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D.B. LUND
EDITOR

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NATURAL AND APPLIED WAX COATINGS ON ORANGES

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ABSTRACT

Scanning electron microscopy was used to examine the wax coating on oranges. The natural wax platelets are irregular in shape and size, have a rough surface and increase in numbers as the orange matures. Most natural platelets can be removed by dipping the orange in a chloroform-methanol solution and rubbing the surface. Store-bought oranges have natural wax platelets 1–2 microns thick covered with a 2–5 micron layer of commercial wax. Wax applied over platelets may be ineffective if the platelets break off in handling and expose the orange's surface. Removal of natural wax platelets prior to commercial waxing allows uniform wax application and consequently better storage life. Drying with air at 2.5 m/s produced uneven wax thickness while 14 m/s air produced a complete, uniform wax layer.

INTRODUCTION

Wax is commercially applied to many fruits and vegetables to reduce dehydration and improve consumer appeal (Hall 1981). Hardenburg in 1967 listed 292 references of research publications, waxing materials and commercial application equipment. Equipment, including waxing, commonly used in Florida citrus packing houses is described by Grierson *et al.* (1978). Navel oranges dipped in a 2.5% emulsion of wax lost 40% less weight than unwaxed samples (Hall and Trout 1944). Concentrations up to 15% wax reduced weight loss further to 75%. Certain waxes were more effective in reducing orange weight loss (Eaks and Ludi 1960). Waxing increased the carbon dioxide content and decreased the oxygen content of the orange's internal atmosphere.

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Oranges were sprayed (Albrigo and Brown 1970) two months before harvest with an antitranspirant plastic. Using the scanning electron microscope they observed the nonuniform deposition of the natural wax and the relatively continuous spray coating applied earlier. A thin coat of wax was not as effective (Ben-Yehoshua 1967) in reducing shrinkage while a thicker coating produced off-flavors later.

The thickness of emulsion wax applied to other fruit such as apples was determined to be 2–3 microns (Trout *et al.* 1953). The effectiveness of wax in maintaining fruit conditions is dependent on temperature. Apples stored at 18°C were improved by adding a single coating of wax while at 1°C the wax layer needed to be thicker or more concentrated to make the same percentage reduction in respiration rate. The ability to reduce respiration and weight loss is affected most by storage temperature and then secondarily by the application of a wax coating. Certain varieties of easily polished apples displayed (Hall 1966) wax removal and relocation when wiped with paper wrappers. Loss in natural wax, and thus increased transpiration, may occur during growth on the tree by abrasion, picking, handling, and packing.

Wax on fruit like prune plums (Bain and McBean 1967) consists of a two component layer. The inner component is thin platelets while the outer component is a mass of random projections which became more complex with maturity. Again the wax layer thickness was estimated to be 3–5 microns.

The surface moisture of citrus fruit is commonly removed by mechanical and air drying methods. The surface drying rate is affected most by the air velocity and the absolute humidity difference (Miller 1978). In his experiments, air velocity was varied from 2.0 to 4.2 m/s. For Hamlin oranges, the drying rate was proportional to the air velocity raised to the 0.17 power. Because of this fractional exponent, further increases in air velocity produce smaller increases in drying rate. Water vapor transmission rate was not (Miller and Grierson 1983) related to the precent solids of commercial citrus coatings.

The objective of this investigation was to study the natural and artificial wax coatings on the orange using scanning electron microscopy in order to determine the effect of air velocity on the drying rate and uniformity of the wax coating.

MATERIALS AND METHODS

For study of the naturally occurring wax layer, Valencia oranges were hand-picked from the University of California Experiment Station Farm

at Winters, California on July 1. The oranges were brought to campus and placed in cold storage. The next day, after being brought to 25°C, 18 oranges were selected, three for each of the following treatments: (1) naturally occurring waxed orange, (2) dewaxed, (3) dewaxed plus thick layer of artificial wax dried at high air velocity, (4) dewaxed plus thick layer of artificial wax dried at low air velocity, (5) dewaxed plus thin layer of artificial wax dried at high air velocity, and (6) dewaxed plus thin layer of artificial wax dried at low air velocity. The naturally occurring wax was removed by dipping the orange in a solvent solution of 2:1 chloroform-methanol at room temperature for 1 min, manually rubbing the surface, rinsing in running water and wiping the fruit dry. To add artificial wax, the orange was rolled around in a petri dish of Sta Fresh 223 (FMC) wax. A thin layer of wax was obtained by wiping off the excess liquid. Drying occurred in an airstream of either 2.5 or 14 m/s air at 29°C and 30–40% RH. The lower air velocity was selected because it was in the mid-range of Miller's (1978) tests while the higher flow was selected from figures suggested by industrial representatives. Miller (1978) found that increasing the air velocity from 2 to 4 m/s had a minimal effect on the drying rate and yet industry reported significant increases in drying at higher air velocities. The fruit was removed from the air stream when a small strip of tissue paper would no longer stick to the orange's surface. Drying time varied from 1–20 min.

Two samples of the peeling were taken from each orange 90° from the stem end and 180° apart with a 5 mm cork borer. Each sample was frozen in liquid nitrogen and placed in a vacuum chamber for two days. The samples were gold misted in preparation for viewing with an ISI/DS130 scanning electron microscope.

RESULTS

The observed time to dry wax on an orange at air velocities of 3.4 to 16.4 m/s is shown in Fig. 1. The trend differs from Miller's (1978) results for surface moisture. Miller's work showed that further increasing air velocity has a diminishing effect on drying rate, i.e., drying rate is proportional to air velocity raised to the 0.17 power. The difference in our results at high air velocity is caused by hydrodynamic movement of the wax, which yields a thin wax layer and this dries faster. This divergence from strictly drying only, occurs at a particular air velocity depending on physical properties of the wax. For this specific wax and air conditions, uniform drying over the fruit's surface occurred for air velocities up to 5 m/s. At air velocities above 7 m/s there were visible ripples in the liquid wax

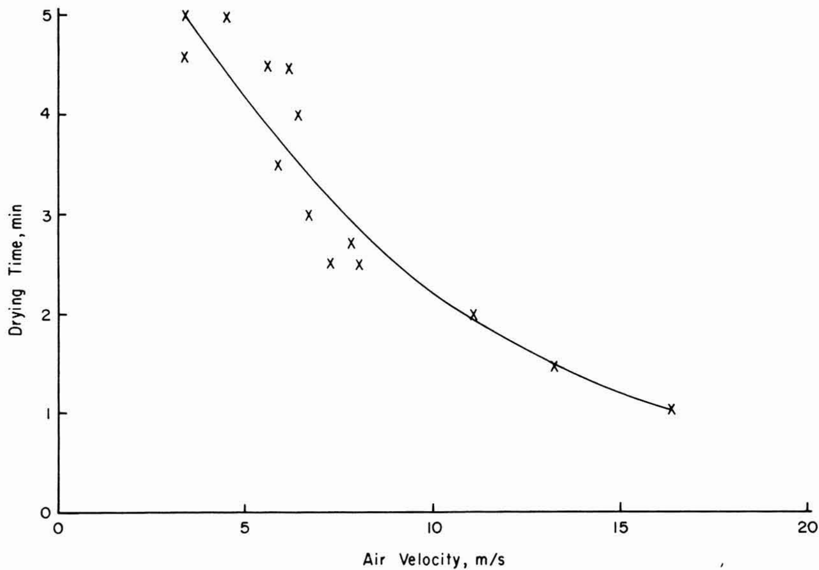

FIG. 1. EFFECT OF DRYING AIR VELOCITY ON WAX DRYING TIME. Air is at 29°C and 40% RH. Sta-Fresh 223 wax on Valencia orange. Regression equation in Min = $7.6895\ e^{-0.1241\ m/s}$ with $R^2 = 0.928$.

surface and above 15 m/s the liquid was blown off the orange. At 14 m/s increasing the wax temperature from 5 to 50°C reduced the drying time from 1.2 to 0.8 min, a less significant amount than the effect due to air velocity.

The surface of an orange is far from being smooth. A view of the orange peeling's cross-section with a SEM at 20–100 magnification suggests that the surface area due to microscopic roughness is about 15% greater than that of a smooth sphere. In estimating an orange's surface area available for wax application, this should be the upper limit relative to the fruit's macroscopic surface area as computed from diameter measurements.

Natural wax platelets (Fig. 2) are irregular in shape and size with a rough surface. Platelets vary in their degree of attachment, in thickness with respect to location and change in thickness with maturity (Nagy *et al.* 1977). As the orange matures, there is an increase in the area covered by platelets. After dipping the field picked orange in the chloroform-methanol solution, rubbing, rinsing and wiping dry, nearly all natural wax platelets were removed (Fig. 3). With the surface cleared, open stomata were visible.

A store-bought, commercially waxed, Valencia orange displayed the results of applying wax over the natural platelets (Fig. 4). The raised area in the left side of the photo shows both layers while the right side shows

FIG. 2. NATURAL WAX PLATELETS ON HAND-PICKED ORANGE. SEM-100X.

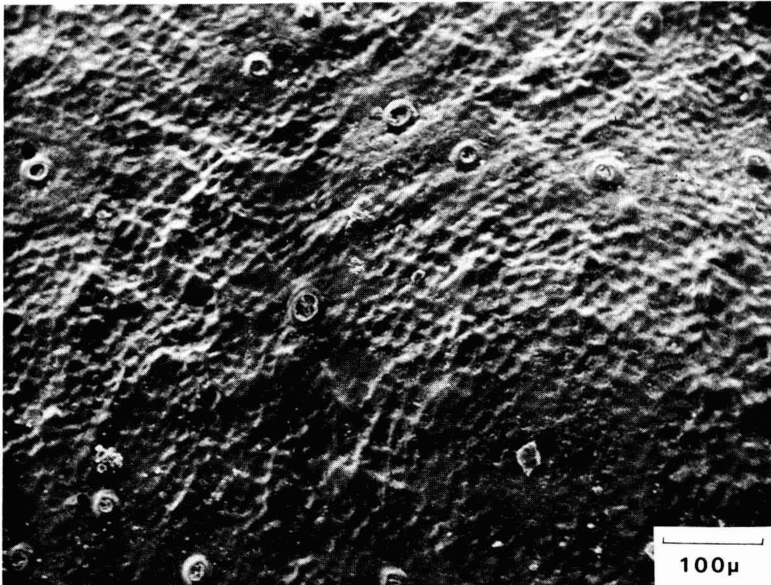

FIG. 3. ORANGE WITH WAX PLATELETS REMOVED. SEM-120X.

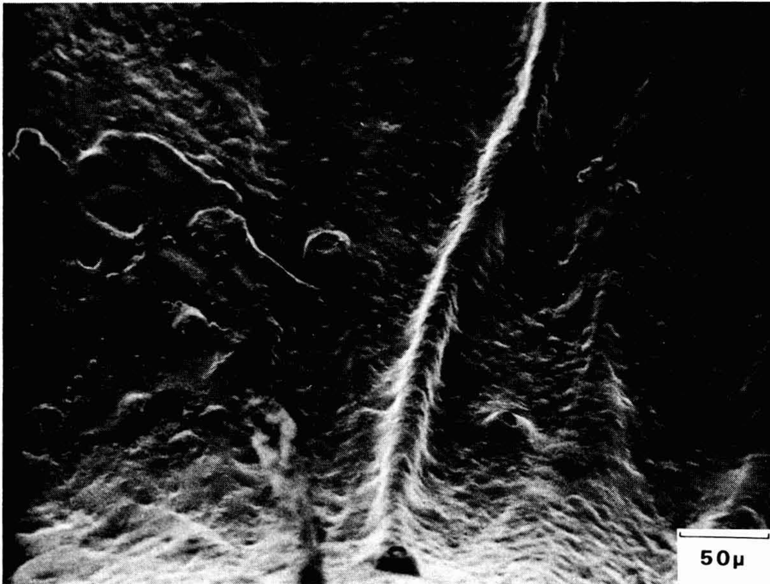

FIG. 4 STORE-BOUGHT ORANGE WITH WAX APPLIED OVER NATURAL PLATELETS. SEM-200X.

an area covered only with artificial wax. The ridge down the center of the photo is likely all artificial wax relocated during drying. Commerically applied wax is commonly 2–5 microns thick while natural platelets are 1–2 microns thick. By dipping oranges in liquid wax and drying without forced air, it was possible to create a wax layer up to 30 microns thick. In order to completely cover the stomata, the wax layer has to be about 20 microns, a thickness which may not be economically justified.

Applying a thick layer of wax and air drying at 2.5 m/s produces incomplete, nonuniform coverage (Fig. 5). Most of the surface appeared to be wetted except for the bottoms of the "valleys" and peaks of the "mountains", i.e., the stomata. The thin layer of wax with 2.5 m/s air drying produced a coating which covered the entire surface with a reduced thickness. The applied wax layer's upper surface was smooth from forced air drying while the under surface was rough, an imprint of the orange's surface.

Wax drying with 14 m/s air produced a rippled surface (Fig. 6). As long as there was sufficient wax, the entire surface was covered. The amount of wax applied did not display any topographical differences.

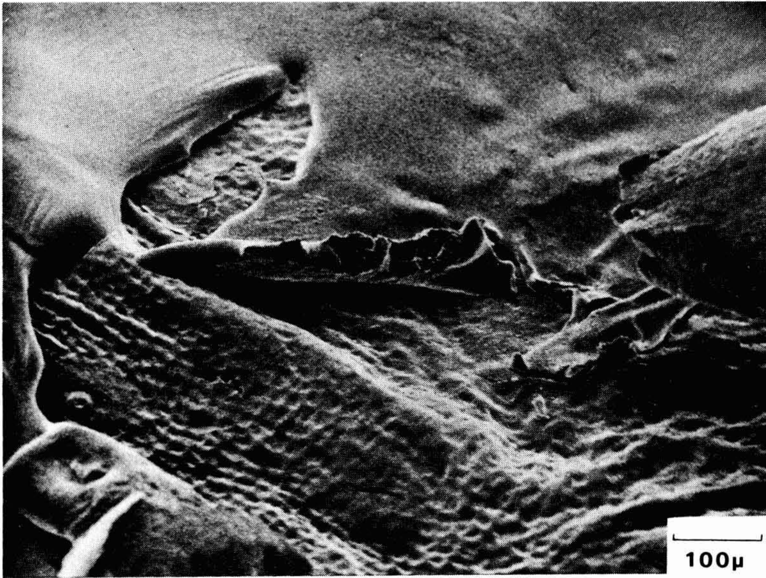

FIG. 5. THICK INCOMPLETE LAYER OF STA-FRESH 223 WAX DRIED AT 2.5 m/s. SEM-100X.

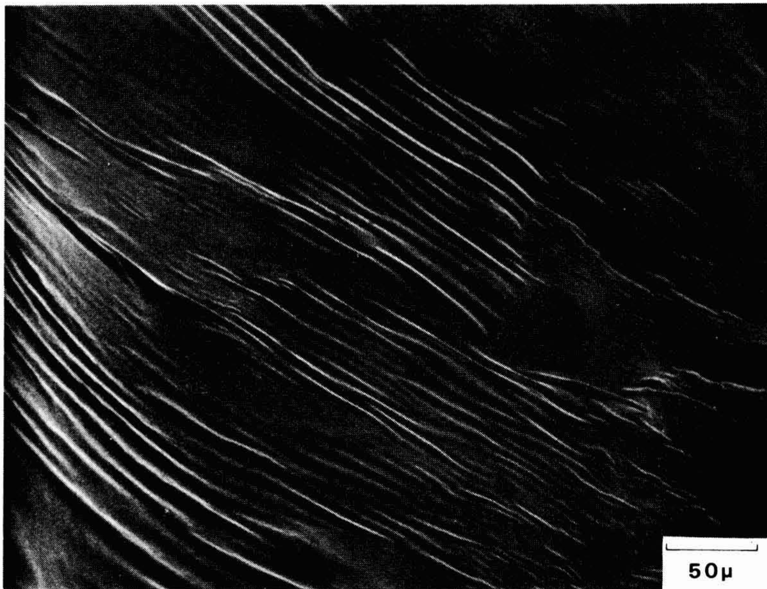

FIG. 6. RIPPLED SURFACE OF STA-FRESH 223 WAX DRIED AT 14 m/s. SEM-190X.

DISCUSSION

Natural wax platelets do not protect the orange adequately during numerous handling operations. Wax applied over platelets may become ineffective if the platelets break off in handling and expose part of the fruit surface to dry air. Because platelets are so irregular in shape, size, roughness and degree of attachment and because they do not serve as an adequate base, they should be removed prior to application of an artificial wax. With a clean, smooth fruit surface the applied wax can be more efficiently used to form a stable, uniform, complete coating.

Depending on wax properties there is an air velocity above which wax is redistributed on the fruit. This redistribution and wax thickness reduction is the primary cause for decreased drying time. High air velocity, for whatever reasons, does reduce drying time but increases energy consumption. With the same air conditions, higher air velocity will require more energy per fruit since air volume (which is proportional to energy consumption) increases disproportionally more than drying rate. To insure a thin, complete wax coating it would be more economical to control the wax's physical properties (i.e., viscosity) than use high air velocity alone.

CONCLUSIONS

This study produced the following conclusions: (1) Viewing the fruit's microsurface features with the scanning electron microscope provided a comparison of natural platelets and applied wax layers. (2) Removing natural wax platelets prior to waxing provides a more stable base for application of a wax coat. (3) Wax application methodology and drying air velocity affect uniformity and completeness of the wax coating. (4) Drying with too high air velocity can redistribute the wax over the fruit's surface. (5) High air velocity is beneficial for certain conditions but is not the sole answer to more effective, reduced cost wax drying.

ACKNOWLEDGMENTS

The authors recognize Dr. Jack Pangborn, Director of the Facility for Advanced Instrumentation at University of California, Davis for his assistance with the scanning electron microscope.

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STORAGE STABILITY OF MILKFAT GLOBULE MEMBRANE

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ABSTRACT

To investigate changes in structure and function of milk fat globule membrane (MFGM) components due to oxidation of membrane lipids, freeze-dried MFGM were stored under controlled temperature and relative humidity (RH) conditions. It was found that while membranes underwent extensive lipid oxidation when stored under air, fluorescent compounds were formed. These compounds exhibited fluorescence with excitation maxima at 350 nm and emission maxima at 440 nm; which were similar to those of the fluorescent substances derived from the reaction of oxidized fatty acids and primary amines. Formation of high molecular weight proteins was detected by SDS-PAGE in samples stored under air, but not in samples stored under nitrogen and was also affected by time and relative humidity. Activity of xanthine-oxidase, an important prooxidative agent, was greatly influenced by temperature and complete inactivation was observed when MFGM's were stored at 37°C, 50% RH for 1 day.

INTRODUCTION

Milk fat globule membrane (MFGM) has an important role in final properties of dairy products. Structurally, MFGM separates the fat globule from the aqueous phase of the milk and consists of a complex mixture of proteins, glycoproteins, triglycerides, cholesterol and phospholipids. MFGM phospholipids, containing unsaturated fatty acids, are highly susceptible to oxidation, and can play a role in the initiation of lipid oxidation and its mediation to the triglyceride core of the fat globule.

Although it is reported that milk susceptible to spontaneous oxidation is not very common anymore (Schroder 1982), metal contamination (especially copper and iron) introduced from metal fittings and pipelines can catalyze the oxidation of unsaturated fatty acids of MFGM phospholipids. Another reactive oxidative species which can cause extensive lipid peroxidation problems is superoxide, a product of the reaction catalyzed by

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xanthine oxidase. Since xanthine oxidase is abundant in the MFGM's, it is possible that initiation of lipid peroxidation could be caused by this enzyme (McPherson and Kitchen 1983). It is also important to note that milk normally becomes saturated with 0_2 between milking and processing, and that this oxygen is not subsequently removed (Schroder 1982).

Stability and integrity of MFGM is important to prevent "leakage" of globule fat content and its further oxidation. Oxidation-induced changes can cause severe membrane damage leading to fat content "leakage" and hence to the stability and acceptability problems.

We studied lipid oxidation-induced changes in freeze-dried MFGM to clarify some changes that this membrane undergoes during processing and storage.

MATERIALS AND METHODS

Preparation And Storage Of MFGM

Fresh, raw cow milk was obtained from a local Jersey herd at the time of normal milking. Isolation of MFGM was started within 5 h of milking. We used a modification of the procedures by Kitchen (1977; 1974) as outlined in Fig. 1.

Cream was separated by centrifugation of the whole milk (4000 rpm; 10 min, room temperature) and was washed 3 times with 3 volumes of 10 mM tris HCl buffer (pH 7.5) containing 0.25 M sucrose and 0.15 M NaCl and 1 time with distilled water. Washing steps remove all materials entrapped between fat globules. The washed cream was suspended in distilled water to give a final fat concentration of about 30%. After cooling to 10-12°C, the cream was churned to break the fat globule membranes. MFGM is primarily in this aqueous phase. To remove any membrane entrapped in the butter, it was extracted 2 times with distilled water (1 ml/g butter) at 45°C, and the aqueous extracts were combined with the butter milk which contains the crude membrane. Membranes were isolated from this aqueous phase by ultracentrifugation at 100,000 g for 60 min (5°C) (Beckman L5-75 Ultracentrifuge, Type 70 Ti fixed angle rotor, Beckman Instruments Inc., Wakefield, MA). Upon resuspension of the pellet in 10 m M tris-HCl buffer (pH 7.5), the ultracentrifugation step was repeated. The final pellet, MFGM, which consisted of brown-red, pink and fluffy white layers, was resuspended in 10 mM tris-HCl buffer by 4–6 strokes of a teflon pestle in a glass homogenizer and sonicated for 10 min (Bransonic 32 ultrasonic cleanser, Branson Cleaning Equipment Co., Parrot Dr., Shelton, CT). Upon isolation of membranes, 2 ml of MFGM suspension were placed in 20 ml vials, frozen in liquid nitrogen and freeze-dried in a

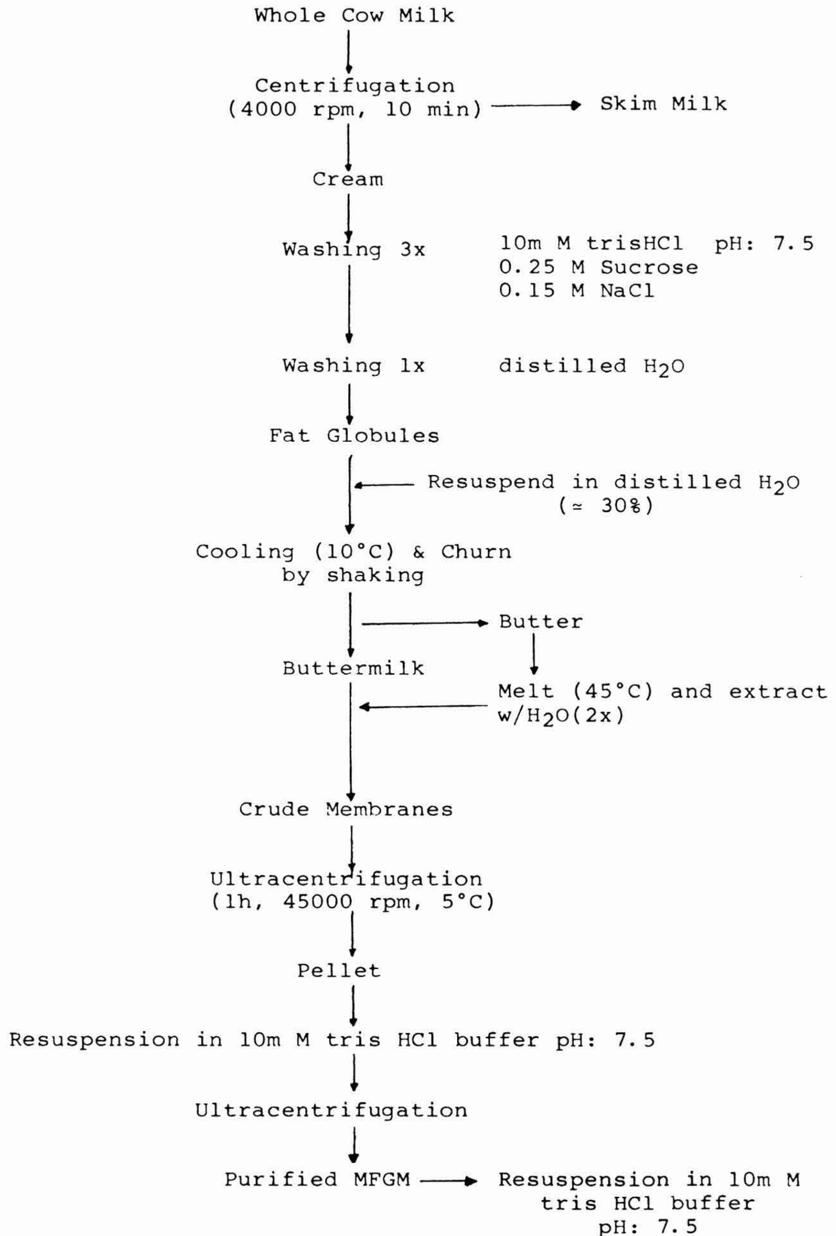

FIG. 1. EXPERIMENTAL FLOW DIAGRAM FOR THE PREPARATION OF MILK FAT GLOBULE MEMBRANES.

Virtis laboratory freeze drier (The Virtis Company, Inc., Gardiner, NY) (P $\leqslant$ 200 mTorr, condenser temperature of -50°C) for two days. Vacuum was released with dry air. Freeze dried samples were stored at 37°C and 50.4% RH, unless otherwise stated, for suitable time intervals (up to 4 weeks) when duplicate samples were removed from storage and the following studies were performed. Control freeze-dried membranes were placed at -40°C.

Fluorescence Assay

A modification of the procedure described by Chung and Toyomizu (1976) was used. Duplicate stored samples were treated as outlined in Fig. 2. The organic extract was used to obtain the fluorescence spectra with an Aminco-Bowman spectrophotofluorometer (American Instruments Co., Silver Spring, MD). Standard solution was quinine sulfate (1 ppm in 0.1 H_2SO_4). The slit arrangement was as follows: excitation exit slit 2 mm; emission entrance slit 2 mm; emission exit slit 2 mm. The meter multiplier was set at 10. Scanning was performed at 120 nm/min (wavelength) and 5 cm/min (recorder).

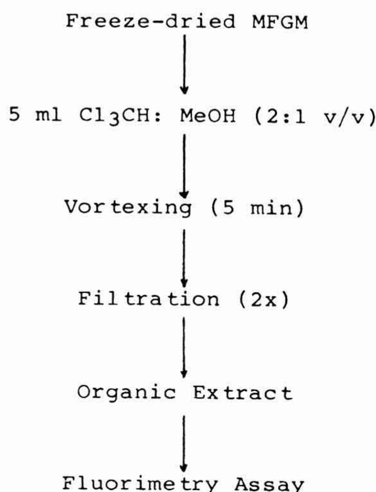

FIG. 2. EXPERIMENTAL FLOW DIAGRAM FOR THE EXTRACTION OF FLUORESCENCE COMPOUNDS PRODUCED DURING STORAGE OF FREEZE-DRIED MILK FAT GLOBULE MEMBRANES.

SDS-Polyacrylaminde Gel Electrophoresis (SDS-PAGE)

SDS-PAGE (slab gels) was performed in a Bio-Rad protean double slab electrophoretic cell, (BioRad Laboratories, Richmond, CA) using a Buchler power supply model 3-1500 (Buchler Instruments, Inc., Fort Lee, NJ) (constant current). The procedures followed the methodology described by Nakhost and Karel (1983). Phosphorylase 6, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and α-lactalbumin (Pharmacia Fine Chemicals, Piscataway, NJ) were used as protein markers. 100 μl of reconstituted (to original moisture content) membranes were added to 200 μl of sample solution (approx. 1 mg protein per ml of final solution) with the final concentration of 8 M urea, 0.3% SDS and 20 mM dithiothreitol (DTT) and then incubated at 50°C for 30 min. This resulted in complete solubilization and therefore a clear solution was obtained. Alliquots of such solution (25 μl) were loaded on the top of the stacking gel using bromophenol blue as tracking dye. SDS-PAGE was performed at 20 mA/slab gel (constant current) until the tracking dye reached the separating gel and then 30 mA/slab gel for the rest of the run. Total run time was about 5 h.

Moisture Sorption Isotherm

Freeze-dried MFGM moisture sorption isotherm was determined following the procedure used by Labuza (1984). Freeze-dried membranes were placed in controlled relative humidity chambers (dessicators) at 37°C and the weight gain was measured until equilibrium occurred. To create constant RH, we used various saturated salt solutions as shown in Table 1 (Wexler and Hasegawa 1954). These saturated salt solutions produced a constant water vapor presure in the headspace at constant temperatures. For zero RH, dry calcium sulfate (Drierite, W. A. Hammond, Drierite Co., Xenia, OH) was used. The resulting type III isotherm is shown in Fig. 3.

Table 1. Saturated salt solutions used during the moisture sorption isotherm determination (Wexler and Hazegawa 1954)

SATURATED SALT SOLUTIONS	%RH(37°C)
$LiCl.H_2O$	11.7
$MgCl_2.6H_2O$	32.3
$Na_2Cr_2O_7.6H_2O$	50.4
NaCl	75.5
KNO_3	88.7

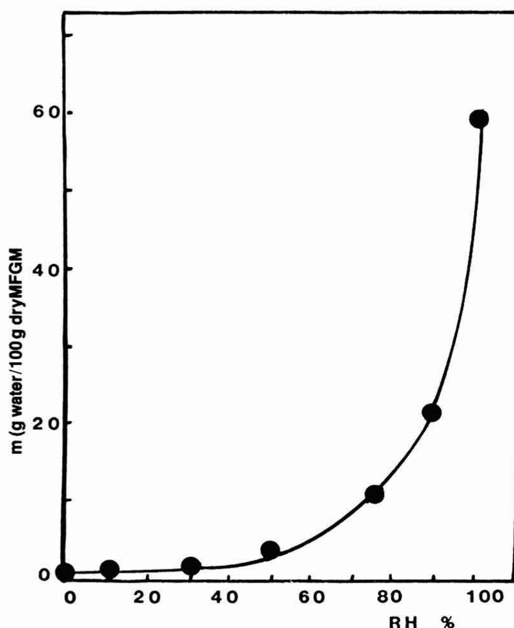

FIG. 3. FREEZE-DRIED MFGM SORPTION ISOTHERM AT 37°C.

Xanthine Oxidase Activity

Xanthine oxidase activity was measured spectrophotometrically following absorbance increase at 290 nm upon aerobic oxidation of hypoxanthine to uric acid (Kalkar 1947; Roussos 1967, 1964).

All measurements were made with a Hitachi 200-Perkin Elmer spectrophotometer with cuvettes having a light path of 1.0 cm. For standards we used purified xanthine oxidase (Sigma Chemical Co., St. Louis, MO).

The samples incubation mixture contained 1.5 ml of reconstituted and diluted (200 times) MFGM and 1.5 ml of Hypoxanthine (3 mg/ml) prepared in 0.1 M glycylglicine buffer pH 7.5. The control reaction mixture, reference cuvette, had the same composition as above, except that hypoxanthine was omitted. The reaction was initiated by addition of the hypoxanthine solution after adjusting the zero with MFGM dilution. The increase in absorbance at 290 nm (formation of uric acid) was recorded as function of time.

Xanthine oxidase activity of stored MFGM was calculated as μ moles of uric acid produced per minute (extintion coefficient: 1.22×10^4 mol^{-1} l^{-1}) assuming the activity of freeze-dried MFGM stored at -40°C as a reference value (100% activity).

RESULTS

Fluorescence

Detection of lipid oxidation products by fluorescence techniques has been used in biological tissues (Gray 1978; Logani and Davies 1980), in freeze-dried meats (Kamarei and Karel 1984), on liver hepatocytes (Koster *et al.* 1982) and in erythrocyte membranes (Hochstein and Jain 1982). In earlier experiments, we found that traditional methods for lipid oxidation assessment, such as TBA and diene conjugation, did not work in our freeze-dried system, confirming reports by Melton (1982) and Chipaults and Hawkins (1971). We therefore chose fluorescence as the methodology to assess lipid oxidation in our system.

Excitation at 350 nm resulted in maximum emission at 440 nm and emission wavelength set at 440 nm resulted in an excitation spectrum with a maximum at 350 nm. Both showed formation of a single peak.

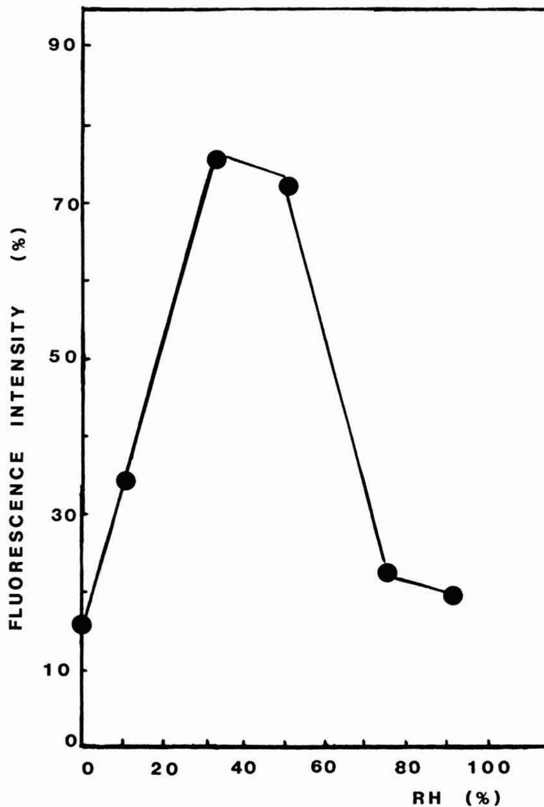

FIG. 4. DEPENDENCY OF FLUORESCENCE INTENSITY WITH RELATIVE HUMIDITY IN MFGM STORED AT 37°C FOR 4 WEEKS.

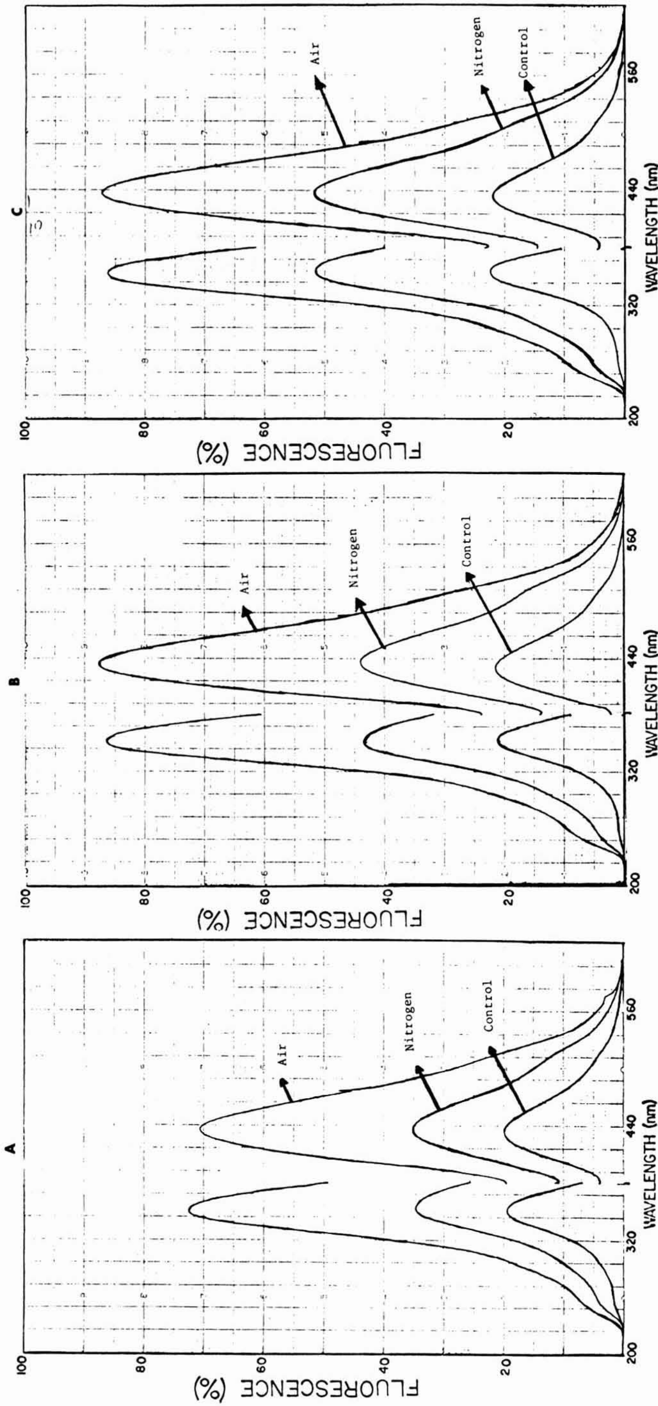

FIG. 5. FLUORESCENCE EXCITATION AND EMISSION SPECTRA OF CHLOROFORM-METHANOL (2:1 v/v) EXTRACTS OF FREEZE-DRIED MFGM STORED AT 37°C, RH = 50%, FOR (A) 5 days: (B) 10 days: (C) 20 days. The lower curves were obtained from similar extracts of control MFGM. Excitation 350 nm, emission 440 nm, and meter multiplier 10.

As shown in Fig. 4, there is a strong dependence of fluorescence intensity with RH when freeze dried MFGM was stored at controlled T (37°C) under air for 4 weeks. We therefore stored our samples at the RH (30-50%) which the maximum oxidation (fluorescence intensity) on MFGM would be expected. The Spectra corresponding to samples stored for 5, 10, and 20 days at 37°C (50% RH) are shown in Fig. 5a, b and c, respectively.

Samples stored under nitrogen also showed some increase in fluorescence intensity (possibly due to oxidizing compounds, or oxygen entrapped in the dry membrane preparations) but much lower than those of the samples stored under air (RH: 50%, 37°C). Freeze-dried MFGM kept at −40°C in sealed flasks were included as controls, but slight intensity increase was also found.

SDS-Polyacylamide Gel Electrophoresis

SDS-PAGE was used to elucidate changes in the MFGM protein profile during storage under the previously described conditions. Figure 6 shows the pattern of MFGM protein upon storage for 2 and 4 weeks under air and nitrogen. Freeze-dried MFGM, kept at −40°C, were used as controls and contained three major size classes of polypeptides with molecular weights approximately 150,000, 60,000, and 43,000. In the stored sam-

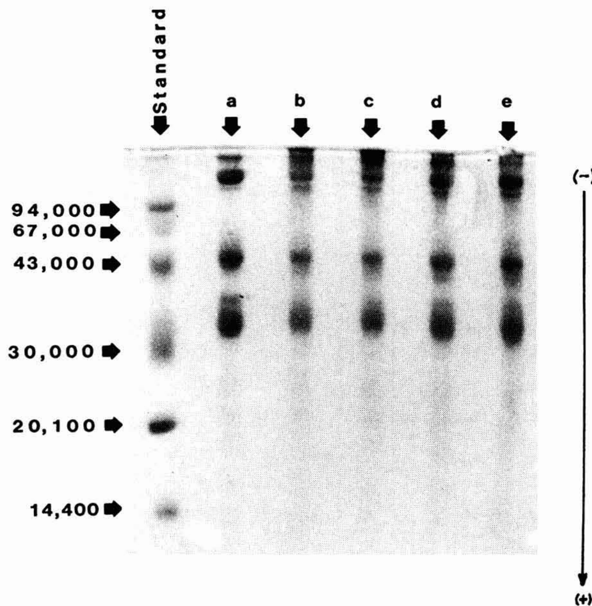

FIG. 6. SDS-PAGE OF F.D. MFGM STORED UNDER THE FOLLOWING CONDITIONS: (A) control −40°C: (B) 2 weeks, R.H. = 50%, under air: (C) 4 weeks, R.H. = 50%, under air: (D) 2 weeks, R.H. = 50%, under nitrogen: (E) 4 weeks, R.H. = 50%, under nitrogen.

ples, we detected the formation of High Molecular Weight Proteins (HMWP) observed mostly on the top of the separating gel, without being able to penetrate in the resolving gel.

The bands corresponding to the major polypeptides faded considerably compared with the control sample. Membranes stored for 4 weeks showed more important changes and those stored under N_2 showed negligible change.

Xanthine-Oxidase Activity

Specific involvement of xanthine oxidase in lipid peroxidation has been demonstrated by Allen and Humphries (1977). Therefore, knowing the important role of this enzyme in the development of lipid oxidation, the MFGM enzyme activity was studied.

As shown in Fig. 7a, there is a strong dependence of membrane's enzyme activity with temperature and storage time. Xanthine-oxidase activity is almost completely lost after 1 day storage at 37°C and only 25% of the original activity was lost when membranes were stored at 4°C. Results at 20 and 30°C were, as expected, between the above mentioned values.

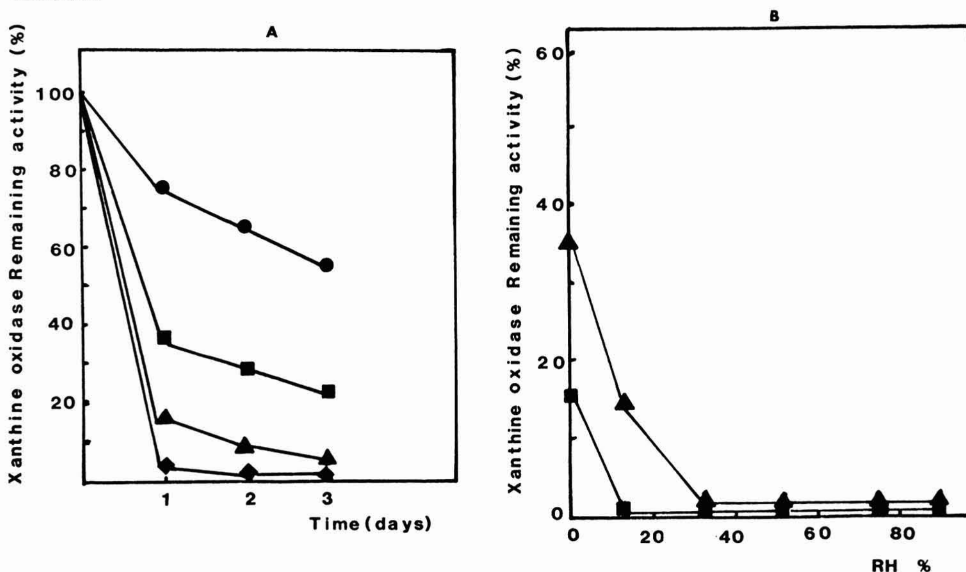

FIG. 7. MFGM XANTHINE OXIDASE ACTIVITY DURING STORAGE UNDER DIFFERENT CONDITIONS. (A) R.H. = 50%; (●) 4°C; (■) 20°C; (▲) 30°C, (◆) 37°C: (B) 37°C; (▲) 2 weeks; (■) 4 weeks. Enzyme activity was expressed as µmoles of uric acid produced per minute (extinction coefficient: 1,22 × 10^4 mol^{-1} 1^{-1}) using the activity of freeze-dried MFGM (at −40°) as a reference value (100%).

The enzyme behavior as RH was increased from 0 to 90% is shown in Fig. 7b. As relative humidity increased, enzyme activity decreased for the same storage time (2 or 4 weeks), with a maximum deactivation when RH was higher than 31% after 2 weeks storage and higher than 11% after 4 weeks storage. Samples stored under nitrogen (37°C, RH: 50%) showed negligible activity after 1 and 2 weeks storage (data not shown).

DISCUSSION

Fat globule membrane plays a role in fat oxidation since it forms a region where the lipids are in contact with most catalyst and other substances that may affect the reactions. The membrane phospholipids contain a large portion of polyunsaturated fatty acids residues, which are susceptible to oxidation. Consequently, fat oxidation probably begins and is most intense in the membrane (Mulder and Walstra 1974).

For lipid oxidation assessment, we developed a fluoresence technique, since fluorescence is 10 to 100 times more sensitive than other colorimetric assays in lipid oxidation detection (Dillard and Tappel 1971).

As shown by the fluorescence spectra (Fig. 5) during storage freeze-dried MFGM underwent an extensive oxidation when the headspace was air. Although samples stored under nitrogen also showed fluorescence, it was to a much lesser extent. It may have been due to entrapped oxygen or to some temperature-induced nonenzymatic browning products. Control membranes kept at −40°C showed much lesser fluorescence.

The pattern of fluorescence intensity upon storage shows considerable increase in fluorescent compounds formation at very early storage, approaching the maximum after 10 days (Fig. 5). However, between 10 and 20 days storage, negligible increase was found, probably due to the fact that fluorescent chromophores started decomposing under the experiment conditions. It is also important to note the role of RH in lipid oxidation-induced fluorescence. As shown in Fig. 4, lipophilic fluorescent compounds developed in a much lesser extent at "low" and "high" RH compared to intermediate RH's. Two possible explanations can be considered: first, we can assume that although lipid oxidation rates in different systems used to be higher at low and high RH (Karel 1975), the rate of formation of lipid soluble compounds having the structure -R-N=C-C=C-N-R, which are reported to be responsible for fluorescence (Dillard and Tappel 1971; Malshet and Tappel 1973), in different biological materials and could be higher at intermediate RH's in MFGM under study. Second, there is another influencing factor that should be responsible for that behavior and this could be the presence of the enzyme xanthine

oxidase in a great extent. It has been found that xanthine oxidase for MFGM has a prooxidative role and also that the oxidative capabilities of this enzyme were greatly enhanced by heat denaturation (Allen and Humphries 1977; Bruder 1980). Allen and Humphries postulated that this may have been caused by release of bound nonactive Cu or more probably by an increased exposure of metal containing prostetic groups following denaturation. As shown in Fig. 7, xanthine oxidase activity was very dependent on temperature and RH. A great loss of activity was detected when MFGM's were stored for 1 day at 37°C, RH = 50% and no activity was found after 2 weeks in the same storage conditions. For MFGM stored under dry conditions (37°C, Calcium Sulfate), 35% of activity was found after the same period. According to our results, enzyme denaturation was more important at intermediate RH's. It is interesting to note that the formation of HMWP, detected by SDS-PAGE, as shown in Fig. 6, was affected by time, RH and the atmosphere under which MFGM were stored.

Increases were observed only in samples exposed to air, and no polymers were found in those stored under nitrogen, indicating a correlation between HMWP formation and lipid oxidation. Studies on different biological systems as on rat liver microsomes and hepatocytes (Koster and Lee 1980; Koster *et al.* 1982), in red cell membranes exposed to peroxides (Koster and Lee 1983) and exposed to sonicated lipid vesicles (Alloisio *et al.* 1983) reported a close association between HMWP formation and lipid oxidation, since lipid oxidation inhibition resulted in lack of HMWP's formation. Bouzas and Karel (1984) and Funes and Karel (1984) also reported HMWP formation when red cell membranes were stored under different conditions. Moreover, Hochstein and coworkers (Jain and Hochstein 1980, Corey *et al.* 1980) have stated that the formation of HMWP's is subsequent to peroxidation of membrane lipids, although they suggest that other polymerization reactions are also possible.

Consequently, it seems that MFGM protein polymerization is induced by lipid oxidation of membrane lipids and the mechanism of polymerization is possibly similar to that described by Gamage *et al.* (1973).

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FACTORS AFFECTING SULFUR DIOXIDE BINDING IN DRIED APPLES AND APRICOTS

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ABSTRACT

The effect of sulfuring on bisulfite binding in apricots and apples was determined. Sulfuring methods had no effect on binding. Dehydration resulted in less conversion of the sulfur dioxide to the sulfate. An oxygen scavenger added to the packaged dried apricots reduced both the rate of sulfur dioxide conversion to sulfate and the total sulfur dioxide loss, resulting in the product remaining lighter in color for a longer time. In resulfuring, a progressively larger percentage of the added sulfur dioxide was bound as the storage time before resulfuring increased. The pH effect on sulfur dioxide binding was different in model solutions than in particulate fruit. In the former, there appeared to be a direct relationship to bisulfite dissociation but not in the latter.

INTRODUCTION

Sulfur dioxide treatment is used widely in the food industry to reduce fruit darkening rate during drying and storage. The chemical reactions of sulfur dioxide when it is added to fruits and other food products are complex, and considerable research has been done in this area. When sulfur dioxide is absorbed into fruit, or a product of similar pH, it is converted mainly to the bisulfite ion. The bisulfite ion can remain free and available to retard the formation of Maillard-type compounds, and it can also reversibly bind to certain compounds, such as the carbonyl groups of aldehydes (Gehman and Osman 1954). This bound sulfite is considered to have no retarding effect on product deterioration (Burroughs and Sparks 1973), thus it is important to know the factors that influence binding.

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Binding of sulfur dioxide in model systems has been studied by Ingram and Vas (1950), McWeeny (1981, 1982), and others. Burrough (1964, 1973) investigated the compounds that cause binding in apple juice and wines. McBean (1967) studied sulfur dioxide binding in apricots and peaches sulfured in a controlled gas sulfuring chamber and dried in a hot-air dehydrator.

The present study was initiated to determine the effects that product variability, commercial sulfuring and drying procedures, and storage have on sulfur dioxide binding and its availability for retarding non-enzymatic browning. This study comprises the main commercial sulfuring procedures for apricots, along with sun-drying, and a controlled experimental sulfuring procedure for apples.

MATERIALS AND METHODS

Apricot Sulfuring and Drying

Apricots were sulfur treated by submitting the freshly cut fruit to sulfur dioxide gas, provided by either bottled gas or burning sulfur. Sun-drying of the apricots was accomplished by the regular commercial procedure of placing the fruit on trays in the sun for 2–5 days and then stacking the trays until dry. One experimental lot was hot-air dehydrated at 68°C to determine the effect of drying method on sulfate formation.

Apple Sulfuring and Drying

Apple pH was varied by soaking the fresh Granny Smith apple slices for 20 min in juice that had been freshly made from the apples and in which the pH had been adjusted by adding either potassium hydroxide or citric acid. Apples were also dipped into a 1% bisulfite solution before dehydrating at 68°C to a water activity (A_w) of 0.6. The final SO_2 and A_w levels were adjusted by injection into sealed pouches of the dried product a predetermined mixture of sulfur dioxide dissolved in water.

Oxygen Scavenging in Packages

Oxygen was removed from some of the packaged dried apricots by adding an oxygen scavenger mixture into the package. A small polyethylene pouch containing a mixture of 0.7 g ferric chloride, 2.3 g activated carbon, and 0.9 g sodium bisulfite in 1.2 ml. of 3% hydrogen peroxide solution was sealed inside the package of fruit to absorb the oxygen. This mixture was shown by Stanley and Block (1963) to absorb over 98% of the oxygen in a one liter volume of air within 12 h.

Composition of Model Solutions

Model solutions consisted of 17% glucose and 0.4% sodium bisulfite in citrate/phosphate buffer of the desired pH.

Analytical Methods

Total sulfur dioxide was determined by the iodine tritration procedure of Ponting and Johnson (1945), while free SO_2 was determined by adding 3 ml of 1M HCl to 50 ml of the filtrate obtained in the above method followed by iodine titration. Bound sulfur dioxide was calculated from the difference between the two. Sulfate was determined gravimetrically by adding hot barium chloride solution to the boiling liquid phase obtained from the activated carbon filtration of the residue in the Monier-Williams sulfur dioxide analysis procedure (AOAC 1980). The AOAC (1980) procedure was used for moisture determination.

RESULTS AND DISCUSSION

Sulfur Dioxide Binding and Losses in Dried Apricots

In following the sulfuring operation, only about 10% of the absorbed sulfur dioxide was bound in the initial sulfuring. During sun-drying, more of the sulfur dioxide became bound and some was totally lost from the system by vaporization and by combination with oxygen to form the sulfate. About 30–40% of the sulfur dioxide was in the bound form in the final dried product (Fig. 1). This is lower than the 55–80% bound SO_2 found by McBean (1967) in fruit after 10–14 h dehydration. The reason for his higher percentage is probably because of the use of an experimental sulfuring chamber where the fruit was exposed to a constant high concentration of SO_2 gas (2%) during the entire sulfuring time of 6–8 h, and also his use of hot-air dehydration. In commercial sulfuring, the gas is produced in the chamber for only a short initial period, peaking for a few minutes at about 2%, after which it drops off, ending at a concentration of 0.4% or less after 4–5 h (Bolin *et al.* 1983). Sulfuring fruit by burning elemental sulfur or by injecting sulfur dioxide gas directly into the chamber gave similar results.

Studying the influence of storage on binding indicated that frozen storage (−10°C) had no consistent effect (Fig. 2). When the storage termperature was raised to 21°C, the total sulfur dioxide loss increased as did the degree of binding. After 12 months storage, about 30% of the total sulfur dioxide had been lost and the proportion bound had increased to 75%. At this time, only a slight change in visual appearance could be noticed.

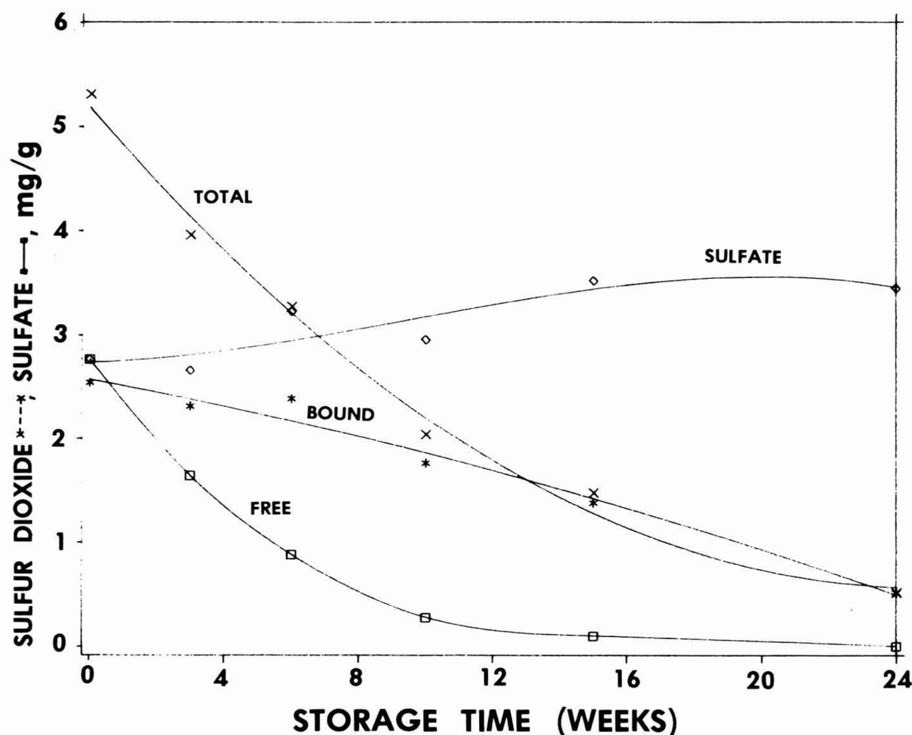


FIG. 1. CHANGE IN SULFITE AND SULFATE CONTENT OF DRIED SULFURED APRICOT HALVES STORED AT 32°C (EXPRESSED ON MOISTURE FREE BASIS, MFB)

Storage of the dried apricots at 32°C resulted in a further rate increase in the sulfur dioxide loss. The initial change was a rapid loss of free sulfur dioxide (Fig. 1), which resulted in a concurrent loss in total sulfur dioxide, since the bound sulfur dioxide level held fairly constant. As more of the free SO_2 was lost, the bound sulfur dioxide content became lower because some of it was converted back to the free form. At the point where the product had darkened visually, which was at about 11 weeks, 90% of the free SO_2 had been lost. At this point, 70% of the bound SO_2 was still present as a bisulfite pool, but its dissociation rate was too slow to provide enough free bisulfite to inhibit the Maillard reaction.

One factor that influenced SO_2 binding was the availability of oxygen to the system. With oxygen present, some of the sulfur dioxide present can react irreversibly to form the sulfate. By removing these molecules of free SO_2 from the system, the equilibrum is shifted and more bound SO_2 is released. The rate of free sulfur dioxide oxidation varied during drying, depending on the drying procedure used, with a 50% greater rate occur-

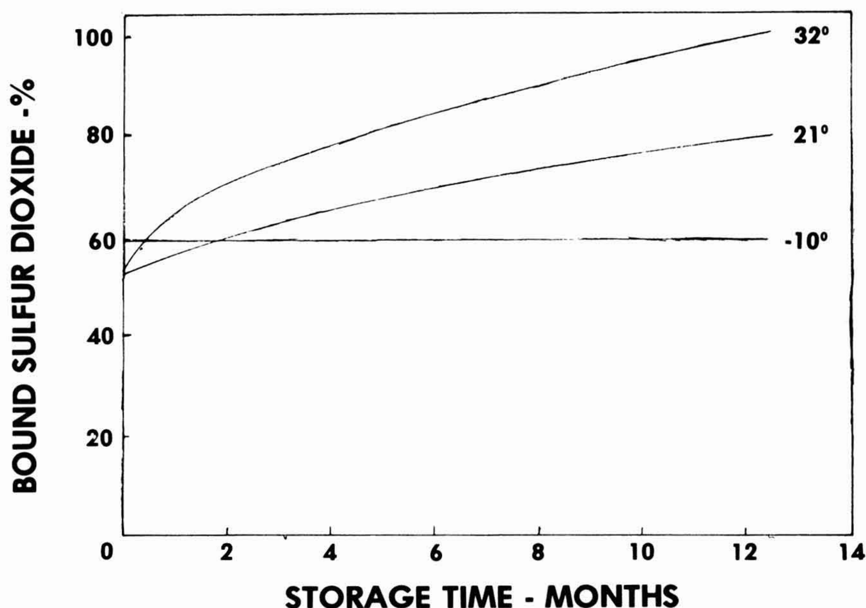


FIG. 2. EFFECT OF STORAGE TEMPERATURE ON SULFUR DIOXIDE BINDING IN APRICOTS

ring in sun-dried fruit than dehydrated. Because oxygen is required in formation of sulfate reaction, any reduction in the availability of oxygen should result in a decreased reaction rate.

One way of removing oxygen from a sealed package of fruit is to add a scavenger material to reduce oxygen availability. This oxygen reduction slows down the detrimental loss of sulfur dioxide, but it does not completely eliminate it. In this study, dried sulfured apricots stored with an oxygen scavenger produced sulfate, but at a much slower rate than untreated samples (Fig. 3). Oxygen scavenged samples also lost total sulfur dioxide at a slower rate than the control, which resulted in the fruit product retaining its light color longer. After 16 weeks storage, the oxygen scavenged samples were a considerably lighter color than the control. This lightness difference would be expected since a higher concentration of free bisulfite would be available in the product for a longer period.

Since sulfur dioxide is lost from a product during storage, resulting in an increase in the rate or formation of Maillard type compounds, and also darkening, dried fruits are commercially resulfured before packaging for distribution. In samples that were moderately resulfured immediately after drying, about 60% of the equilibrated sulfur dioxide was bound. In samples that were stored six weeks at 32°C before resulfuring, 70% was bound, and when the storage time was increased to 26 weeks before re-

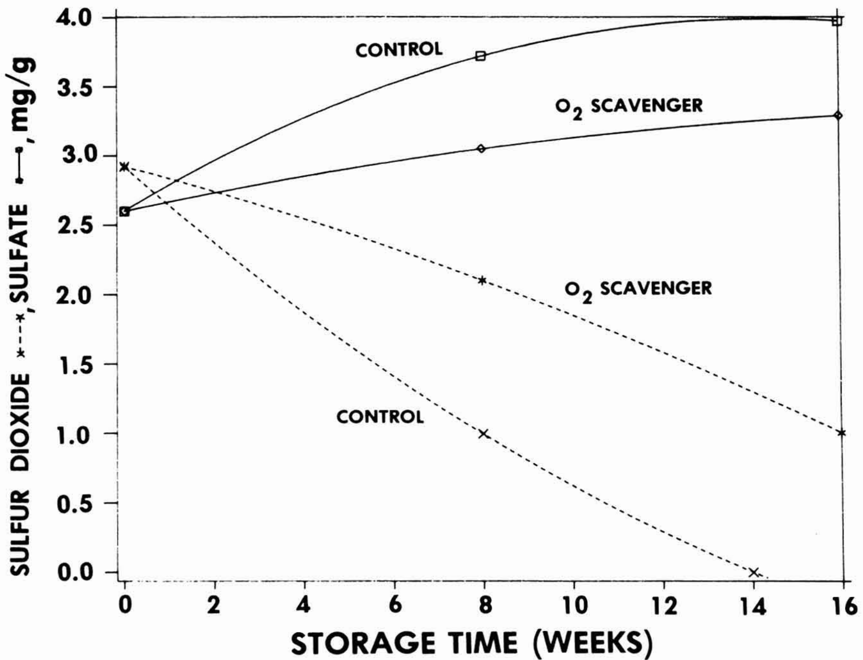


FIG. 3. CHANGE IN TOTAL SULFUR DIOXIDE AND SULFATE IN APRICOT HALVES STORED AT 32°C WITH AND WITHOUT AN OXYGEN SCAVENGER SYSTEM.

sulfuring, essentially 100% of the added sulfur dioxide became quickly bound. This indicates the proclivity for the Maillard compounds that are formed in the product during storage to strongly bind sulfur dioxide.

Sulfur Dioxide Binding in Dried Apples

The effect of fruit pH and sulfur dioxide concentration as well as storage time on binding was studied in dried sulfured apples, since these parameters influence the degree of SO_2 binding. In pH adjusted dried apples, more sulfur dioxide binding occurred initially as the product pH decreased from 3.9 to 2.8 (Table 1), with the continued loss rate relationships during storage being similar (Fig. 4). The increase in binding at the lower pH level occurs in the pH area where there is a decrease in bisulfite dissociation.

Model Solutions

Since the magnitude of pH range adjustments in fruit is limited, a model set of solutions was prepared to determine if sulfur dioxide binding over a broader pH range could possibly relate to sulfur dioxide dissocia-

Table 1. Influence of pH on sulfur dioxide binding in dried apples

pH	SULFURING LEVEL			
	LOW		HIGH	
	Total SO_2 (mg/g)	Bound SO_2 (%)	Total SO_2 (mg/g)	Bound SO_2 (%)
2.8	0.86	59	2.72	69
3.4	0.69	43	2.59	57
3.9	0.42	48	2.16	51

tion. Analysis of equilibrated glucose-bisulfite solutions showed that there was an increase in percent binding as the solution pH value increased, with a maximum being reached at about pH 4, followed by a reduction in binding (Fig. 5). This binding curve has a similar shape to the bisulfite ion dissociation curve, indicating that there is a possible

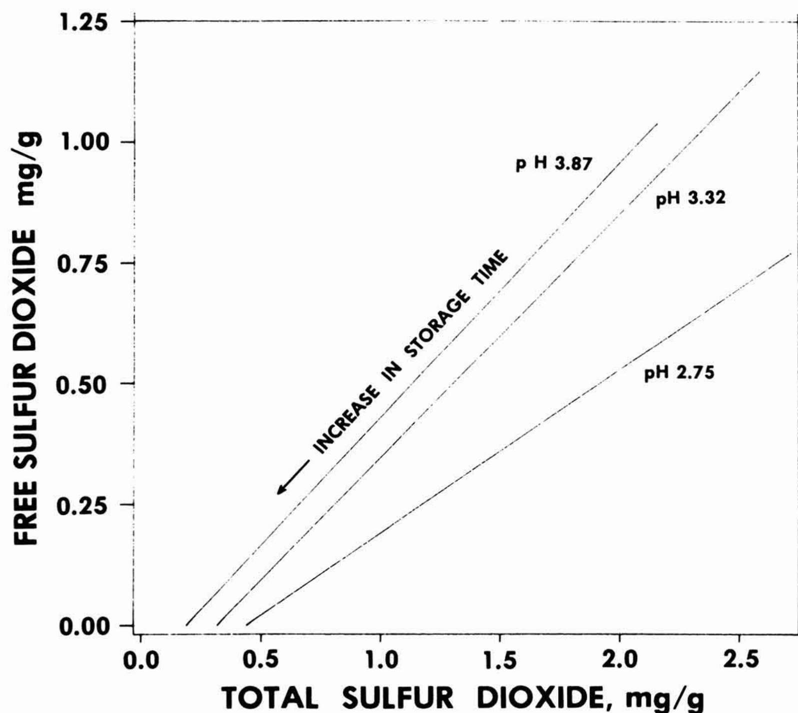


FIG. 4. RATIO OF FREE TO TOTAL SULFUR DIOXIDE IN DIFFERENT PH DRIED APPLES DURING STORAGE AT 36°C.

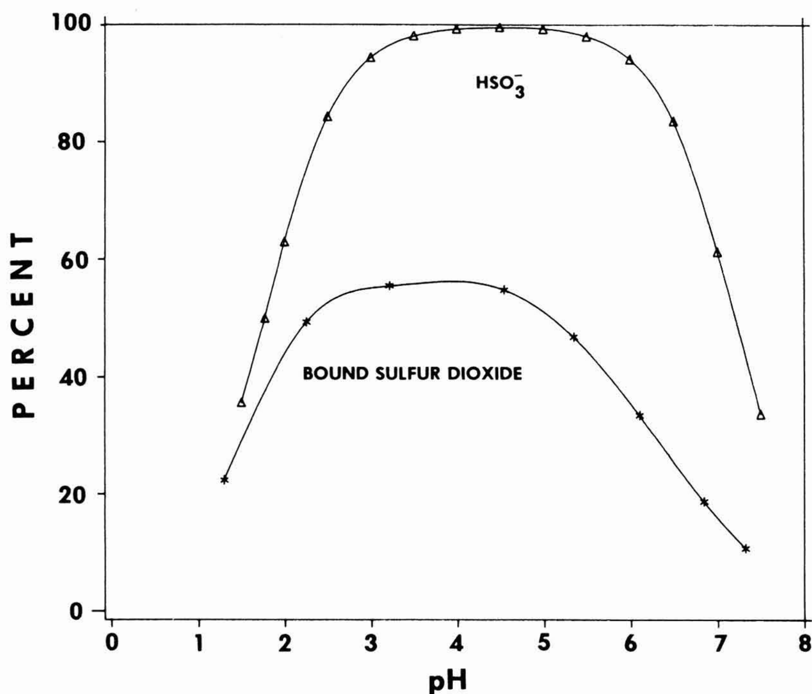
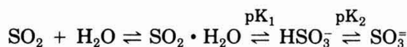


FIG. 5. COMPARISON OF BOUND SULFUR DIOXIDE IN MODEL GLUCOSE/BISULFITE SOLUTIONS TO CALCULATED BISULFITE. The dissociation curve calculations were made using $pK_1 = 1.77$ and $pK_2 = 7.20$ (King *et al.* 1981) in reaction



$$SO_2 + H_2O \rightleftharpoons SO_2 \cdot H_2O \overset{pK_1}{\rightleftharpoons} HSO_3^- \overset{pK_2}{\rightleftharpoons} SO_3^=$$

relationship between the two. Vas (1949) in his work with model systems also found that SO_2 binding varied with pH, but he did not relate this to SO_2 dissociation.

The model solution did not react the same as the particulate dried apple. In going from pH 2.8 to 3.9 in the model solutions, the binding increased from 48% to 57%. However, in dried apples, the binding at these same pH levels reacted differently, by dropping from about 69% down to 51%. This difference could not be caused by the small variations in the total sulfur dioxide content of these apple samples, since Vas (1949) found that even large differences in SO_2 cause only a minor change in binding. This difference of the higher percentage of binding occurring in the lower pH fruit could be caused by the multiplicity of different binding components other than glucose in the fruit, each of which could have different binding characteristics. For example, two strong binding compounds, acetaldehyde and pyruvic acid, behave very differently (Burroughs and

Sparks 1973). In acetaldehyde, as the pH is raised from 3 to 4, the equilibrium constant of the bisulfite compound decreases. However, with pyruvic acid, the reverse occurs; it greatly increases. Apples contain both of these compounds, plus a multiplicity of others such as pectins, which bind strongly, and also various sugars. Sugars also have a different binding capacity, from sucrose which exhibits no binding, up to mannose which binds three times greater than glucose (Ingram and Vas 1950).

The differences in these model solutions and dried apples illustrates how research results obtained from abbreviated model solutions cannot always be extrapolated to relate directly to reactions in a complex chemical system such as is present in an agricultural product.

In these series of experiments gaseous sulfuring procedures of the fruit, whether by burning sulfur or direct injection, did not affect SO_2 binding or product stability during storage, but temperature did. Sulfur dioxide binding in dried apples does not appear to be directly related to bisulfite ion dissociation, as it is in the model solution. However, even the effect of dissociation on binding does not tell the whole story since all the bound sulfur dioxide cannot be accounted for by the quantity of bisulfite ion or molecular sulfur dioxide present.

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EVALUATION OF EXTRUSION COOKED COTTONSEED/SOYBEAN BLENDS OF DIFFERENT PROPORTIONS

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ABSTRACT

Cottonseed kernel/full-fat soy flour blends of different proportions were cooked in a Brady low-cost extruder. Total and free gossypol, available lysine, proximate analysis, and PER and NPU values varied directly with composition. Reduction in free gossypol as a result of extrusion was constant, and independent of composition (ca. 90%). The 25 and 50% cottonseed blends exhibited low free gossypol (within limits permitted for human consumption), high available lysine, high PER and NPU values, and amino acid patterns equalling or exceeding the FAO/WHO (1973) children's pattern, except for moderate deficiencies in total sulfur amino acids and small to negligible deficiencies in valine. Both blends resembled full-fat soy products in proximate analysis. These results indicate that extension of soy with cottonseed products up to a level of 50%, utilizing the procedure described in this work, would be feasible.

INTRODUCTION

Soybeans and soybean products are known to be excellent sources of high-quality protein (FAO 1970; Wolf and Cowan 1971). Cottonseed is also an abundant protein source whose cost is approximately 60% that of

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soybeans (Anon. 1984). Cottonseed protein, however, is of lower quality than soybean protein (Harper *et al.* 1978). Also, glanded cottonseed, by far the most common variety, contains gossypol, a substance which is known to be toxic to man and other monogastric animals (Harper 1969; Smith 1970).

If cottonseed and soybean proteins were combined and the resulting blend heat-treated, the following results would be expected: (1) the free gossypol contained in cottonseed would be substantially reduced by reaction with the lysine from both proteins (Bressani and Elias 1968); (2) the excessive lysine contained in soybean protein (FAO 1970), however, would keep the available lysine of the blend at a high level, thus assuring good protein quality; (3) cottonseed products, currently used mostly for feeding of ruminants due to their high free gossypol, would be made available for human consumption; and (4) low-cost cottonseed protein would serve as an extender for higher-cost soybean protein.

The purpose of this work was to evaluate the above hypothesis.

MATERIALS AND METHODS

Raw materials utilized in preparing the cottonseed/soybean blends were dehulled cottonseed kernels, obtained from a local plant, and recently produced (approximately one month old) full-fat soy flour, manufactured by Productos Alimenticios Delicias, S. A. de C. V. in Delicias, Chihuahua, Mexico, utilizing a low-cost extrusion process (Harper and Del Valle 1979). Total and free gossypol contents of the cottonseed kernels were determined utilizing AOCS Official Methods (1975).

Blends of soy and cottonseed containing 25, 50, 75 and 100% cottonseed were prepared and cooked in a Brady low-cost extruder (Harper *et al.* 1978; one pass, 90 s at 140°C). The following analyses were conducted on the cooked blends: total and free gossypol contents (also in blends before cooking, AOCS 1975); available lysine content (Conkerton and Frampton 1959); proximate chemical analysis (AOAC 1970); amino acid analysis (Spackman *et al.* 1958; Kohler and Palter 1967); and Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) (Munro and Allison 1964; Miller 1963), utilizing casein as a reference protein. PER and NPU were also determined on the same sample of full-fat soy flour utilized in the blends.

Further information on the PER and NPU methods is as follows. All determinations were carried out at Instituto Nacional de la Nutrición in

Mexico City, utilizing animals from its rat colony. Five male weanling rats, 22–23 days old and weighing 27–28 g, were used in each determination. Before initiation of feeding, all animals were standardized by starving for 24 h. Protein levels in diets were adjusted to 10%. PER and NPU were determined for each rat at the end of the test period, which was 28 days, after which average values, with corresponding standard deviations, were calculated for each group of animals fed the same diet. For the NPU determinations, body nitrogens were measured by sacrificing the animals, homogenizing the carcasses and analyzing the homogenates by the Kjeldahl method (AOAC 1970). Body nitrogen at the beginning of the test period was taken equal to average body nitrogen of five of the same weanling rats (same population, age and average weight) as those used in the feeding tests. All animals were housed in standard rat cages (one animal per cage), located in Instituto Nacional de la Nutrición's animal laboratory.

RESULTS AND DISCUSSION

Total and free gossypol contents of the cottonseed kernels were 9840 and 8946 ppm, respectively.

Results obtained with the extruded blends are reported as follows: Table 1, total and free gossypol and available lysine contents, also of the unextruded blends; Table 2, PER and NPU values; Table 3, proximate chemical analyses; and Table 4, amino acid analyses.

Table 1. Total and free gossypol and available lysine contents of cottonseed kernel/full-fat soy flour blends of different compositions, before and after extrusion

PERCENT COTTONSEED IN BLEND	TOTAL GOSSYPOL EXTRUSION, PPM		FREE GOSSYPOL EXTRUSION, PPM		REDUCTION IN FREE GOSSYPOL, PERCENT	AVAILABLE LYSINE AFTER EXTRUSION, G/100 G PROTEIN
	BEFORE	AFTER	BEFORE	AFTER		
25	2530	1920	2267	281	88	5.3
50	4967	3910	4494	423	91	4.6
75	7404	5350	6719	714	89	4.3
100	9840	7060	8946	1091	88	3.9

Table 2. P.E.R. and N.P.U. values of extruded cottonseed kernel/full-fat soy flour blends of different proportions

PERCENT COTTONSEED IN BLEND	P.E.R.[a]	CORRECTED P.E.R.[b]	N.P.U.[a]
0	2.83 ± 0.48	2.08	48.9 ± 7.25
25	2.64 ± 0.79	1.94	44.8 ± 5.84
50	2.20 ± 1.68	1.68	41.7 ± 11.58
75	2.15 ± 5.84	1.58	37.0 ± 4.74
100	1.95 ± 1.42	1.43	30.9 ± 0.46
CASEIN	3.41 ± 0.29	2.50	51.8 ± 8.37

[a]Results reported as average ± standard deviation.
[b]Corrected P.E.R. reported on basis of casein P.E.R. = 2.5.

Concerning Table 1, the Protein Advisory Group (PAG) of the United Nations has set a limit on the maximum free gossypol content of cottonseed products which may be considered suitable for human consumption and which is 600 ppm (PAG 1975). Under these conditions, only the 25 and 50% cottonseed blends, possessing 281 and 423 ppm of free gossypol, respectively, would be acceptable. The available lysine contents of these products, 5.3 and 4.6 g/100 g protein, respectively, were appreciably higher than is usual for 100% cottonseed products of adequate free gossypol levels, values for which vary from 2.9 to 3.9 g/100 g protein (Rao *et al.* 1963). Table 2 shows that the high available lysine and low free gossypol contents of these blends were reflected in their corrected PER (1.94 and 1.68 for the 25 and 50% blends, respectively) and NPU (44.8 and 41.7, respectively) values: all were appreciably higher than those for the extruded 100% cottonseed product, which were 1.43 and 30.9, respectively.

The low free gossypol contents of the 25 and 50% cottonseed blends were due to two factors, i.e., dilution by the soybean component and reduction by heat treatment applied in the extruder. The dilution effect is clearly seen in Table 1, which shows that total and free gossypol contents decreased, while the available lysine content increased, all with increas-

Table 3. Proximate analyses of extruded cottonseed kernel/full-fat soy flour blends of different proportions

PERCENT COTTONSEED IN BLEND	PERCENT PROTEIN	PERCENT FAT	PERCENT ASH	PERCENT MOISTURE
0[a]	39.0	21.1	5.2	7.0
25	37.9	25.0	5.2	5.9
50	37.1	25.7	5.3	4.2
75	36.7	27.4	5.3	3.3
100	35.3	30.2	5.3	2.8

[a]Data correspond to commercial extruded full-fat soy flour (from FAO 1970).

ing blend soybean proportion. The same effect is also apparent in Table 2, which indicates that PER and NPU values decreased with increasing proportion of the cottonseed component in the blend. Heat treatment applied in the extruder was equally effective in reducing free gossypol in all blends, since Table 1 shows that percent reduction of this substance, after passing through the extruder, was approximately the same in all cases, of the order of 90%.

Table 3 shows that proximate analyses of the different blends were dependent upon composition, varying between extreme values for 100% cottonseed and 100% soybeans. Since the latter materials did not differ much in proximate analysis, all blends were fairly similar in this respect. In like manner, Table 4 shows that amino acid analyses reflected blend composition between the extreme values of 100% cottonseed and 100% soybeans.

Table 4 includes the FAO/WHO (1973) amino acid children's pattern for comparison. It may be seen that with the exception of sulfur amino acids and valine, the 25 and 50% blends either equalled or exceeded the pattern. Moreover, although the deficiencies in sulfur amino acids were considerable, those in valine were negligible for the 25% blend and small in the case of the 50% blend.

Table 4. Amino acid analyses of extruded cottonseed kernel/full-fat soy flour blends of different proportions

PERCENT COTTONSEED IN BLEND	AMINO ACID CONTENT, G/16 G NITROGEN ISO	LEU	LYS	TOTAL SULFUR	TOTAL AROM.	THREO	TRY	VAL
0[a]	5.02	8.18	6.30	2.59	8.08	4.44	1.37	5.31
25	4.32	7.86	5.66	2.65	8.73	4.10	1.24	4.57
50	3.77	7.26	5.88	2.71	8.99	3.36	1.09	3.91
75	3.84	7.44	5.10	2.78	9.27	3.28	0.84	4.17
100	3.27	6.74	4.57	2.85	10.92	4.02	0.82	4.08
FAO/WHO (1973) CHILDREN's PATTERN	4.0	7.0	5.5	3.5	6.0	4.0	1.0	5.0

[a]Data correspond to commercial extruded full-fat soy flour (from FAO 1970).

SUMMARY AND CONCLUSIONS

Summarizing, the results of this work indicate that extension of full-fat soy products with cottonseed kernels, up to a level of 50%, would be feasible. Such blends would have the following characteristics: (1) free gossypol content within limits established by the PAG (1975); (2) proximate analysis similar to that of full-fat soy products; (3) fairly high PER and NPU values, considerably exceeding those for 100% cottonseed; and (4) amino acid analysis equalling or exceeding the FAO/WHO (1973) children's pattern in all amino acids, with the exception of a moderate deficiency in sulfur amino acids and a small to negligible deficiency in valine.

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THE EFFECT OF POSTHARVEST HANDLING TECHNIQUES ON CANNERY YIELDS OF PIMIENTO PEPPERS

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ABSTRACT

A damage classification scheme was developed and used to assess postharvest handling of pimiento peppers. Mass losses due to evaporation were lowered by removing the stem at the calyx. Greater moisture losses were observed at 27°C and 45% RH than 37°C and 65% RH in all damage classes except microbial invasion. Greatest peeling-yield losses were observed in classes which permitted lye penetration of the cuticle (mechanical, bacterial, and fungal damage). A model equation was developed to predict yield based on the lye concentration, average pepper weight and the percentages of fruit in each of five damage classifications (whole sound, shrivel, mechanical, bacterial and fungal). Removal of the stem during harvest, careful screening of fruit at the receiving station, and damage evaluation of incoming loads should result in improved pimiento processing yields.

INTRODUCTION

Competition from overseas suppliers and increasing field labor costs have stimulated pimiento processors to seek ways to improve cannery yields. Although a pimiento pepper harvester has been developed (Fullilove and Futral 1972), it has not found commercial acceptance. The major growing areas of pimiento peppers have been shifting away from the major processors who have remained in the same location. As a result, pepper-receiving stations have been established by the processors in the growing areas for sorting and grading prior to long distance transport (150–500 miles) in either open-mesh metal trailers or pallet-bins on flatbed trailers. Although in-plant yields have been improved by the adoption of lower lye concentrations during peeling and alternate coring techniques (Heaton 1984), no previous research has focused on the potential for improving processing yield by modifying postharvest handling.

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Thompson (1978) has noted that up to 50–70% of commercial packs of peppers suffer some form of physical damage. Damage can be incurred by the pepper fruit as the result of physiological disorders during growth and development, infestation by insects or microbes, and mechanical damage by handlers, machinery or stem punctures. Lengthened transportation and holding times have increased moisture losses between harvesting and processing.

New techniques of evaluating changes during postharvest handling of fruits and vegetables such as loss assessment (NRC/NAS 1978), the systems approach (Shewfelt *et al.* 1984) and damage classification (Studer *et al.* 1981) have been introduced. The purpose of this study was to assess and evaluate factors in postharvest handling of pimiento peppers that would affect processing yield.

MATERIALS AND METHODS

A damage classification scheme for pimiento peppers was devised based on personal observation by the authors and consultation with field, receiving station and processing plant personnel. The classes were mutually exclusive and classification was performed by the same experienced judge using the visual criteria described in Table 1. Preliminary studies included the 8 classes plus all possible combinations. Since less than 5% of the fruit showed combined damage, none of the combinations were tabulated. In cases where 2 or more types of damage were observed in the same pepper, it was classified by the predominant defect.

Mass Loss

Two hundred freshly harvested pimiento peppers were randomly divided into 2 equal groups. In one group, the entire stem was removed from each pepper by clean cuts with a sharp knife. The remainder of the peppers were wounded by removal of ⅛" of the stem. Both whole stem and no stem treatments were further subdivided for storage at 37°C, 65% RH or 27°C, 45% RH in Lab-Line Environrooms.

Additional raw pimiento peppers, obtained from the local processing plant, were then sorted by damage class. Twenty peppers were randomly selected from each damage class (except insect damage as there was little insect infestation in this particular load) labeled and weighed. Five peppers from each of the damage classes were stored in Lab-Line Environrooms programmed to maintain the following conditions: (1) 5°C, 65% RH; (2) 19°C, 55% RH; (3) 27°C, 45% RH; and (4) 37°C, 65% RH.

Table 1. Damage classification scheme used in this study

CLASS	ABBREVIATION	DESCRIPTION
Whole Sound	WS	No defects, not soft, shrivelled, limp pliable, may yield to slight pressure
Growth Cracks	GC	Obvious epidermal ruptures with complete or partial suberization
Mechanical	M	Fresh cuts, bruises, punctures, abrasions, crushing or cracking; no suberization
Shrivelled	SH	Distinctly soft or flabby, often wrinkling of epidermis without tissue breakage
Bacterial	B	Weakened, depressed, watery lesions; slight flesh discoloration; epidermis may be ruptured
Insect	I	Obvious chewing damage, frass around calyx or small circular holes in fruit wall
Fungal	F	Soft, weakened, depressed areas, distinctly discolored by aerial hyphae and fruiting bodies
Sunscald	SU	White-yellow necrotic and water-soaked blisters, which eventually become papery

Concurrent storage studies with other commodities in the environmental chambers precluded selection of other temperature/relative humidity regimes. Peppers were individually labeled and weighed. Each pepper was reweighed after 12, 24 and 48 h of storage.

Handling Losses

Pimiento pepper damage was monitored at three major steps in the postharvest handling system: (1) freshly harvested as they arrived at the receiving station and were being held prior to dumping and receiving; (2) after dumping, sorting and grading at the receiving station prior to loading on the truck to the processing plant; and (3) after receiving and unloading at the processing plant prior to washing, peeling and coring. Pepper samples were taken randomly in 4.5 gallon plastic containers holding 80–100 peppers and each pepper was classified using the scheme shown in Table 1. Recording of the data was aided by the use of a Hewlett Packard HP-97 programmable printing calculator as a data logger programmed for entry by a single keystroke. This technique enabled classification of approximately 500–600 peppers/h by a single judge (Esensee 1984).

Sampling occurred on 3 receiving dates during August, 1983 which is typically the earliest and hottest month of the pepper harvesting season. Totals of peppers evaluated at each step represent at least 7 containers. In each case, all samples were evaluated from those of a single truckload. Peppers were followed from the receiving station to the processing plant (a distance of 165–200 miles).

Theoretical Yield

Theoretical yield determinations were made based on methodology developed by Heaton (1984). Pimiento peppers were obtained from a local processing plant as they arrived and were separated into the 8 damage classes. Lots of 50 peppers each consisting of 1, 2 or 3 damage classes were subjected to a simulation of commercial peeling and coring. Each lot was washed, weighed and peeled in a small industrial-type batch peeler (Dixie Canner Equipment Co., Athens, GA). Standard peeling conditions were 99°C with a lye concentration of 5.5% for a period of 3.25 min. Since lye concentration could not be maintained at a constant level due to evaporation, concentration was monitored after 3–4 batch peelings by titration with standardized 0.1 N HCl. Skins were loosened with water and removed by hand. Coring was accomplished using an industrial coring machine (Altman Industies Inc., Gray, GA) using the 1⅕" diameter coring knife. After final washing to remove loose seeds and minimal trimming, the peppers were drained and weighed.

Statistical Analysis

Standard analysis of variance and regression techniques were performed using the Statistical Analysis System (SAS 1982) package. Model equations were developed using the backwards stepwise regression procedure.

RESULTS AND DISCUSSION

Mass Loss

The effect of stem length on mass loss during storage is shown in Table 2. Complete removal of the stem results in reducing the losses due to transpiration through the stem. The conditions of the experiment are not unrealistic in terms of actual handling conditions. Pimiento peppers are

Table 2. Effect of stem length and time of storage on percentage mass loss of pimiento peppers during storage at 38° C, 65% RH or 27° C, 45% RH

TIME	STEM	
(h)	Whole	None
12	2.0	1.6
24	3.4	3.1
48	6.7	6.0

harvested during the late summer and early fall months in warm climates with daytime temperature frequently greater than 30°C. Long transportation distances can result in delays of 48–60 h between harvesting and processing.

Pimientos are indeterminate and lack natural fruit abscission which can result in plant defoliation, predisposing remaining fruit to sunscald (Barber and Sharpe 1979). Tugging at the fruit in the conventional manner can also lead to damaging the plant and decrease subsequent yield. In addition to increased moisture loss through transpiration, stems can inflict puncture damage to adjacent fruit during handling and transport. Furthermore, the stem represents greater than 3% of the pepper which is of no use to the processor (Cochran 1963). Thus, it would appear that harvesting of fruit at the calyx with a sharp knife or shears could benefit both the grower and processor. Adoption of such a practice must be implemented with care, however, to prevent mechanical damage to the fruit while ensuring that the entire stem is removed.

The rate of mass loss is affected by the presence and type of damage and the storage conditions (Table 3). Most rapid losses were observed in peppers damaged mechanically (M) or microbially (B and F). Shrivelled (SH) pepper as expected exhibited the least mass loss. Vapor pressure difference (VPD) is known to be an important factor affecting mass loss in fresh fruits and vegetables (Wills *et al.* 1981). In all classes except B and F, greater losses were observed at the lower relative humidity/temperature conditions (27°C, 45% RH, VPD = 0.310) than at the higher relative humidity/temperature regime (37°C, 65% RH, VPD = 0.121). In decayed peppers (B), the greater losses observed at the higher temperature is attributable to more rapid growth of the bacteria resulting in greater availability of the moisture present and increased respiration as a stress response.

Table 3. Interactive effects of damage class × storage time and damage class × storage conditions on percentage mass loss of pimiento peppers

		DAMAGE CLASS						
		WS	GC	M	SH	B	F	SU
TIME (h)								
2		0.3	0.3	0.5	0.2	0.3	0.3	0.3
4		0.6	0.7	1.1	0.6	0.7	0.8	0.6
8		1.2	1.4	2.1	1.1	1.4	1.6	1.2
24		4.2	4.7	6.6	3.9	4.9	5.9	4.0
48		7.0	7.5	10.6	6.4	8.6	10.0	7.0
TEMP (°C)	RH (%)							
5	65	0.6	0.9	1.5	0.7	0.8	0.9	0.7
19	55	2.5	2.7	3.5	2.4	2.9	3.1	2.3
27	45	3.4	3.6	5.5	2.9	3.1	4.2	3.0
37	65	2.4	2.5	3.3	2.3	3.9	4.1	2.7

Damage Incurred During Handling

Changes in 3 major damage classes during handling are shown in Table 4. Although little or no mechanical damage is observed in freshly harvested peppers, each handling operation contributes to the damage. At several locations, peppers are dropped up to 12 feet with nothing to cushion the drop but other peppers. Mechanical fingers that aid flow result in puncture damage to the peppers unless the fingers are equipped with rubber covers. Loading trucks at the receiving station and unloading them at the processing plant are the major sources of mechanical damage.

Culling of diseased fruit at the receiving station is critical. A consistent 3-fold increase in bacterial damage was noted between grading at the receiving station and unloading at the processing plant. Little increase in fungal damage is noted between the receiving station and processing plant, but percentages observed in freshly harvested fruit are high enough to affect processing yields.

Theoretical Yield

The sources of the greatest losses of yield in pimiento unit operations are the peeling and coring steps. Lye concentration is a major factor in peeling losses (Heaton 1984) as the lye rapidly dissolves the fleshy per-

Table 4. Interactive effect of handling step and receiving date on mechanical damage (p = 0.006), bacterial invasion (p = 0.02) and fungal invasion (p = 0.0003)

HANDLING STEP	MECHANICAL			BACTERIAL			FUNGAL		
	A[a]	B	C	A	B	C	A	B	C
Freshly harvested	1.0	1.0	0.5	1.7	2.3	1.3	10.6	12.4	11.3
Graded at receiving station	6.2	2.5	1.5	0.1	1.7	0.8	3.2	12.4	8.7
Transported to processing plant	16.1	6.7	9.9	0.3	4.8	2.4	3.5	19.7	9.8

[a]Each receiving date occurred in the same calendar month representing a truckload of pimiento peppers transported at least 150 miles from receiving station to processing plant.

Table 5. The effects of individual damage classes on yield.

CLASS	REPLICATIONS	YIELD[1] (%)
Whole Sound (WS)	14	54.2 a
Shrivel (SH)	10	54.2 a
Growth Cracks (GC)	6	51.5 ab
Insect Damage (I)	5	49.0 bc
Sunscald (SU)	3	48.2 bcd
Mechanical Damage (M)	8	45.9 cde
Fungi (F)	7	44.5 de
Bacteria (B)	5	42.7 e

[1]Means in the same column bearing a different subscript letter are significantly different ($p<0.05$) using the new Duncan's Multiple Range Test

icarp. The effect of the types of damage observed in the raw product on the yield after peeling and coring is shown in Table 5. Maximum yields were obtained with whole sound and shrivelled (due to either drought or senescence) pepper. Growth cracks occur during rapid uptake of water by the fruit, while attached to the plant, effecting splitting of the skin followed by suberization of the skin. The resultant epidermal layer is apparently partially resistant to penetration by the lye. Sunscald is the result of photodamage causing the epidermis to separate from the pericarp with only slight alteration to the epidermis (Barber and Sharp 1971). Once the epidermis is penetrated during peeling, the injured pericarp may be more readily dissolved than healthy tissue. Obvious breaks in the epidermis

due to insect infestation, mechanical damage, or microbial invasion lead to more rapid disintegration of the pericarp.

The development of predictive equations for theoretical yield could be of benefit to the processor in making decisions on culling rates at the receiving stations. Theoretical yield refers to values obtained under laboratory conditions. Pack-out yields in the processing plants tend to be 10–20% lower than values shown here. Factors leading to processing losses include excessive lye concentration, machinery damage and trimming. It should be emphasized that most of the losses observed in this study were attributable to dissolution of the flesh by lye. Normal trimming would lead to greater losses in yield values. Regression coefficients for model equations developed to predict theoretical yield are shown in Table 6.

Increased yield values are obtained with intact peppers (whole sound and shrivelled) while decreased yield values are obtained when mechanical, bacterial or fungal damage is present. Factors for growth cracks, insect infestation and sunscald were not significant. Using Eq. 3 from Table 6 to calculate the theoretical yield of trucks arriving at the processing plants profiled in Table 3, losses of 1.5, 2.6 and 1.7% are obtained on receiving dates A, B and C, respectively. These losses are notable in themselves and would be magnified by higher lye concentrations and trimming.

Maintenance of lye concentration is critical in obtaining good pepper yields. The lye strength used in this study is a narrow range (5.0–6.0) and is generally lower than standard commercial practice. The importance of lye concentration is emphasized by the large regression coefficient corresponding to a 6.7% loss in yield for every 1.0% increase in lye strength. Lye concentration should never by higher than that needed to effect adequate peeling (Heaton 1984).

Average pepper weight is an additional factor in theoretical yield. The relationship predicts an increase of 1.0% yield for every 4.3 g increase in the weight of the average pepper. Use of a single blade size, not uncommon in commerical practice, lowers yields in the smaller peppers. Thicker fruit walls in larger peppers may also be responsible for higher yields. Potential improvements in yield might result from increasing minimum pepper size at the receiving station or size segregation at the plant prior to coring with knives of differing blade sizes.

The predictive power of the regression equation is improved with the incorporation of lye concentration and average weight as factors. When comparing predicted and actual yield values, all 3 equations tend to overestimate yield rather than to underestimate it. For example, Eq. 3 from Table 6 overestimates the yield on only 10 of 35 trials. Thus, it would appear that these equations would tend to represent minimal expected losses from postharvest damage.

Table 6. Regression coefficients of model equations developed to predict theoretical yield on the basis of percentage of each damage class (Table 1), lye concentration (%) and average pepper weight (g). The coefficient of determination (r^2) is given for each equation as are the correlation (r) and root mean square error (RMSE) for the predicted and actual yield of 35 combinations of damage classes

		DAMAGE CLASS											PREDICTED vs ACTUAL	
	INTERCEPT	WS	GC	M	SH	B	I	F	SU	LYE	WT	r^2	r	RMSE
1	49.9	0.043	NS	-0.040	-0.043	-0.072	NS	-0.053	NS	NI	NI	0.58	0.56	4.48
2	85.9	0.038	NS	-0.039	-0.042	-0.068	NS	-0.050	NS	-6.5	NI	0.64	0.62	4.39
3	75.6	0.032	NS	-0.042	-0.047	-0.067	NS	-0.050	NS	-6.7	0.23	0.73	0.70	3.70

NS—Not Significant NI—Not included in the model

Damage Classification

The damage classification scheme developed in this study has potential use as a quality assurance tool in postharvest handling of pimiento peppers and similar commodities. The results presented suggest that a scheme with only five classes: whole sound (includes shrivelled), minor (includes growth cracks, insect and sunscald), mechanical, bacterial, and fungal, is needed. Sampling of the loads from each grower could be used as a basis of payment on a sliding scale such as that developed by Kramer (1958) for corn. Maximum allowable damage from each class could be specified by the processors to the receiving stations based on yield predictions. At the processing plant, production schedules could be based on the damage profiles of waiting trucks. For example, a truck with more bacterial damage might be advanced in the schedule particularly in hot, humid weather. Damage classification schemes have been proposed by Leonard *et al.* (1977) and Studer *et al.* (1981) for tomatoes. These schemes would appear to be appropriate for any commodity that is processed and relatively stable at ambient temperatures.

SUMMARY AND CONCLUSIONS

Postharvest handling practices for pimiento peppers were investigated to determine possible factors in ultimate processing yields. Harvesting by a clean cut with a knife or shears to leave the entire stem on the plant would lead to less moisture loss during handling, less damage from stem punctures, and less damage to the plants in the field. Stricter sorting at the receiving stations to eliminate peppers evidencing mechanical, bacterial or fungal damage would lead to improved yields at the processing plant. Lower yields from these damage classes are attributed to greater losses of moisture during holding and flesh during lye peeling.

A regression equation was developed to predict theoretical yield based on damage classes, lye concentration and average pepper weight. The damage classification scheme and the predictive model could be used by the processor to evaluate the effectiveness of receiving stations, set stricter grading standards at receiving stations, develop a sliding scale for payment to the growers based on damage condition, and aid in process scheduling of waiting truckloads.

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BOOK REVIEW

Analysis of Food Contaminants. John Gilbert, Editor. 1984. Elsevier Applied Science Publishers. pp. 386. $74.00.

This book contains seven chapters that deal with selected aspects of food contaminant analysis. The chapters, each written by a different author, include the topics (1) gel chromatography as a sample clean-up technique, (2) immunoassays for drugs in meat, (3) headspace gas chromatography, (4) trace metals, (5) mycotoxins by HPLC, (6) selected ion monitoring in mass spectrometry and (7) N-nitrosamines using chemiluminescence. The sections range from 30 to 60 pages in length. Each has a very detailed organization and table of contents making the chapters easy to follow and somewhat uniform in presentation.

Although the focus of the book is contaminant analysis, the impact extends beyond the food analyst and includes the food chemist. The style avoids the strict "how to" approach and the multiple tables of data. Rather, the chemical aspects of the analyses and the chemistry of the analytes are emphasized. Food scientists in general would benefit from the information about these powerful analytical techniques.

The book has value to the food chemist because the choice of topics, both the analyses and the techniques, is appropriate and timely for today's concerns about food contamination. The reviews are current, objective and balanced, and contain extensive reference lists. The chapters provide good background and seem very readable. This adds appeal for the casual reader as well as someone specifically interested in performing the given analysis. This style of writing and editing broadens the potential audience and improves the value of the book.

The book is of very high quality and is probably one of the best of its kind published recently. For someone with interests in food chemistry and a curiosity about the seven above-mentioned areas, the book can be considered an excellent source of current, objective information.

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