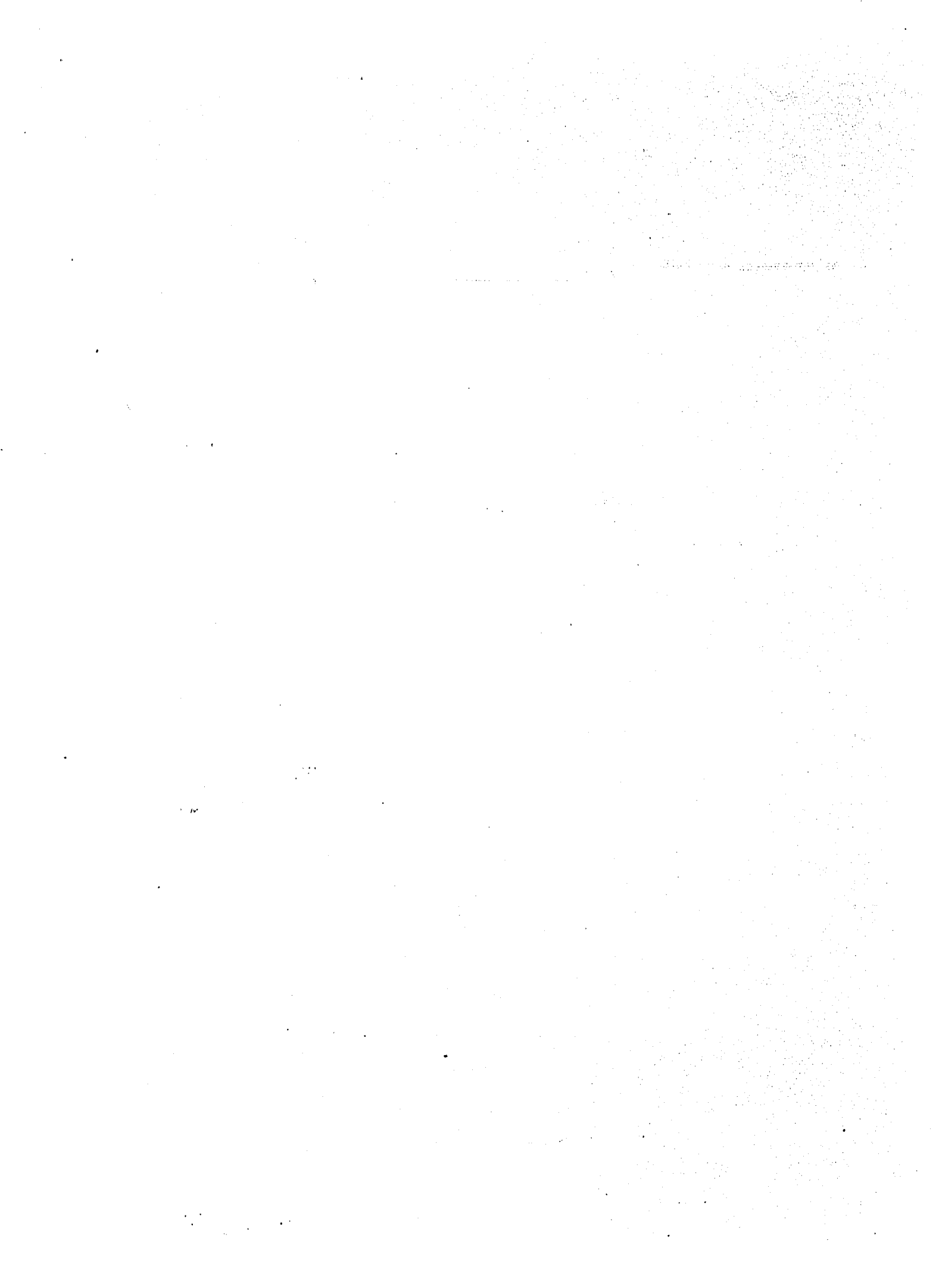
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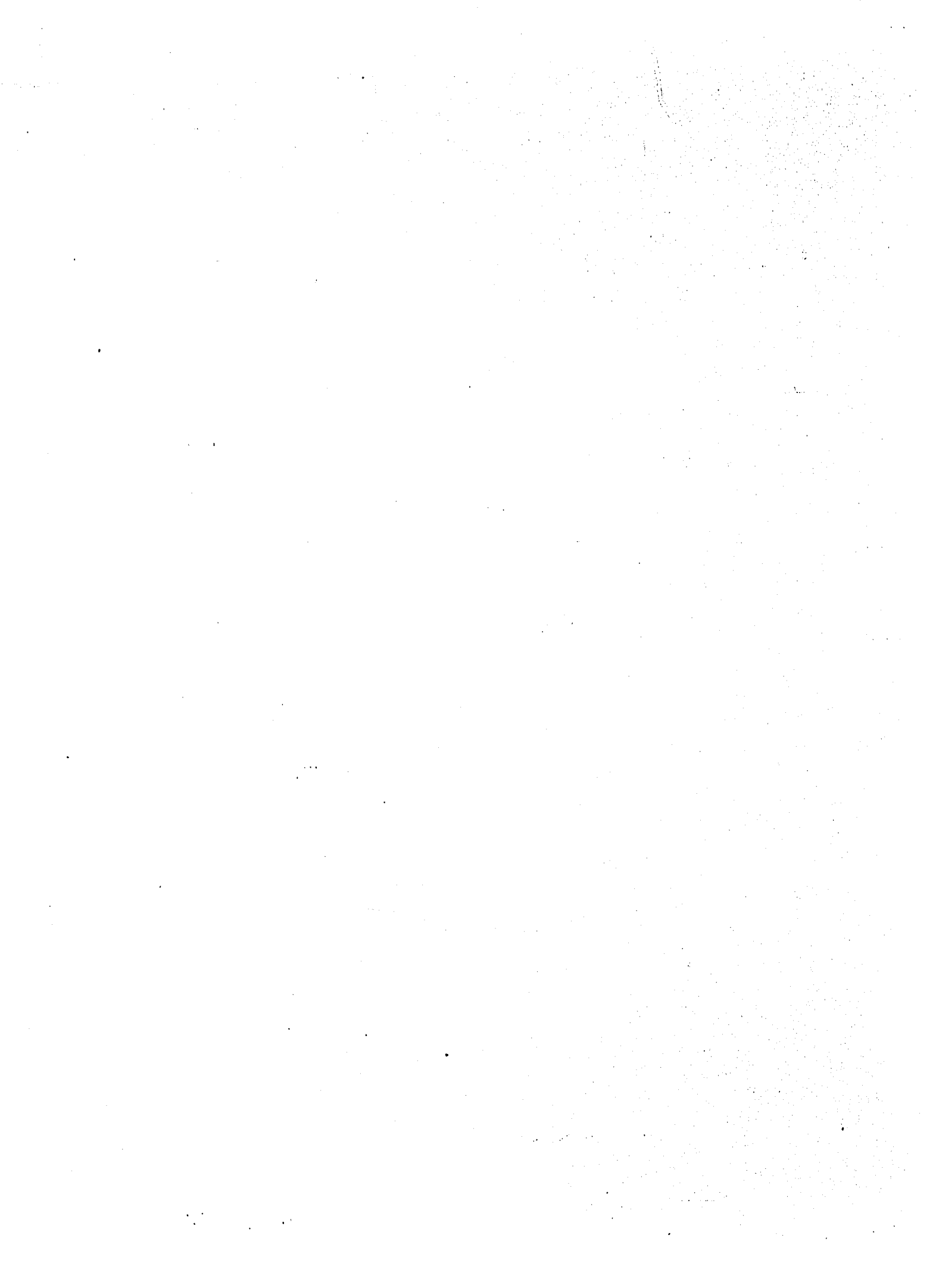
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**Properties of soybean-corn mixtures processed by low-cost  
extrusion**

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**Iowa State University, 1989**

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Properties of soybean-corn mixtures  
processed by low-cost extrusion

by

Gabriel Jaime Guzman

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Major: Food Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University  
Ames, Iowa  
1989

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v

DEDICATION

To my parents

## INTRODUCTION

The utilization of low-cost extruders (LCE) for human purposes has centered on the production of inexpensive, highly nutritious, ready-to-eat foods products. Successful products have been based on combinations of cereals and legumes whose proteins are complementary in terms of amino acid balance. Less effort has been devoted to determine the characteristics of extruded products in which the legume:cereal ratios vary to optimize parameters such as protein digestibility and enzyme deactivation.

## The Insta Pro Extruder

Low-cost extruders (LCE) are single-screw autogenous extruders that operate at low moisture (below 20% feed moisture) and require minimal auxiliary equipment. Autogenous means that all cooking heat is developed by friction through dissipation of mechanical energy applied to the shaft of the extruder.

In the case of the Insta Pro, there is no means of adding or removing heat. The temperature profile along the barrel (Figure 1) is determined by the characteristics of the feed materials, the configuration of the extruder, the feeding rate, and the back pressure, controlled manually at the exit of the extruder. During extrusion of low-starch, high-oil materials, temperature can be controlled by injecting water at the inlet chamber, by changing the feeding rate, or

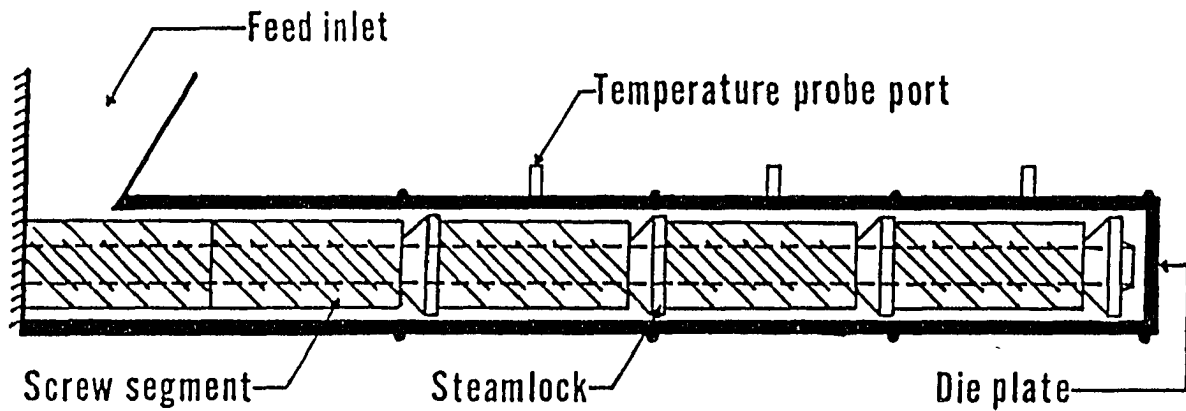


Figure 1. Components of the Insta Pro Model 600 extruder (Kuipers, 1987)

by rotating the nose cone located at the center of the die plate. Rotating the nose cone changes the gap between itself and a nose bullet at the end of the shaft (not shown in Figure 1). This action changes the back pressure and, hence, temperature changes. Primary operational adjustments include the use of restrictions called steam locks of different diameters, a choice between single-flight and double-flight screws, and the use of different die areas.

Kuipers (1987) looked at the effects of extruder configuration on the operation of the Insta Pro extruder. He reported that increasing restrictions generally result in longer retention times inside the barrel and higher exit temperatures.

Corn/Soybean-Derived Products Made  
with Low-Cost Extrusion Technology

Since 1974, work has been done at Colorado State University (CSU) to evaluate the extent that low-cost extrusion can be used to process cereals grains with or without the presence of oilseeds or legumes to produce nutritious foods for less developed countries (Harper et al., 1977). In a summary report (Harper and Jansen, 1981), the CSU group stated that instant products could be produced having a high caloric density, protein quality similar to milk and good shelf life. Three types of LCE were evaluated: the Brady (model 2106), the Insta Pro (500 and 2000), and the Anderson extruders. Currently, LCE's are being used in developing countries to produce nutritious precooked foods based on legumes and cereals (Akinyele, 1987).

An extrusion process for making edible full-fat soy flour was first reported in 1964 (Mustakas et al., 1964). Full-fat soy flours prepared by extrusion have been shown to have good nutritional value, flavor and stability (Bookwalter et al., 1971b; Mustakas et al., 1964). Extrusion-cooked full-fat soybean products have been used to improve the nutritional quality, acceptability and/or stability of corn-based foods (Bookwalter, 1977; Bookwalter et al., 1971a), of breads (Harper and Jansen, 1981) and cookies (Tsen et al., 1975).

Extrusion of whole or degermed corn using nonlow-cost systems (de Muelenare and Buzzard, 1969) and LEC (Harper et al., 1977) has been done previously. Extrusion-cooked corn became a major ingredient in corn-

soy-milk blends distributed by the Food for Peace Program (Harper and Jansen, 1981).

Bressani and Elias (1966) evaluated the protein quality of various combinations of corn and soybean flour. This and other studies (Bressani et al., 1974) have shown that when a corn:soy mixture is prepared in a proportion that approximately half of the protein comes from the cereal and the other half from the soybean, the mixture results in higher protein quality food than the sum of the individual components. The proportion is obtained using approximately seven parts by weight of corn and three parts of soybeans.

The majority of the studies on corn-soybean blends has been performed using the Brady LCE and a corn to soybeans ratio of seven to three. The United States Department of Agriculture's (USDA) corn-soybean blend (CSB) specifications for export indicate a maximum moisture content of 10% and minimum requirements of 16.7 and 6.0% for protein and fat, respectively (Harper et al., 1977). All the of the 70:30 whole corn:dehulled soy, corn grits:whole soy and corn grits:dehulled soy samples extruded at 132 or 149°C in an Insta Pro extruder met the USDA specifications in experiments done by the CSU group (Harper et al., 1977).

Extrusion of dehulled soybeans at 138-149°C product exit temperature, using the Insta Pro, reduces trypsin inhibitor activity (TIA) 67 to 97% (Harper and Jansen, 1981). When whole soybeans with 5.9±5% moisture were extruded using the Insta Pro extruder at 146°C, 86% TIA was destroyed (Jansen, 1979). Destruction of soybean TIA increased

as extruder exit temperature increased. The temperature effect also was observed when the Brady LEC was used (Lorenz and Jansen, 1980; Tsen et al., 1975). Ringe and Love (1988) found that processing a 70% cowpea-30% corn blend at 170°C with the Insta Pro extruder resulted in 84% TIA destruction.

Lorenz and Jansen (1980) recommended an extrusion temperature of 143°C for production of full-fat soy flour on a LEC. At this temperature, the corrected protein efficiency ratio (PER) was 1.94 compared with PERs of 1.01 for unextruded soy and 2.5 for casein. The highest PER, 2.15, obtained for full-fat soy flour processed in a pilot-plant plastics extruder appeared in products whose trypsin inhibitor had been 89% inactivated by the process (Mustakas et al., 1970).

The CSU group found that extrusion temperatures for cereal-based products in the Insta Pro extruder ranged between 150 and 170°C (Harper and Jansen, 1981). Nitrogen solubility indices (NSI) for all of the 70:30 corn-soybean extruded samples were low, ranging from 2.6 to 6.0%, opposed to 14.4% for the commercial CSB. A lowering in the NSI values of similar mixtures through an increase in the extrusion temperature has been reported by Molina et al. (1983). Decreased NSI values indicate a higher degree of cooking suggesting a higher protein digestibility (Molina et al., 1983).

Phillips and Baker (1987) reported values of 77.8% protein digestibility (PD) for raw cowpea meal and 83 to 85% PD for extruded cowpea products. Different extrusion temperatures (150, 175, and 200°C) did not cause significantly different PDs.

Protein quality evaluated by the PER method was affected favorably by increasing the temperature of extrusion of soybeans (Lorenz and Jansen, 1980) and of a 70:30 corn:soy mixture (Molina et al., 1983). Lorenz and Jansen (1980) also observed a drop in PER at high extrusion temperatures.

Lysine retention during extrusion cooking is strongly influenced by process temperature (Bjorck and Asp, 1984). Cereal mixtures extruded without addition of sugars may experience a loss of available lysine of 32% (170°C, 10 or 14% moisture). At mild extrusion conditions, chemical methods using the analysis of 1-fluoro-2,4-dinitrobenzene (FDNB)-lysine and even total lysine predicted the loss of biologically available lysine in biscuits (Bjorck et al., 1983). However, at more severe conditions, the chemical methods underestimated lysine (Asp and Bjoerck, 1984).

#### Storage Stability of Extrusion-Cooked Foods

Extrusion-cooking of legumes and cereals has proven effective in destroying microorganisms and inactivating undesirable biological factors consequently improving in the stability of the extrudates (Mustakas et al., 1970; Bookwalter et al., 1971a, 1971b; Molina et al., 1983).

Mustakas et al. (1964) showed that an extrusion-cooked full-fat soy flour had a low peroxide value (PV) (3.0 meq/Kg of oil) and low free fatty acids (FFA) content (0.99% of fat as oleic) at the end of 39 weeks



when it was stored at 38°C and 45% RH, but the PV was 6.4 and FFA were 4.84% at the end of 15 weeks, respectively, when the sample was stored at 45°C and 25% relative humidity (RH). In a related optimization study (Mustakas et al., 1970) where lipoxygenase was inactivated before extrusion, 'acceptable' PV and flavor scores were reported for adequately extruded full-fat soy flours stored for up to 12 months in glass bottles in the dark at room temperature. Peroxide values and panel flavor scores followed similar trends. Rancidity scores ranged from slight to moderate and FFA levels remained low for full-fat soy flours extruded with an Insta Pro at different temperatures and stored at 22 and 38°C with 40 and 70% RH (Harper and Jansen, 1981).

Harper and Jansen (1981) stored blends of cereals and soybeans, raw or processed by low-cost extrusion. The raw soy-corn mixture showed a slight degree of rancidity (evaluated by a panel) after six months at 38°C with 40 or 70% RH in spite of very high FFA levels (75% of the fat). In contrast, the same blend after extrusion and storage had a moderate rancidity score but low FFA levels (1.46% of the fat). When Molina et al. (1983) stored mixtures of whole soybeans:whole corn (30:70) processed by low-cost extrusion at 35°C (no RH specified), FFA increased from about 1 to 10% of the fat after 12 weeks. The residual lipase activity in these products has never been studied.

Destruction of natural antioxidants caused by extreme processing conditions is thought to be responsible for the susceptibility of extruded full-fat soy flour to lipid oxidation. But native tocopherols are stable during extrusion at 149°C, with a retention time of 2 min and

feed moisture of 15 to 30% (Mustakas et al., 1970).

### Analysis of Tocopherols in Foodstuffs

There are eight naturally occurring forms of vitamin E which belong to two distinct series of compounds: the tocopherols and the tocotrienols. Tocopherols have a saturated isoprenoid side-chain of sixteen carbons whereas tocotrienols have a triply unsaturated side chain. Members within one series differ from one another in the number and position of methyl groups attached to the aromatic ring.

Many values for vitamin E levels in foods have been based on total tocopherol determinations (Ames, 1972). These are a measure of the total reducing materials remaining after a series of purification procedures. The Association of Official Analytical Chemists' (AOAC) method (AOAC, 1980) for example, deals only with one of the eight vitamers,  $\alpha$ -tocopherol. The procedure involves grinding, extracting the lipids with hot ethanol for 4 to 16 hr, and separating between water and hexane. The resultant extract is dried under vacuum and saponified using ethanol, KOH and ascorbic acid. After a series of washings and filtrations with water and hexane, a working extract is obtained.  $\alpha$ -Tocopherol is isolated by thin layer chromatography (TLC) and determined colorimetrically.

The possibility of creating artifacts during the AOAC procedure is large. Besides, it is based on the fact that  $\alpha$ -tocopherol has the highest biological activity of the several vitamin E compounds. It has

been proposed by Bieri and Poukka Evarnts (1974) that the average American diet has twice as much  $\gamma$ -tocopherol as  $\alpha$ -tocopherol, and that  $\alpha$ -tocopherol contributes as much as 20% of the vitamin E activity of U.S. diets. In general, each of the eight compounds has different vitamin E activities and antioxidant properties. The concentration of each individual compound must be known before an assessment of the vitamin E and antioxidant activity of a sample can be made.

In many foods, some of the vitamin E seems to be bound in a protein complex (Voth and Miller, 1958). Thus, the more polar the extracting solvent, the more assurance there is of extracting all the E vitamers. A combination of solvents is generally used. The sample is initially extracted with ethanol or acetone followed by addition of water to the solvent extract and re-extraction with hexane. Saponification is widely used to split associated triglycerides and thus prepare a tocopherol concentrate (Contreras-Guzman and Strong, 1982). But tocopherols are very unstable in the presence of alkali, specially when oxygen is present (Ames, 1972). Use of adsorption chromatography to remove contaminants such as carotenoids has been described (Ward, 1958). This step may result in substantial tocopherol losses if strong adsorbants are used.

High-Performance liquid chromatography (HPLC) has become the preferred technique for the separation and quantitation of tocopherols. The first HPLC systems involved a silica column with a mobile phase consisting of hexane and a polar modifier such as diisopropyl ether (Van Niekerk, 1973). Because of the inherent instability of silica columns,

the more stable reversed-phase systems became more commonly used (Combs and Combs, 1985). Polar bonded phases have been used for chromatography of vitamin E to a limited extent (Westerberg et al., 1981). In polar bonded phases, functional amino groups or a combination of amino and cyano groups are covalently attached to the silica support. An advantage of polar bonded phases over plain silica is less susceptibility to water. Compared to reversed-phase systems, polar bonded phases give better separation of the tocopherols (Westerberg et al., 1981).

#### Explanation of dissertation format

This dissertation is divided in three parts each being a complete paper already published (Part I), submitted (Part II) or to be submitted (Part III) to professional journals. Part I reports a new, simplified procedure for the extraction, separation, and measurement of tocopherols from soybeans and a soybean-based food. The second part examines the effect of substituting increasing percentages of soybeans with whole corn during low-cost extrusion at different exit temperatures. The protein nutritional quality and the potential for flavor problems during storage and use in high moisture foods of the extruded blends was analyzed. Part III reports a systematic shelf stability test of a 80% soybeans-20% corn extruded mix.

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PART I. TOCOPHEROLS OF SOYBEAN SEEDS AND SOYBEAN CURD  
(TOFU)



TOCOPHEROLS OF SOYBEAN SEEDS AND SOYBEAN CURD  
(TOFU)

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Journal of Agricultural and Food Chemistry

34:791-795

## ABSTRACT

$\alpha$ -,  $\gamma$ -, and  $\delta$ -Tocopherols from soybean seeds and soybean curd (tofu) were extracted, separated and quantitated by high-performance bonded normal-phase liquid chromatography with ultraviolet (UV) detection at 280 nm. Two successive extractions with ethanol at a solvent to dry matter ratio of about 20:1 were performed. Saponification was not necessary. The tocopherols were separated in 18 min in an amino column by using 1% isopropyl alcohol in hexane as the mobile phase. Tocopherol content varied significantly from one soybean variety to another. The amounts of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols in the soybeans ranged from 10.9 to 28.4, 150 to 191, and 24.6 to 72.5  $\mu\text{g/g}$  (dry basis), respectively. Processing of soybeans into tofu results in a loss of vitamin E (47% and 30% for two varieties analyzed), but the tofu is a greater source of tocopherols than the soybeans on a dry basis. Storage of the curds for 15 days under commercial conditions does not affect vitamin E content.

## INTRODUCTION

The good nutritional properties of soybean food products rest largely on the high content and quality of the soybean protein. A number of nitrogen balance studies have suggested that the digestibility and biological value of soybean protein for humans are satisfactory and compare favorably with animal proteins (Liener, 1972). Tofu, a highly hydrated cheeselike product made by coagulation of the protein in a soybean extract or soy milk, is a regular item in American supermarkets now. A high proportion of polyunsaturated lipids and lack of cholesterol are additional nutritional characteristics used in the marketing of tofu.

The level of vitamins and minerals in tofu is not well documented in the literature even though it is occasionally referred to in the popular literature. Miller et al. (1952) studied the retention of calcium, iron, thiamin, riboflavin, and niacin in commercially prepared soybean curd. Values for the content of some vitamins and minerals in soybeans and tofu are listed in USDA Handbooks No. 8 (Agricultural Research Service, 1968) and No. 456 (Agricultural Research Service, 1975). These values are the averages of two samples that are made from unknown soybean varieties, by unknown processing methods, and for which older analytical methodology is employed. One of the vitamins not listed in the handbooks is vitamin E. Vitamin E activity in foods derives from two distinct series of compounds, the tocopherols and the

tocotrienols. Many values for vitamin E levels in foods have been based on total tocopherol determinations based upon the Emmerie and Engel reaction (Ames, 1972). Practically, total tocopherol values thus obtained are a measure of the total reducing materials remaining after a series of purification procedures. Separation and quantitation of the individual tocopherols and tocotrienols became more common with the refinement of chromatographic techniques.

$\alpha$ -Tocopherol has the highest biological activity. In the past, it was the only compound considered in dietary calculations (National Research Council, 1980). Changing dietary fat patterns in the United States have resulted in soybean oil becoming the predominant dietary fat, and, consequently, average diets may have twice as much  $\gamma$ -tocopherol as  $\alpha$ -tocopherol (Bieri and Poukka Evarts, 1974). In general, each one of the vitamin compounds has different vitamin E activities and antioxidant properties, making it necessary to consider the concentration of vitamers other than  $\alpha$ -tocopherol before an assessment of the vitamin E and antioxidant activity of a sample can be made.

Separation and quantitation of the tocopherols in soybean oil have been achieved by various methods. Paper chromatography (Brown, 1952; Green et al., 1955; Ward, 1958; Herting and Drury, 1963), thin-layer chromatography (Herting and Drury, 1967), and more recently high-performance liquid chromatography (Van Niekerk, 1973; Carpenter, 1979; Cort et al., 1983; Rammell and Hoogenboom, 1985; Speek et al., 1985) have been used for this purpose with good results. Although a variation

in total and individual tocopherol content from one sample of soybeans to another has been observed, there has not been an attempt to determine whether varietal differences exist or whether the observed variation is due to other factors. Varietal differences have been recently reported for corn (Combs and Combs, 1985) and winged beans (de Lumen and Fiad, 1982).

In this paper, we report the content of the tocopherol vitamers in five soybean varieties grown under identical conditions. We also have examined the vitamin E content of tofus made from these varieties and the loss of vitamin E during refrigerated storage of tofus from two of the soybean varieties.

## MATERIALS AND METHODS

## Soybeans

Five soybean varieties (Corsoy, Strayer, Vinton, Weber, 2325) were donated by Strayer Seed Co., Hudson, IA. The varieties were all grown in Iowa in the summer of 1984. The seeds were ground with a coffee mill to pass a No. 60 sieve. Four grams of ground soybeans was extracted with 100 mL of ethanol in a stoppered flask, under gold fluorescent light, for 30 min. The suspension was filtered through Whatman filter paper No. 4 and the residue reextracted with 50 mL of ethanol for 1 hr. The ethanol extracts were combined and dried in a rotary vacuum evaporator at 40°C. The extract was rinsed out of the round-bottom flask with two 25-mL portions of hexane and filtered through anhydrous Na<sub>2</sub>SO<sub>4</sub>. The hexane was evaporated under vacuum at 35°C and the extract rinsed out with three 1-mL portions of isooctane into 10-mL graduated test tubes. The samples were centrifuged in a bench top centrifuge for 10 min.

## Tofu Manufacture

Tofu was made by following the procedure of Johnson (1984). Nine hundred grams of soybeans was soaked in tap water for 10-12 h at room temperature. The hydrated beans were drained, combined with 6 L of tap

water, and then ground to a slurry with a Cherry-Burrell Vibroreactor. The slurry was transferred to a steam jacketed kettle, and an additional 1 L of tap water was added. The slurry was brought to 95°C and stirred constantly. After 7 min, the cooked slurry was poured into a coarse-mesh filtering sack. One liter of water was added to the residue, and the combined filtrates were filtered through a fine cloth. A 30-mL aliquot of soy milk was removed to measure the solids content by using the light-scattering technique of Johnson and Snyder (1978). The solids content of the soy milk was then adjusted to the desired solids level by using tap water. The soy milk was brought up to 85°C, and calcium sulfate was added for coagulation at this point. The amount of calcium sulfate added was such that its concentration in the soy milk would be 0.019 N. After settling for 5 min, the resulting coagulum was cut and poured into a cheesecloth-lined stainless-steel box with perforations on all sides to allow drainage. After it was pressed for 15 min, the tofu was placed in a water-filled plastic container and stored at 4°C until tested.

#### Tofu Extraction

Forty grams of finely chopped tofu was blended with 100 mL of ethanol for 30 s. The resultant slurry was filtered through four layers of cheesecloth and a Whatman filter paper No. 4 under vacuum. The residue was extracted with 100 mL of ethanol, and the filtrates were

combined and dried under vacuum at 40°C. The remaining steps were identical with the ones described for the beans.

Ground soybeans and tofus were analyzed for moisture by drying in a convection oven at 70°C for 18 h. Lipid content was determined in dried samples with hot hexane for 4 hr in a Goldfish extraction apparatus. Moisture content ranged from 7.9% to 9.4% for the soybeans and from 74.0% to 81.3% for the tofus, respectively. Lipid content ranged from 22.5% to 25.9% for the soybeans and from 24.6% to 26.0% for the tofus, respectively (dry weight basis).

#### Storage Study

Tofus made from the varieties 2325 and Vinton were stored in a display case at 5°C. Triplicate samples were taken after 0, 2, 4, 6, 9, 12, and 15 days of storage.

#### High-Performance Liquid Chromatography (HPLC)

The chromatographic system consisted of a normal-bonded phase 250 x 4.6 mm Ultrasil NH<sub>2</sub> (Beckman) column with a 3-cm amino guard column (Brownlee Labs). The mobile phase was 1% 2-propanol in hexane at a flow rate of 2.0 mL/min. Twenty-microliter samples were injected by using a 20- $\mu$ L loop.  $\alpha$ -Tocopherol and  $\gamma$ -tocopherol were purchased from Eastman Organic Chemicals (Rochester, NY).  $\delta$ -Tocopherol was isolated from



commercial soybean oil. The soybean oil was extracted twice with 100% ethanol in a ratio 1:1 oil to ethanol. The ethanol was evaporated under vacuum, and the dried extract was dissolved in methanol. For separation and purification, a preparative reversed-phase HPLC system was used. The system consisted of a 250 x 9.4 mm Partisil ODS-3 C<sub>18</sub> (Whatman) semipreparative column with a mobile phase of 3% water in methanol. Total chromatographic analysis time was 12 min. The technique of "heart cutting" was used for the collection of the peaks. A minimum of three passes through the system was necessary before  $\delta$ -tocopherol of acceptable purity could be obtained.

The tocopherols were monitored by the absorbance at 280 nm with a fixed-wavelength UV detector (Beckman/Altex) equipped with one of two flow cells: an analytical cell for analytical work and a preparative cell for collection of compounds. Purity of the compounds used as standards was determined by using silica gel TLC with chloroform as the mobile phase, analytical HPLC, and spectrophotometric scans.

For analysis of recovery efficiency, standards dissolved in ethanol were added to the ground beans and the tofus before extraction. Recoveries for  $\alpha$ -,  $\gamma$ -, and  $\delta$ - tocopherol were 101, 104, and 100% from the ground beans and 94, 107, and 104% from the tofus, respectively.

## RESULTS AND DISCUSSION

Large-scale separation of  $\alpha$ - and  $\gamma$ -tocopherols from a synthetic mixture of tocopherols has been achieved by HPLC using a preparative silica column (Anon., 1984). Isolation of  $\delta$ -tocopherol in milligram quantities from a natural source requires higher resolution than is necessary for a mixture of pure tocopherols because of the presence of other tocopherols and interfering compounds. In soybean oil, separation of  $\gamma$ -tocopherol (present in larger quantities than  $\alpha$ -tocopherol) from  $\delta$ -tocopherol is the critical purification step.  $\gamma$ -Tocopherol has a more similar polarity to  $\delta$ -tocopherol than does  $\alpha$ -tocopherol. Figure 1A shows the chromatogram of an ethanol extract from commercial soybean oil. Two major peaks occupy most of the area under the chromatogram. Peaks 1 and 2 were collected, purified, and identified as  $\delta$ - and  $\gamma$ -tocopherol, respectively. Parts B and C of Figure 1 are chromatograms of the tocopherols during the last heart-cut step.

The first HPLC system for the separation of tocopherols involved silica column with hexane/diisopropyl ether as the mobile phase (Van Niekerk, 1973). Since then, other eluant mixtures for silica columns as well as the more stable reversed-phase systems, normal-bonded phase systems exhibit a better stability and higher resolution of tocopherols (Westerberg et al., 1981), respectively. Chromatograms of a mixture of standards (Figure 2A), of an extract from ground soybeans (Figure 2B), and of an extract from tofu (Figure 2C) reveal satisfactory

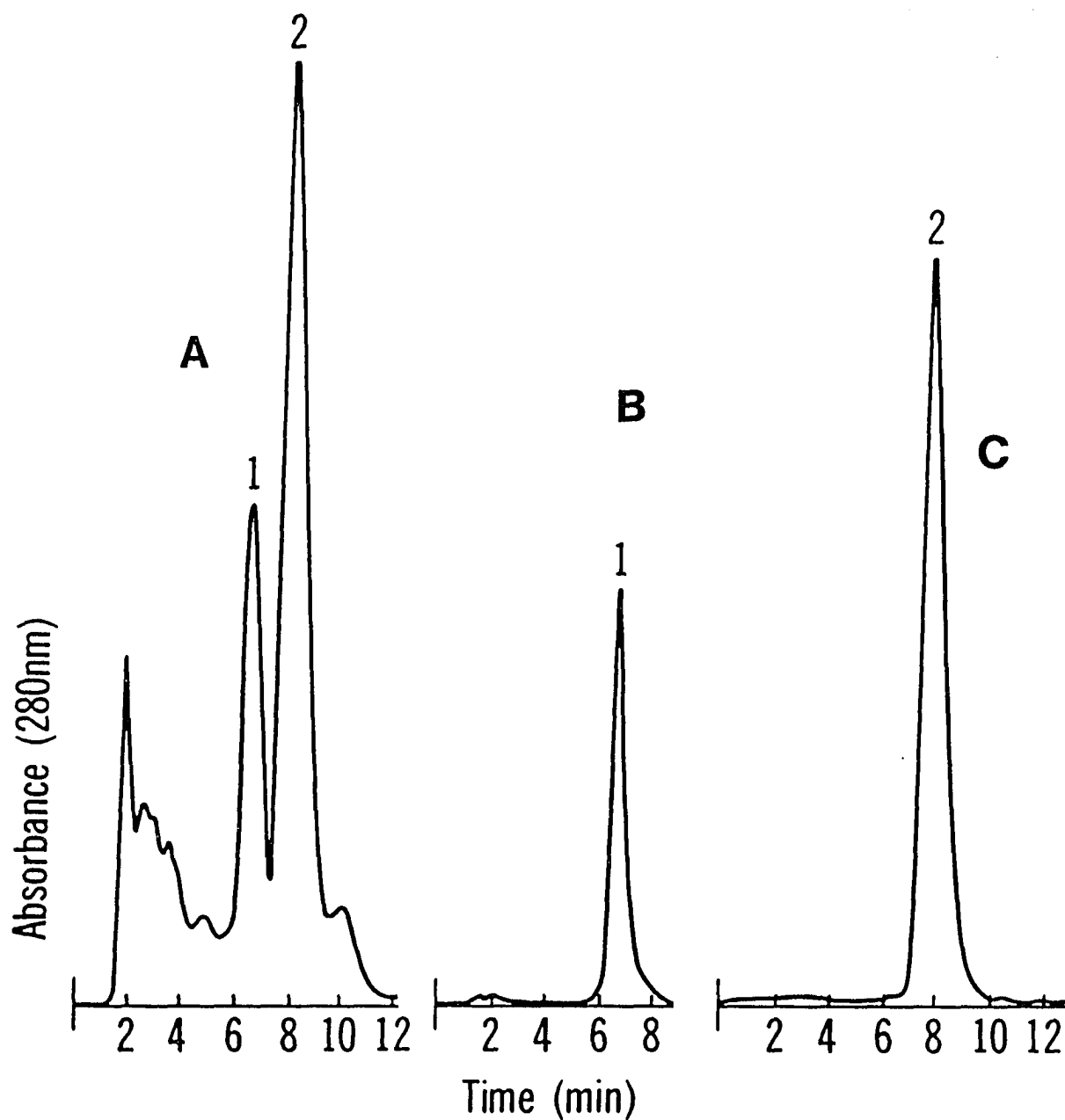


Figure 1. Isolation of  $\delta$ -tocopherol (peak 1) and  $\gamma$ -tocopherol (peak 2) by preparative reversed-phase HPLC. Part A shows an ethanol extract of commercial soybean oil. Chromatograms B and C correspond to isolated  $\delta$ - and  $\gamma$ -tocopherols, respectively, on the reversed-phase preparative column

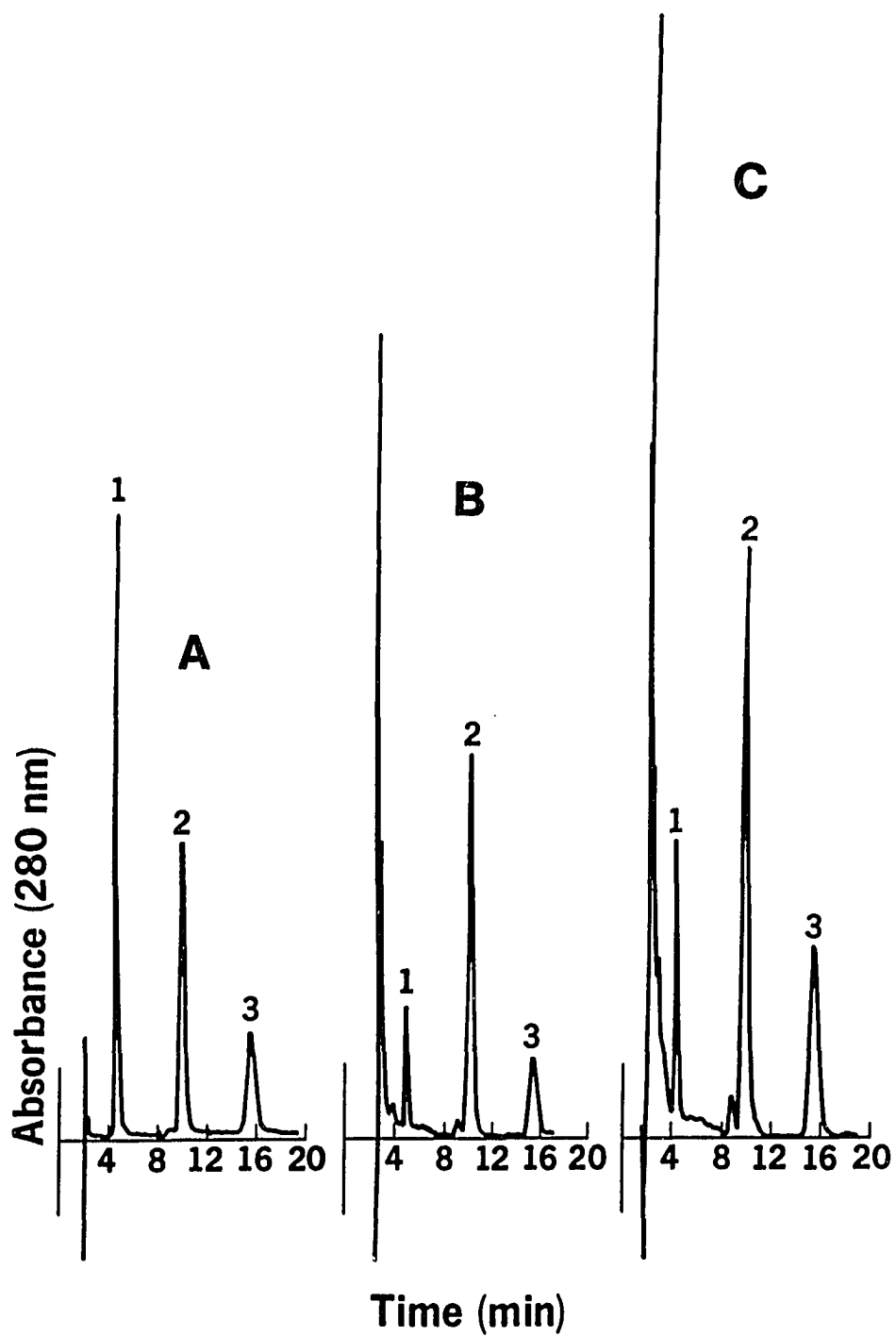


Figure 2. Normal-phase (amino) HPLC separation of  $\alpha$ - (peak 1),  $\gamma$ - (peak 2), and  $\delta$ -tocopherol (peak 3) in a mixture of pure tocopherols (A), an extract from ground soybeans (B), and an extract from tofu (C)

resolution of the three main tocopherols.  $\alpha$ -Tocopherol tends to be eluted early and adjacent to a large peak containing UV light-absorbing compounds of nonpolar nature. Another tocopherol that has been found in soybeans in amounts of about 1% of the total tocopherol is  $\beta$ -tocopherol (Rammell and Hoogenboom, 1985). The small peak eluting earlier than  $\gamma$ -tocopherol probably is  $\beta$ -tocopherol according to the retention time of this compound in polar-bonded HPLC systems similar to the one described herein (Matsuo and Tahara, 1977; Jansson et al., 1980; Westerberg et al., 1981; Rammell and Hoogenboom, 1985). Because of its minimal presence in our soybean varieties, quantitation of  $\beta$ -tocopherol was not included in the present work. For purposes of calculating the total vitamin activity of a sample, however,  $\beta$ -tocopherol is considered half as active as  $\alpha$ -tocopherol and 5 times as active as  $\gamma$ -tocopherol (National Research Council, 1980). In wheat germ,  $\beta$ -tocopherol may be as much as 40% of the total tocopherol content (Quaife, 1948).

In the preparation of foods and feedstuffs for analysis, lipid extraction with hot solvent generally followed by saponification using heat, KOH, and an antioxidant used to be a widespread practice. Extraction at room temperature with nonpolar solvents has been lately used in soybeans (Priestley et al., 1980) and in winged beans (de Lumen and Fiad, 1982). It has been proposed that some of the vitamin E in foods is bound up as a protein complex (Voth and Miller, 1958). With the use of a polar solvent such as ethanol, the investigator has more assurance of extracting all the E vitamers. Saponification is used to

cleave triglycerides and remove impurities of a reducing nature (Contreras-Guzman and Strong, 1982). But, the tocopherols are very unstable in the presence of alkali, particularly if oxygen is present (Ames, 1972). Saponification may be necessary when a sample contains tocopheryl esters, and gas chromatography with fluorescent detection is used for analysis (Van Niekerk, 1982). The presence of triglycerides does not seem to offer interference in the analysis of tocopherols by HPLC. For vegetable oils, injection of a few microliters dissolved in the mobile phase or another appropriate solvent has become accepted practice (Van Niekerk, 1973; Carpenter, 1979; Rammell and Hoogenboom, 1985). A similar approach on extracts from legume beans in which no added tocopheryl esters exist has been applied successfully (de Lumen and Fiad, 1982). Speek et al. (1985) have recently reported the absence of esterified E vitamers in several seed oils including soybean oil. Our extraction procedure was developed with the simplicity required for routine analysis. We took into account the sample size required, time involved, amount of solvent, diversity, and amount of reagents and number of extraction steps. Table I lists the tocopherol contents of the five soybean varieties analyzed and of the tofus made from soybeans of these varieties. The content of the three individual tocopherols is significantly different (99% confidence limit) among ground soybeans of the five varieties analyzed and among their tofus.

A rigorous comparison is not possible using most values in the literature because either the analyses are performed in refined, commercial oil or no reference is given as to the origin of the oil.

Table I. Tocopherol content<sup>1</sup> and vitamin E activity<sup>2</sup> of five soybean varieties and tofus<sup>3</sup>

Variety	$\alpha$ -tocopherol		$\gamma$ -tocopherol <sup>4</sup>		$\delta$ -tocopherol		Vitamin E activity	
	Beans	Tofu	Beans	Tofu	Beans	Tofu	Beans	Tofu
Vinton	28.4 <sup>a</sup>	30.5 <sup>a</sup>	171	187	48.7 <sup>b,c</sup>	61.1 <sup>b</sup>	46.0 <sup>c</sup>	49.8 <sup>b</sup>
2325	10.9 <sup>b</sup>	19.7 <sup>a,b</sup>	145	217	35.5 <sup>c,d</sup>	64.3 <sup>b</sup>	25.9 <sup>a</sup>	42.0 <sup>a</sup>
Corsoy	12.4 <sup>b</sup>	10.5 <sup>b</sup>	161	153	24.6 <sup>d</sup>	35.8	28.7 <sup>a</sup>	26.1
Weber	25.9 <sup>a,b</sup>	22.5 <sup>a,b</sup>	191	215	60.6 <sup>a,b</sup>	76.9 <sup>a,b</sup>	48.7 <sup>b,c</sup>	44.8 <sup>a</sup>
Prize	27.7 <sup>a</sup>	32.6 <sup>a</sup>	150	156	72.6 <sup>a</sup>	85.8 <sup>a</sup>	43.4 <sup>b</sup>	49.1 <sup>b</sup>

<sup>1</sup>Tocopherol content ( $\mu\text{g/g}$  of dry matter).

<sup>2</sup>Vitamin E activity (mg of  $\alpha$ -tocopherol equivalents/kg of dry matter).

<sup>3</sup>Means in a column followed by a common letter are not significantly different at the 5% level.

<sup>4</sup>No significant differences at the 5% level were found for  $\gamma$ -tocopherol means.

Our values, nonetheless, agree with most of the previous reports on the tocopherol content of soybean oil (Brown, 1952; Green et al., 1955; Ward, 1958; Herting and Drury, 1963; Herting and Drury, 1967; Carpenter, 1979; Cort et al., 1983). Refined corn oil contained half as much  $\alpha$ -tocopherol and one-fourth as much total tocopherol as the lipid from freshly extracted seed corn (Herting and Drury, 1963). Refined safflower oil, on the other hand, had  $\alpha$ -tocopherol and total tocopherol levels similar to crude oil.

Tofus are better sources of tocopherol than ground soybeans on a dry basis, reflecting the changes in composition undergone by the beans as they are transformed into tofu. When the soymilk is obtained, the material that is retained by the coarse and fine filters consists mainly of seed hulls. These structures are likely to contain smaller amounts of any vitamin than the internal structures of the soybean seeds.

The loss in tocopherols caused by the processing of soybeans into tofu is described in Table II. As much as 50% of the tocopherols originally present in the soybeans may be lost in the process. Miller et al. (1952) reported the retention of some nutrients in commercially prepared soybean curd. Retention of thiamin was 13-25%; riboflavin, 15-27%; and niacin, 18-47%. Tocopherol retention in our study ranged from 52 to 78%. As a fat-soluble vitamin, it is expected that vitamin E will be lost to a lesser degree than the water-soluble vitamins when the newly formed curd is drained and pressed. The effects of food processing and storage on total tocopherols (Harris, 1962) and specifically on  $\alpha$ -tocopherol (Ames, 1972) in foods have been described



Table II. Total milligrams of tocopherols in 900 g of starting soybeans and in the resulting tofus

Variety	Vitamer	Soybeans	Tofu	% Retention
2325	$\alpha$ -tocopherol	9.0	7.0	78
	$\gamma$ -tocopherol	119.4	76.9	64
	$\delta$ -tocopherol	29.2	22.8	78
Vinton	$\alpha$ -tocopherol	23.4	12.2	52
	$\gamma$ -tocopherol	140.5	75.0	53
	$\delta$ -tocopherol	40.1	24.5	61

in some detail. Some of the processes in which a significant tocopherol loss has been determined are the polishing of rice, the milling and bleaching of wheat flour, the manufacture of breakfast cereals, and the canning of beans, corn, and peas (Ames, 1972). The tocopherols are unstable in the presence of unsaturated fats, oxygen, alkalis, ultraviolet light, and metal ions (Nelis et al., 1985). Wet heating at 80°C for 4 hr had no effect on the tocopherols of wheat flour, but dry heat for 4 hr at 150°C caused measurable destruction (Harris, 1962). The loss in tocopherols observed in the manufacture of tofu may be attributed mainly to the discarding of tocopherol-containing structures and partly to the peroxidation of the unsaturated oils and the contact with oxygen and metal ions (soybeans are high in zinc, iron, and copper) during the heating steps.

The vitamin activity expressed as milligrams of  $\alpha$ -tocopherol equivalents per kilogram of dry matter of ground beans or tofu is shown in Table I. To calculate vitamin activity, the milligrams of  $\gamma$ -tocopherol were multiplied by 0.1 (National Research Council, 1980) and those of  $\delta$ -tocopherol by 0.01. (Bieri and McKenna, 1981), and the sum was added to the milligrams of  $\alpha$ -tocopherol. Even with only 10% of the biological activity of  $\alpha$ -tocopherol,  $\gamma$ -tocopherol contributes 34-59% of the vitamin E activity of the soybean varieties analyzed.

The determination coefficients of the linear regression analysis of the plots of tocopherol content ( $\mu\text{g/g}$  on a dry basis) vs. time (days) of storage of tofu were all not statistically significant ( $P < 0.05$ ). Priestley et al. (1980) aged mature soybean seeds by "natural aging" (long-term storage) and by "accelerated aging" (exposure to high temperature and humidity). The levels of the three main tocopherols ( $\alpha$ ,  $\gamma$ ,  $\delta$ ) and the levels of free radicals were fairly constant after 8 years of natural aging and 4 days of accelerated aging. The manufacture of tofu involves several processes that result in a rearrangement of the seed structural components, with consequent exposure of internal structures to the environment. In addition, considerable heating is employed, and calcium is added in the form of calcium sulfate. It seems, however, that, under the conditions of storage utilized, the tocopherol content of tofu is not significantly affected after a period of 2 weeks.

Kodicek et al. (1959) reported retention of 60% of the  $\alpha$ -tocopherol and one-third of the total tocopherol of corn after storage

for 12 weeks at room temperature. In tortilla prepared from steeped corn in 1% lime water, 95% of the tocopherol was destroyed after the same storage treatment. The stable content of tocopherols during storage of tofu may be attributed to the short storage time and to the environment of reduced oxygen pressure in which the product is held.

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## ACKNOWLEDGMENT

This project was supported by the Iowa Agriculture and Home Economics Experiment Station. The article is published as Journal Paper N. J-12167 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; projects 2433 and 2164, the latter a contributing project to North Central Regional Research Project NC-136. The project was funded in part by the Iowa Soybean Promotion Board. We thank Dr. Lester Wilson for providing technical assistance and Dennis Strayer, Strayer Seed Co., Hudson, IA, who donated the soybean seeds used in this study.

PART II. PROPERTIES OF SOYBEAN-CORN MIXTURES PROCESSED BY  
LOW-COST EXTRUSION



PROPERTIES OF SOYBEAN-CORN MIXTURES PROCESSED BY  
LOW-COST EXTRUSION

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Running title: Properties of Extruded Soybean-Corn...

Submitted to: Journal of Food Science

## ABSTRACT

Ground soybeans and corn at ratios of 60:40, 70:30, 80:20 and 100:0 soybean:corn were extruded with an Insta Pro 600 extruder. Initial moisture content was adjusted to 10%. Extruder exit temperatures were 127, 138, 149 and 160°C. Soybean trypsin inhibitor activity was destroyed 48.9 to 98.8% as exit temperature increased from 127 to 160°C. Extrusion had no major effect on the tocopherol isomers. Lipoxygenase was completely inactivated by extrusion. In vitro protein digestibilities of samples extruded at 127°C were significantly lower than in samples extruded at higher temperatures. Residual lipase activity ranged from 2.7 to 63.7 micromoles H<sup>+</sup>.min<sup>-1</sup>.g<sup>-1</sup>. Lipase may contribute to unique flavor problems.

## INTRODUCTION

An extrusion process for making edible full-fat soy flour was first reported in 1964 (Mustakas et al., 1964). Full-fat soy flours prepared by extrusion have shown to have good nutritive value, flavor and stability (Bookwalter et al., 1971b; Mustakas et al., 1964). Extrusion cooking produced a full-fat soy flour that was successfully added to northern corn bread (a leavened product) and chapatti (unleavened) at levels higher than toasted defatted or commercial steam-processed full-fat soy flour (Bookwalter et al., 1971a). Extrusion-cooked full-fat soybean products have also been used to improve the nutritional quality, acceptability and/or stability of corn-based foods for aid programs (Bookwalter, 1977), breads (Bookwalter et al., 1971b; Harper and Jansen, 1981), and cookies (Tsen et al., 1975).

Low-cost extruder cookers (LCEs) are being used in developing countries to produce nutritious precooked foods based on legumes and cereals (Harper and Jansen, 1985). Low-cost extruders are single-screw autogenous extruders that operate at low moisture (<20%) and require minimal auxiliary equipment. All heat for cooking is developed by friction through viscous dissipation of mechanical energy applied to the shaft of the extruder.

Extrusion of dehulled soybeans at 138-149°C (product exit temperature) using the Insta Pro LCE reduced trypsin inhibitor activity (TIA) from 67 to 91% (Harper and Jansen, 1981). Ringe and Love (1988) found that processing cowpea/corn blends at 170°C with the Insta Pro

extruder results in 84% TIA destruction. Lorenz and Jansen (1980) recommended an extrusion temperature of 143°C for production of full-fat soy flour on a LCE. At this temperature, the corrected protein efficiency ratio (PER) was 1.94 compared with PERs of 1.01 for unextruded soy and 2.5 for casein.

Destruction of natural antioxidants caused by extreme processing conditions is thought to be responsible for the susceptibility of extruded full-fat soy flour to lipid oxidation. But native tocopherols are stable during extrusion at 149°C, with a retention time of 2 min and feed moisture of 15 to 30% (Mustakas et al., 1970). An increase in fat acidity values during storage of extruded mixtures of corn and soybeans has been reported (Harper and Jansen, 1981; Molina et al., 1983), but the residual lipase activity in these products has not been studied.

The utilization of LCEs has centered on the production of inexpensive, highly nutritious, ready-to-eat food products. Successful products have been based on combinations of cereals and legumes whose proteins are complementary in terms of amino acid balance. The purpose of this study was to investigate the effects of substituting increasing percentages of soybeans with whole corn during extrusion at different extruder exit temperatures. Specifically, we wanted to examine the nutritive properties and the potential for flavor problems during storage in high-moisture foods of various extruded blends of soybeans and corn.

## MATERIALS AND METHODS

## Soybeans and Corn

Corsoy 79 soybeans were donated by Strayer Seed Farms (Hudson, IA). Pioneer 3183 corn kernels were donated by Insta Pro (Des Moines, IA). Soybeans and corn were cracked by using a roller mill equipped with rollers having 14 horizontal corrugations per inch. The gap between the rollers was 0.43 mm for soybeans and 0.50 mm for corn. Soybeans and corn were mixed and the moisture of the mixes was adjusted to 10%. Mixes were allowed to equilibrate overnight at 4°C and extruded the next day.

## Extrusion Process

Extrusion was carried out by using an Insta Pro Model 600 extruder, which was operated at a constant screw speed of 550 rpm. The barrel and screw were made up of segments. The screw segments slip over a central keyed shaft with steam locks (ring-like restrictions) placed between them. Primary operation adjustments of the Insta Pro extruder involved the use of steam locks with different diameters, a choice between single-flight and double-flight screws and the use of different die sizes. These restrictions control the temperature profile along the barrel. During extrusion, temperature can be controlled by rotating the nose cone located at the center of the die plate, by changing the

feeding rate and by injecting water at the inlet chamber. The last option was not used in this study.

Table 1 shows the extruder configurations used to process the mixtures. Double-flight screws were used in all instances. Samples were extruded, allowed to cool and ground with the roller mill.

#### Tocopherol Content

The presence of naturally occurring tocopherols was measured by using a modification of a procedure developed in our laboratory (Guzman and Murphy, 1986). Samples were extracted with hexane for 3 hr, and the residue was reextracted with hexane two consecutive times for 1 hr. The extracts were combined, evaporated and analyzed by high performance liquid chromatography (HPLC). The defatted meals were ground and utilized for analysis of in vitro protein digestibility (PD), TIA and lipoxxygenase activity. Extraction of tocopherols was more efficient if ethanol was used for materials that had not undergone extrusion.

#### Trypsin Inhibitor Activity

Trypsin inhibitor activity was determined according to a modification of the AACC method developed by Hamerstrand et al. (1981).

Table 1. Insta Pro 600 extruder configurations for the processing of whole soybeans-whole corn mixtures

Temperature (°C)	Soybean:corn ratio			
	60:40	70:30	80:20	100:0
127	10P-10P-10P-10P	10P-10-8-8	10P-10-10-8	10-8-5-5
138	10P-10P-10P-10P	10P-10-8-8	10P-10-8-5	10-8-5-5
149	10P-10P-10P-10P	10P-10-8-8	10P-10-8-5	10-8-5-5
160	10P-10P-10P-10P	10P-10-8-8	10P-10-8-5	10-5-5-5

### Lipoxygenase Activity

The activities of soybean lipoxygenase 1 and 2 were determined polarographically by using a YSI Oxygen monitor model equipped with a Clark electrode (Yellow Springs Instruments). A solution of linoleic acid was prepared every day by adding 0.1 mL grade III linoleic acid (Sigma Chemical Co.) to 0.1 mL Tween 20. Three-tenths of a milliliter of 0.1 N NaOH was slowly added during stirring, and the dispersion was brought to a final volume of 25 mL with deionized water. The linoleate solution was diluted with 50 mM sodium borate buffer, pH 9.0 or 50 mM sodium phosphate buffer, pH 6.8 for lipoxygenase 1 and lipoxygenase 2 assays, respectively, giving a final concentration of 2.57 mM linoleic acid. Defatted samples were mixed with deionized water, stirred for 3 hr and centrifuged at  $13776 \times g$  for 10 min. The substrate was added to the reaction chamber and aerated by vigorous stirring for 3 min. Enzyme solutions were added to the chamber and the initial velocity of the enzymatic reaction was recorded. Enzyme activity was recorded in units of micromoles  $O_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  of sample.

### Lipase Activity

Lipase activity was measured by using a modification of the method of Moscovitz et al. (1977). A reaction mixture containing 50 mL of 50 mM Tris-HCl buffer, pH 8.3, 10 g olive oil (Sigma Chemical Co.) and 5 g sample was homogenized for 2 min by using a Virtis homogenizer. The



homogenate was incubated at 40°C in a water bath with vigorous stirring. Four-milliliter aliquots were taken every minute and mixed with 16 mL ethanol to stop the reaction. Free fatty acids released were determined by titration to pH 9.5 with 0.02 N NaOH. The enzymatic reaction followed a negative exponential growth curve. The curves were analyzed by using the NLIN (NonLINEar regression) procedure of the Statistical Analysis System (SAS). This procedure yields least-squares estimates of the parameters of a nonlinear model. The model used was  $Y=B_0*(1-EXP(-B_1*X)) + B_2$ , where Y was micromoles of acid titrated and X was time in min.  $B_0$ ,  $B_1$  and  $B_2$  were constants. A lipase activity unit was defined as the amount of enzyme required to produce 1 microequivalent of  $H^+ \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  of sample. All samples were assayed in duplicate.

#### In Vitro Protein Digestibility and Proximate Analysis

In vitro protein digestibility and moisture, crude protein and oil contents were determined according to AOAC methods (AOAC, 1984). Oil content was determined by using the acid hydrolysis method (AOAC method 7.063, AOAC, 1984).

#### Experimental Design

A 4 (soybean/corn ratios) x 4 (extruder exit temperatures) x 2 (replications) factorial design was used. The soybean corn ratios were

60:40, 70:30, 80:20 and 100:0. Extruder exit temperatures were: 127, 138, 149 and 160°C. Fifty-pound batches of ground soybeans and ground corn were extruded, and at least five-kg portions of the product were collected. Samples were stored at -5°C until analyzed. Statistical analyses were performed by using the Statistical Analysis System (SAS). The General Linear Model (GLM) procedure was used to test for linearity and equality of slopes. Probability levels of  $p \leq 0.05$  were considered significant for all statistical procedures.

## RESULTS AND DISCUSSION

Extrusion caused decreases in moisture ranging from 37.5 to 58.5%. The temperature effect on residual moisture content was linear, with higher extrusion temperatures resulting in lower moisture contents (Figure 1). The slopes of the lines for the different mixes were not statistically different.

$\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol contents were 1.2, 18.4 and 5.5 mg per 100 g of soybeans and 1.3, 3.8 and 0.1 mg per 100 g of corn (dry-weight basis). The tocopherols were not affected by the extrusion process. In some instances, the amount of tocopherols extracted by extensive mixing with large volumes of solvent was greater for samples extruded at higher temperatures (Table 2). Greater extractability may have been the result of more tissue disruption at the higher temperatures. No significant differences were found for  $\gamma$ -tocopherol values and these data were not included in Table 2. Retentions greater than 100% for total tocopherols after low-cost extrusion of corn of corn/soybean blends have also been reported by Harper and Jansen (1981).

Inactivation of TIA increased with extrusion temperature (Figure 2). Analysis of variance indicated an overall linear effect of extruder exit temperature on TIA inactivation if the data for the 60:40 mixture was excluded from the analysis. The slopes of the regression lines were significantly different from each other. Trypsin inhibitor deactivation was most temperature dependent in the 70:30 blend and least dependent in the 80:20 blend. Jansen and coworkers (Harper and Jansen, 1981; Lorenz

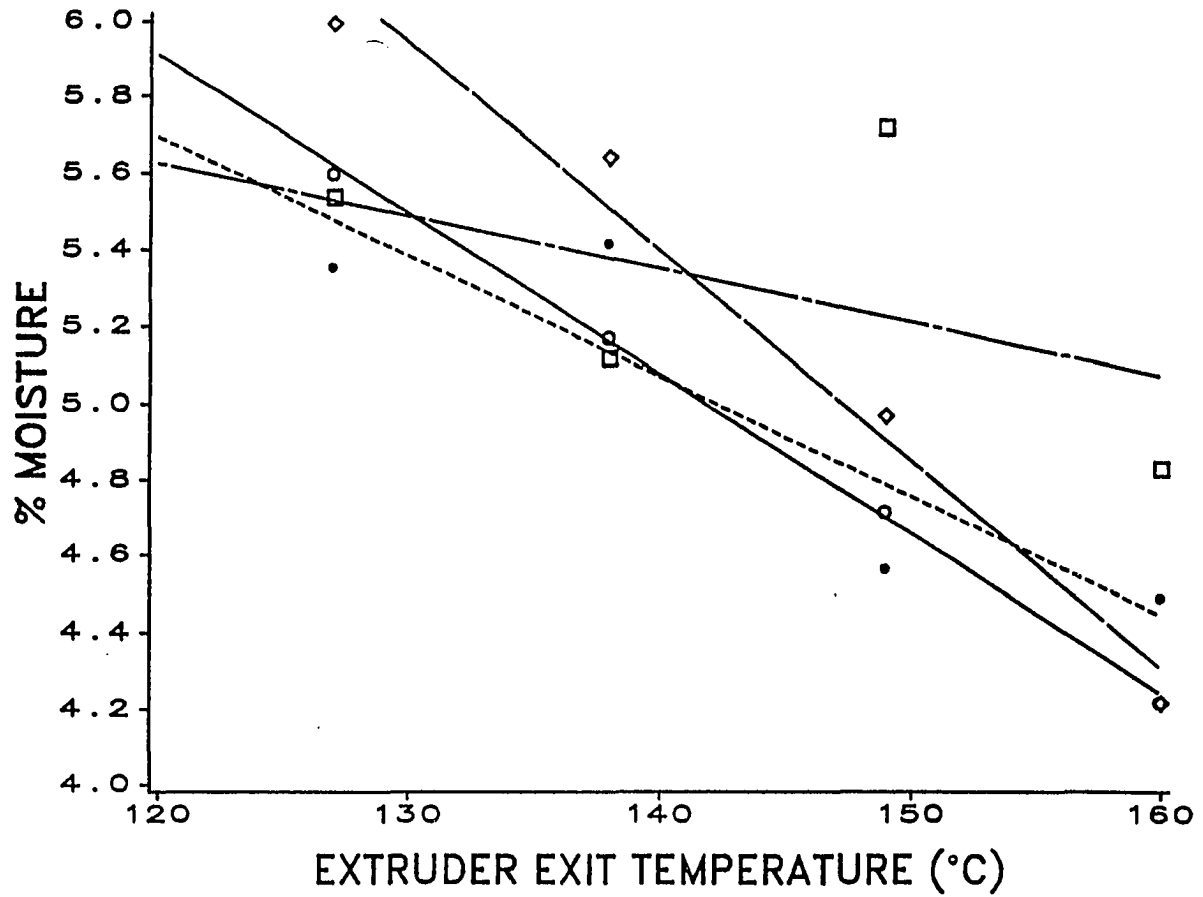


Figure 1. Effect of extruder exit temperature on moisture content.  
□——□ 60:40, ◇——◇ 70:30, ●-----● 80:20,  
○——○ 100:0 soybeans:corn

Table 2. Tocopherol content in mg per 100 g of sample

	Temperature (°C)	Soybean:corn ratio <sup>1,2</sup>			
		60:40	70:30	80:20	100:0
α-Tocopherol	127	0.6 <sup>a</sup>	0.5 <sup>a</sup>	0.8	1.1
	138	0.6 <sup>a</sup>	0.9 <sup>a,b</sup>	1.1	1.1
	149	0.8 <sup>a</sup>	1.1 <sup>b</sup>	0.9	1.1
	160	1.2 <sup>b</sup>	1.2 <sup>b</sup>	0.9	0.9
	MSE	0.2	0.2	0.1	0.3
γ-Tocopherol	127	6.5 <sup>a</sup>	8.5 <sup>a</sup>	16.7	18.3
	138	11.9 <sup>b</sup>	14.1 <sup>b</sup>	16.4	18.9
	149	11.5 <sup>b</sup>	14.1 <sup>b</sup>	16.0	18.2
	160	14.8 <sup>b</sup>	15.5 <sup>b</sup>	13.9	17.5
	MSE	23.7	7.4	7.3	8.3

<sup>1</sup>Values with the same letter within a column are not statistically significant ( $\alpha=0.05$  level).

<sup>2</sup>Columns whose numbers are not lettered indicate a nonsignificant F value ( $\alpha>0.05$  level).

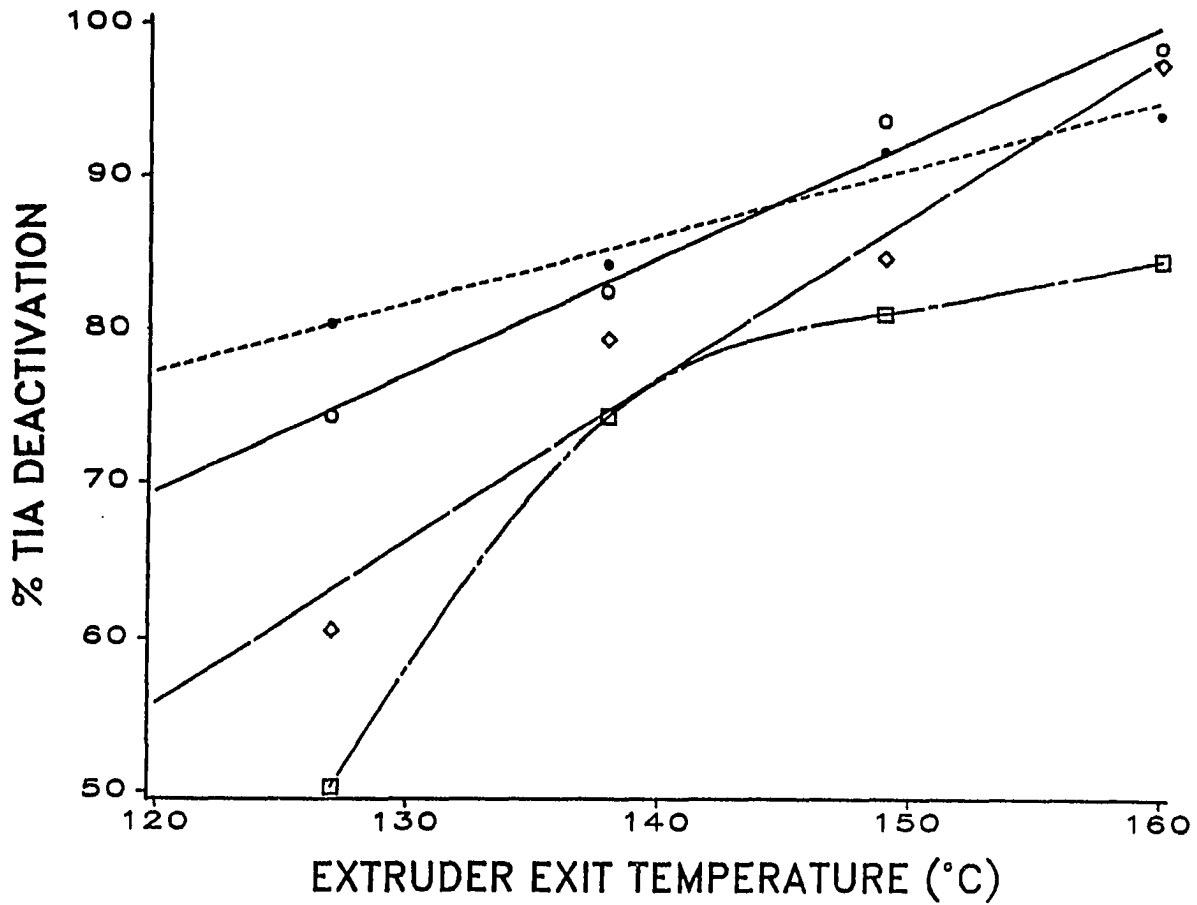


Figure 2. Effect of extruder exit temperature on TIA deactivation.  
 □ — □ 60:40, ◇ — ◇ 70:30, ● — ● 80:20,  
 ○ — ○ 100:0 soybeans:corn

and Jansen, 1980; Jansen, 1979) evaluated the residual TIA in full-fat soybean flour prepared by using different LCEs. They found that 86% TIA was destroyed when whole soybeans with  $5.9 \pm 5\%$  moisture were extruded by using the Insta Pro model (exit temperature,  $149^{\circ}\text{C}$ ) (Jansen, 1979). Our results agreed with their finding (Figure 2). Jansen's group also observed that destruction of soybean TIA increased as extruder exit temperature increased. The temperature effect was observed when the Brady extruder, another LCE, was used (Lorenz and Jansen, 1980; Tsen et al., 1975).

The presence of corn resulted in a protective effect against the thermal deactivation of soybean TIA (Figure 2). The high content of oil in the soybeans acted as a lubricant during the extrusion process. As a result, replacement of a portion of the soybeans with materials that contain less oil (maintaining a constant moisture content) required changing the extruder configuration to less restrictive settings to achieve a similar temperature profile in the extruder. Fewer restrictions to feed flow generates decreased friction and shear. Because the extruder operates at a constant rpm, the decreased shear results in a shorter average residence time (Kuipers, 1987). A similar protective effect was observed by Bressani et al. (1978). They extruded a 70:30 corn:soybean mixture in the Brady LCE. When the moisture content was as low as 13.6%, TIA inactivation was only 15.3%. Inactivation increased to 80% with addition of water to the raw mixture. Likewise, removal of the hulls resulted in improved destruction of TIA in soybeans (Lorenz and Jansen, 1980).

Increasing extrusion temperatures increased in vitro protein digestibilities (PDs) (Figure 3). The lack of fit for a linear model (PD vs. extruder exit temperature) was significant indicating a nonlinear effect of temperature on PD. Average PDs were significantly lower for samples extruded at 127°C ( $\alpha=0.05$  level). Improvements in PD with increasing extrusion temperatures were larger and more consistent for the 70:30 soybean:corn combination. Protein digestibilities of the raw mixtures were 83.5%, 83.8%, 76.7% and 77.0% for the 60:40, 70:30, 80:20 and 100:0 mixes, respectively. Extrusion at all temperatures significantly enhanced the protein quality of the 80:20 and 100:0 mixes ( $\alpha=0.05$  level). The drop in PD of the 100% soybean sample after 149°C probably was due to the extremely restrictive extruder configuration required to achieve higher temperatures. We do not have an explanation for the same phenomenon in the 60:40 blend. Phillips and Baker (1987) reported values of 77.8% PD for raw cowpea meal and 83 to 85% PD for extruded cowpea products. Different extrusion temperatures (150, 175 and 200°C) did not cause significantly different PDs. Protein quality evaluated by the PER (protein efficiency ratio) method was affected favorably by increasing the temperature of extrusion of soybeans (Lorenz and Jansen, 1980) and of a 70:30 corn:soy mixture (Molina et al., 1983). Lorenz and Jansen (1980) also observed a drop in PER at high extrusion temperatures.

When the protein digestibility was plotted against TIA deactivation, a linear trend was observed (Figure 4): In general, samples whose TIA was deactivated to a larger extent exhibited a better



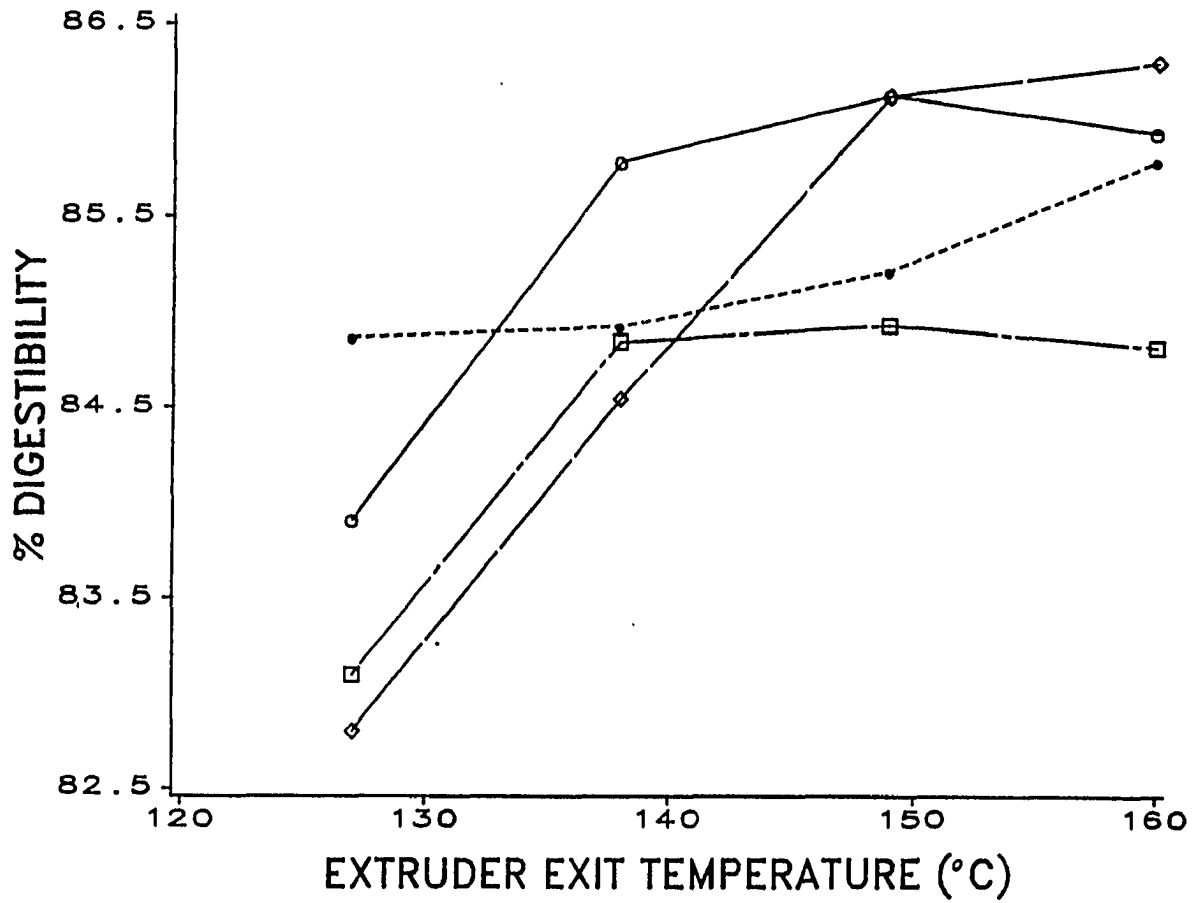


Figure 3. Effect of extruder exit temperature on in vitro protein digestibility. □—□ 60:40, ◇—◇ 70:30, ●—● 80:20, ○—○ 100:0 soybeans:corn

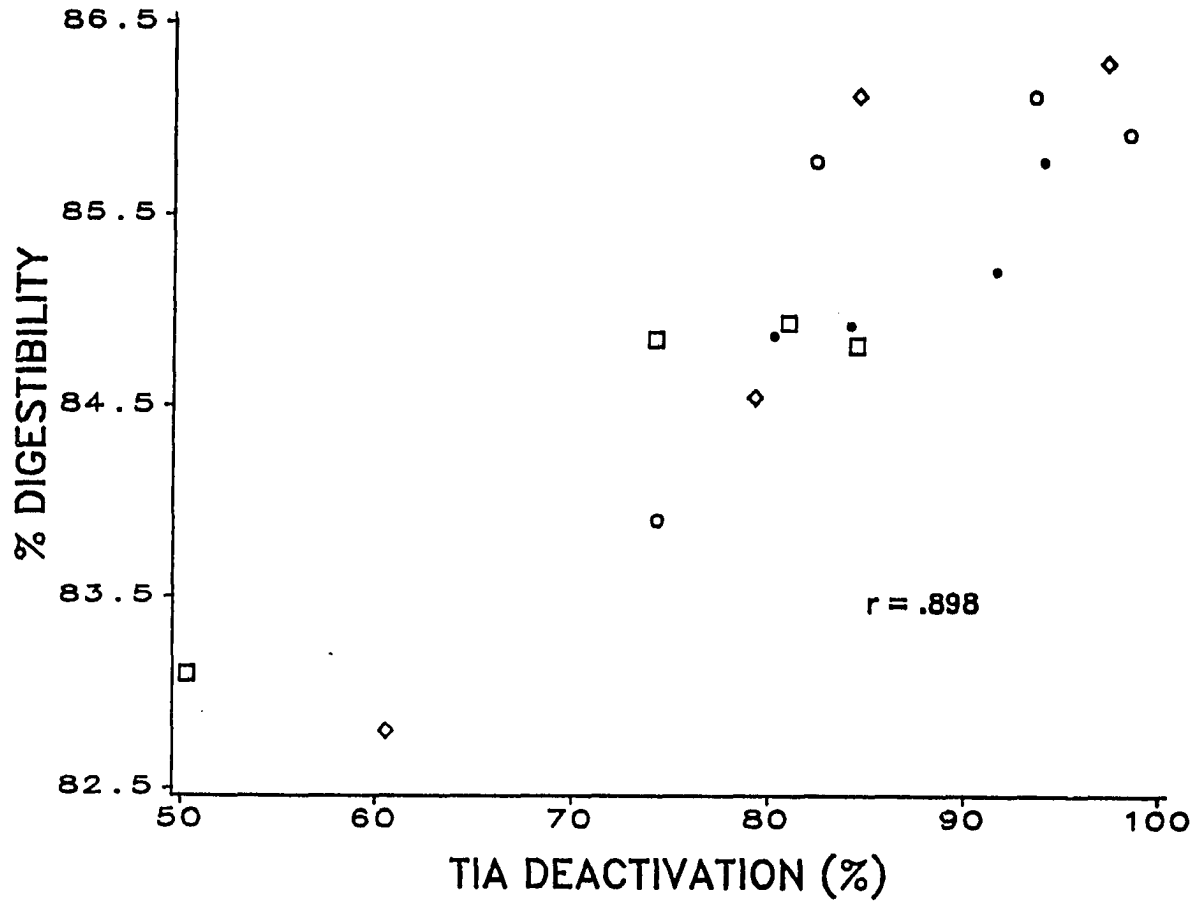


Figure 4. In vitro protein digestibility versus %TIA deactivation.  
□ 60:40, ◇ 70:30, ● 80:20, ○ 100:0 soybeans:corn

PD. The lack of fit could be indicative of factors other than TIA destruction that are responsible for enhanced PD.

Activities of lipoxygenase 1 and 2 were 1420 and 733 units in the raw soybeans and 2.83 and 0.84 units in the raw corn, respectively. The activities of native soybean lipoxygenases 1 and 2 were completely destroyed in all extruded samples.

Fatty acid production at pH 8.3 and 40°C followed a negative exponential curve (Figure 5). The constants  $B_0$  and  $B_1$  in the equation indicate the amplitude of the curve on the Y axis and the rate of change of the slope, respectively. The derivative of the curve at time zero ( $B_0 \cdot B_1$ ) is an estimate of the initial activity. Initial activity of lipase was 14.2 and 4.4 micromoles  $H^+ \cdot \text{min}^{-1} \cdot g^{-1}$  of sample for raw soybeans and corn, respectively. The means of lipase initial activity for all combinations of blends and temperature are shown in Table 3. The effects of extrusion temperature on lipase activity were not clear. Samples processed at 160°C had the highest initial activity in the 60:40, 80:20 and 100:0 blends. Equivalent plots of initial activity and of parameter  $B_1$  (indicative of the rate of change of the slope) followed the same pattern for each blend. Plots of  $B_0$  (indicative of total activity in the first 8 min of reaction) did not show any trend. When the average lipase initial activity of each blend was plotted against the percentage of soybeans, a linear trend was observed between 70% and 100% soybeans. The average for 60% soybeans fell out of the curve.

Harper and Jansen (1981) observed increases in the free fatty acid (FFA) content upon storage of 70:30 corn:soy mixtures, raw or processed

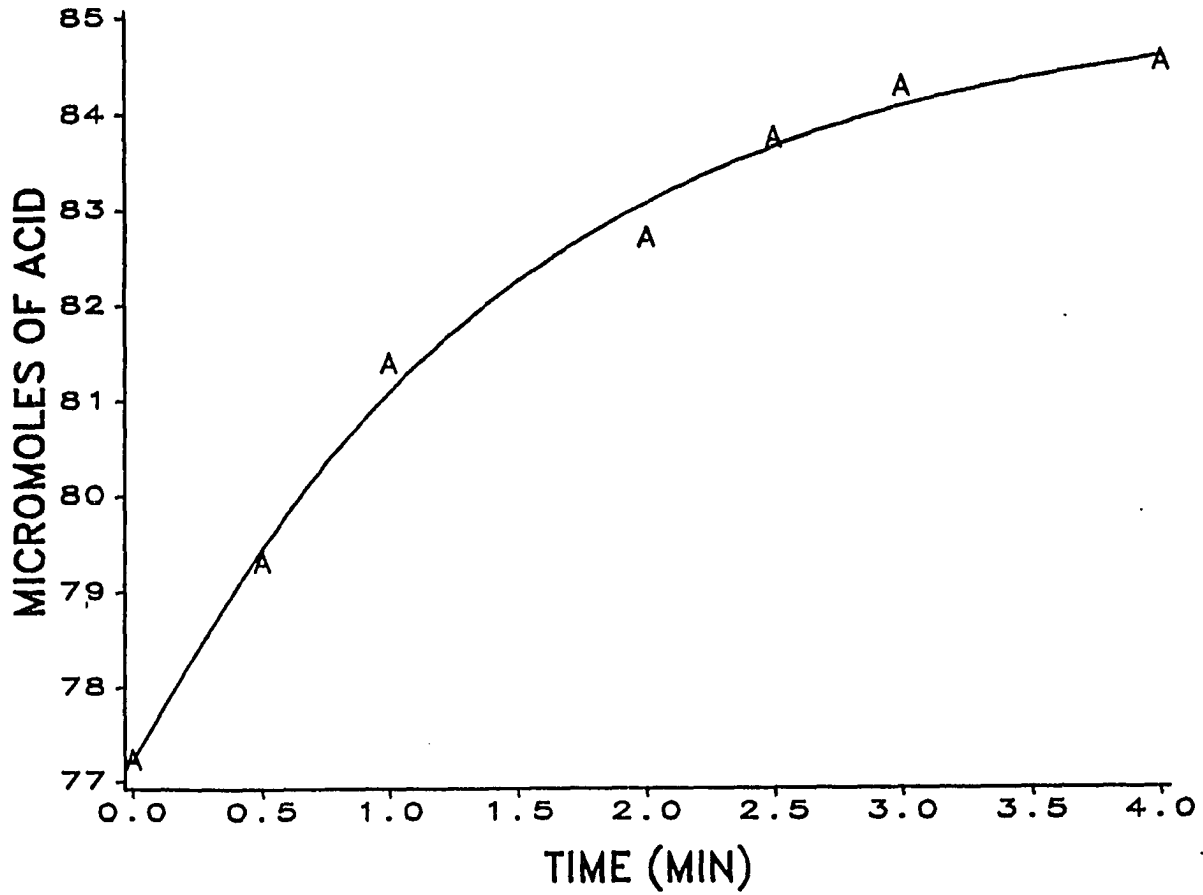


Figure 5. Typical reaction curve for lipase. Conditions of the assay: 40°C, pH 8.3. A, actual observed values

Table 3. Lipase initial activity<sup>1</sup>

Temperature (°C)	Soybean:corn ratio			
	60:40	70:30	80:20	100:0
127	19.5	6.3	18.4	22.3
130	7.2	13.2	8.7	13.5
149	27.8	11.0	21.2	9.0
160	31.7	3.2	22.3	56.6

<sup>1</sup>In micromoles H<sup>+</sup>.min<sup>-1</sup>.g<sup>-1</sup>.

with an Insta Pro extruder, and packed in two different types of bags. In most cases the extruded samples showed minimal increases (up to less than 2% total fat as FFA) after 6 months. Two extruded samples (stored at 38°C and 40% relative humidity), however, had an increase in FFA from less than 1% to more than 20% of the total fat. The authors attributed this unexpected result to the probable deterioration of the bag. Levels of FFA remained low for ground soybeans, raw or extruded, and stored under the same conditions. Increases in FFA during storage of corn/soy and rice/soy extruded mixtures packed in polyethylene bags that were inside a triple paper bag were observed by Molina et al. (1983). Samples stored at 35°C had from 7 to 10% of their fat as FFA. Our results showed that a heat-resistant lipase activity remains in soybeans extruded alone or in combination with corn. Under certain storage or

handling conditions, the enzyme may cause a deleterious change in the quality of these products.

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## ACKNOWLEDGMENT

This project was supported by the Iowa Agriculture and Home Economics Experiment Station. The article is published as Journal Paper N. J-1311 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; projects 2433 and 2164, the latter a contributing project to North Central Regional Research Project NC-136.

PART III. DETERIORATION OF A SOYBEAN-CORN  
MIXTURE PROCESSED BY LOW-COST EXTRUSION

DETERIORATION OF A SOYBEAN-CORN  
MIXTURE PROCESSED BY LOW-COST EXTRUSION

by

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Running title: Deterioration of Soybean-Corn...

Submitted to: Journal of Food Science

## ABSTRACT

The effects of temperature and relative humidity on the physicochemical and aroma changes for a soybean-corn mixture processed by low-cost extrusion were determined. An Insta Pro 600 extruder-cooker was used to process a blend of 80% soybeans and 20% corn with a moisture content of 10%. Four temperatures (15, 25, 35 and 45°C) and two relative humidities (RH) (11 and 65%) were used during a 15-week storage study. Production of volatiles followed apparent zero-order reaction kinetics up to week 12 of storage. Activation energies ( $E_a$ ) ranged from 4.2 to 37.4 Kcal/mol and were higher at the lower RH than at the higher RH for the majority of compounds analyzed. Free fatty acid (FFA) production was observed at 35 and 45°C, respectively. The aromas of all samples stored at 65% RH were judged undesirable by an untrained panel by week six of storage. Samples stored at 25 and 15°C with 11% RH were judged acceptable by more than 60% of the panelists after 14 weeks of storage.

## INTRODUCTION

One of the main purposes of thermally processing foods is to render the final product more resistant to deterioration during storage. Extrusion cooking of legumes and cereals has proven effective in destroying microorganisms and inactivating undesirable biological factors with a consequent improvement in the stability of the extrudates (Mustakas et al., 1970; Bookwalter et al., 1971a, 1971b; Molina et al., 1983). Mustakas et al. (1964) showed that an extrusion-cooked full-fat soy flour had a low peroxide value (PV) (3.0 meq/kg of oil) and low FFA (0.99% of fat as oleic) at the end of 39 weeks when stored at 38°C and 45% RH, but, the PV was 6.4 after 15 weeks and FFA was 4.84% after 39 weeks, respectively, when the sample was stored at 45°C and 25% RH. Sensory evaluation was not performed on their samples after storage. In a related optimization study (Mustakas et al., 1970) where lipoxygenase was inactivated before extrusion, Mustakas' group reported 'acceptable' peroxide values and flavor scores for adequately extruded full-fat soy flours stored for up to 12 months in glass bottles in the dark at room temperature. Peroxide values and panel flavor scores followed similar trends. Rancidity scores ranged from slight to moderate and FFA levels remained low for full-fat soy flours extruded with an Insta Pro extruder at different temperatures and stored at 22 and 38°C with 40 and 70% RH (Harper and Jansen, 1981).

Harper and Jansen (1985) stored raw or low-cost extrusion-processed blends of cereals and soybeans. The raw soy-corn mixture showed a

slight degree of rancidity (evaluated by a panel) after 6 months at 38°C with 40 or 70% RH in spite of very high FFA levels (75% of the fat). In contrast, the same blend after extrusion had a moderate rancidity score but low FFA levels (1.46% of the fat).

The inconsistencies that come up in the sensory analyses of legume/cereal-based extruded products during storage studies point to the fact that several deleterious reactions take place at the same time. A typical example of the occurrence of interactive reactions in foods is the oxidation of lipids with concomitant nonenzymatic browning. The carbonyls formed in lipid oxidation can increase the rate of browning (Pokorny, 1981) and the rate of oxidation may be decreased by compounds produced in the browning reaction (Lingnert, 1980). Labuza (1985) describes two hypothetical case studies of shelf life where microbiological, chemical and physical changes that take place upon storage of frozen pizza and dehydrated mashed potatoes are considered. Application of numerical techniques to predict the storage life of potato chips undergoing spoilage by water uptake and lipid oxidation was shown by Quast and Karel (1972a).

Although previous storage studies involving extruded mixtures of cereals and legumes have provided valuable information, a systematic approach is required before predictions of shelf life can be made. A representative illustration of such an approach was given by Ringe and Love (1988). They stored an extruded cowpea-corn flour blend at 3 RH's and 4 temperatures for the purpose of elucidating the kinetics of lysine loss through nonenzymatic browning (NEB).

We have reported elsewhere the complete inactivation of soybean lipooxygenases 1 and 2 after extrusion of mixtures of soybeans and corn at various temperatures (Guzman et al., 1988). Native tocopherols were resistant to thermal breakdown, but there was significant lipolytic activity after extrusion. We have noticed serious undesirable odors in extruded blends that were kept at room temperature. In this study, we conducted a systematic shelf stability test of a selected extruded soybean-corn mix.

## MATERIALS AND METHODS

## Soybean-Corn Meal Production

Corsoy 79 soybeans were donated by Strayer Seed Farms (Hudson, IA). Pioneer 3183 corn kernels were donated by Insta Pro (Des Moines, IA). Soybeans and corn were cracked with a roller mill, mixed at a ratio of 80% soybeans and 20% corn, and the moisture was adjusted to 10%. The mix was allowed to equilibrate overnight at 4°C and extruded the next day. A 50-kg batch was extruded with an Insta Pro Model 600 extruder at an extruder exit temperature of 150°C. The cooled extrudate was transferred to plastic bags that were put in 5-kg hard plastic buckets with snap-on lids and stored at -30°C under N<sub>2</sub> until used.

A sorption isotherm for the product was obtained by the proximity equilibration cell technique (PEC) described by Lang et al. (1981). The sample used for determination of the sorption isotherm was vacuum-dried for 12 hr at 25°C.

## Storage Study

Approximately 700 g soybean-corn meal was vacuum-dried for 12 hr at 25°C. The dried meal had a moisture content of 3.57%. Fifty 20-mL scintillation vials were filled with 8.0 g of dried meal and were put in a plastic desiccator which contained a saturated salt solution. Saturated salt solutions of LiCl and NaNO<sub>2</sub> (for a<sub>w</sub> 0.11 and 0.65,



respectively) were prepared in the desiccators by slowly adding salt to 100 mL deionized water that was being constantly stirred. The desiccators were placed in temperature-controlled chambers and allowed to equilibrate. During the next two weeks, the desiccators were opened and the salt solutions were adjusted by adding water or salt. Four temperatures of 15, 25, 35 and 45°C were used to give a total of eight storage conditions. The samples were pulled out of the desiccators for analysis at increasing weekly intervals for up to 16 weeks. Sampling intervals were determined on the basis of temperature of storage after observing the rate of deterioration of the sample stored at 45°C.

#### Tocopherol Content

The presence of naturally occurring tocopherols was measured by using a modification of a procedure described by Guzman and Murphy (1986). Samples were extracted with hexane for 3 hr, and the residue was reextracted two consecutive times for 1 hr each. The extracts were combined, evaporated and analyzed by high performance liquid chromatography.

#### Lipoxygenase Activity

The activities of soybean lipoxygenase 1 and 2 were determined polarographically by using a YSI Oxygen monitor model equipped with a Clark electrode (Yellow Springs Instruments).

### Lipase Activity

Lipase activity was measured by using a modification of the method of Moskowitz et al. (1977). A reaction mixture containing 50 mL of 50 mM Tris-HCl buffer, pH 8.3, 10 g olive oil (Sigma Chemical Co.) and 5 g sample was homogenized for 2 min with a Virtis homogenizer. The homogenate was incubated at 40°C in a water bath with vigorous stirring. Four-milliliter aliquots were taken every minute and mixed with 16 mL ethanol to stop the hydrolytic reaction. Free fatty acids released were determined by titration to pH 9.5 with 0.02 N NaOH. Initial activity of lipase was estimated from the slope of the reaction curve at time zero and its units are given in micromoles  $\text{H}^+ \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  of sample.

### Aroma Panels

Five vials were pulled at every sampling time, capped, fitted into an aluminum foil wrapper, coded with three-digit random numbers and stored at -30°C under  $\text{N}_2$ . An untrained panel of at least 20 judges evaluated the samples by using a multiple comparison test (Larmond, 1977). Evaluations were made at room temperature (22-25°C) in isolated booths under gold-fluorescent lights. The judges were asked to compare each of three or four different samples with a reference sample. The reference was prepared from a batch of extruded mix that had been stored at -30°C under  $\text{N}_2$ . The judges determined whether the odor of the samples was similar to or different from the reference. The judges were

also asked to evaluate the amount of difference by using a scale that included none, slight, moderate, large and extreme rankings. The judges were finally asked to indicate whether the odor was unpleasant or not and to give any comments.

### Analysis of Volatiles

The concentration of volatiles in the stored samples was determined by using a modification of a method developed by Fritsch and Gale (1977). Four grams of sample were weighed into a 100-mL headspace vial that was kept in a water bath with boiling water. A test tube containing 1 mL of a  $10^{-5}$  M aqueous solution of 4-heptanone (internal standard), prepared on the previous day and stirred overnight, was introduced in the headspace vial. Fifty milliliters of boiling distilled water was poured into the vial and the vial was capped with a teflon/rubber septum and sealed. After one minute, a 100-microliter volume was drawn from the headspace and injected into a Varian 2400 gas chromatograph (GC) equipped with a flame ionization detector and a Beckman 427 integrator. A 30-m fused-silica megabore (0.53 mm i.d.) DB-1 column with a 1.5 micron-thick coating (J&W Scientific, Rancho Cordova, CA) was used. Oven, injector and detector temperatures were 100, 200 and 250°C, respectively.

Identification of the compounds present in the headspace of the stored samples was approached by different methods. First, retention times (RT) of appropriate standards were compared to the peaks obtained

in the storage study by using the same conditions that were utilized in the storage study. Since such conditions were developed for quick, routine analysis based on little knowledge of the progress of the sample through storage, they were not optimized for peak resolution and identification. Second, a Hewlett Packard 5970 series Mass Spectrometer (Hewlett Packard Company, Palo Alto, CA) coupled with a Varian Aerograph series 1520 Gas Chromatograph was used to identify each peak, when possible. The mass spectrometer was less sensitive than the flame ionization detector so samples were concentrated using the following techniques. Nitrogen was used to purge the headspace of samples placed in a Wheaton purge and trap glass system (Wheaton Scientific, Millville, NJ), and trapping the volatiles in a TENAX (Analabs, North Haven, CT) column. The column was made by packing a 3-cm long bed of TENAX between two glass wool plugs inside a disposable Pasteur pipet. A styrofoam cup was pierced and the column inserted in the cup through the orifices. The entry and exit sites on the cup were sealed with vaseline. The resin was cooled with iced water during the volatile collection. Nitrogen was gently blown through the sample for 2 hr. The column was then removed and the adsorbed compounds were eluted from the column by washing it with dry ethyl ether to collect 0.5 mL of eluant in a glass centrifuge tube. Third, two microliters of the ether extract were injected (splitless injection) in a Varian 3740 GC equipped with a 30 m DB-5 fused silica (0.25 mm ID, 1.0-micron thick coating) capillary column (J&W Scientific, Rancho Cordova, CA). The column was held at 60°C for 4 min and programmed to 150°C at 10°C/min. The

detector sensitivity was set at  $1 \times 10^{-12}$  amps/sec. Retention times of appropriate standards injected under the same chromatographic conditions were compared to RTs in the chromatogram for a sample. Some compounds were further identified by enriching the ether extract by mixing 0.1 microliter of standards in the syringe before injection in the GC.

A final approach to compound characterization was smelling the eluant from the column. The detector tower was removed, the flame turned off and the make-up gas shut off. Three microliters of the ether extract were injected. The eluants were smelled and characterized by two of the authors.

#### Fat Acidity

Fat acidity was determined according to a modification of the AOAC method 14.071 (AOAC, 1984). Eight grams of sample were extracted with hexane in a goldfish extraction apparatus for 12 hr. The hexane was evaporated, and the extract was dissolved with phenolphthalein-containing solvent and titrated with KOH.

## RESULTS AND DISCUSSION

The sorption isotherm for the soybean-corn meal is shown in Figure 1. The BET monolayer value, calculated according to Labuza (1968), was 0.032 g H<sub>2</sub>O per g of solids which corresponds to an  $a_w$  of 0.12.

## Enzymatic Activity

Activities of lipoxygenase 1 and 2 in the raw soybeans were 1420 and 733 micromoles O<sub>2</sub>.min<sup>-1</sup>.g<sup>-1</sup> of sample. Soybean lipoxygenase activity was thoroughly destroyed by extrusion. Initial activity of lipase was 14.3 and 4.4 units in raw soybeans and raw corn, respectively. Lipase activity in the extruded blend was 17.8 micromoles H<sup>+</sup>.min<sup>-1</sup>.g<sup>-1</sup>. Lipase activity after 16 weeks of storage at 65% RH and 45, 35, 25, and 15°C was 6.7, 19.1, 13.3, and 7.9 units, respectively, and 48.4 and 4.4 units in samples stored at 11% RH and 25 and 15°C, respectively. The reproducibility of the method described here for initial lipase activity in these extruded soybean-corn samples was small. The coefficient of variability for seven runs of one sample was 83%. A refined version of this method has been worked out in our laboratory. Preliminary results obtained using the new method are close to the values reported in this paper.

Release of fatty acids during storage was detectable in samples stored at 65% RH and 45 and 35°C only (Figure 2). The rates of FFA release were  $0.135 \pm 0.009$  and  $0.050 \pm 0.014$  meq FFA per week per kg

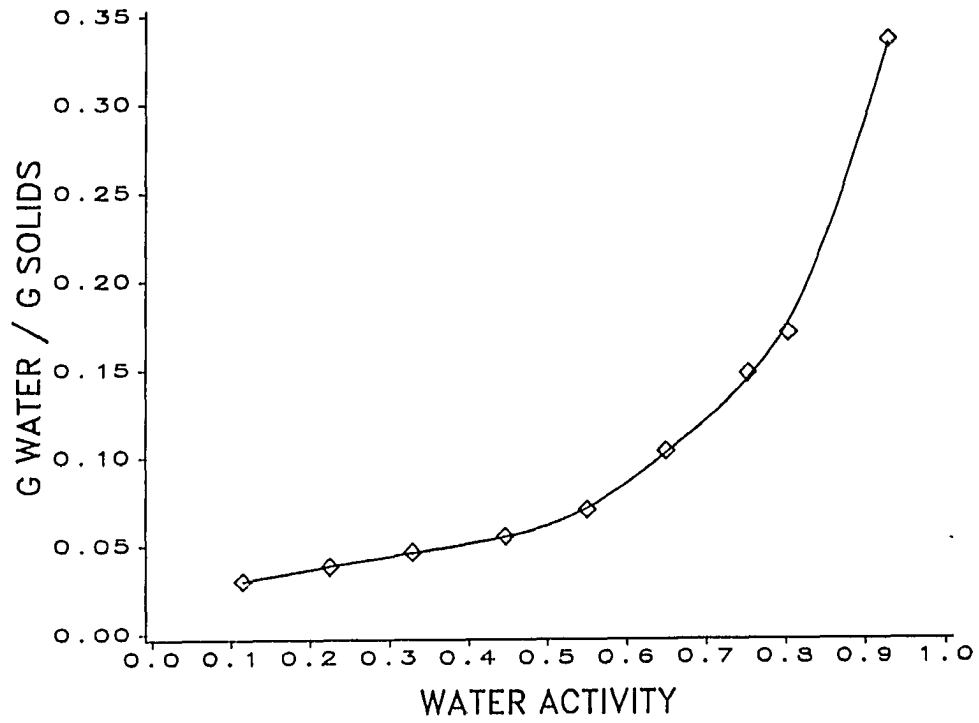


Figure 1. Sorption isotherm at 25°C for the extruded soybean-corn blend

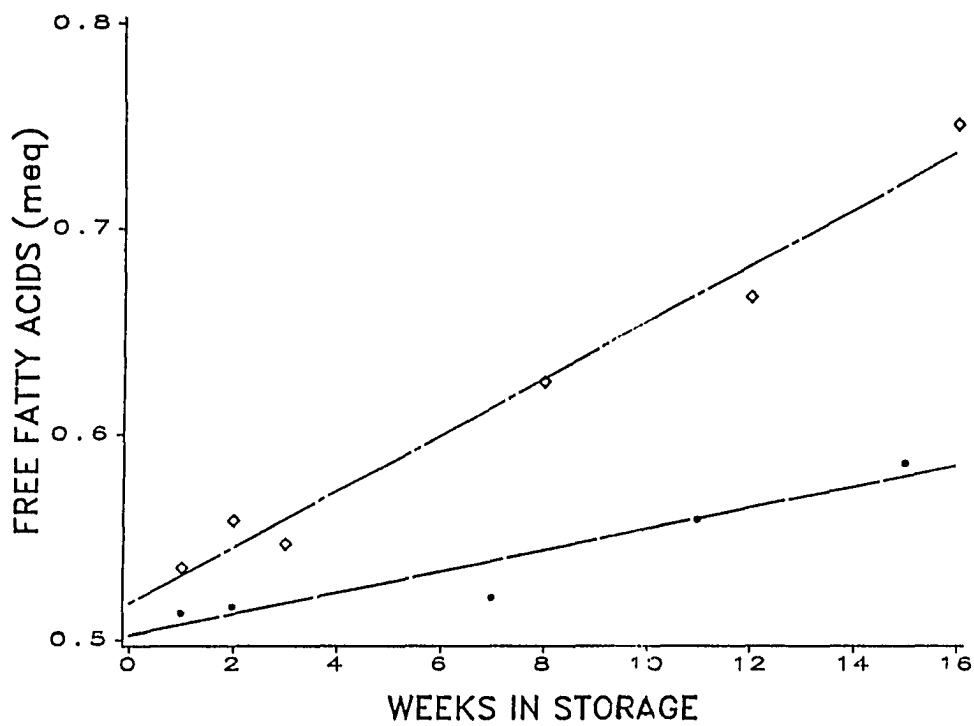


Figure 2. Free fatty acid production during storage at 65% RH.  
● — — — ● 35°C, ◇ — - — ◇ 45°C



(dry weight) of sample at 45 and 35°C, respectively. The values given correspond to an increase in FFA (calculated as oleic) from 0.77 to 1.09 as percent fat. Our results were similar to those obtained by Harper and Jansen (1985) for full-fat soy flour and soybean-corn blends processed with an Insta Pro extruder using several different processing conditions. The largest increases in FFA during storage were found in flours stored at 38°C and 70% RH compared to samples stored at lower temperatures and/or lower RH. The values for FFA after four months at 38°C and 70% RH ranged between 0.66 and 1.76 as percent of fat. In contrast, when Molina et al. (1983) stored mixtures of whole soybeans:whole corn (30:70) processed by low-cost extrusion at 35°C (no RH specified), FFA increased from about 1 to 10% of the fat after 12 weeks. Considering that low-cost extruded materials exhibit an  $a_w$  close to the monolayer value, Molina's samples probably had very high lipase activities.

#### Gas Chromatographic Analysis and Lipid Oxidation

$\alpha$ ,  $\gamma$  and  $\delta$ -Tocopherol contents after extrusion were  $1.0 \pm 0.1$ ,  $15.0 \pm 0.5$  and  $5.7 \pm 0.2$  mg per 100 g of dry sample. Approximate tocopherol values after 16 weeks of storage are given in Table 1. Samples stored at 65% RH had decreasing  $\gamma$ -tocopherol contents as storage temperature increased.

A chromatogram of a sample stored for eight weeks at 45°C and 65% RH is shown in Figure 3. A total of 14 peaks appear in the chromatogram

shown. A chromatogram for a control (not shown) contains only two peaks which correspond to hexanal and to the internal standard, 4-heptanone. The peak corresponding to hexanal has a retention time of 1.94 min.

Table 1. Approximate tocopherol content<sup>a</sup> after 16 weeks of storage

	RH = 11%			RH = 65%			
	15°C	25°C	35°C	15°C	25°C	35°C	45°C
α-Tocopherol	0.9	1.0	1.0	1.2	1.0	0.7	0.9
γ-Tocopherol	13.7	14.9	14.0	15.0	11.9	7.2	5.3
δ-Tocopherol	5.7	5.5	5.8	5.8	7.5	3.8	3.5

<sup>a</sup>Tocopherol content in mg per 100 g of dry matter.

Peak resolution for samples in advanced stages of deterioration was poor. Use of a capillary column for peak identification demonstrates a better resolution than the megabore column.

Figure 4a and 4b show the development of hexanal in the samples stored at 11 and 65% RH, respectively. Because compounds produced during storage coeluted with the internal standard, the peak areas were adjusted instead by using the formula

$$\text{Adjusted area} = \frac{\text{Peak area} * \text{Total chromatogram area}}{\text{Average of total areas}}$$

where 'Total chromatogram area' is the sum of the areas of all the peaks

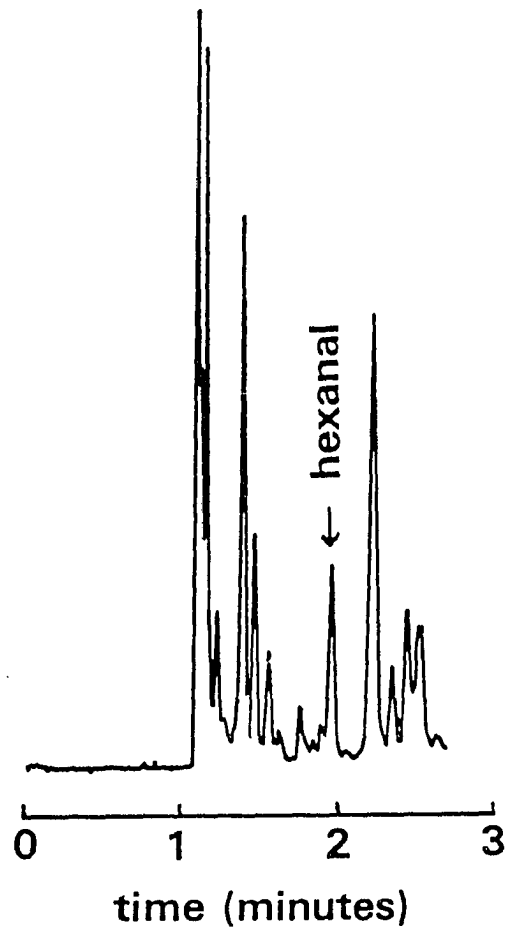


Figure 3. Gas chromatograph of a sample stored at 45°C and 65% RH for 8 weeks

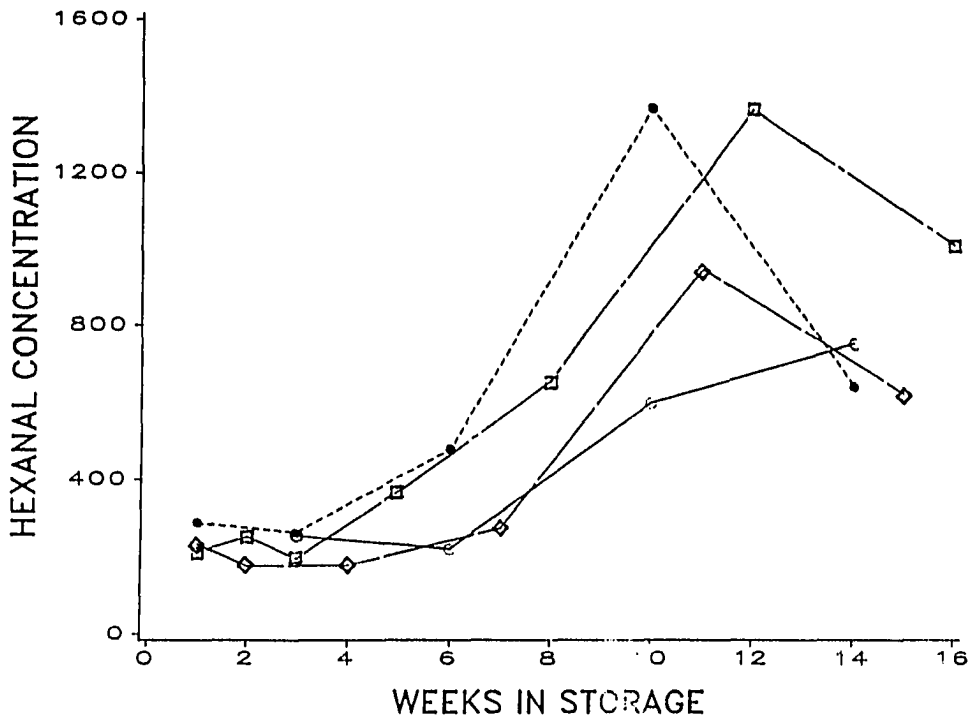


Figure 4a. Hexanal development during storage at 11% RH.

○ — 15°C, ● — 25°C,  
 ◇ — 35°C, □ — 45°C

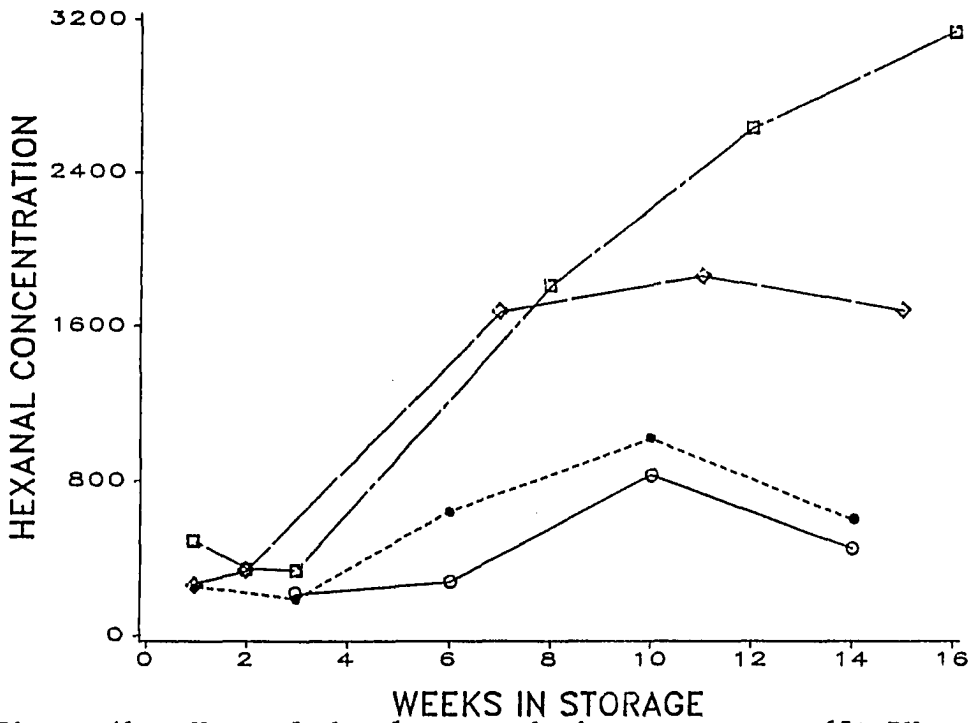


Figure 4b. Hexanal development during storage at 65% RH.

○ — 15°C, ● — 25°C,  
 ◇ — 35°C, □ — 45°C

on the respective chromatogram, and 'Average of total areas' is the average of the total chromatogram areas of the four replicates of the respective sample in the respective sampling day. All samples exhibited an induction period and, with two exceptions, the concentration of hexanal reached a maximum at 10 to 12 weeks and then decreased. The average coefficient of variability of four injections from one sample in a sampling day was  $39 \pm 23\%$ . Apparent zero-order reaction rate constants calculated from the slopes of best-fit regression lines for hexanal production are given in Table 2. Regression lines were calculated from data between the end of the induction period and the maximum hexanal production. Percent errors were largest for 65% RH at 15 and 25°C. Increased variability in data derived from mild storage conditions has been observed in storage studies (Ringe and Love, 1988). Reaction rates increased generally with the temperature increase and with the  $a_w$  increase within each temperature. Table 3 shows the activation energies ( $E_a$ ), and the free energies, entropies and enthalpies of activation for hexanal production. The standard error for  $E_a$  at 11% RH was large due in part to the reaction rate at 25°C (160) which does not follow the trend of the other  $k$ 's. If  $k$  for 25°C was excluded from the regression analysis, the activation energy for 11% RH became 5.69 kcal/mol and the determination coefficient was 0.987. The newly calculated  $E_a$  is about 1 kcal/mol smaller than  $E_a$  for 65% RH. The sensitivity to temperature for hexanal formation was, therefore, higher for samples stored at 65% RH. The values for free energy of activation were similar for both RHs which

Table 2. Apparent zero-order rate constants (k) for hexanal

Relative Humidity	Temperature (°C)	k <sup>a</sup>	Standard error	Percent error <sup>b</sup>	R <sup>2</sup> , <sup>c</sup>
11%	15	49	11	22	0.61
11%	25	160	29	18	0.75
11%	35	86	14	16	0.75
11%	45	128	25	20	0.74
65%	15	90	36	40	0.39
65%	25	116	51	44	0.34
65%	35	238	41	17	0.83
65%	45	242	21	9	0.92

<sup>a</sup>k values are in peak area units per week.

<sup>b</sup>Percent error is (Standard error/k) \* 100.

<sup>c</sup>R<sup>2</sup> is the coefficient of determination for the regression lines of peak area units versus week.

indicated that hexanal was being produced through the same mechanism.

The destruction of lipoygenase, the decrease in the tocopherol contents and the production of hexanal indicate that lipid autoxidation is taking place. The large negative entropies of activation are expected from bimolecular (as opposed to monomolecular) reactions because bringing two molecules together results in more ordering (Price and Dwek, 1979). Bimolecular reactions take place during initiation, propagation and termination of lipid oxidation when oxygen

Table 3. Thermodynamics of hexanal production at two relative humidities

Relative Humidity	$E_a^1$	$\pm$ Standard error	$H^{o\neq 1}$	$G^{o\neq 1}$				$S^{o\neq 2}$
				15°C	25°C	35°C	45°C	
11%	4.21	$\pm$ 4.23	3.61	2.21	2.27	2.33	2.40	-64.0
65%	6.70	$\pm$ 1.62	6.09	2.19	2.24	2.30	2.35	-54.8

<sup>1</sup>Values given are in kcal/mole.

<sup>2</sup>Values given are in cal/mole.

is not limiting (Maloney et al., 1966). The effects of temperature and RH on lipid autoxidation have been investigated in several different food products and model systems (Labuza et al., 1972; Quast and Karel, 1972b). The results indicated that the rate of oxidation was high at very low  $a_w$  and decreased with  $a_w$  until it reaches a minimum. At higher  $a_w$  the rate of oxidation increases with water activity. Lipid oxidation in some foods is applicable to zero-order kinetics (Labuza, 1982). Fritsch and Gale (1977) used the concentration of hexanal to determine oxidative rancidity in breakfast cereals. Production of hexanal was first-order and the  $E_a$ 's for wheat and corn cereal were 14.5 and 19.9 Kcal/mol, respectively, as shown by Labuza (1982). Quast and Karel (1972b) found that the rate of oxygen uptake of potato chips at 37°C was about 50% higher at 62% RH than at 11% RH. The  $E_a$  for oxygen uptake was 10 kcal/mol.

Table 4 lists  $E_a$ 's, free energies, enthalpies and entropies of

Table 4. Thermodynamics of production of volatiles at two relative humidities

Peak number	RT <sup>1</sup> (min)	RH (%)	E <sub>a</sub>	H <sup>0±2</sup>	G <sup>0±2</sup>			S <sup>0±3</sup>
					25°C	35°C	45°C	
4	1.63	11	37.39	36.00	24.23	23.81	23.39	42.08
4	1.63	65	35.22	34.61	24.19	23.84	23.49	34.96
5	1.76	11	21.88	21.27	23.36	23.43	23.50	-7.01
5	1.76	65	16.92	16.31	23.40	23.64	23.87	-23.79
11	2.24	11	12.78	12.17	21.85	22.18	22.50	-32.47
11	2.24	65	15.10	14.49	22.15	22.41	22.66	-25.70
12	2.37	11	10.34	9.72	23.00	23.45	23.89	-44.54
12	2.37	65	7.26	6.65	23.19	23.74	24.30	-55.46
14	2.54	11	9.09	8.47	22.48	22.95	23.42	-46.96
14	2.54	65	6.93	6.32	22.58	23.12	23.67	-54.53

<sup>1</sup>RT is the retention time in the megabore column.

<sup>2</sup>Values given are in kcal/mole.

<sup>3</sup>Values given are in cal/mole.

activation for five of the 14 peaks shown on Figure 3. Although identification of all the peaks was attempted but not achieved, some observations concerning the values shown in Table 4 can be helpful in the overall analysis. In contrast to hexanal, the E<sub>a</sub>'s and the entropies of activation for four of the compounds described in Table 4 were smaller for 65% RH than for 11% RH. These peaks are probably being



produced by a different mechanism. However, the differences observed may not be statistically significant. Error estimates for the peak whose thermodynamic parameters were similar to those of hexanal and whose area showed the greatest increase over time were calculated. The percent error for the zero-order rate constants ranged from 3.8 to 18.8% with an average of 9% compared to an average of 23% for hexanal. However, the percent errors for  $E_a$  were 20 and 33% for 11 and 65%, respectively.

A chromatogram of a headspace concentrate from a sample stored at 45°C and 65% RH is shown in Figure 5. The chromatograms obtained in the storage study included peaks that eluted before 6.85 min in Figure 5. A total of 78 peaks were obtained using this technique. Although during the first 6.85 min of the GC run we consistently detected odors which we defined as solvent-like, alcohol, aldehyde, burnt and stale, strong odors were also detected up until 13 min into the run. Other descriptions after 6.85 min include burnt toast, graphite, musty, barny, mildewy, stale grassy, floury and cereal-like. Table 5 lists four compounds that were identified.

#### Odor Panels

The judges in the odor panels were asked to evaluate the amount of difference between the stored samples and a control at every sampling time. The scale descriptors included: none, slight, moderate, large and extreme difference. The evaluations of the judges were transformed

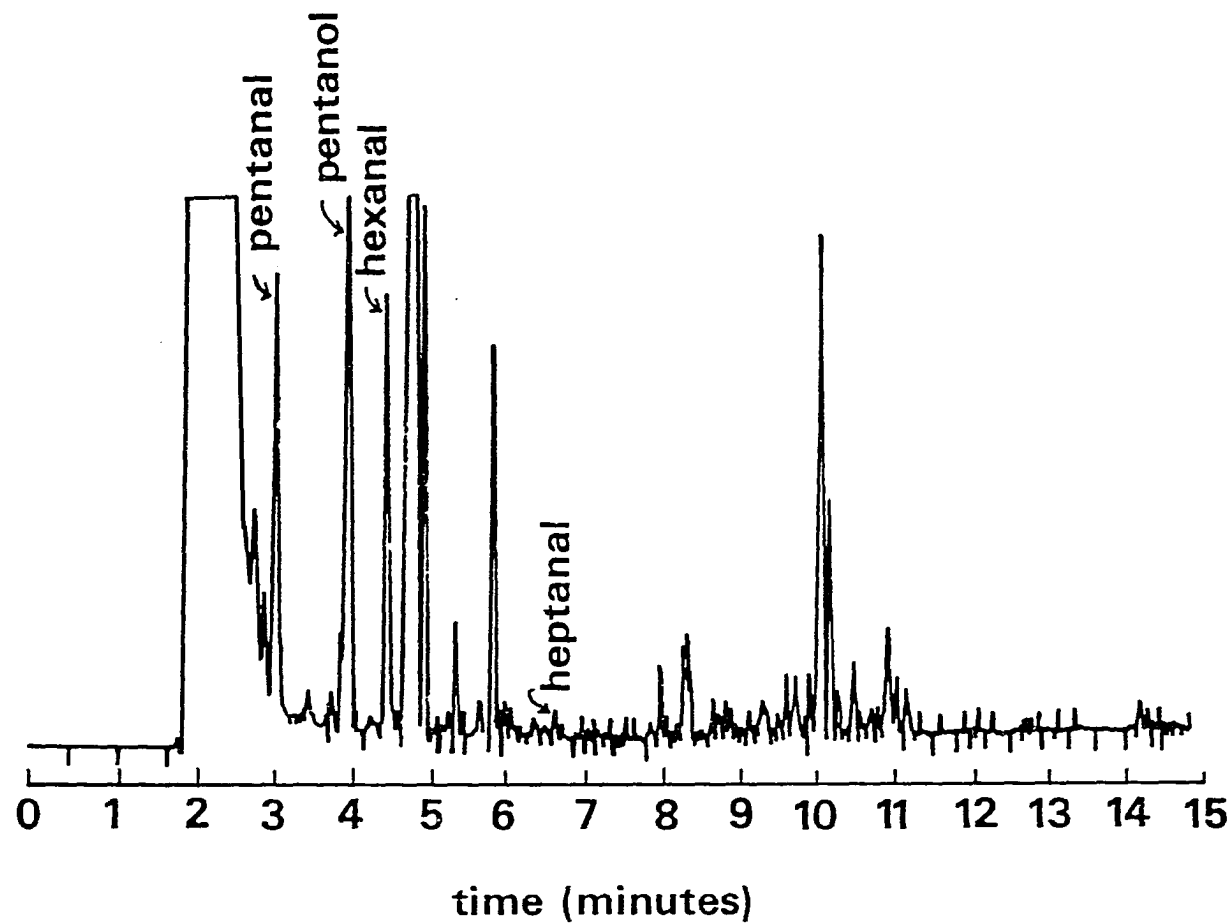


Figure 5. Gas chromatograph of an ethyl ether extract from a concentrated headspace. The sample was stored at 45°C and 65% RH

Table 5. Identification of some flavor components in the headspace of stored samples

RT <sup>a</sup> (min)	Compound	Evidence <sup>b</sup>	RT <sup>c</sup> (min)
3.16	Pentanal	C, M, O, E	1.61
4.05	1-pentanol	C, M, MS	1.83
4.65	Hexanal	C, M, O, E, MS	1.94
6.84	Heptanal	C, M, O, E	2.64

<sup>a</sup>Refers to Figure 5.

<sup>b</sup>C and M = Capillary and megabore columns, respectively (identical RT as chemical standard). O = Odor identification. E = Enrichment. MS = Mass spectrum.

<sup>c</sup>Refers to Figure 3.

to a scale from 1 (none) to 5 (extreme) and plotted versus time. A steady increase in the score (i.e., difference from the control) for samples stored at 15°C with 11% RH was observed (Figure 6a). The other samples stored at 11% RH generally had higher scores at higher temperatures and, after reaching a maximum score (2.7 to 3.3), were judged moderately different from the control. Samples stored at 25 and 15°C with 11% RH were judged acceptable by more than 60% of the panelists after up to 14 weeks of storage (Figure 6b). Samples stored at 35 and 45°C with 11% RH were considered undesirable at four and eight weeks of storage, respectively (Figure 6b). The scores given to samples stored at 65% RH were always higher than the scores for the

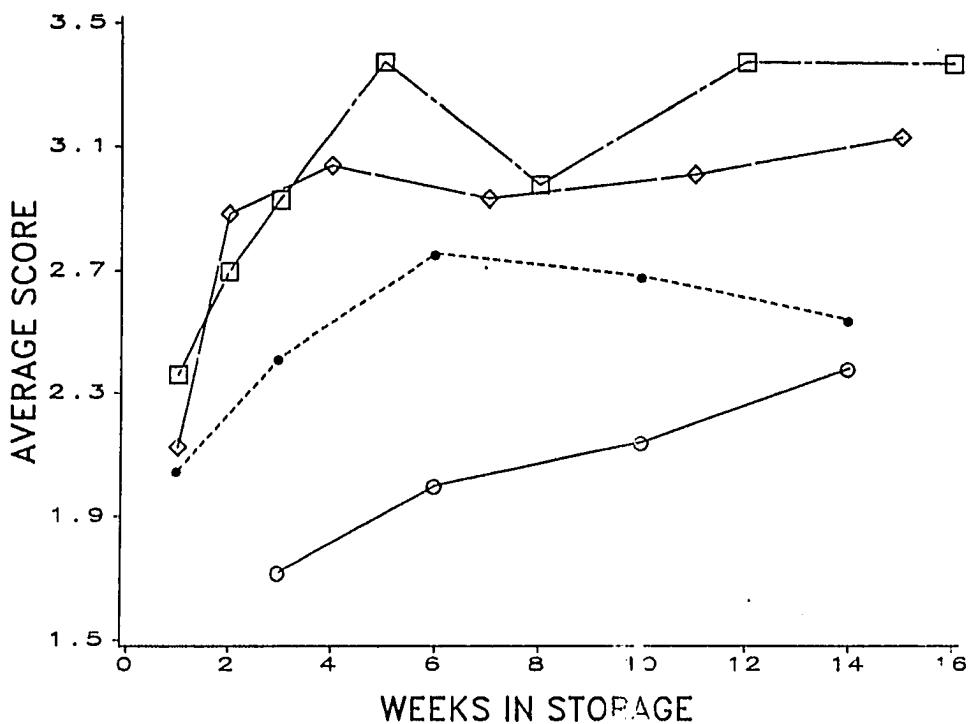


Figure 6a. Average scores for samples stored at 11% RH.

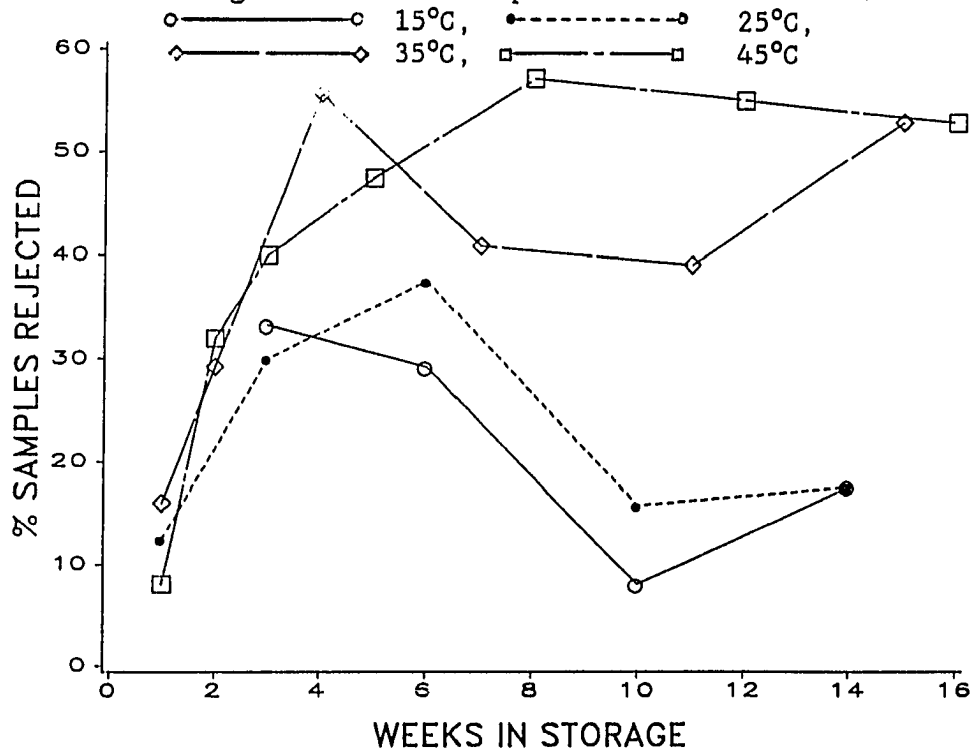


Figure 6b. Percent of panelists that judged the sample stored at 11% RH undesirable. 15°C, 25°C, 35°C, 45°C

corresponding samples at 11% RH (Figure 7a). Again, it took longer for samples stored at lower temperatures to receive their highest score. The aromas of all samples stored at 65% RH were judged undesirable by at least 50% of the panelists by week six of storage (Figure 7b). The average standard deviation for the scores given to one sample in a sampling day was  $1.08 \pm 0.18$ .

When the average score was plotted against the respective values for hexanal, there was no correlation. Similar results were obtained when the percent of replicates judged undesirable was plotted versus hexanal. The absence of correlation is due in part to the fact that samples were judged undesirable early in the study whereas hexanal and the other compounds increase up to 10 to 12 weeks of storage.

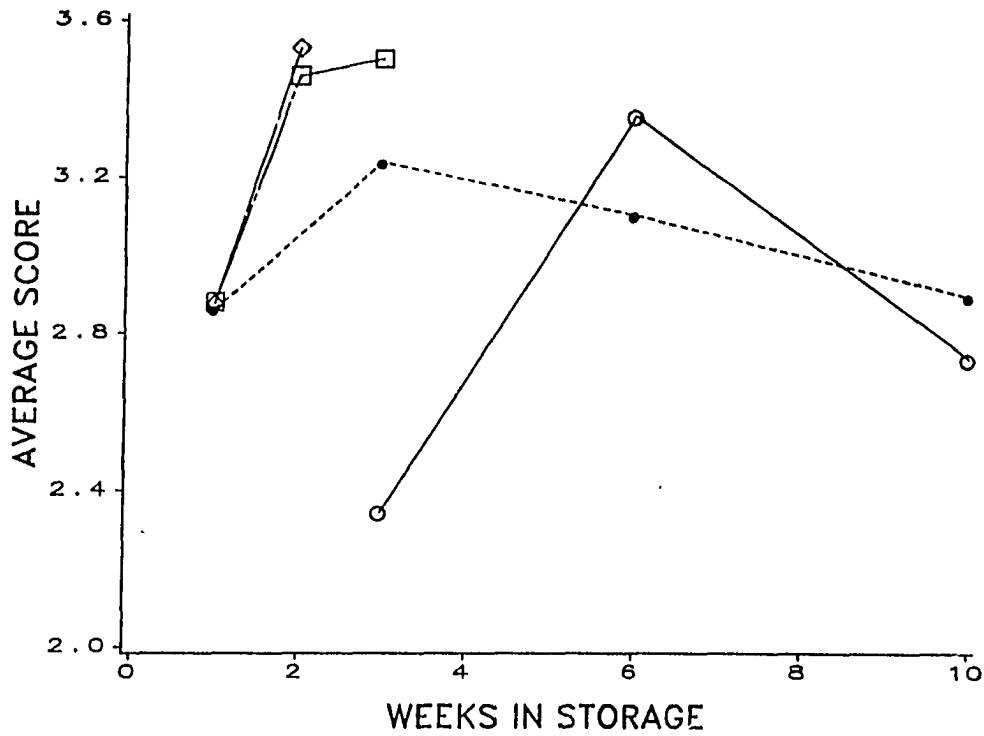


Figure 7a. Average scores for samples stored at 65% RH.

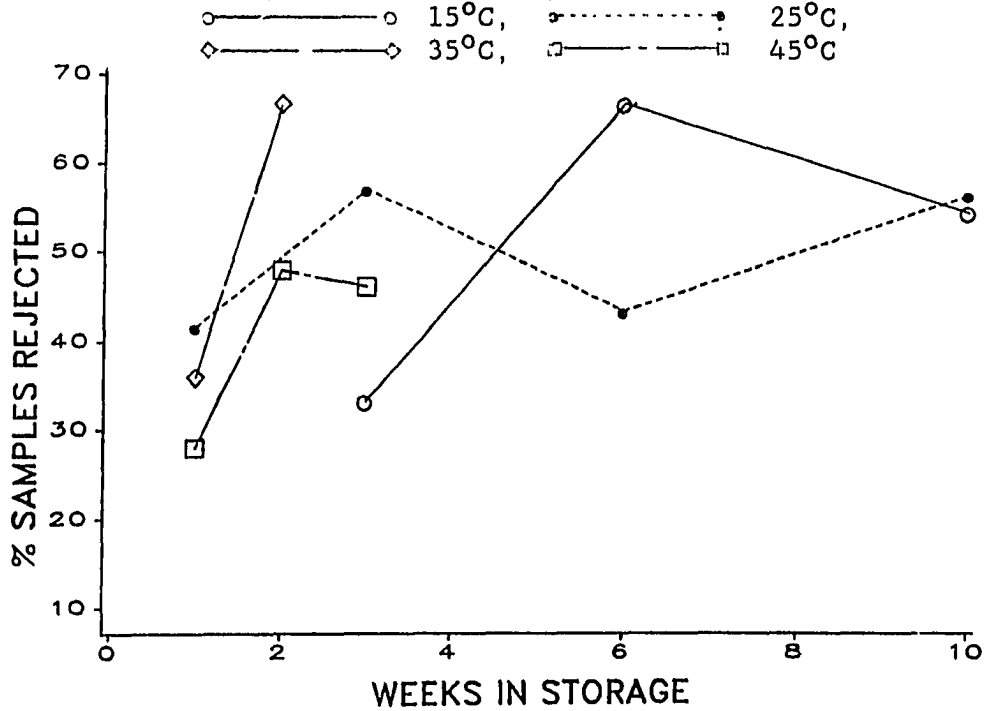


Figure 7b. Percent of panelists that judged the sample stored at 65% RH undesirable. ○—○ 15°C, ●—● 25°C, ◇—◇ 35°C, □—□ 45°C

## CONCLUSION

Lipid oxidation and lipid hydrolysis took place in a blend of soybeans and corn processed by extrusion. Deterioration of the blend occurred very early during storage at 65% RH at temperatures as low as 15°C. Deterioration was retarded by lowering the  $a_w$  to just below the monolayer value but a storage temperature of 35°C rendered the sample undesirable after four weeks from a sensory standpoint. With the use of a method to follow nonenzymatic browning and with careful refinement of the techniques described here, prediction of the shelf life of this product can be made. The technique employed for gas chromatographic analysis of volatiles was a very useful one. Peak resolution increased by using a capillary column, and a broader temperature program. Additional research may tell us what exactly the compounds measured in this study. Such information, together with the kinetic and thermodynamic analysis, may enable us to identify the oxidizing substrates and the mechanisms of oxidation. Based on the results obtained, we consider that the extruded blend should be protected from deterioration by a combination of a barrier to moisture, light and oxygen, and be held at low storage temperatures whenever possible.

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## ACKNOWLEDGMENT

This project was supported by the Iowa Agriculture and Home Economics Experiment Station. The article is published as Journal Paper No. J-13328 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; projects 2433 and 2164, the latter a contributing project to North Central Regional Research Project NC-136. We thank Dr. Earl Hammond and Dr. Jane Love for providing technical assistance.

## SUMMARY

Low-cost extrusion has become a viable alternative for transforming cereals, legumes and other nutrient sources into highly nutritious precooked foods or ingredients. Low-cost extruders are being used throughout the United States and the world. The development of new products continues, partly sponsored by local governments in developing countries.

Low-cost extruders are, in comparison with more expensive models used by the food industry, very inflexible machines. Generally, LCEs were created for a specific use. For example, the Brady and the Insta Pro models were originally designed to cook soybeans for use as animal feed. Consequently, processing of materials that have very different composition requires a lot of experimentation and empirical work.

The results presented here are part of an effort to apply a scientific and engineering approach to understand the processing mechanism and describe the quality characteristics of the products obtained.

In this study, we observed that extruder exit temperature alone can be used as an independent variable affecting relevant food properties. Moisture losses which ranged from 38 to 59%, dependent on extrusion temperature, produces foodstuffs with different shelf stabilities. Lipid oxidation in foods is accelerated by decreasing moisture contents below the monolayer value. Our shelf stability study included a relative humidity of 11%, just below the monolayer value for the product

analyzed. Serious deterioration was observed for all samples stored at 35°C and 45°C. Further insight into the shelf stability of these products could involve the use of a range of RHs around the monolayer value.

Soybean lipoxygenases were inactivated and the native tocopherols were resistant to the extrusion process. Nevertheless, lipid deterioration took place rapidly suggesting a combination of factors that gave rise to autoxidation. The presence of a resistant lipase activity may have contributed to the process of lipoxidation by releasing free fatty acids and facilitating oxygen access to them. The apparent decrease in  $\gamma$ -tocopherol after storage for 4 months under abusive conditions adds evidence to the proposed autoxidation theory.

With a few improvements, the technique employed for gas chromatographic analysis of volatiles can become a very useful one. Peak resolution can be increased by using a column with a small diameter, and a broader temperature program. Additional research may tell us what exactly the compounds measured were. Such information, together with the kinetic and thermodynamic analysis, may enable us to identify the oxidizing substrates and the mechanisms of oxidation.

The activity of lipase will become a significant problem during the utilization of LCEs for pre-extraction processing of oilseeds. We know very little of the nature of this lipase. The degree of activity may be insignificant in the overall process. But, the work done in this study will, at least, provide us with a tool to trace rancidity problems if the LCEs are used as an oil-extraction pretreatment.

The susceptibility of the soybean trypsin inhibitor to thermal destruction was exceptional in the temperature range studied. It is possible to control the residual TIA by manipulating the extruder exit temperature. The protective effect of corn upon soybean TIA during extrusion should be studied more in depth.

All extruded samples had high in vitro protein digestibility compared to the control, NARC reference protein (93% in vitro PD). The effect of extrusion temperature on PD will be of particular importance in the instances in which soybean-corn blends are used as major contributors to the diet of infants. A higher concentration of corn caused PD to remain close to the pre-extrusion value, after extrusion. At a high soybean/corn ratio, extrusion resulted in increases in PD from 8.5 to 10.8%. The Insta Pro extruder can be utilized to produce acceptable foodstuffs with varying nutritional and sensory characteristics, starting with the same raw ingredients.

The procedure developed for tocopherol analysis represents an improvement over the AOAC official method. Time and reagents are saved, the probability of artifacts is reduced, and individual isomers are separated. When utilizing this procedure we found that it is important to disrupt the structure of the tissues to yield the free tocopherols. Processing steps such as tofu manufacture and extrusion exert an excellent disruptive force. In the case of whole seeds, the materials must be ground to the finest particle possible without generating excessive heat.

## ACKNOWLEDGMENTS

I wish to thank Dr. Patricia Murphy for her support and guidance throughout my graduate studies.

To the members of my committee, Dr. Pamela J. White, Dr. Mark H. Love, Dr. Lawrence A. Johnson, Dr. Lester A. Wilson, and Dr. Kenneth Hsu, I wish to acknowledge their invaluable contributions.

I am grateful to my friends in 149 Food Technology for their friendship and understanding, especially to Cathy Hauck and Alfred Fratzke who also helped me analyze my numerical data and prepare my presentations for professional meetings.

I also thank Dr. Earl Hammond for his help with the mass spectrometer.