# JOURNAL OF FOOD PROCESSING AND PRESERVATION

D.B. LUND
EDITOR

FOOD & NUTRITION PRESS, INC.

VOLUME 15, NUMBER 3 JULY 1991

JOURNAL OF FOOD PROCESSING AND PRESERVATION



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One volume of six issues will be published annually. The price for Volume 15 is $125.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $144.00 per year via surface mail, and $153.00 per year via airmail.

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The *Journal of Food Processing and Preservation* is listed in *Current Contents/Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (ISSN: 0145-8892) is published bimonthly by Food & Nutrition Press, Inc. — Office of Publication is 2 Corporate Drive, Trumbull, Connecticut 06611 USA.

Second class postage paid at Bridgeport, CT 06602.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., P.O. Box 374, Trumbull, CT 06611.

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TRUMBULL, CONNECTICUT 06611 USA

Food & Nutrition Press, Inc.
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ISSN 0145-8892

Printed in the United States of America

# CONTENTS

# SHELF AND MICROBIOLOGICAL STABILITY OF ACIDIFIED NONINOCULATED AND INOCULATED (*ALTERNARIA TENUIS*) TOMATO JUICE

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Accepted for Publication January 29, 1991

## ABSTRACT

*Tomato juice was pasteurized (86°C for 10 min) and subjected to 3 different conditions: control (initial pH 4.55), acidified-noninoculated (initial pH 4.00) and acifidified-inoculated (initial pH 4.00) with* Alternaria tenuis. *These were evaluated for chemical, physical and microbiological stability during ambient (29 ± 2°C) storage for 28 days. Acifidied-inoculated and control samples showed appreciable increase in microbial population; attaining Log* $_{10}$ *7.5 and 6.9 cfu* $mL^{-1}$, *respectively, at the end of storage, but no apparent change occurred in acidified-noninoculated. Similar trends occurred in Lactobacilli counts. Conversely, yeast counts increased almost at the same rate in all three treatments.* Alternaria *count and pH increased in acidified-inoculated tomato juice with storage, but total soluble solids decreased. Mycelial mat and settling out of insoluble suspended solids occurred in acidified-inoculated sample by day 16, but these adverse changes were not observed in the other 2 samples.*

## INTRODUCTION

Tomatoes are highly perishable produce, but the juice is relatively less susceptible to deterioration especially in the absence of molds (Huhtanen *et al.* 1976; Odlaug and Pflug 1979). The pH of the juice is one of the major parameters that influences deterioration. Because of this importance, the concept of acidification has become well accepted in the food industry and is of research interest to various workers (Sapers *et al.* 1977; Mudahar *et al.* 1986). Although tomato

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Journal of Food Processing and Preservation **15** (1991) 159–165. 

juice is an acid food with pH ranging between 4.0 and 4.6 (Mundt and Norman 1982), lower acidity values have been reported in various parts of the world, including Nigeria (Aworh *et al.* 1983).

*Alternaria* spp. are common contaminants of tomatoes worldwide (Prusky and Ben-Arie 1981). However, there is paucity of information on quality and microbiological changes associated with *Alternaria* spp. inoculation of tomato juice following processing under high temperature ambient storage.

The purpose of this study, therefore, was to investigate the effects of acidification and postprocess inoculation (using *Alternaria tenuis*) of tomato juice on changes in chemical composition and physical quality as well as microbiological stability during ambient tropical storage.

## MATERIALS AND METHODS

### Tomato Juice: Extraction, Acidification and Inoculation

Tomato juice was extracted from firm ripe tomatoes using hot break method (Gould 1983) by heating (with occasional agitation) quartered tomatoes to 82°C for 1 min. The resulting pulp was blended and filtered using two layers of sterile muslin cloth. The obtained juice was divided into 2 portions (100 mL and 200 mL, respectively) and treated as follows:

(1) Into each of 5 sterile McCartney bottles was dispensed 20 mL juice, capped, pasteurized (86°C for 10 min) using water bath and cooled immediately before storage at 29 ± 2°C for 28 days. The initial pH of juice was 4.55 and this served as control.

(2) The 200 mL portion was acidified (i.e., from pH 4.55 to pH 4.00) using 0.4% (w/v) citric acid (BDH, Poole, England) (Gould 1983) and each of 10 sterile McCartney bottles was aseptically filled (20 mL) and treated as described for control (1). After cooling, 5 of the filled bottles were each aseptically inoculated using 0.1 mL *Alternaria tenuis* spore suspension (approximately $5 \times 10^3$ spores $mL^{-1}$ as determined using Haemocytometer (Weber Scientific International Ltd., England). The spore suspension was prepared (Kiel *et al.* 1981) using stock cultures incubated at 27°C for 7 days. The remaining 5 bottles filled with acidified (pH 4.00) juice were not inoculated. All samples (i.e., control, acidified-inoculated and uninoculated) were analyzed for microbiological, chemical and physical (visual) changes every 7 days and replicated twice.

### Microbiological Evaluation

Following serial dilutions (using phosphate buffer pH 7.0) of samples, aliquots (0.1 mL) were spread plated on plate count agar and acidified (pH 3.5) malt extract agar before incubation at 37°C and 27°C for 2 (microbial load) and 5

(yeasts and molds) days respectively (Harrigan and McCance 1976). Man Rogosa Sharpe (MRS) agar plates were inoculated using 1.0 mL aliquots, overlaid and incubated at 37°C for 3 days (Lactobacilli). The media used were Oxoid products. After incubation, the colonies (in duplicate plates showing 30–300 colonies) were counted and examined for cultural and morphological (Gram or lactophenol cotton blue staining and motility test) characteristics. Biochemical tests using glucose, fructose, galactose, lactose, sucrose, mannitol, arginine and catalase production were carried out following purification of bacterial isolates and identified (Buchanan and Gibbons 1974).

### Chemical (pH and Total Soluble Solids) and Physical Analyses

The pH of the samples was determined using a pH meter (Pye Unicam Ltd., England) standardized against pH 4 and 7 buffers. Total soluble solids content was measured using a hand refractometer (Atago, Co. Ltd., Japan) calibrated in % Brix and the readings were corrected to 20°C.

Physical (visual) examination of juice for settling out of insoluble suspended tomato solids, consistency (flow characteristics as measured by improvised Bostwick Consistometer; Gould 1983) and mycelial mat development on the surface of the juice was carried out at each interval of analysis.

## RESULTS AND DISCUSSION

Figure 1 shows the microbial load of the three treatments during storage. The trends indicate the differences in behavior of the microorganisms as affected by the treatments. Apparently, the nondiscernible change in acidified-noninoculated juice suggests shelf-stability. In contrast, the marked increase in microbial load in acidified-inoculated juice is an indication of microbial shelf-instability. Similar trends occurred in *Lactobacillus, Leuconostoc* and *Streptococcus* ssp. counts. Thus, the Lactobacilli counts, being the most predominant are presented (Fig. 2). Lactic acid bacteria (LAB) are commonly associated with spoilage of tomato products (Juven and Wesslowicz 1981), and this was confirmed in the present work. However, the pronounced increase (approximately 11 fold) in Lactobacilli counts at the end of storage in acidified-inoculated sample (Fig. 2) suggests alteration of the juice (due to the inoculation) from an adverse to a favorable medium. This occurrence, therefore, supports the concept of metabiosis (Huhtanen *et al.* 1976).

Whereas the trends exhibited by either the microbial load or Lactobacilli counts varied with treatments, yeast counts showed different patterns; i.e., gradual increase occurred in all 3 samples from $\log_{10}$ 4.0 (day 0) to about $\log_{10}$ 4.75 (day 28). These trends, particularly in acidified-noninoculated juice, may be attributable to the ability of yeasts to outgrow bacteria and dominate in acid

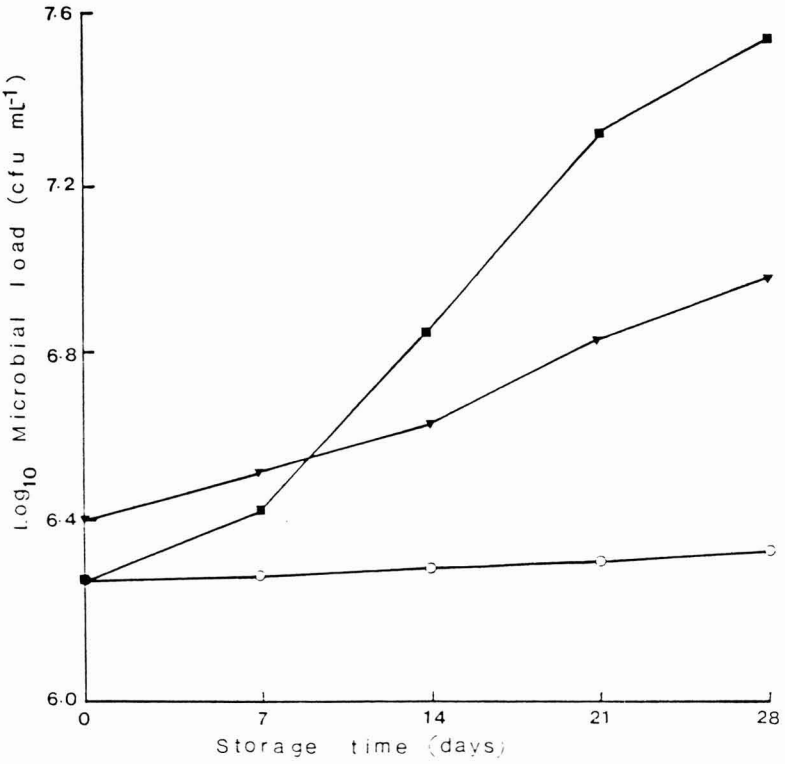


FIG.1. MICROBIAL LOAD OF CONTROL (▼), ACIDIFIED-NONINOCULATED (○) AND ACIDIFIED-INOCULATED TOMATO (■) JUICE DURING STORAGE
Each data point represents the mean of 4 determinations.

environment (Birnbaum *et al.* 1977). Conversely, molds were highly retarded in control and acidified-noninoculated samples and low counts ($< 30$ cfu $mL^{-1}$) were observed, but much higher counts (Fig. 3) occurred in acidified-inoculated juice. Thus, this compares favorably with the findings by Beuchat *et al.* (1990) that showed nondetection of molds in sour cream or salad dressing (acidic products just like tomato juice) but relative high (15–150 colonies per plate) population of yeasts.

The chemical compositional changes in foods are closely related to the microbial activity. Figure 3 illustrates such occurrences, being characterized by decrease in total soluble solids (TSS) with increase in pH and mold counts during storage. This probably indicates utilization of TSS by the mold, especially as from day 10 (Fig. 3). These findings are similar to the observed decrease in reducing sugar content and increase in pH of tomatoes inoculated with *Alternaria solani* (Efiuvwevwere and Hobson 1989).

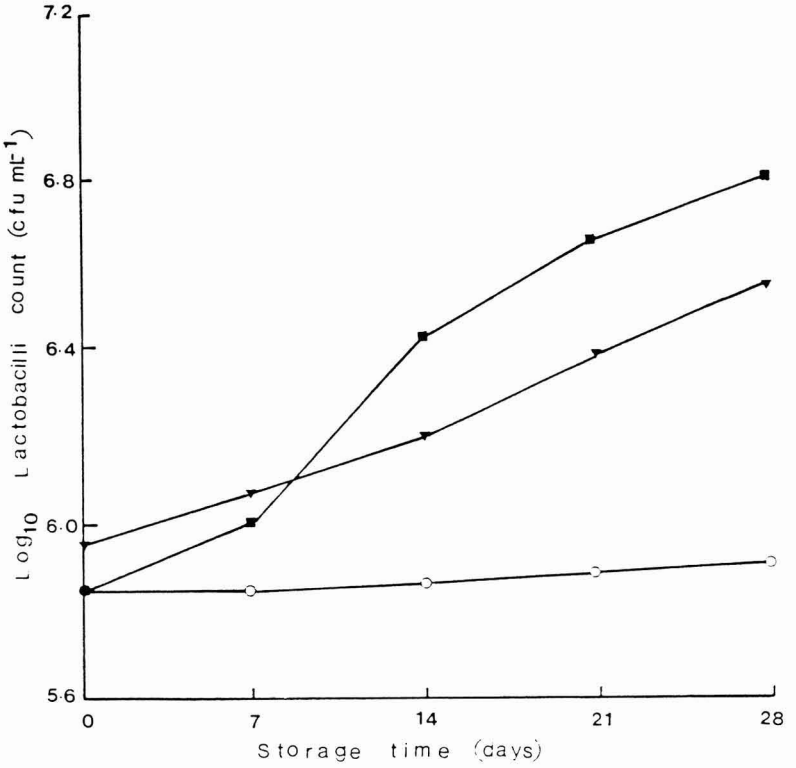


FIG.2. LACTOBACILLI COUNTS OF CONTROL (▼), ACIDIFIED-NONINOCULATED (○) AND ACIDIFIED-INOCULATED TOMATO (■) JUICE DURING STORAGE
Each data point represents the mean of 4 determinations.

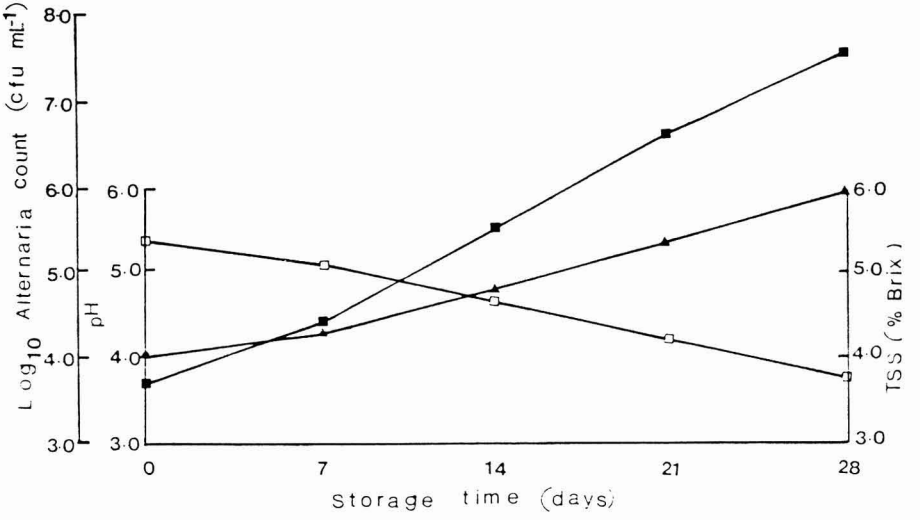


FIG. 3. CHANGES IN pH (▲), TOTAL SOLUBLE SOLIDS (TSS) (□), AND *ALTERNARIA* COUNT (■) IN ACIDIFIED-INOCULATED TOMATO JUICE DURING STORAGE
Each data point represents the mean of 4 determinations.

Mycelial mat and settling out of insoluble suspended solids occurred in acidified-inoculated sample resulting in formation of three layers: the upper was mostly greyish/brown mycelial mat; the middle was uncharacteristic in color and consistency; the bottom consisted of settled out insoluble solids and poor consistency. But control and acidified-noninoculated samples showed no adverse physical changes within 28 days of storage.

The present work has demonstrated that postprocess mold (*Alternaria tenuis*) inoculation (simulating contamination) of tomato juice enhanced shelf-instability within 16 days of ambient tropical storage and possible occurrence of public health hazards due to elevation of pH beyond the critical 4.6. This, therefore, indicates the need to follow Good Manufacturing Practices to avoid postprocess contamination during processing of tomato products in spite of acidification.

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# STABILITY OF LIPIDS AND OMEGA-3 FATTY ACIDS DURING FROZEN STORAGE OF ATLANTIC SALMON[1,2]

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Accepted for Publication February 14, 1991

## ABSTRACT

*Although it is known that dietary omega-3 fatty acids can be beneficial in preventing certain diseases, the stability of omega-3 fatty acids during frozen storage of fish is an area of considerable uncertainty. In Atlantic salmon muscle it was found that omega-3 fatty acids are relatively stable even under adverse storage conditions. Frozen storage of salmon fillets for 3 months at −12°C showed very little loss of triglyceride and no selective change in triglyceride fatty acid composition, but an increase in LPL (lysophospholipids) and in free fatty acids. Stereospecific analysis of both triglycerides and phosphatidylcholine before and after frozen storage was executed. Nonrandom lipolysis of phospholipid did occur to a certain degree, resulting in selective enrichment of free fatty acids in omega-3 fatty acids. Although this preferential hydrolysis of the omega-3 fatty acids was accompanied by formation of polar (presumably oxidized) material, it was determined that most of the omega-3 fatty acids remained nutritionally available after 3 months in frozen storage.*

[1]This work represents part of the M.Sc. thesis submitted by S. M. Polvi to the Technical University of Nova Scotia.
[2]Supported by a grant from the Natural Sciences Engineering and Research Council of Canada and the Atlantic Salmon Federation through contributions of Henry G. Walter Jr., of the Monel Corporation.
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Journal of Food Processing and Preservation **15** (1991) 167–181. 

## INTRODUCTION

Omega-3 fatty acids and their beneficial health effects have become a popular research subject in the past decade. Interest in long-chain marine omega-3 fatty acids was initiated when reports by Dyerberg *et al.* (1975) showed a lower incidence of thrombotic and immunologically related diseases in Greenland Eskimos than in Danes or Eskimos living in Denmark. Since that time many studies have documented this fact (Goodnight *et al.* 1982; Leaf and Weber 1988; Harris *et al.* 1985; Phillipson *et al.* 1985; Harris 1989). All studies in man have shown a reduction of plasma triglyceride levels and some studies have shown a lowering of hyperlipidemia, cholesterol and lipoprotein levels (Harris 1989). Burr *et al.* (1989) have provided definite evidence of the benefits of increasing consumption of fatty fish by patients with cardiovascular problems.

Fish and shellfish are the main sources of omega-3 acids (Ackman 1989) and one parameter of interest to nutritionists is the effect of frozen storage on EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), the two important omega-3 fatty acids in fish muscle. Ackman (1967) noted that up to 70% of fatty acids in Atlantic cod (*Gadus morhua*) flesh could be liberated from phospholipids. Hardy *et al.* (1979) found that during frozen storage at $-10°C$, the neutral lipid of cod tended to remain unchanged, while the major change in lipids was through lipolysis of the phospholipids. This was in agreement with Oehlenschlager and Schreiber (1988) who found that free fatty acids increased rapidly in redfish (*Sebastes marinus*) frozen at $-10°C$, while their formation from phospholipids was almost inhibited at $-30°C$. Recently de Koning and Mol (1990) found an increase in free fatty acid formation and a decrease in certain phospholipids in cape hake (*Merluccius capensis*). On the other hand, Whitsett *et al.* (1987) found that in Pacific salmon fatty acids increased during frozen storage with a corresponding decrease in triglycerides and slight increases in the mono- and diglycerides. The reasons for this difference in lipids subject to hydrolysis is not clear. Apparently muscle triglycerides of many species are subject to hydrolysis, but this is less common than lipolysis of phospholipids (Lovern and Olley, 1962). It is also known that temperatures have a marked effect on the hydrolysis of lipids in fish tissues. Lower temperatures ($-30°C$) seem to slow lipolysis down considerably.

An increasing proportion of salmon now comes from aquaculture practiced in several countries. It is possible, using diet manipulation, to produce Atlantic salmon (*Salmo salar*) with high proportions of omega-3 fatty acids (Hardy *et al.* 1987), and also, as we have demonstrated in our study, to modify fatty acid composition in other respects to some extent. The effect of different dietary fatty acids on the deterioration of the lipids and fatty acids during frozen storage of fish has not to our knowledge been reported. Since much of the world's fish is

now distributed in frozen form, the highly unsaturated fatty acids of fish must be proven to be stable during frozen storage before they can be promoted as "beneficial" to the health of the public. The distribution of omega-3 fatty acids on glycerol is of considerable interest from a biomedical point of view (Ackman 1988; Ackman and Ratnayake 1989), as well as of interest to nutritionists.

## MATERIALS AND METHODS

### Experimental Design: Fish

Atlantic salmon (weighing between 400–500 g) were sampled in October, 5 months after being placed on 4 experimental diets containing herring oil, canola oil, EPA/DHA concentrate or egg lipid acids. Diets were made up several times during the course of the study. A typical result for fatty acid composition is given in Table 1. One fillet was analyzed fresh or after being frozen for a short period at $-30°C$, while the other fillet was stored in a polyethylene bag (no special atmosphere) at $-12°C$ for 3 months. These fillets were analyzed by the same procedure after this period of frozen storage.

### Experimental Design: Analytical Methods

Analysis of tissue followed published procedures for lipid recovery (Bligh and Dyer 1959). Lipid classes were separated by plate thin layer chromatography (TLC) as described by Eaton *et al.* (1975). TLC was carried out on "Prekote" Adsorbosil-5 silica gel plates (920 cm × 20 cm), 200 μm particle size (from Applied Science Laboratories, College Park, PA) developed in hexane:diethyl ether:acetic acid::85:15:1.

An Iatroscan TH-10 Mark III TLC/FID analyzer equipped with a flame ionization detector was used for quantitation of the lipid classes as described by Ackman (1981) and Parrish and Ackman (1985). The air flow was 2 L/min, the detector hydrogen flow rate 160 mL/min, and the scanning speed 0.42 cm/s. Area measurements were derived from a SP4200 computing integrator with a recording chart speed of 16 cm/min. A 97:3:1 ratio of hexane:diethyl ether:formic acid was used to separate free sterols, triglycerides, free fatty acids, and sterol esters from phospholipids. Acetone:water:acetic acid::100:1:1 and a second development of chloroform:methanol:acetic acids:water 25:15:2:1 were used to separate phospholipids.

Fatty acids were methylated and analyzed by GC (gas chromatography) using a Perkin Elmer 900 gas chromatograph. A DB-Wax (polyethylene glycol) fused silica column, dimensions 320 m × 0.25 mm I.D. (J&W Scientific Inc., Folsom, CA) was used for this analysis. Helium pressure was set at 26 psig and an oven

temperature of 200°C was used. Electronic integrator areas were converted to w/w% by FID correction (Craske and Bannon 1988) and thence to mole %.

The positions of the fatty acids in the triglyceride molecules before and after frozen storage were determined essentially by following the methods of Brockerhoff (1965), Breckenridge (1978), and Christie and Moore (1969).

After the polar lipids had been isolated and separated, phospholipase $A_2$ was used to determine the fatty acids in the 1 and 2 positions. Although both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were examined in detail (Polvi 1989), only results for PC, the major phospholipid, will be presented here. The procedure for PC was as follows: *Ophiophagus hannah* snake venom (2.5 mg) was dissolved in 0.1 M Tris buffer solution (0.5 mL; pH 7.5) containing $CaCl_2$ (4 mM). Phosphatidylcholine (5–50 mg) was dissolved in freshly distilled diethyl ether (2 mL), 100 μL of the snake venom solution was added and the mixture shaken vigorously for 1 h. The mixture was washed into a conical flask with methanol (10 mL) followed by chloroform (20 mL) and the solution dried over anhydrous sodium sulfate. After filtering and removing the solvent on a rotary evaporator, the products were applied to a silica gel TLC plate which was developed first in hexane:diethyl ether:formic acid::60:40:2. The top half of the plate was sprayed with 2′,7′-dichlorofluorescein solution, and the free fatty acid band was scraped off and the acids esterified. When the plate was dry, it was redeveloped in chloroform:methanol:acetic acid:water::25:15:4:2 and the lysophosphatidylcholine (Rf approx. 0.2) was recovered and transesterified for GC analysis.

## RESULTS

Muscle from farmed salmon which had been fed each of 4 experimental diets of a base diet supplemented with different fats for the previous 5 months was analyzed before and after 3 months frozen storage at −12°C. The fatty acid compositions of the diets can be found in Table 1. For practical purposes weight and mole percentage are almost identical. Diet 1 (herring oil) was high in 20:1 and 22:1. Diet 2 (canola oil) contained the relatively high levels of 18:1 and 18:2ω6 typical of vegetable oils together with more 18:3ω3 than is common in the ocean (Ackman 1989). Diet 3 was an EPA/DHA (redfish oil fatty acids) enriched diet with high levels of these long-chain PUFA. The fourth diet contained egg lipid and was similar to the canola oil diet in some ways, but contained relatively high levels of 20:4ω6 (arachidonic acid).

In most samples there was a measurable loss of phospholipid during frozen storage. This loss of phospholipid corresponded to part of an increase in free fatty acids (Table 2). The amount of free fatty acids increased from trace amounts to as much as 6.6% of the total lipid and the mass of the free fatty acids (Table

TABLE 1.
FATTY ACID COMPOSITION (m/m%) TYPICAL OF LIPIDS IN DIETS FED TO ATLANTIC SALMON

| Fatty Acid | Diet 1 Herring Oil | Diet 2 Canola Oil | Diet 3 EPA+DHA Concentrate[1,2] | Diet 4 Egg Lipid[1] |
|---|---|---|---|---|
| 14:0 | 8.5 | 2.1 | 3.5 | 2.4 |
| 16:0 | 14.3 | 9.2 | 8.8 | 8.7 |
| 18:0 | 1.3 | 1.8 | 1.0 | 1.5 |
| 16:1ω7 | 7.4 | 1.9 | 2.7 | 4.9 |
| 18:1ω9 | 9.9 | 41.1 | 6.9 | 29.7 |
| 20:1ω9+11 | 12.2 | 3.7 | 3.6 | 3.6 |
| 22:1ω11+13 | 17.8 | 4.6[3] | 5.3 | 4.5 |
| 18:2ω6 | 6.9 | 19.2 | 7.6 | 25.0 |
| 18:3ω3 | 1.2 | 7.1 | 1.0 | 3.5 |
| 18:4ω3 | 1.9 | 0.5 | 4.3 | 0.5 |
| 20:4ω6 | 0.3 | 0.1 | 0.5 | 3.3 |
| 20:4ω3 | 0.3 | 0.1 | 0.9 | 0.1 |
| 20:5ω3 | 5.2 | 1.7 | 18.3 | 1.9 |
| 22:5ω3 | 0.6 | 0.2 | 0.9 | 0.4 |
| 22:5ω6 | 0.05 | 0.05 | 0.5 | 0.9 |
| 22:6ω3 | 5.7 | 3.2 | 23.4 | 4.6 |
| Others | 6.45 | 3.45 | 10.8 | 4.5 |

[1] Fed as ethyl esters.
[2] Details in Ratnayake *et al.* (1988).
[3] Primarily from herring meal; canola oil contains the 22:1ω9 isomer but no 22:1ω11+13 fatty acids.

2) appeared to be derived ½ from phospholipids and ½ from triglycerides. The lipolysis of phospholipid was not random and the two major phospholipid species were hydrolyzed at different rates (Table 3). There was a large decrease in the amount of PE and a more modest decrease in PC. There was a compensating increase in total lysophospholipids. The amount of SPH (sphingomyelin) remained unchanged and the PS (phosphatidylserine), PI (phosphatidyl-inositol) and phosphatidic acid were found only in trace amounts before and after frozen

TABLE 2.
LIPID CLASSES (g/100g tissue) OF MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE) FED DIFFERENT DIETS AND ANALYZED BEFORE AND AFTER FROZEN STORAGE [1]

| | | LIPID CLASS[2] | | | |
|---|---|---|---|---|---|
| | Treatment | PL | CH | TG | FFA |
| Herring | F | 0.67 ±0.03 | 0.07 ±0.01 | 5.14 ±0.03 | TR |
| | S | 0.58 ±0.05 | 0.02 ±0.01 | 5.09 ±0.15 | 0.19 ±0.1 |
| Canola | F | 0.66 ±0.11 | 0.02 ±0.01 | 5.95 ±0.20 | TR |
| | S | 0.65 ±0.03 | 0.02 ±0.01 | 5.73 ±0.12 | 0.23 ±0.01 |
| EPA+DHA Concentrate | F | 0.54 ±0.15 | 0.05 ±0.01 | 3.76 ±0.13 | 0.04 ±0.01 |
| | S | 0.43 ±0.11 | 0.04 ±0.01 | 3.75 ±0.15 | 0.16 ±0.03 |
| Egg Lipid | F | 0.56 ±0.06 | 0.06 ±0.01 | 3.81 ±0.15 | 0.01 ±0.005 |
| | S | 0.44 ±0.01 | 0.05 ±0.01 | 3.72 ±0.12 | 0.23 ±0.05 |

[1] F; frozen briefly at -30°C. S; first frozen at -30°C, then stored at -12°C for 3 months.
[2] PL=phospholipid; CH=cholesterol; TG=triglyceride; FFA=free fatty acids.
Note: Generally 8 Chromarods were used. Standard deviations are given for lipid from one fish, but results are averaged for 2 fish.

storage. There was also the appearance after frozen storage of a polar type material on the TLC/FID Chromarods (Table 3), possibly an oxidative polymer (Kaitaranta and Ke 1981).

There were very few differences in the profiles of the total lipid fatty acids of the fish muscle before and after frozen storage (Table 4) in any of the 4 experimental groups. There was even less difference in the fatty acid profiles of the triglyceride fraction before and after frozen storage (Table 5). Any obvious change in fatty acids from deterioration during frozen storage was confined to the phospholipid portion of the lipid (Table 6).

TABLE 3.
PHOSPHOLIPID SPECIES (w/w%) OF MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE) FED SEPARATE DIETS ANALYZED BEFORE AND AFTER EXTENDED FROZEN STORAGE[1]

| | | LIPID CLASS[2] | | | | | |
|---|---|---|---|---|---|---|---|
| Diet | Treatment | PE% | PC% | SPH% | LPL% | PS,PI,PA | Polymer |
| Herring | F | 20.0 ±2.1 | 69.9 ±2.1 | 2.3 ±0.2 | 8.1 ±0.13 | TR | ND |
| | S | 15.3 ±1.3 | 65.4 ±3.2 | 2.1 ±0.3 | 16.1 ±0.1 | TR | 1.0 |
| Canola | F | 21.2 ±1.9 | 71.2 ±2.4 | 1.9 ±0.1 | 5.8 ±0.1 | TR | ND |
| | S | 17.1 ±1.3 | 67.0 ±2.1 | 1.2 ±0.1 | 13.6 ±1.0 | TR | 1.0 |
| EPA+DHA Concentrate | F | 18.8 ±1.8 | 72.8 ±1.9 | 3.0 ±0.04 | 5.4 ±0.21 | TR | ND |
| | S | 16.1 ±0.8 | 71.1 ±1.8 | 3.2 ±0.04 | 9.0 ±0.5 | TR | 0.5 |
| Egg Lipid | F | 23.9 ±2.5 | 67.1 ±1.9 | 2.9 ±0.1 | 6.1 ±0.1 | TR | ND |
| | S | 20.1 ±2.0 | 64.8 ±3.2 | 3.8 ±0.05 | 10.3 ±0.1 | TR | 0.9 |

[1] F; frozen briefly at -30°C. S; first frozen at -30°C, then stored at -12°C for 3 months.
[2] PE=phosphatidylethanolamine; PC=phosphatidylcholine; SPH=sphingomyelin; LPL=lysophospholipids; PS=phosphatidylserine; PI=phosphatidylinositol; PA=phosphatidic acid.
Note: Generally 8 Chromarods used. Standard deviations are given for lipids from one fish, but results are averaged for 2 fish.

The position of the fatty acids on the triglyceride molecule was determined before and after frozen storage. No change was found in the proportions of the fatty acids in any position (data not shown). Phospholipase $A_2$ was used to determine the fatty acids in the two positions of the phosphatidylcholine. Differences were found before and after frozen storage (Table 7). There was a decrease in the levels of the omega-3 fatty acids, primarily DHA (22:6ω3). The liberated omega-3 fatty acids showed up to a certain extent in the composition of the free fatty acids (data not shown).

TABLE 4.
FATTY ACID COMPOSITION (m/m%) TYPICAL[1] OF TOTAL LIPID IN MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE), FED FOUR EXPERIMENTAL DIETS, AND ANALYZED BEFORE AND AFTER EXTENDED FROZEN STORAGE[2]

| Fatty Acid | Herring Oil | | Canola Oil | | EPA/DHA Concentrate | | Egg Lipid | |
|---|---|---|---|---|---|---|---|---|
| | F | S | F | S | F | S | F | S |
| 16:0 | 17.3 | 18.6 | 13.3 | 13.5 | 14.6 | 13.4 | 15.3 | 15.1 |
| 18:0 | 2.3 | 2.2 | 2.8 | 2.6 | 2.4 | 2.4 | 2.5 | 2.2 |
| 16:1ω7 | 8.4 | 9.1 | 2.7 | 3.2 | 4.3 | 4.0 | 5.8 | 7.0 |
| 18:1ω9 | 12.9 | 12.0 | 40.3 | 38.4 | 8.2 | 8.0 | 39.3 | 38.6 |
| 20:1 | 12.4 | 11.4 | 4.4 | 4.5 | 3.9 | 4.4 | 4.6 | 4.5 |
| 22:1 | 12.4 | 12.3 | 2.7 | 3.3 | 3.6 | 4.5 | 2.9 | 3.4 |
| 18:2ω6 | 7.3 | 7.1 | 6.6 | 16.1 | 8.2 | 7.9 | 12.5 | 11.9 |
| 18:3ω3 | 1.1 | 1.6 | 4.5 | 4.5 | 1.0 | 1.0 | 0.7 | 0.7 |
| 18:4ω3 | 1.6 | 1.1 | 1.4 | 1.4 | 3.8 | 3.6 | 0.1 | 0.7 |
| 20:4ω6 | 0.4 | 0.4 | 1.2 | 0.3 | 0.6 | 0.6 | 1.5 | 1.5 |
| 20:5ω3 | 3.3 | 3.1 | 0.4 | 1.2 | 15.0 | 14.2 | 1.3 | 1.1 |
| 22:5ω3 | 1.0 | 1.0 | 1.2 | 0.4 | 2.9 | 3.1 | 0.5 | 0.5 |
| 22:6ω3 | 9.2 | 7.7 | 5.0 | 5.0 | 25.1 | 24.9 | 6.1 | 6.1 |
| Others | 10.4 | 12.4 | 2.9 | 5.6 | 6.4 | 8.0 | 6.9 | 7.1 |

[1] Two extracts were each analyzed twice. Confirmation of the above results were obtained in all cases.
[2] F; frozen briefly at −30°C. S; first frozen at −30°C, then stored at −12°C for 3 months.

## DISCUSSION

The fatty acid content and composition of salmonids can be quite variable (Ackman 1989; Hardy and King 1989), and variations of the fatty acid compositions through zonal distribution of tissues in fish muscle must be considered (Aubourg *et al.* 1990). In many cases cultured fish can have a lower ratio of ω3 to ω6 fatty acids than wild fish (Van Vliet and Katon 1990). However, our question is whether the level of omega-3 fatty acids present in fresh fish indicates similar values in frozen fish.

Deterioration of important fatty acids such as omega-3 fatty acids during frozen storage is perhaps of less interest to the general public than to those concerned with fish "quality." Oxidation products of free fatty acids can produce off-

TABLE 5
FATTY ACID COMPOSITION (m/m%) TYPICAL[1] OF TRIGLYCERIDES IN MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE) FED FOUR EXPERIMENTAL DIETS, AND ANALYZED BEFORE AND AFTER EXTENDED FROZEN STORAGE[2]

| Fatty Acids | Herring Oil | | Canola Oil | | EPA+DHA Concentrate | | Egg Lipid | |
|---|---|---|---|---|---|---|---|---|
| | F | S | F | S | F | S | F | S |
| 16:0 | 18.6 | 17.5 | 13.6 | 12.7 | 15.9 | 15.3 | 14.3 | 14.1 |
| 18:0 | 2.8 | 2.6 | 2.9 | 3.0 | 2.4 | 2.6 | 2.4 | 2.5 |
| 16:1ω7 | 9.5 | 9.6 | 3.0 | 3.2 | 5.7 | 5.7 | 5.9 | 6.5 |
| 18:1ω9 | 16.1 | 15.8 | 43.3 | 44.1 | 11.1 | 11.0 | 43.8 | 44.0 |
| 20:1 | 12.4 | 14.2 | 4.5 | 5.1 | 4.3 | 4.9 | 5.0 | 5.6 |
| 22:1 | 11.9 | 14.6 | 2.7 | 3.3 | 3.7 | 4.7 | 3.6 | 4.2 |
| 18:2ω6 | 7.6 | 7.0 | 16.7 | 15.9 | 9.4 | 9.1 | 12.6 | 11.9 |
| 18:3ω3 | 1.0 | 1.0 | 3.9 | 4.0 | 1.1 | 1.1 | 0.7 | 0.7 |
| 18:4ω3 | 1.3 | 1.2 | 1.2 | 1.1 | 3.2 | 3.8 | 0.6 | 0.6 |
| 20:4ω6 | 0.3 | 0.3 | 0.3 | 0.2 | 0.6 | 0.6 | 1.0 | 0.9 |
| 20:5ω3 | 2.5 | 1.9 | 0.8 | 0.7 | 13.2 | 12.7 | 0.9 | 0.7 |
| 22:5ω3 | 1.0 | 0.7 | 0.4 | 0.4 | 2.7 | 2.8 | 0.6 | 0.4 |
| 22:6ω3 | 5.5 | 4.3 | 2.9 | 3.3 | 19.2 | 19.4 | 4.2 | 4.0 |
| Others | 9.5 | 9.3 | 3.8 | 3.0 | 7.5 | 6.3 | 4.4 | 3.9 |

[1] Two extracts were each analyzed twice. Confirmation of the above results were obtained in all cases.
[2] F; frozen briefly at −30°C. S; first frozen at −30°C, then stored at −12°C for 3 months.

flavors (Hardy *et al.* 1979) and create toughening and texture problems (Gill *et al.* 1979). In high fat fish such as salmon, with a fairly strong flavor, the impact of the storage problems prevalent in lean fish (Hardy 1979) is probably less critical, but must be considered if overproduction of farmed fish leads to extended frozen storage. The question of the stability of unsaturated fatty acids, primarily the omega-3 fatty acids, was examined in this study. We have observed some deterioration when the fish are stored at −12°C. This is not good commercial practice, but it is a temperature close to that used by many distributors and consumers.

It was found that after frozen storage under these "adverse" conditions there were small changes in the fatty acid composition of the total lipid. When the lipid was separated into triglyceride and phospholipid portions, there was very

TABLE 6.
FATTY ACID COMPOSITION (m/m%) TYPICAL[1] OF TOTAL PHOSPHOLIPIDS IN MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE) FED FOUR EXPERIMENTAL DIETS AND ANALYZED BEFORE AND AFTER EXTENDED FROZEN STORAGE[2]

| Fatty Acids | Herring Oil | | Canola Oil | | EPA+DHA Concentrate | | Egg Lipid | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 16:0 | 16.7 | 24.7 | 12.1 | 20.1 | 13.9 | 18.3 | 12.8 | 10.5 |
| 18:0 | 3.0 | 5.0 | 1.9 | 2.2 | 3.3 | 2.6 | 3.4 | 3.7 |
| 16:1ω7 | 5.4 | 5.0 | 1.9 | 2.2 | 3.3 | 2.6 | 3.4 | 3.7 |
| 18:1ω9 | 10.6 | 11.9 | 25.1 | 23.1 | 9.2 | 8.2 | 25.5 | 23.9 |
| 20:1 | 5.4 | 3.6 | 2.1 | 2.1 | 2.4 | 1.9 | 2.3 | 2.3 |
| 22:1 | 2.8 | 1.5 | 0.7 | 0.8 | 1.0 | 1.0 | 0.9 | 0.9 |
| 18:2ω6 | 4.5 | 6.7 | 11.2 | 10.0 | 4.9 | 2.0 | 7.7 | 8.1 |
| 18:3ω3 | 0.9 | 0.8 | 3.5 | 2.8 | 0.7 | 1.1 | 0.7 | 0.7 |
| 18:4ω3 | 1.3 | 1.2 | 1.3 | 1.4 | 2.5 | 0.8 | 0.5 | 0.7 |
| 20:4ω6 | 0.8 | 1.1 | 1.3 | 1.3 | 0.7 | 0.7 | 3.7 | 4.2 |
| 20:5ω3 | 6.8 | 7.4 | 3.5 | 4.4 | 8.6 | 9.4 | 2.6 | 4.1 |
| 22:5ω3 | 1.9 | 1.7 | 1.9 | 1.0 | 2.8 | 2.0 | 1.3 | 1.8 |
| 22:6ω3 | 33.6 | 22.4 | 27.2 | 22.0 | 42.6 | 39.7 | 30.2 | 30.2 |
| Others | 6.3 | 7.0 | 6.3 | 6.6 | 4.1 | 9.7 | 5.0 | 5.2 |

[1] Two extracts were each analyzed twice. Confirmation of the above results were obtained in all cases.
[2] F; frozen briefly at −30°C. S; first frozen at −30°C, then stored at −12°C for 3 months.

little or no change in the fatty acid profile of the triglyceride, but selective lipolysis had definitely modified the fatty acids in the phospholipid portion. Since the phospholipid comprises only approximately 10% of the total lipid in these fish, the change was not immediately obvious in the total lipid. The content of free fatty acids indicated an origin from both triglycerides and phospholipids (Table 2).

Lovern and Olley (1962) suggested that lipolysis does not favor any particular fatty acids on glycerol. This was not found to be the case in this study. From Tables 6 and 7 it can be seen that the fatty acid composition of the phospholipid changed during frozen storage. Lipolysis did not appear to be random and the loss of omega-3 fatty acids is particularly obvious in the DHA. There were

TABLE 7.
FATTY ACID COMPOSITION (m/m%) OF PHOSPHATIDYLCHOLINE IN MUSCLE OF ATLANTIC SALMON (OCTOBER SAMPLE), FED THE EPA/DHA CONCENTRATE AND ANALYZED BEFORE AND AFTER EXTENDED FROZEN STORAGE AT -12°C[1]

| | SAMPLE 1 | | | | | | SAMPLE 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before Frozen Storage | | | After Frozen Storage | | | Before Frozen Storage | | | After Frozen Storage | | |
| Fatty Acid | Total | Pos 1 | Pos 2 | Total | Pos 1 | Pos 2 | Total | Pos 1 | Pos 2 | Total | Pos 1 | Pos 2 |
| 14:0 | 2.2 | 1.6 | 2.6 | 5.3 | 4.1 | 6.7 | 3.1 | 4.7 | 1.3 | 5.1 | 6.1 | 4.2 |
| 16:0 | 23.3 | 42.7 | 3.9 | 32.5 | 58.8 | 6.2 | 21.6 | 38.2 | 5.0 | 28.5 | 35.1 | 21.8 |
| 16:1ω7 | 2.6 | 2.4 | 2.6 | 6.8 | 9.6 | 4.0 | 3.8 | 7.4 | 0.4 | 5.5 | 8.3 | 2.1 |
| 18:0 | 1.7 | 3.4 | ND | 3.5 | 6.3 | 0.9 | 2.5 | 3.4 | 1.7 | 3.0 | 5.3 | 0.9 |
| 18:1ω9 | 20.3 | 30.3 | 10.4 | 13.2 | 4.3 | 22.1 | 19.3 | 26.3 | 12.3 | 12.7 | 18.7 | 6.9 |
| 18:1ω7 | 1.9 | 2.9 | 0.9 | 0.7 | 1.3 | 0.1 | 0.4 | 0.4 | 0.4 | 2.3 | 3.9 | 0.7 |
| 18:2ω6 | 3.8 | 6.8 | 0.8 | 2.9 | 3.1 | 2.5 | 4.0 | 6.6 | 1.6 | 4.8 | 5.8 | 3.4 |
| 20:1 | 1.3 | 2.1 | 0.1 | 2.7 | 4.5 | 0.9 | 1.6 | 2.8 | 0.4 | 1.5 | 2.5 | 0.5 |
| 20:5ω3 | 7.9 | 3.9 | 11.9 | 7.3 | 2.3 | 12.3 | 12.3 | 5.3 | 19.1 | 6.6 | 1.7 | 11.7 |
| 22:1 | 0.4 | 0.9 | ND | 0.4 | 0.8 | 0.1 | 0.8 | 1.3 | 0.3 | 1.7 | 2.5 | 0.9 |
| 22:5ω3 | 1.5 | 0.4 | 2.6 | 1.0 | 0.3 | 0.7 | 1.8 | 0.6 | 2.8 | 1.6 | 0.9 | 2.4 |
| 22:6ω3 | 33.7 | 2.8 | 64.2 | 23.6 | 3.9 | 42.9 | 39.0 | 13.6 | 64.5 | 27.4 | 9.9 | 44.5 |

[1] Each sample represents lipids from one fish.

differences in the way the two samples were hydrolyzed during frozen storage. This could be due to deterioration and rupture of the membranes during the three months of storage. Hardy *et al.* (1979) also found that a slight but relatively consistent loss of the C-22 fatty acids such as DHA was detectable in cold storage cod samples. It was suggested that this might be expected if some oxidation of the liberated C-20 and C-22 polyenoic acids had occurred. This was consistent with our Chromarod-Iatroscan studies which showed that a small amount of polar material appeared only in the lipids extracted from frozen material. The greater percentage of the fatty acids liberated from both triglycerides and phospholipids appeared, however, as free fatty acids (3–6% of total lipid).

Bligh and Scott (1966) showed that cod frozen for 9 months at −12°C had an increase in free fatty acids from 5 to 326 mg/100 g tissue due to the hydrolysis of phosphatidylethanolamine and phosphatidylcholine. De Koning and Mol

(1990) also found an increase in free fatty acid formation and a decrease in certain phospholipids in cape hake (*M. capensis*) frozen at −5°C and −18°C. This agrees with our work showing a decrease in PC and especially in PE (Table 3), and an increase in FFA (Table 2).

Lovern and Olley (1962) suggested that after one fatty acid was released from the phospholipid molecule, the other fatty acid was immediately set free as well. However, in our study there was an increase in lysophosphatidylcholine (Table 3) as well as in free fatty acids. This was in agreement with Ohshima and Koizumi (1983) who found that in skipjack muscle the contents of PC, PE, SPH and PS decreased while those of LPC, LPE and FFA increased. Atlantic salmon frozen at −12°C for 3 months lost up to 20% of the phospholipid (Table 2). From other studies (Ohshima and Koizumi 1983; Ohshima *et al.* 1984; Bligh and Scott 1966) we can conclude that lipolysis will continue to occur for some time. As with all other enzyme reactions, the overall rate is conditioned by factors such as concentration of substrate and enzyme, pH, temperature and cofactors such as calcium ions. The diet fed to the fish did not seem to be a factor in phospholipid hydrolysis (Table 3).

In conclusion, we observed very little lipolysis occurring in Atlantic salmon triglyceride, but a nonrandom lipolysis of the total phospholipid occurred during frozen storage. A nonrandom lipolysis in phosphatidylcholine alone was also confirmed by HPLC (Polvi 1989). The significance of our findings is that even though lipolysis does occur in phospholipids, most of the nutritionally desirable omega-3 fatty acids of Atlantic salmon fillets are available in the form of triglyceride. In leaner salmon a further contribution is nutritionally available either in the phospholipids, in the lysophospholipids, or as free fatty acids. It can be stated with confidence that although good commercial freezing practice is not always maintained, salmon remains a good source of omega-3 fatty acids even after a reasonable length of time in frozen storage

## ACKNOWLEDGMENTS

The authors would like to thank Dr. W.M.N. Ratnayake for his assistance and expertise in analyzing the data. Thanks are also due to Derek Knox who reared the fish and administered the dietary lipid feeding program at the St. Andrews Biological Station, Fisheries and Oceans.

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# EFFECT OF PHYSICAL STRUCTURE OF STARCH MATERIALS ON WATER DIFFUSIVITY

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Accepted for Publication February 18, 1991

## ABSTRACT

*The physical structure, developed during drying, affects the transport of water and the quality of processed solid foods. The effective diffusivity of water in starch-based systems was estimated from drying data at moisture 3–50% and temperatures 40–100°C. Hydrated granular or gelatinized starches and their mixtures with sugars or inert particles were used to produce different structures during air drying. The physical structure was characterized by the bulk porosity, estimated from the bulk and solid densities of the samples. The water diffusivity was influenced strongly by the porosity, developed during drying. Mechanical compression, starch gelatinization or addition of water-soluble sugars reduced the water diffusivity in granular starches. Incorporation of inert particles or extrusion cooking resulted in higher water diffusivities, due to higher porosities of the starch/inert mixtures or the expanded extrudates.*

## INTRODUCTION

The transport of water in solid foods is very important in dehydration and rehydration processes, where very high rates are desirable. On the other hand, low transport rates are necessary in preventing physicochemical or microbial deterioration of stored dehydrated or intermediate moisture foods.

In the drying of foods, the transport of water within the solid matrix is normally the controlling mechanism, since the interphase mass transfer is relatively high, due to high air velocities or to vacuum around the product (Saravacos 1986). The mechanism of transport may be by molecular diffusion of liquid or vapor

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Journal of Food Processing and Preservation **15** (1991) 183–195. 

water, capillary flow of liquid water, hydrodynamic flow or thermal diffusion (Bruin and Luyben 1980). The effective or apparent water diffusivity is a useful transport property in calculations of dehydration and sorption processes in foods. The water diffusivity can be estimated by applying the diffusion equation to experimental drying or sorption data.

The method of drying has a profound effect on the water diffusivity, due to differences in food structure. Freeze-drying of foods results in higher water diffusivities than air-drying or puff-drying (Saravacos 1967).

Starch materials have been used as model systems in investigations of physical and engineering properties of foods. Isothermal sorption of water in starch gels gave low water diffusivities which decreased at lower moisture contents (Fish 1958). Low water diffusivities have been found in extruded pasta, due to low porosity (Andrieu and Stamatopoulos 1986).

The transport of water in foods is a complex process and application of thermodynamic principles requires the porosity of the material and the equilibrium and transport properties of the components of the system. Semiempirical equations of water diffusivity as a function of moisture content, temperature and physical structure (porosity) are useful in the design of food processes for products of improved quality.

## MATERIALS AND METHODS

### Materials

Two native corn starches were used in granular and gelatinized form, Hylon 7 (63% amylose) and Amioca (98% amylopectin). The starch powders had an initial moisture content of 11% (wet basis), and they were supplied by National Starch and Chemical Corp. (Bridgewater, NJ). Granular starch samples were prepared at moisture contents 0–1 kg water/kg dry solids by drying in vacuum oven at 70°C or mixing with distilled water and equilibrating at room temperature for 24 h. Alternatively, starch samples were equilibrated to a fixed moisture content in constant relative humidity jars (saturated salt solutions or sulfuric acid) at 25°C for 2 weeks. Gelatinized samples were prepared by heating mixtures of Amioca starch (1 part)/water (1 part) at 100°C for 30 min. Mixtures of 75% starch and 25% glucose, sucrose or dextrin were also prepared in granular or gelatinized form (Marousis *et al.* 1989). Dextrin of dextrose equivalent 15% (Lo-Dex 15) was supplied by American Maize (Hammond, IN). Mixtures of 75% granular starch 25% inert particles were prepared using glass beads, 380 mesh (Ace Scientific Supply Co., East Brunswick, NJ), silica (240 mesh), alumina (80–200 mesh) and carbon black (60 mμ, Fisher Scientific, Springfield, NJ).

The particle density ($\rho_p$) of the granular starches at various moisture contents was determined by gas displacement (Marousis and Saravacos 1990), using a

SPY-2 stereopycnometer (Quantachrome Corp., Syosset, NY). The stereopycnometer, which measures the particle volume, excluding the interparticle space, was operated with helium gas at an absolute pressure of 2.3 bar. The bulk density ($\rho_b$) of the starch samples was determined from the mass and the bulk volume, which were estimated either from the sample dimensions (spherical diameter or slab thickness) or by volumetric displacement with glass beads, 200 μm diameter.

The bulk porosity (E), defined as the volume fraction of the void (air) in the sample, was estimated from the equation,

$$E = 1 - \rho_b/\rho_p \quad (1)$$

The bulk porosity, E, calculated from Eq. (1) is the volume fraction of open pores, which are accessible to the helium molecules (larger than 3.5 Å). This method of porosity measurement cannot differentiate between ''connected'' and ''disconnected'' pores, which might both exist in the starch material.

The particle size distribution was determined by two methods, sieve analysis and Coulter counter (Elzone Particle Analyzer). Starch powder of 11% moisture was sieved in standard US sieves No. 150 to No. 200 (openings 104 to 74 μm). Dilute suspensions of starch granules in salt solution were analyzed for particle size distribution in the Coulter counter.

Compressed starch samples were prepared using a laboratory compression Instron model TM (Instron Engineering Corp., Canton, MA). The granular starch, equilibrated at a given moisture, was compressed at pressures up to 50 bar in an aluminum ring 33 mm diameter and 3.5 mm thick. The porosity and bulk density of the compressed samples were estimated from the mass and the dimensions of the starch slab.

Extrusion-cooked starches were prepared using a laboratory single-screw extruder (Brabender Instruments, South Hackensack, NJ), operated at 50–250 RPM. Starch extrudates were produced at moisture contents 0.35–0.15 kg water/kg dry solids and die (exit) temperatures 90–180°C. The extrudates were used for the determination of water diffusivity by air-drying the expanded samples, or the pressed extrudates (low porosity).

The porosity development during drying was observed using a stereomicroscope (Bausch and Lomb, Rochester, NY). A number of spherical samples (2 cm diameter) of initial moisture content $X_o$ = 1 kg water/kg dry solids were dried at 60°C for various times. The samples were separated into two hemispheres and the internal structure was photographed (Marousis and Saravacos 1990).

## Estimation of Effective Water Diffusivity

Two types of granular and gelatinized starches were used, spheres 2 cm diameter and slabs 57 mm diameter and 3.5 mm thick. A pilot plant air-dryer (C. G. Sargent's Sons Corp., Graniteville, MA) was used at temperatures 40–

100°C, relative humidity 10% and air velocity 2 m/s. The sample weight was measured periodically using a PE160 Mettler balance (Mettler Instrument Corp., Hightstown, NJ). The moisture content of the samples (X, kg water/kg dry solids) was based on the weight of dry solids, determined by vacuum at 70°C for 24 h. The mean moisture ratio $(X - X_e)/(X_o - X_e)$ of each sample was calculated at various drying times (t). $X_o$ is the initial moisture content. The equilibrium moisture content in the dryer ($X_e$) was estimated by extrapolation of the drying curve, X versus t (Marousis *et al.* 1989).

The effective water diffusivity in the starch materials was estimated by applying the unsteady-state diffusion equation to the drying data (Perry and Green 1984; Karathanos *et al.* 1990). Since the initial moisture content was relatively low ($X_o<2$), all drying took place in the falling rate period. Due to the high air velocity, the main resistance to mass transfer was assumed to be within the sample, justifying the application of the diffusion equation (Saravacos 1986). Assuming a constant water diffusivity (D) within small moisture intervals, the following solutions of the diffusion equation were used for the slab and spherical samples respectively (Perry and Green 1984):

$$W = \frac{X-X_e}{X_o-X_e} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp\left[-\frac{(2n-1)^2\pi^2 Dt}{4L^2}\right] \quad (2)$$

$$W = \frac{X-X_e}{X_o-X_e} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left[-\frac{n^2\pi^2 Dt}{r_o^2}\right] \quad (3)$$

where L is the thickness of the slab sample (drying from the one side) and $r_o$ is the radius of the spherical sample. The dimensions of the samples were measured at various drying times, and they were used for the estimation of water diffusivity (equations 2 and 3) and the bulk density and porosity as functions of moisture content (Marousis and Saravacos 1990).

The water diffusivity values at various moisture contents, obtained from the simplified solutions of the diffusion equation (2 and 3), gave similar results with the numerical solution, using a computer simulation method (Karathanos *et al.* 1990).

## RESULTS AND DISCUSSION

### Bulk Porosity

Mechanical pressure reduced the bulk porosity of granular starches, particularly at moisture contents higher than 0.3 kg water/kg dry solids, as shown in Fig. 1 for Hylon 7 (high-amylose starch). Similar results were obtained with

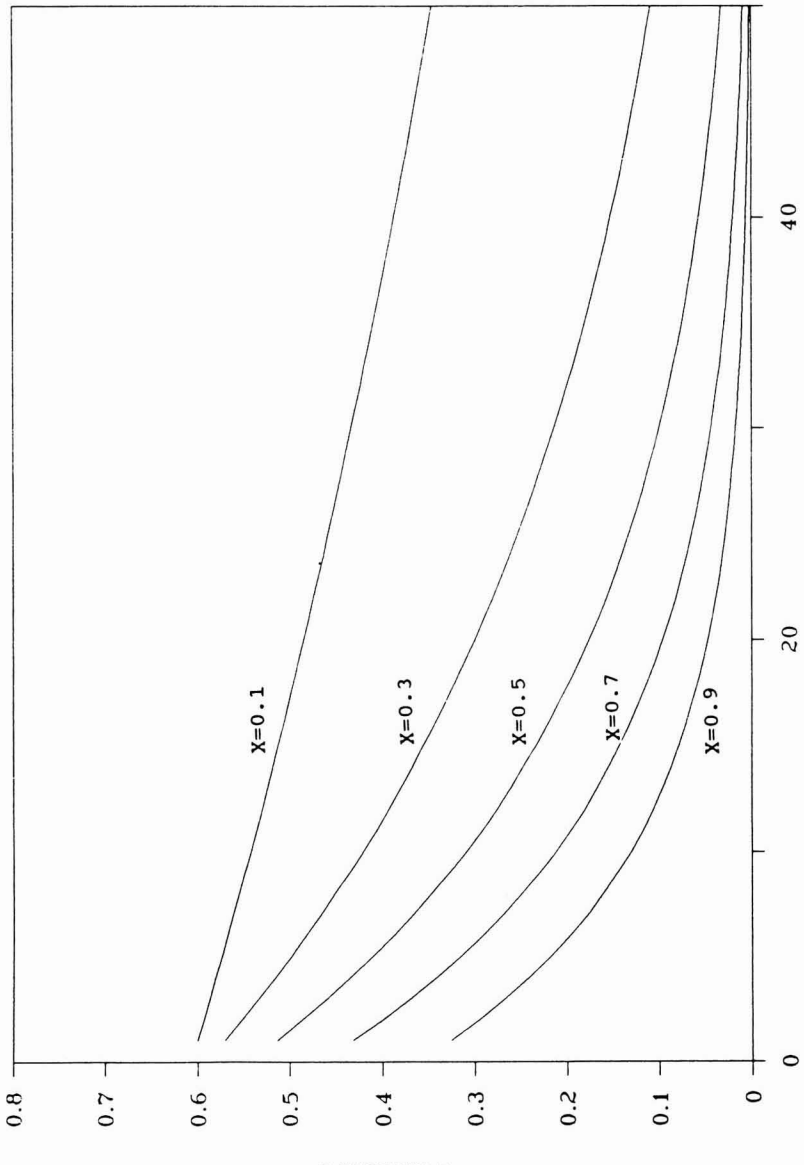


FIG. 1. POROSITY OF GRANULAR STARCH SLABS (AMIOCA) AS A FUNCTION OF MOISTURE CONTENT X (kg WATER/kg DRY SOLIDS) AND MECHANICAL PRESSURE P (bar, 1 bar = $10^5$ Pa)

Amioca (high-amylopectin) starch. The high-moisture starch granules were more deformable (compressible) than the dry powders, and thus low bulk porosities were obtained at pressures lower than 50 bar. Regression equations were obtained for the porosity (E) as a function of moisture content, dry basis (X), and pressure P (bar). For granular Amioca (high-amylopectin starch) the following equation was derived with a coefficient of determination $R^2 = 0.98$:

$$E = (0.600 + 0.0643 \cdot X - 0.370 \cdot X^2) \cdot \exp(0.0077 - 0.113 \cdot X \cdot P) \quad (4)$$

Similar results were obtained for granular Hylon 7 (high-amylose starch) samples. Coulter particle analysis and microscopic observations showed that the mean size of granules was 10.5 μm for Amioca and 8.7 μm for Hylon 7.

**Water Diffusivity in Granular Starches**

The effective water diffusivity in hydrated granular starches, estimated from drying data at 40–100°C, was relatively high, ranging from $5 - 50 \times 10^{-10}$ $m^2/s$. Figure 1 shows the water diffusivity in three starch slabs of Amioca of different initial porosity (E), estimated from drying data at 60°C. A maximum in water diffusivity was observed in granular starches near moisture content X = 0.15 kg water/kg dry solids, evidently due to the high porosity developed during drying (Marousis *et al.* 1989). In the early stages of drying, liquid water diffusion may be the main transport mechanism, because of the low porosity. As drying progressed, vapor diffusion appeared to become important in granular starches, due to the increased porosity of the samples. Since the diffusivity of water vapor is 4 orders of magnitude higher than liquid diffusivity, the effective (overall) diffusivity is expected to increase considerably. At very low moisture contents ($x < 0.1$) the water diffusivity decreased sharply, due to the lower mobility of the strongly adsorbed water on the starch surfaces.

Regression analysis of the effective water diffusivity values (D) versus moisture content (X), temperature (T) and bulk porosity (E) resulted in the following semiempirical equation:

$$D = \left( 4842 + 0.5735\ X^{-4.3369} + 34212 \left[ \frac{E^3}{(1-E)^2} \right] \right) e^{-\frac{4.5}{RT}} \quad (5)$$

In Eq. (5) D is the effective water diffusivity ($\times 10^{-10}$ $m^2/s$), which is a measure of various transport mechanisms, which might include liquid and vapor diffusion, surface diffusion, Darcy's flow, evaporation and condensation (Bruin and Luyben 1980). The water diffusivity increased with the temperature in the range 40–100°C, following an Arrhenius relationship with an energy of activation for diffusion $E_a = 4.5$ kcal/mol.

The effect of bulk porosity (E) was correlated best with the term $E^3/(1 - E)^2$ of the Carman-Kozeny equation:

$$\frac{Q}{A} = K \frac{\Delta P}{\mu L} \qquad K = \frac{1}{5 \rho_p^2 S^2} \frac{E^3}{(1-E)^2} \tag{6}$$

The permeability (K) of the Carman-Kozeny equation of slabs of granular starch was determined in a permeation cell using helium gas flow. The water diffusivity increased significantly with increasing gas permeability.

Figure 2 shows the effective water diffusivity in granular high-amylose starch (Hylon 7) as a function of moisture content at 30 and 60°C (Eq. 5). Porosity is the most important parameter, while moisture content becomes significant below 0.15 kg water/kg dry solids.

## Effect of Sugars

Incorporation of water-soluble sugars in granular starches reduced significantly the water diffusivity. The decrease in diffusivity was related to the molecular weight of the sugar, with dextrin (mixture of water-soluble polysaccharides)

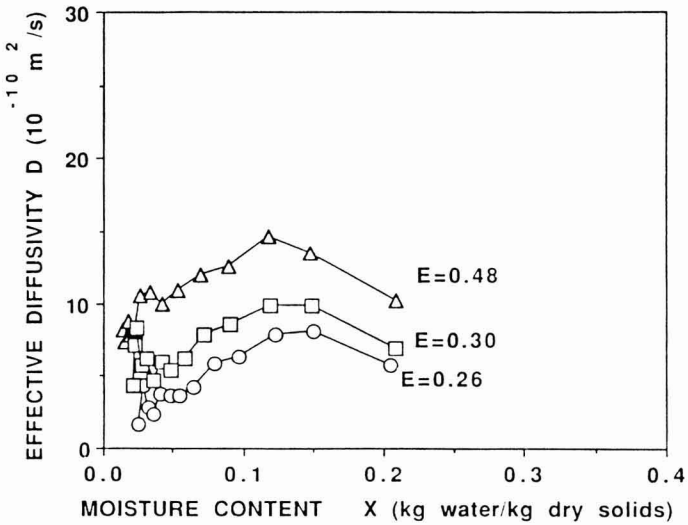


FIG. 2. EFFECTIVE WATER DIFFUSIVITY (D) OF GRANULAR HYLON 7 AS A FUNCTION OF MOISTURE CONTENT X (kg WATER/kg DRY SOLIDS) (AIR-DRYING OF SLABS AT 60°C)
Initial moisture content $X_o$ = 0.3 kg water/kg dry solids.
Initial porosities E = 0.37, 0.43, 0.49

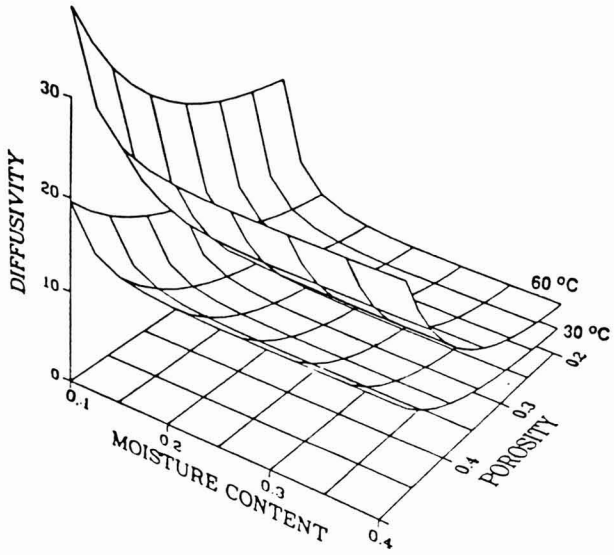


FIG. 3. EFFECTIVE WATER DIFFUSIVITY (D) AS A FUNCTION OF POROSITY AND MOISTURE CONTENT IN HYDRATED STARCH (HYLON 7) SAMPLES

having the strongest effect (Marousis *et al.* 1989). The sugars reduced significantly the porosity of the dried granular starches (Table 1), demonstrating the direct relationship between porosity and water diffusivity. The effect of sugars was stronger in high-moisture starches, in which water is transported by liquid diffusion.

The sugars had little effect on the water diffusivity in gelatinized starches. The sugar molecules may be less mobile in gelatinized than in granular starches, having little effect on the transport of water.

## Inert Particles

Incorporation of inert particles in granular starches tended to increase the effective water diffusivity. The effect of inert particles was more pronounced in starch/carbon mixtures, possibly due to the smaller size and the hydrophobicity of the carbon particles. The inert particles prevented the shrinkage and collapse of the starch structure during drying, resulting in higher bulk porosities (Table 1).

Microscopic observations of spherical samples of starch showed the formation of characteristic patterns of porosity during the drying process (Marousis and Saravacos 1990). In granular starches, radial channels were formed extending from the surface to the center of the sample and increasing the porosity and the

TABLE 1.
POROSITY OF MIXTURES OF GRANULAR STARCH 75%/ SUGARS OR INERT MATERIALS 25%, DRIED AT 60°C

| MATERIAL | POROSITY, (E) | |
|---|---|---|
| | AMIOCA | HYLON 7 |
| Starch | 0.45 | 0.48 |
| Starch 75%/ Glucose 25% | 0.34 | 0.40 |
| Starch 75%/ Sucrose 25% | 0.36 | 0.40 |
| Starch 75%/ Dextrin 25% | 0.30 | 0.33 |
| Starch 75%/ Glass 25% | 0.46 | 0.49 |
| Starch 75%/ Carbon 25% | 0.57 | 0.55 |
| Starch 75%/ Alumina 25% | 0.52 | 0.56 |
| Starch 75%/ Silica 25% | 0.50 | 0.52 |

effective water diffusivity. Incorporation of inert particles did not change the porosity pattern. An entirely different structure was observed during drying of gelatinized starches, without the characteristic radial channels and with irregular cracks formed throughout the mass of the sample. Sugars had little effect on the microscopic structure of the gelatinized starches.

## Extrusion Cooking/Gelatinization

Extrusion cooking causes important physical and physico-chemical changes in starch-based food materials. The water diffusivity is affected significantly by compression, heat and shear phase changes (gelatinization), depolymerization (dextrinization), and expansion of the extrudates. The range of water diffusivities in Amioca (high-amylopectin) starch, subjected to various extrusion conditions, is shown in Fig. 4. The starch extrudates were obtained using a laboratory single-screw extruder, operated at 140°C (die temperature) and a screw speed 150 RPM. The high water diffusivities in the expanded extrudate (e.g., $48 \times 10^{-10}$ m$^2$/s at $X = 0.2$ and 60°C) were caused by its highly porous structure (bulk porosity of the dried extrudate was about 0.8). Reduction of porosity by compressing the hot extrudate resulted in lower water diffusivities (e.g., $1 \times 10^{-10}$ m$^2$/s at $X = 0.2$ and 60°C) in samples of Amioca, which were completely gelatinized during extrusion.

In extrusion cooking, phase changes (from crystalline to amorphous) of starch materials can be caused by thermal gelatinization and/or polymer melting. Heat gelatinization reduces significantly the water diffusivity (Saravacos *et al.* 1990),

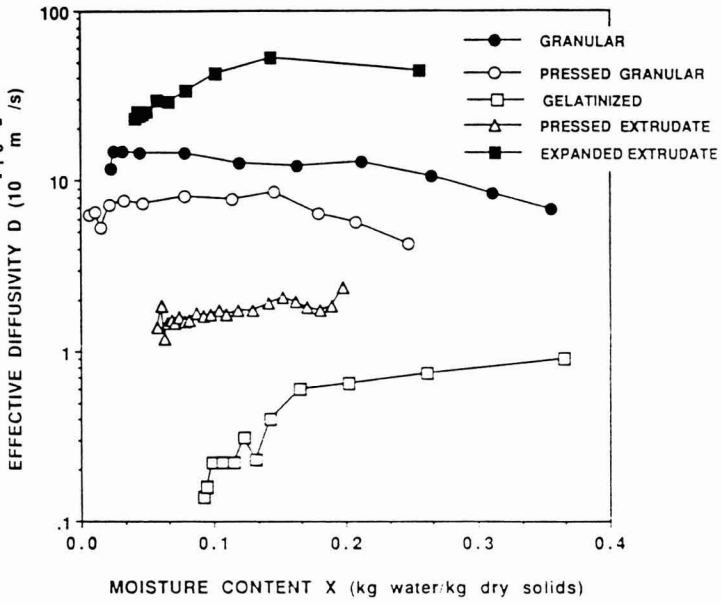


FIG. 4. EFFECTIVE WATER DIFFUSIVITIES (D) IN GELATINIZED AND IN EXPANDED OR PRESSED EXTRUDATES OF AMIOCA STARCH (AIR-DRYING OF SLAB SAMPLES AT 60°C)
Single-screw extrusion at 140°C, 150 RPM

and polymer melting appears to have a similar effect. Gelatinization has been found to increase significantly the thermal conductivity of the starch materials.

Differences in water diffusivity between Amioca (high-amylopectin) and Hylon 7 (high-amylose starch) were evidently caused by the different physical structure of the samples, developed during extrusion and drying. The Amioca samples gelatinized readily and behaved like viscoelastic materials which tended to collapse during drying at high temperatures, resulting in lower water diffusivities. Hylon 7 starch gelatinized with more difficulty, requiring higher water content than Amioca, forming elastic gels, which tended to crack during drying, increasing the water diffusivity. The collapse of physical structure (decrease in porosity) is undesirable in freeze-dried foods, where a highly porous structure must be maintained for rapid dehydration. High relative humidities or moistures and high temperatures may cause collapse and reduction in water diffusivity of freeze-dried and other porous foods (Saravacos 1967). Sugars and other water-soluble components tend to increase the collapsing process.

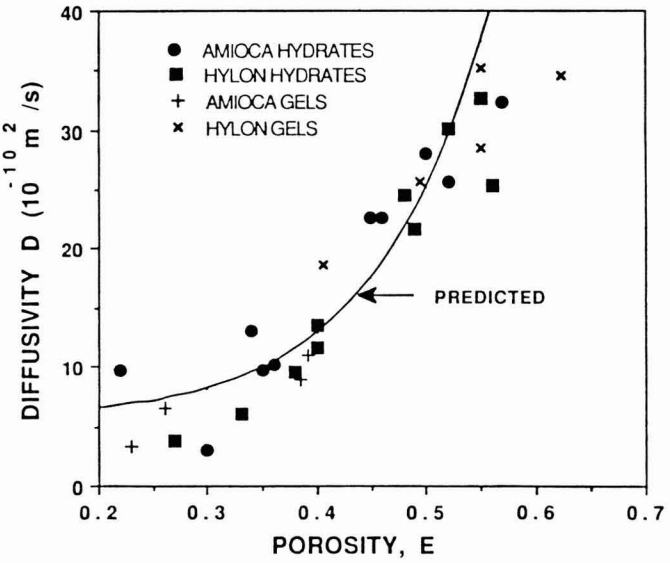


FIG. 5. EXPERIMENTAL AND PREDICTED (EQ. 5) EFFECTIVE WATER DIFFUSIVITIES IN STARCH MATERIALS AS A FUNCTION OF POROSITY

## CONCLUSIONS

The importance of physical structure on the transport of water during the drying process has been demonstrated in model starch systems. High porosities, developed during drying of granular or gelatinized starches, resulted in high effective water diffusivities. Lower porosities and lower water diffusivities were obtained by mechanical compression, addition of sugars or gelatinization of the starch materials. A semiempirical model was developed (Eq. 5) that can predict with fair accuracy the effective water diffusivity from the porosity of the starch material, as shown in Fig. 5. The diffusion of water is related to physical or physico-chemical changes of stored foods. Control of the physical structure can improve the shelf-life and the quality of the food products.

## NOMENCLATURE

A Cross-sectional area of flow
D Effective diffusivity
E Bulk porosity
$E_a$ Activation energy for diffusion
K Permeability constant
L Slab thickness

P Pressure
$r_o$ Radius of spherical sample
S Specific area of particles
t Time
T Absolute temperature
X Moisture content, dry basis
$X_o$ Initial moisture content, dry basis
$X_e$ Equilibrium moisture content, dry basis
W Moisture ratio
$\Delta P$ Pressure drop
$\mu$ Viscosity
$\rho_b$ Bulk density
$\rho_p$ Particle density

## ACKNOWLEDGMENT

New Jersey Agricultural Experiment Station publication No. D-10535-16-90, supported by State funds and the Center for Advanced Food Technology. The Center for Advanced Food Technology is a New Jersey Commission on Science and Technology Center.

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# SHELF-LIFE EXTENSION OF MICHIGAN APPLES USING SUCROSE POLYESTER

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Accepted for Publication March 6, 1991

## ABSTRACT

*Golden Delicious, Ida Red and McIntosh apples were treated with the sucrose polyester (SPE) Semperfresh™, to determine its influence on apple maturity parameters, assess its value to delay ripening and to improve shelf life during cold storage. Apple quality objective and sensory measurements included: Hunter color differences, Magness-Taylor texture and Kramer Shear Press firmness, soluble solids, total acidity, sensory difference from control and consumer acceptability tests. The influences of Semperfresh on storage quality of the apple cultivars varied. SPE retarded apple ripening as shown by persistence of green tissue colors, and increased tissue firmness and titratable acidity. Soluble solids were not significantly affected by Semperfresh treatments. SPE treated apples exhibited sensory differences when compared to the controls, but SPE treatments did not have the same sensory effects on each cultivar. SPE treatment improved consumer acceptability ratings for Golden Delicious and McIntosh apples, but had no significant improvement for Ida Red apples.*

## INTRODUCTION

Quality is at the forefront of importance in the food industry because of consumer demand. Therefore, it is imperative that processors meet the consumer challenge of fresh and fresh quality at minimal cost in order to survive in the marketplace. In terms of fresh fruits, quality is no longer a seasonal event but a year-long phenomenon (Freeman 1990).

Numerous postharvest physiological reactions occur in apples which influence their quality attributes such as appearance, texture, flavor and nutritional value.

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Journal of Food Processing and Preservation **15** (1991) 197–214. 

While some of the physiological changes are positive, other reactions are detrimental to the quality of the commodity. The shelf-life of a commodity can be lengthened by placing in refrigeration or controlled atmosphere that retards respiration, reduces the autocatalytic production and accumulation of ethylene and diminishes microbial decay (Haard 1985).

Controlled atmosphere (CA), routinely used for apple storage, influences the rate of compositional changes (Kader 1986). Pigment and fruit acidity losses are diminished, delays in fruit ripening and softening are slowed and improved nutrient retention can occur with CA storage. But CA is not without problems. Closely related species, different cultivars of the same species, and tissue maturation variations result in dissimilar and often unpredictable oxygen and carbon dioxide tolerance limits (Haard 1985; Kader 1986). Physiological disorders induced by CA include internal browning and surface pitting of apples (Kader 1986). Economically, CA storage units are costly to construct, maintain, and are limited or unavailable in many countries. In addition, commodities must be quickly used following the opening of the chamber.

Several investigators have used sucrose polyesters (SPE) as a postharvest preservation treatment for apples in conjunction with a refrigerated storage environment (Banks 1984; Smith and Stow 1984; Chu 1986; Drake *et al.* 1987; Santerre *et al.* 1989). "Cox's Orange Pippin" apples were treated poststorage with SPE. During 21 days of a simulated marketing period, apple tissues had increased $CO_2$ concentrations, decreased yellow color production and increased firmness (Smith and Stow 1984). Likewise, Banks (1984) found that "Cox's Orange Pippin" apples treated with SPE and stored at 4°C resulted in increased internal $CO_2$ and decreased internal $O_2$ tissue concentrations. However, in apples treated with a 1.25% SPE formulation and stored at 3.5°C for up to 5 months, SPE did not affect texture, color or weight loss (Smith and Stow 1984). Succeeding "low-oxygen" storage of McIntosh and CA storage of Delicious apples, a poststorage application of SPE reduced tissue softening during a 3 week extended storage period at 15°C and 90–95% relative humidity (RH) but did not influence the firmness of CA stored McIntosh apple (Chu 1986). Both Drake *et al.* (1987) and Santerre *et al.* (1989) applied Semperfresh™, an improved formulation of earlier SPE products, to apples. Poststorage application of Semperfresh on Golden Delicious apples following CA and refrigerated storage resulted in retarded color development, higher acid, greater firmness, increased internal $CO_2$, and decreased internal ethylene values when compared to untreated controls. CA stored, treated apples displayed similar attributes but no difference in firmness was evident (Drake *et al.* 1987). Santerre *et al.* (1989) further demonstrated delayed color development and increased firmness in both Golden Delicious and McIntosh apple cultivars during 4 months of storage at 5°C. Not affected by the Semperfresh treatment were pH, total acidity, and soluble solids measurements. No significant flavor and textural differences were found sen-

sorially when apples treated with 1.2% Semperfresh were compared to untreated apples after two month storage time (Santerre *et al.* 1989).

This study extends previous work conducted in this laboratory (Santerre *et al.* 1989) and focuses on the use of several concentrations of Semperfresh to prolong cold storage shelf-life of three Michigan cultivated apples (Golden Delicious, Ida Red, McIntosh). Shelf-life extension of SPE treated apple cultivars without the need of CA storage will lower costs associated with CA apples stored less than six months. Because the consumer is the ultimate assessor of whether treatment differences are of any consequence (Smith and Churchill 1983), consumer acceptability studies were conducted following sensory discrimination tests.

## MATERIALS AND METHODS

Ida Red, McIntosh and Golden Delicious apples were harvested in the fall 1989 from the Horticulture research orchards of Michigan State University, East Lansing, MI. General quality of the apples was good, although McIntosh and Golden Delicious apples had a small amount of bruising. Apples were stored at 5°C prior to application of SPE treatments. Following refrigerated storage, apples were washed in cold water, air dried and subjectively selected for uniformity according to size, color, general appearance, and freedom from external defects.

### Semperfresh Formulation and Apple Treatments

Semperfresh™ powder (Inotek International Co., Painesville, OH) was used to prepare a 3.6% (w/v) SPE stock solution as described previously by Santerre *et al.* (1989), except that solutions were blended for 5 min. Apples from each cultivar were randomly sorted into four 25 kg lots for treatment. Three groups were treated with either a 3-s dip in 0.6%, 0.9% or 1.2% SPE solutions. An untreated lot dipped in water served as the control. Apples were air dried, stored at 5°C with 90–95% RH and then removed from storage after 2, 3 and 4 month intervals to be analyzed for physical, chemical and subjective parameters.

### Objective Evaluations

Whole apple external and internal reflectance color differences were monitored using a Hunter Color Difference Meter (Model D25-2, Hunter Associates Laboratory Inc., Fairfax, VA). Skin color was measured after standardization with a pink tile ($L_L = 68.8$; $a_L = 23.2$; $b_L = 9.4$) for Ida Red and McIntosh cultivars and a yellow tile ($L_L = 78.4$; $a_L = -3.0$; $b_L = 22.7$) for the Golden Delicious cultivar. Flesh color was measured after standardizing the instrument with a white tile ($L_L = 92.3$; $a_L = -1.2$; $b_L = 0.5$).

Apple firmness was assessed by two methods. Puncture pressure (lb force) was determined by using a Magness-Taylor Texture tester (D. Ballauf Mfg. Co., Washington, D.C.) with a 5/16 in. diameter flat cylinder plunger. Measurements were conducted following the removal of apple skin (1″ diameter) and determining penetration force at 90° angles along an equatorial plane of 4 apples/treatment for each cultivar. Apple tissue shear force (lb force/g) was measured on a Kramer Shear Press (Model T-2100-C, Food Technology Corp., Rockville, MD) with a 10 blade shear extrusion cell. Attenuation was set at 1/10, with 3000 lb transducer force and 78.5 calibration factor. Center slice firmness was tested after peeling, coring and slicing the top and bottom off of each apple from three apples per treatment.

Soluble solids ($^{O}$Brix) concentrations were determined using an Abbe-3L refractometer (Bausch & Lomb Optical Co.). Apple juice was manually squeezed from longitudinal slices from four apples per treatment from each cultivar. Total acidity (% malic acid) was measured on homogenates produced by blending 100 g apple tissue (25 g from 4 apples/treatment) with 100 mL deionized water for 1 min. Homogenates (20 mL) were titrated with 0.1 N NaOH to pH 8.0 using a digital pH meter (Model 610A, Corning Glass Works, Medfield, MA).

## Statistical Analysis

The SPE storage study was a completely cross-classified 3-factorial design (cultivar, Semperfresh concentration, and storage time). Analysis of variance (ANOVA) was used to determine the main effects and interactions. Polynomial regression analysis was used to determine the specific affects of SPE concentrations and storage times on chemical and physical parameters. The optimal regression model was selected by RSQUARE and STEPWISE procedures considering high $R^2$ (squared multiple correlation coefficient) and suitable $C_p$ (a statistic for a p variable subset of k candidate variables) statistics (Gill 1987). Data were analyzed using SAS software (SAS 1985).

## Subjective Evaluations

Sliced apples were visually examined for brown core. To evaluate sensorially the control and Semperfresh treated apples after storage, two types of sensory evaluation tests were employed. Difference from control tests were used to ascertain whether a difference existed between the SPE treated apples and the untreated cultivars and to estimate the magnitude of any differences. The difference testing procedure was selected since quality assurance/quality control and storage studies are cases in which the size of the difference as compared to the control is important for decision making (Meilgaard *et al.* 1987a). Following difference testing, affective tests were used to assess the personal responses of

potential SPE apple product users to determine if the Semperfresh treated apples would achieve parity relative to the control apples.

## Sensory Test Methods

The difference from control procedure was followed according to the Meilgaard *et al.* (1987a) method. A 10-point category numerical scale with verbal end anchors (0 = no difference to 10 = extreme difference) was used to rate the size of the overall sensory difference between treatments. The panel consisted of 24 untrained students, faculty and staff from Michigan State University with an age range from 18–55 yrs. Subjects evaluated three replications of each apple cultivar (24 subjects × 3 cultivars × 3 reps). The test was a completely cross-classified 5-factorial design (storage time, cultivar, SPE treatments, panelist and replication).

To determine the affective status of treated and untreated apple cultivars, consumer acceptance tests were used according to Meilgaard *et al.* (1987b). A numerical and verbal anchored 9-point hedonic scale was employed (1 = dislike extremely to 9 = like extremely) to determine the overall acceptability of the apples. The sensory panel was composed of a total of 270 Michigan State University consumers, including students, faculty and staff. Every 30 subjects evaluated one apple cultivar at a time. The test was replicated 3 times (30 subjects × 3 cultivars × 3 reps). Consumers attending each repetition were different. The experimental design was a 5-factorial mixed (nested-factorial) classification with the panelist factor nested within 3 other factors (storage time, cultivar and replication) and the SPE treatment factor was cross-classified with the other 4 factors.

## Environmental Conditions

Sensory tests were held in the sensory evaluation laboratory of the Department of Food Science and Human Nutrition at Michigan State University. This laboratory is equipped with 15 isolated testing booths, temperature regulated positive air flow and constant illumination. Panelists evaluated apple samples under white fluorescent lighting.

Sensory tests were conducted on 0% (control), 0.6% and 1.2% Semperfresh treated apples, to minimize the number of consumer tests and to obtain physical and chemical data concomitantly with the sensory data. Apple cultivars were stored in cold storage chambers on open racks at 5°C for 8 weeks and 12 weeks prior to sensory evaluation.

## Apple Preparation/Preservation

Whole apples were removed from refrigerated storage chambers and held at ambient temperature for approximately 1 h preceding sensory evaluations. The

apples were sliced by hand into 1-inch thick vertical slices, making certain the apple core was not part of the sensory evaluation slice. Weights of each apple slice were not determined. Apples were prepared and served as soon as subjects were in the testing booth to minimize enzymatic discoloration of the apples.

One apple slice was placed in an unlidded two-ounce plastic container labeled with a 3-digit random number with the exception of the control (0% SPE) apples, which were labeled with "C" for the difference testing method (when required). Apple slice presentation orders were randomized and balanced. Subjects were instructed to drink the ambient temperature deionized water *ad libitum* prior to and between apple evaluations. Panelists were also allowed to either expectorate or swallow the apple slices. The difference tests were held 5 days preceding consumer acceptablility testing. The former tests were held midmorning and midafternoon. Consumer acceptability tests were held continuously from morning until late afternoon for three days. Three replications of sensory tests were conducted.

### Sensory Statistical Analysis

ANOVA was used to test the main effects and interactions. Nonorthogonal designed contrasts (Bonferroni t statistics) were used to compare the differences among treatment means (Gill 1987).

## RESULTS AND DISCUSSION

### External/Internal Color

External green skin colors (measured as the inverse of Hunter $a_L$ values) in 3 apple cultivars were greater ($P = 0.01$) as SPE treatment concentrations increased (Fig. 1). The Ida Red cultivar had a greater green skin color retention (4.73 unit/1% SPE) when compared to the Golden Delicious (3.42 unit/1% SPE) and McIntosh cultivars. Unlike the other two cultivars, the effect of Semperfresh treatments on McIntosh apple skin color Hunter $a_L$ values was not a declining linear relationship. SPE concentrations of >0.6% retarded the change of green skin color of McIntosh apples more strongly than did lower concentrations. Nonlinearity may be the reason for the significant interaction between Semperfresh concentration and storage interval for McIntosh apples stated by Santerre *et al.* (1989). These data suggested that Semperfresh applications inhibited yellow color development. SPE concentrations did not affect apple skin brightness (Hunter $L_L$), yellow color (Hunter $b_L$) and chroma $(a_L^2 + b_L^2)^{1/2}$ values.

Since apple skin colors are basically in the red-yellow-green quadrants, hue angle of skin color was defined as $\tan^{-1}(b_L/a_L)$ while it was in the yellow-red quadrant $(+b_L/+a_L)$ and $\tan^{-1}(b_L/a_L) + 180$ while in the yellow-green quadrant

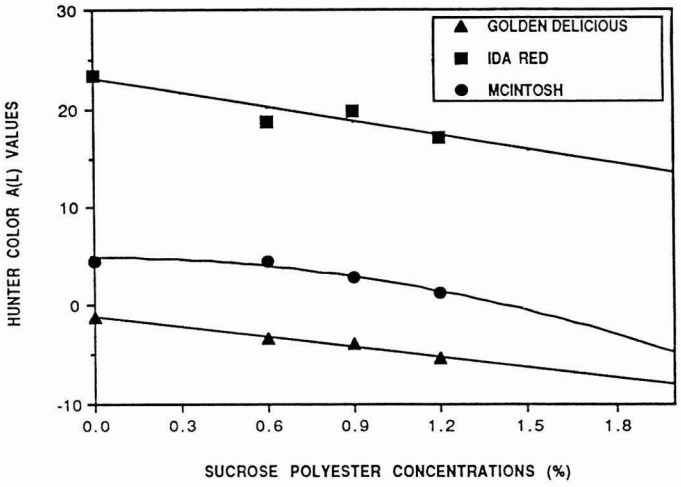



FIG. 1. EFFECT OF SUCROSE POLYESTER CONCENTRATIONS ON APPLE SKIN GREEN COLOR

$(+b_L/-a_L)$. This definition was derived from the concepts of Little (1975) and Francis (1975) who described the function $b_L/a_L$ as a hand sweeping counter-clockwise on a dial, starting at $0\pi r$ (red), to $\pi r/2$ (yellow), to $\pi r$ (green), to $3\pi r/2$ (blue) and at $2\pi r$ back to red.

Semperfresh concentrations significantly ($P = 0.05$) influenced apple skin color hue angles, $\tan^{-1}(b_L/a_L)$. Santerre *et al.* (1989) also reported marked SPE treated McIntosh variations on hue angles and chroma during storage. SPE-treated apples had larger hue angles than untreated apples (Fib. 2). As SPE concentrations were increased, larger impacts on hue angles were apparent for Ida Red and McIntosh cultivars compared to the Golden Delicious cultivar. Skin hue angles of Golden Delicious apples increased at 5.95°/1% SPE and were located in the yellow-green quadrant. Skin hue angles for the Ida Red and McIntosh cultivars were situated in the yellow-red quadrant. The observations of hue angles coincided with the Hunter $a_L$ measurements. Retention of greener skin colors implied an inhibition of ripening and increased shelf-life (Drake *et al.* 1987). A change of green to greenish yellow to yellow hues is one of the best apple maturity indexes (Magness and Diehl, 1924).

Cultivar differences were evident regarding the influence of SPE treatments on internal flesh colors. Internal flesh color of Ida Red apples was not affected by the Semperfresh treatment in contrast to the other two cultivars. Brightness (Hunter $L_L$) and green (measured as the inverse of Hunter $a_L$) values were significantly ($P = 0.01$) influenced by the SPE concentration, storage interval, or both, in McIntosh and Golden Delicious apples. As storage time progressed,

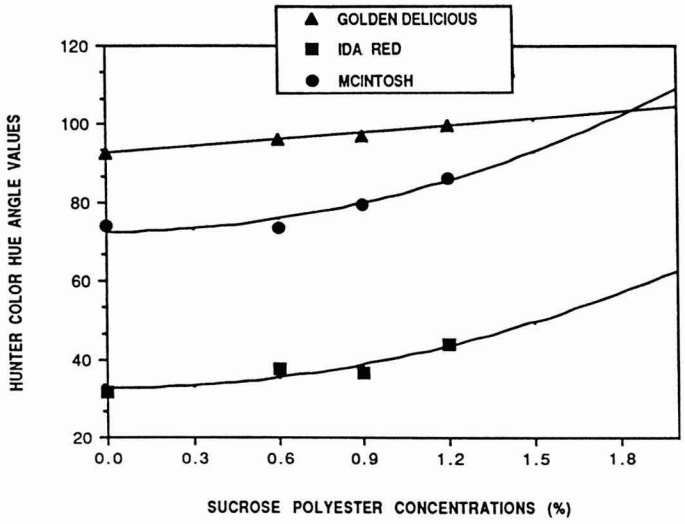



FIG. 2. EFFECT OF SUCROSE POLYESTER CONCENTRATIONS ON APPLE SKIN HUE ANGLE VALUES

an increased brightness of flesh color was observed in McIntosh and Golden Delicious cultivars, however, the rates of brightness development differed between cultivars (Fig. 3). During the first two months of storage, the McIntosh apple flesh brightened at a faster rate than Golden Delicious apple flesh. After three months storage, the internal flesh brightness values did not change signif-

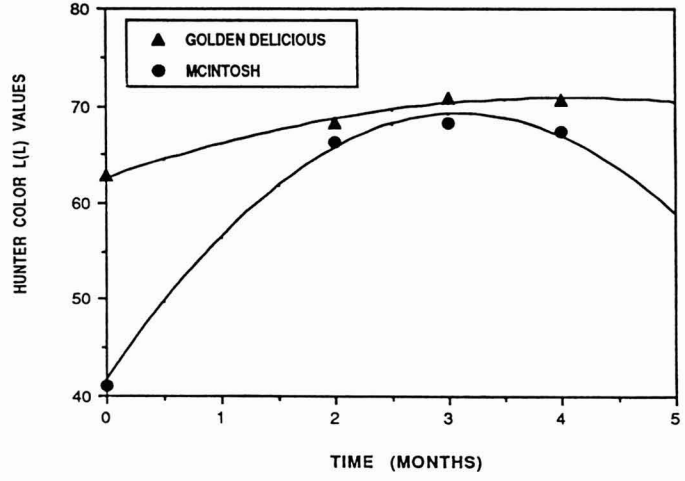
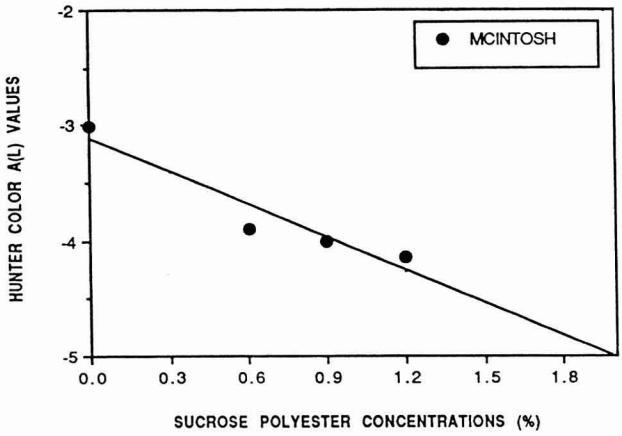



FIG. 3. EFFECT OF STORAGE TIME ON APPLE FLESH BRIGHTNESS VALUES



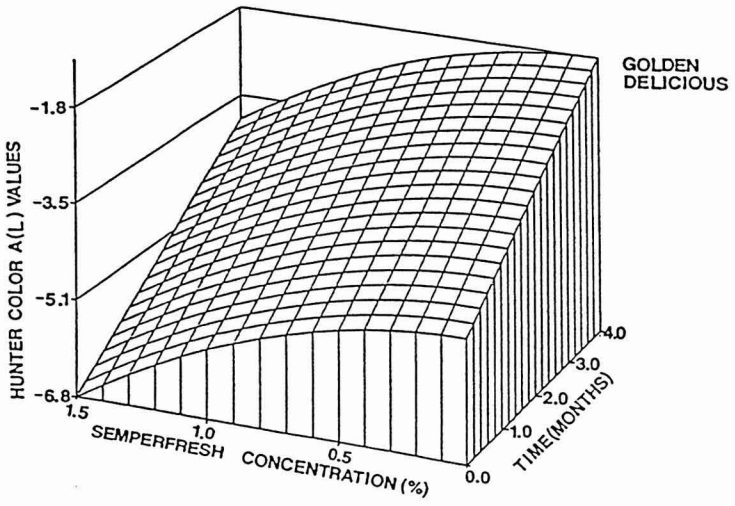




FIG. 4. EFFECT OF STORAGE TIME AND/OR SUCROSE POLYESTER CONCENTRATIONS ON APPLE FRESH GREEN COLOR

icantly for Golden Delicious apples. However, McIntosh apple flesh started losing brightness.

McIntosh green color retention in the apple flesh was significantly influenced ($P = 0.01$) by the application of Semperfresh. The effect of Semperfresh treatment on McIntosh flesh green color retention was 0.95% unit/1% SPE (Fig. 4, upper). Golden Delicious apples were significantly influenced by SPE concentration and storage time (Fig. 4, lower). As storage time increased, Golden Delicious flesh

was less green in color which agrees with Santerre *et al.* (1989). Both treated and untreated Golden Delicious apples lost flesh green color at the same rate (0.71 unit/month) during storage. Green color retention of Golden Delicious apple flesh was more apparent when >0.6% SPE concentrations were applied. Semperfresh treatments did not affect apple flesh yellow color (Hunter $b_L$) and chroma values, but both characteristics were influenced by storage time ($P = 0.01$). Flesh yellow hues increased throughout storage, whereas chroma decreased. McIntosh and Golden Delicious SPE treated cultivars implied retarded disappearance of apple flesh green color.

## Firmness

McIntosh and Golden Delicious apples exhibited significant ($P = 0.01$) interactions between treatment with Semperfresh and storage times for puncture pressure firmness (Fig. 5, upper and middle), but the application of SPE treatments did not affect the firmness of Ida Red apples (Fig. 5, lower). SPE concentrations consistently increased the firmness (2.16 lb force/1% SPE) of Golden Delicious apples despite the length of storage, whereas a declining firmness rate of 1.57 lb. force/month due to advanced storage time for treated and untreated apples was noted. Semperfresh-treated McIntosh apples were also firmer than the untreated controls. Firmness data for the SPE treated Golden Delicious and McIntosh cultivars coincide with the results of Chu (1986) and Santerre *et al.* (1989). However, the importance of SPE concentrations on puncture firmness of the McIntosh cultivar was highly dependent on storage interval. As storage time increased, smaller Semperfresh concentrations resulted in firmer apple tissues. After one month storage, McIntosh apples treated with 1.2% SPE were the most firm in comparison to the other McIntosh treatments. In contrast, following 4 months storage, the 0.6% SPE treated McIntosh apples exhibited the firmest texture. Thus, McIntosh apples treated with the higher Semperfresh concentrations lost firmness more quickly than apples treated with lower SPE concentrations. Tissue firmness declined more rapidly in the SPE-treated McIntosh apples than in the untreated controls. Therefore, puncture pressure firmness values from 1.2% SPE treated McIntosh apples (13.00 lb force) were not significantly different from the untreated apples (13.06 lb force) following 4 months storage. However, SPE-treated McIntosh apples remained firmer than untreated apples throughout the storage period. Ida Red apples revealed a loss of firmness at a rate of 1.3 lb force/month throughout the refrigerated storage period.

Semperfresh treatments of apple cultivars did not significantly affect flesh tissue shear forces. However, there was a trend indicating that as storage time advanced apple tissue shear forces declined.

Firmness results, demonstrating increased apple tissue firmness following SPE treatment, are different from the results of Smith and Stow (1984) who used

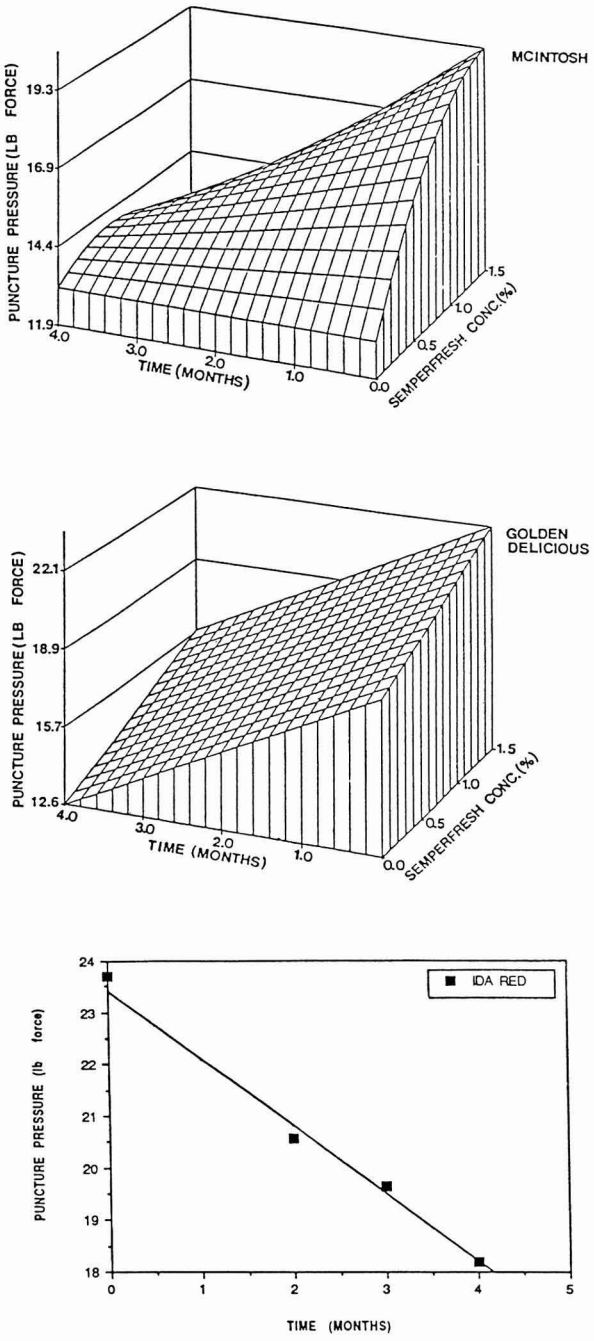



FIG. 5. EFFECT OF SUCROSE POLYESTER CONCENTRATIONS AND/OR STORAGE TIME ON PUNCTURE PRESSURE

another SPE (Prolong™) applied to "Cox's Orange Pippin" apples prior to 3.5°C storage for 5 months. Apple firmness, yellowing and weight loss were not reduced with 1.25% Prolong treatment. Differences from our results may be attributed to differences between the cultivars, storage environments, SPE chemicals, and firmness determination methods used between the studies. However, following 5 months controlled atmosphere storage, Smith and Stow (1984) noted that by increasing SPE treatment concentrations (1–4%), firmness was retained in apples stored for 3 weeks at various temperatures (3.5–18°C).

## Total Acidity

Cultivar differences in titratable acids or total acidity were observed. Semperfresh-treated Golden Delicious and Ida Red apples had greater total acidity than untreated apples of both cultivars, but not the McIntosh cultivar (Fig. 6). Santerre *et al.* (1989) observed that total acidity concentrations varied over time in both the Golden Delicious and McIntosh cultivars. In the present study, McIntosh total acidity concentrations decreased sharply after one month storage. A decline in acidity demonstrates maturation development. Applications of increased SPE concentrations to Ida Red apples prevent losses of titratable acids, especially at >0.6% SPE concentrations. A significant interaction ($P=0.01$) between Semperfresh concentration and storage interval was observed for total acidity of Golden Delicious apples. As SPE concentrations increased, total acidity of Golden Delicious was greater throughout storage, but the effect was not significant after four months. Results were consistent with the work of Drake *et al.* (1987) who found greater total acid concentrations in Semperfresh-treated Golden Delicious apples than in untreated apples. This study confirmed previous research showing that titratable acid concentration in Golden Delicious apples increased during the first two months of storage followed by a decline from two to four months storage (Santerre *et al.* 1989). With increasing SPE concentrations, the titratable acid retention in Golden Delicious apples changed as storage time increased. At one month storage total acidity retention by Semperfresh application was $13.84 \times 10^{-2}$% malic acid/1% SPE. This was followed by a reduction to $9.13 \times 10^{-2}$% malic acid/1% SPE at 2 months storage time. The retention in total acidity observed in Golden Delicious and Ida Red cultivars indicates delayed ripening.

## Soluble Solids

Soluble solids contents at the beginning of the storage study for Golden Delicious, McIntosh, and Ida Red cultivars were 10.1°, 10.4°, and 11.9° Brix, respectively. Soluble solids values were not affected by storage time or SPE treatment in apple cultivars. These results are in contrast to Santerre *et al.* (1989)

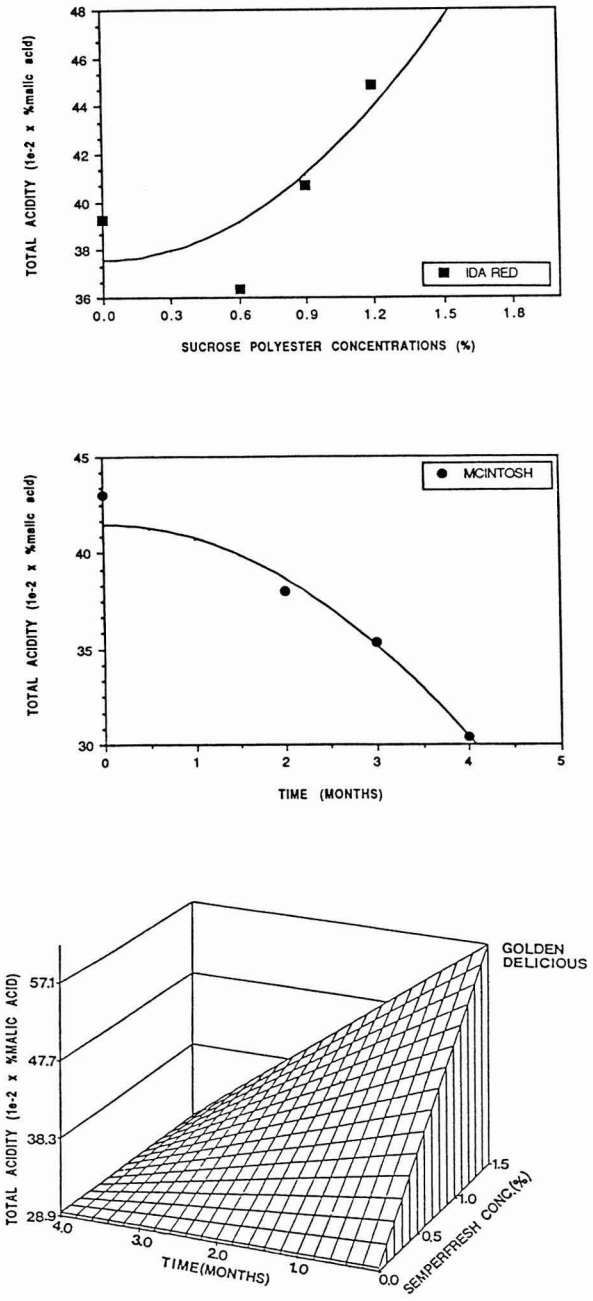



FIG. 6. EFFECT OF SUCROSE POLYESTER CONCENTRATIONS AND/OR STORAGE TIME ON TOTAL ACIDITY

who found decreased soluble solids concentrations at four months for McIntosh apples, while soluble solids concentrations in Golden Delicious apples increased after two months and decreased after four months storage.

## Sensory Evaluation

Minimal sensory research has been reported using SPE-treated apples.

The difference from control test results are shown in Table 1. Apples treated with 0.6% and 1.2% Semperfresh exhibited significant differences ($P = 0.01$) as compared to the untreated controls for the Golden Delicious, Ida Red and McIntosh apple cultivars. Storage time was not a significant factor. Semperfresh treatment resulted in larger differences with the Golden Delicious apple cultivar when compared to the McIntosh and Ida Red cultivars, as noted by the differences among mean intensities (Table 1). Santerre *et al.* (1989), using a triangle testing procedure, detected no significant flavor or textural changes between 0% and 1.2% Semperfresh-treated McIntosh and Golden Delicious apples following two months cold storage (5°C). There were different methods used between the Santerre *et al.* (1989) study and this study, which may account for these conflicting results. Santerre *et al.* (1989) stored apples in plastic bags following SPE treatment, whereas apples were stored openly within the environmental chambers in this study. The sensory difference methods were also dissimilar. Santerre *et al.* (1989) used an overall difference testing technique to measure flavor and texture. Flavor and texture may not vary among the SPE-treated apples as compared to the untreated controls, but apple attributes not measured may be different. In addition, apples have inherent variation in quality attributes over a

TABLE 1.

DIFFERENCE FROM CONTROL APPLE SENSORY TEST[1]

| Concentration | Cultivar | | |
|---|---|---|---|
| SPE | Ida Red | McIntosh | Golden Delicious |
| 0% SPE[2] | 2.16[a] | 1.96[a] | 1.82[a] |
| 0.6% SPE | 3.84[b] | 3.39[b] | 4.45[b] |
| 1.2% SPE | 3.55[b] | 3.44[b] | 4.35[b] |

[1] Within a column, means not followed by the same letter are significantly different at $P \leq 0.01$. Means are based on a ten point category numerical scale with verbal and anchors (0 = no difference to 10 = extreme difference).

[2] Blind control; SPE = sucrose polyester, Semperfresh™

time period (Smith and Churchill 1983). The focus of the difference from control testing method in this study was on whether an overall difference existed between the apples and to estimate the size of any such differences. No attributes were specifically identified.

Consumer acceptability results for the effect of storage time across Semperfresh treatments within cultivar are shown in Table 2. The Ida Red cultivar demonstrated the same degree of liking (like slightly on the hedonic scale) when tested after two and three months refrigerated storage. In contrast, the McIntosh apple cultivar exhibited a significant decrease ($P = 0.01$) in consumer acceptability after three months storage as compared to the two months storage time period. Mean acceptability values ranged from like moderately/like slightly to like slightly on the hedonic scale. Evidence also indicated that lower consumer acceptability for Golden Delicious apples over time ($P = 0.10$). Again, the mean acceptability scores at two and three months revealed that the Golden Delicious apples were liked slightly.

Table 3 reveals the mean consumer acceptability values for the Semperfresh treatment effect across time within cultivar. The application of 0.6% and 1.2% Semperfresh resulted in Golden Delicious apples having a significantly ($P = 0.01$) improved acceptability (like slightly) over the untreated apples. However, as SPE concentration increased, from 0.6% to 1.2%, the acceptability of the Golden Delicious apples was not improved. In contrast, SPE did not change the consumer acceptability of Ida Red apples. Despite the treatment, treated and untreated Ida Red apples were liked slightly. The McIntosh cultivar exhibited acceptability ratings in between the other two cultivars. There were no differences between the untreated McIntosh apples and the 0.6% SPE-treated McIntosh apples in terms of consumer acceptability (like slightly). But, when Semperfresh concentrations were increased to 1.2%, the treated McIntosh apples demonstrated a significantly higher ($P = 0.05$) degree of consumer acceptability as compared

TABLE 2.
CONSUMER ACCEPTABILITY TEST-TIME EFFECT[1]

| Storage Time | Variety | | |
|---|---|---|---|
| | Ida Red | McIntosh | Golden Delicious |
| 2 months | 6.27[a] | 6.38[a] | 6.20[a] |
| 3 months | 6.38[a] | 5.97[b**] | 5.90[b*] |

[1] Within a column, means not followed by the same letter are significantly different. * $P \leq 0.10$; ** $P \leq 0.01$. Means are based on a numerical hedonic scale with verbal and anchors (1 = dislike extremely to 9 = like extremely).

TABLE 3.
CONSUMER ACCEPTABILITY TEST-SUCROSE POLYESTER EFFECT[1]

| Concentration | Variety | | |
|---|---|---|---|
| SPE | Ida Red | McIntosh | Golden Delicious |
| 0% SPE[2] | 6.34[a] | 6.01[a] | 5.69[a] |
| 0.6% SPE | 6.20[a] | 6.11[ab] | 6.19[b**] |
| 1.2% SPE | 6.43[a] | 6.41[b*] | 6.26[b**] |

[1]Within a column, means not followed by the same letter are significantly different. *P≤0.05; **P≤0.01. Means are based on a numerical hedonic scale with verbal and anchors (1 = dislike extremely to 9 = like extremely).
[2]Control; SPE = sucrose polyester, Semperfresh™.

to the untreated controls. Golden Delicious apples were most affected by SPE on consumer acceptability when compared to McIntosh apples, as noted by the level of significance.

Even though all apple cultivars treated with SPE demonstrated significant differences (P = 0.01) as compared to the untreated apples (Table 1), these differences did not influence the degree of liking of the cultivars in the same manner (Table 3). The SPE treatment differences did not affect the degree of liking of Ida Red apples, but the SPE treatment differences generally resulted in improvement of the overall acceptability ratings of the treated McIntosh and Golden Delicious apples as compared to the untreated controls. Sensory data has demonstrated that product differences detectable by trained sensory panels can also be distinguished by untrained product users (Smith and Churchill 1983).

## CONCLUSIONS

### Objective Studies

Greener flesh tissues persisted in Semperfresh-coated McIntosh, Golden Delicious and Ida Red cultivars. SPE effects upon apple tissue firmness and total acidity concentrations were dissimilar among the cultivars. Semperfresh did not increase the firmness of Ida Red apples. Golden Delicious and Ida Red SPE-treated apples had increased total acidities demonstrating delayed ripening. In addition, as storage time advanced, titratable acidity concentrations varied between and within apple cultivars. Although the influences of Semperfresh on the storage quality of apple cultivars varied, SPE delayed ripening during storage. Therefore, the use of Semperfresh in place of CA storage remains a viable alternative to delay apple tissue senescence.

### Sensory Studies

Apple cultivars (Golden Delicious, Ida Red, McIntosh) treated with SPE exhibited significant differences as compared to the untreated controls. The application of SPE to Golden Delicious apples resulted in an improvement in consumer acceptability ratings. In contrast, SPE treatment did not change the consumer acceptability scores for Ida Red apples, and an improvement in acceptability values was seen only when the highest SPE concentration was used for the McIntosh apples. Apple cultivar differences were also apparent when focusing upon the effect of time across SPE treatments within cultivar in terms of consumer acceptability. The Ida Red cultivar during two and three months storage time demonstrated the same consumer acceptability. In contrast, the consumer acceptability scores for McIntosh and Golden Delicious cultivars were decreased with prolonged storage time.

## ACKNOWLEDGMENTS

Acknowledgement is made to the Michigan Agricultural Experiment Station for its support of this research. Thanks is also extended to Dr. John L. Gill, Margaret Conner, Debbie Klein and Suzanne Palmer for their advice and assistance throughout this project.

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# PRECOOLING AND MODIFIED ATMOSPHERE STORAGE OF GREEN ASPARAGUS

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Accepted for Publication March 6, 1991

## ABSTRACT

*In the first study, the effects of 3 precooling techniques on the storability of locally grown green asparagus kept for 21 days at 1.5°C under normal atmosphere (RA) were assessed and compared. In the second study, the keeping quality of green asparagus stored at 1.5°C for 28 days, under RA with 2 levels of relative humidity (RH), and under 3 different modified atmosphere (MA) compositions, was assessed and compared. MA conditions were maintained with silicone membranes installed as windows on laboratory scale containers. Precooling the asparagus prior to storage reduced losses by more than 20%. However, there were no significant differences between the three techniques studied. After 28 days, MA stored asparagus had better color, fresher appearance, firmer texture and lower mass loss (<12%) than that stored for the same period under RA with 80% RH (mass loss of 60%) or with >95% RH (mass loss of 41%). Although the silicone membrane was able to adequately control the $CO_2$ levels, regular injections of $O_2$ were required to prevent anaerobic respiration.*

Journal of Food Processing and Preservation **15** (1991) 215–224. 

## INTRODUCTION

Asparagus (*Asparagus officinalis altilis*) is a perennial plant originating from temperate regions. In Canada, its production averages 300 tonnes per year. This accounts for less than 16% of the total annual consumption (Longmuir 1984). Currently, domestic production cannot meet demand during the growing season, which extends from early May to mid-July. This industry could therefore benefit from increased production and by improved handling and storage practices.

Asparagus is one of the most perishable commodities. With its very high rate of respiration (15 times that of apples), asparagus quickly deteriorates after harvest. At temperatures above 4°C, sugars and vitamins are lost rapidly and coarse fibers develop. It is therefore crucial to rapidly cool the spears right after harvest and to maintain the temperature between 0 and 4°C during transit and marketing (Ehlert and Seelig 1966). Additional benefit can be obtained by packaging the asparagus under modified atmosphere (MA). Increasing $CO_2$ to 10% improves storability and retention of green color and solids, enhances spear tenderness, and retards soft rot propagation. Reduced $O_2$ concentrations have failed to show any advantage (Saltveit 1985). MA is beneficial if spears are held for more than two days at 2°C.

In Québec, it is common for asparagus spears to be handpicked and sorted. They are rarely precooled. The spears are usually packed in 7 or 14 kg wooden boxes, covered with crushed ice and sent directly to the fresh market. Although various studies have demonstrated the beneficial effects of precooling and modified atmosphere (MA) packaging on the keeping quality of asparagus grown in southern United States (Lipton 1977), little has been done to adapt this technology to domestically produced asparagus.

The purpose of this study was twofold:

(1) To assess the effects of different precooling techniques on the keeping quality of domestically produced asparagus; and

(2) To compare the keeping quality of domestically produced asparagus stored under regular atmosphere (RA) to that stored under different compositions of MA, at the same temperature and for the same period of storage.

## MATERIALS AND METHODS

To achieve the above mentioned objectives, the experiment was divided into two parts. Part I consisted of the precooling experiment, while Part II dealt with the RA versus MA storage. Each part of the experiment was set according to a completely randomized block design in which each treatment was replicated four times.

The Martha Washington green asparagus used in the experiment was grown locally on a black sandy clay loam, 15 cm thick on a deep grey clay. It was handpicked, sorted, trimmed, precooled and stored on the same day. The sorting procedure consisted of removing the spears that had a diameter smaller than 5 mm and those that had been damaged or deformed. Some spears were shortened in order to fit into the containers. Aside from small reddish spots on few asparagus spears, no sign of disease was observed.

## Part I: Effects of Different Precooling Techniques on the Keeping Quality of Green Asparagus

In Part I, the effects of different precooling techniques on the keeping quality of green asparagus stored at 1.5°C under RA was investigated. The three techniques studied were forced air precooling, $CO_2$ precooling, and water precooling.

The forced air precooling unit used the air circulating through the cold room evaporator. The asparagus spears were single layered on a screen rack positioned perpendicularly to the air flow path. The average air speed going through the loaded rack was 2–3 m/s with an air temperature of about $-1$°C. The air and spear temperatures were monitored with Type-T thermocouples and recorded with a DORIC Minitrend 205 data logger.

The $CO_2$ precooling unit consisted of an insulated box with an elliptical air flow path, and blowers. The air temperature was controlled by providing the proper amount of dry ice and by opening or closing dampers to obtain the desired air ratio. The racks containing the asparagus were mounted parallel to the air flow path. The air temperature was maintained between $-3$ and 1°C. Here also, the air and spear temperatures were monitored.

Hydrocooling was obtained by immersing the asparagus in a mixture of ice and water containing 200 ppm of chlorine. Chlorine was added to the cooling water to prevent the spread of pathogens. Here again, the water and spear temperatures were monitored with Type-T thermocouples connected to the DORIC Minitrend data logger.

For control purposes, non-precooled asparagus were also stored at 1.5°C under RA for the same storage period.

## Part II: Effects of Storage Conditions on the Keeping Quality of Green Asparagus

In Part II, the keeping quality of green asparagus stored under regular atmosphere with about 85% RH (RA), under regular atmosphere with RH close to saturation (HRA) and with 3 different MA compositions was assessed and compared.

The RA conditions was obtained by directly exposing the paper bag containing the spears to the air in the cold room, which was kept at 1.5°C with about 85% RH. For the HRA treatment, the paper bags containing the spears, were placed in perforated plastic bags. The MA compositions were achieved and maintained with silicone membranes installed as windows on the storage containers shown in Fig. 1. With this system, the MA is maintained through two simultaneous processes: product respiration and membrane permeation. The MA containers were constructed from sections of polyethylene plastic pipe, 335 mm in length by 115 mm in diameter. Clear acrylic top and bottom lids allowed visual inspection of the product throughout the storage period. A screw top cover made it easier to open and close the container. Design $CO_2$ levels of 2% (MA-A), 5% (MA-B) and 10% (MA-C) required the installation of window surface areas of 38, 15 and 8 $cm^2$, respectively. These surface areas were calculated using the following equation:

$$\text{AREA} = \frac{\text{RR} * \text{M}}{\text{K} * \text{CO}_2} \tag{1}$$

where

Area = silicone membrane area, $m^2$
RR = respiration rate of asparagus stored under MA, L. of $CO_2 \cdot kg^{-1} \cdot h^{-1}$
M = mass of stored asparagus, kg
K = permeability of the silicone membrane to $CO_2$, 1750 $L \cdot d^{-1} \cdot m^{-2} \cdot Atm^{-1}$
$CO_2$ = desired partial pressure differential across the membrane, Atm

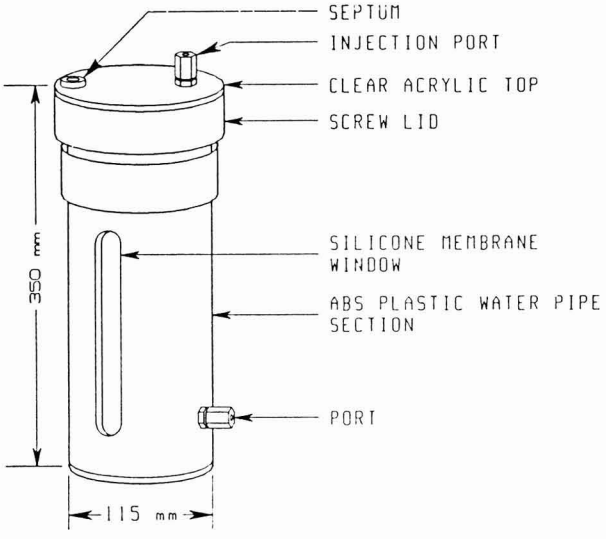


FIG. 1. DIAGRAM OF THE LABORATORY SCALE CONTAINER

At any given temperature, the respiration rate of a commodity is greatly affected by the composition of the surrounding air. However, the respiration rate of asparagus stored under MA could not be found in the literature. Raghavan and Gariépy (1989) have reported that the respiration of vegetables stored under MA or controlled atmosphere is usually reduced by about 50%. Based on these observations, the respiration rate of asparagus under MA was set at 20 mg $CO_2$/kg·h, which is about half the published value at the same temperature under normal air composition (ASHRAE 1986).

In the eventuality that the $O_2$ dropped too low, a port was provided at the top of the container to allow the injection of a gas mixture made of 33% $O_2$ and 67% $N_2$. Periodical analysis of the gas composition in the containers indicated whether or not injection was necessary.

### Procedure

Storage of asparagus was studied using laboratory scale containers. Prior to the experiment, the storage containers were washed with chlorinated water (500 ppm) and checked for airtightness.

The spears were first precooled in batches according to treatment specifications. The containers were then filled with about 500 g of asparagus, sealed and placed in a walk-in cold room maintained at 1.5°C with about 85% RH. Part I and II lasted for 21 and 28 days, respectively.

The gas compositions prevailing in the MA containers were analyzed with Hewlett Packard 5890A gas chromatograph equipped with a thermal conductivity detector. Separation of the gas components was performed at 50°C on a Molecular Sieve 5A column (diameter: 3 mm, length: 1.82 m, 80/100 mesh) and a Poropak Q (diameter: 3 mm, length: 1.82 m, 80/100 mesh) mounted on a time controlled column sequence-reversal pneumatic valve. The carrier gas was Helium. This setup did not allow the separation of Argon from $O_2$. In this paper, the $Ar + O_2$ gas mixture is referred as $O_2$ measured levels. The concentration of Ar in ambient air is about 1%.

Storage, trimming and total mass losses were assessed at the end of the storage period. The trimming operation consisted of removing the spears that were considered unmarketable.

## RESULTS AND DISCUSSION

### Air Temperature and Relative Humidity

During the experiments, the cold room temperature averaged 1.5°C with a relative humidity level of around 80%. These conditions prevailed in the RA chambers since they were opened to the cold room environment. The temperature in the HRA and MA chambers was also 1.5°C and some condensation was

observed in all HRA and MA containers indicating that the RH levels were close to the saturation point.

## Part I: Effect of Different Precooling Techniques on the Keeping Quality of Green Asparagus

Although no serious attempts were made to optimize the performance of the laboratory scale precoolers, the observed cooling rates were around $2°C \cdot min^{-1}$ with the forced air precooler, $2.5°C \cdot min^{-1}$ with the $CO_2$ precooling unit and $2°C \cdot min^{-1}$ with the water precooler. The cooling rates were satisfactory, since all asparagus spears were cooled from 22°C to 2°C in less than 10 min.

In this experiment, it was demonstrated that the keeping quality of domestic asparagus stored under RA can be improved with precooling since the mass losses of precooled asparagus were all significantly lower than the non-precooled spears (Table 1). Precooling the asparagus was found to reduce total losses by 20–30%. Although the lowest total mass loss was recorded for water precooled asparagus, it was not found significantly different from the two other precooling methods tested. The quality of the asparagus was acceptable with good color and appearance. Spear limpness and opened tips were two major causes of losses.

## Part II: Effects of Storage Conditions on the Keeping Quality of Green Asparagus

**Progression of the Gas Compositions in the MA Containers.** The MA composition in each container was determined by gas chromatography on a regular basis. In all MA containers, the $CO_2$ progression was characterized by a sharp increase during the first 4 days of storage followed by a slow decrease over the following 8–10 days (Fig. 2, 3 and 4). From then on, the $CO_2$ levels

TABLE 1.
KEEPING QUALITY OF PRECOOLED AND NON-PRECOOLED GREEN ASPARAGUS AFTER 21 DAYS OF STORAGE UNDER RA

| Precooling Method | Initial Mass, g | Storage Loss, % | Trim Loss, % | Total Loss, % |
|---|---|---|---|---|
| Non | 551.0 | 12.3 a[1] | 54.0 a | 66.3 a |
| $CO_2$ | 553.4 | 8.7 b | 34.0 b | 42.7 b |
| Air | 551.4 | 9.9 b | 34.6 b | 44.3 b |
| $H_2O$ | 551.6 | 9.1 b | 26.0 b | 35.1 b |

[1] Means in the same column with the same letter are not significantly different at 0.05 level.

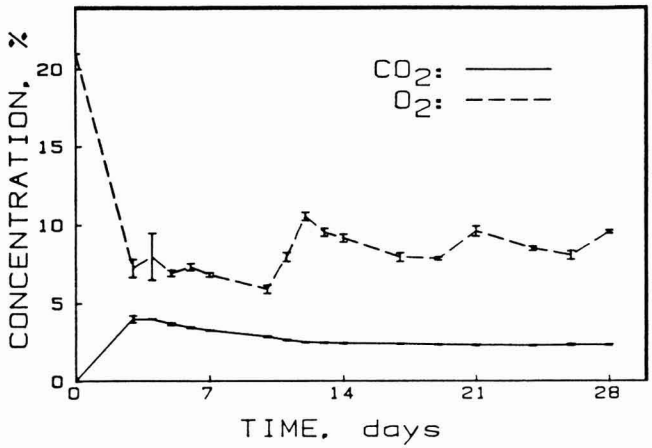


FIG. 2. PROGRESSION OF THE $CO_2$ AND $O_2$ CONCENTRATIONS UNDER TREATMENT MA-A
The Container was designed to maintain 2% $CO_2$.

remained relatively constant until the end of the experiment. In general, the silicone membrane windows were able to adequately control the levels of $CO_2$ in the MA containers.

The progression of $O_2$ levels were much more hectic. Large fluctuations resulted from the injection of the gas mixture into the containers in order to maintain the $O_2$ levels at adequate levels (Fig. 2, 3 and 4). It was also observed that when

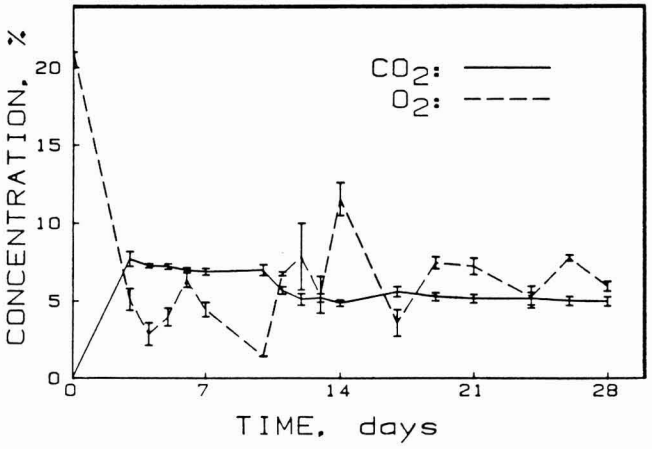


FIG. 3. PROGRESSION OF THE $CO_2$ AND $O_2$ CONCENTRATIONS UNDER TREATMENT MA-B
The container was designed to maintain 5% $CO_2$.

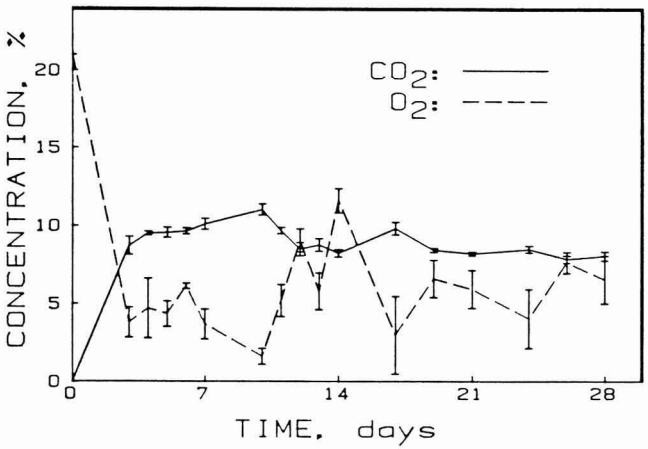


FIG. 4. PROGRESSION OF THE $CO_2$ AND $O_2$ CONCENTRATIONS UNDER TREATMENT MA-C
The container was designed to maintain 10% $CO_2$.

the $O_2$ concentrations dropped below 3%, the $CO_2$ levels had a tendency to increase. This $O_2$ concentration appeared to be the threshold value under which the undesired anaerobic respiration can no longer be inhibited (Bidwell 1979). MA storage of asparagus should therefore be performed in an atmosphere containing no less than 3% $O_2$.

It was noted that fluctuations in both $CO_2$ and $O_2$ levels were more pronounced under treatments MA-B and MA-C. This was attributed to the more frequent $O_2$ injections required to maintain the $O_2$ above the threshold value.

Although it has been demonstrated that the silicone membrane was able to adequately control both the $O_2$ and $CO_2$ concentrations in similar experiments performed with other vegetables (Gariépy *et al.* 1986, 1985), the system was unable to provide enough $O_2$ to the asparagus. The addition of a calibrated orifice to supplement the $O_2$ in the MA container should be investigated. More experiments should also be performed to assess the potential of other polymeric films with lower $CO_2$:$O_2$ selectivity for MA storage of domestically produced asparagus.

## Keeping Quality

Storage, trim and total mass losses were assessed after 28 days of storage and the results are reported in Table 2. MA storage was the best condition for the asparagus, since the total mass losses recorded under the 3 MA storage conditions were significantly lower (0.05 level) than under RA and HRA. It was not possible to determine which gas composition was best suited since the differences in mass

**TABLE 2.**
KEEPING QUALITY OF GREEN ASPARAGUS AFTER 28 DAYS OF STORAGE UNDER RA, HRA AND DIFFERENT MA COMPOSITIONS

| Storage Condition | Initial Mass, g | Storage Loss, % | Trim Loss, % | Total Loss, % |
|---|---|---|---|---|
| RA | 551.4 | 18.1 a[1] | 50.6 a | 68.7 a |
| HRA | 553.1 | 11.5 b | 29.8 b | 41.3 b |
| MA-A (2% $CO_2$)[2] | 549.7 | 1.0 c | 11.0 c | 12.0 c |
| MA-B (5% $CO_2$) | 550.8 | 0.9 c | 5.9 c | 6.8 c |
| MA-C (10% $CO_2$) | 551.2 | 0.8 c | 8.2 c | 9.0 c |

[1] Means in the same column with the same letter are not significantly different at the 0.05 level.
[2] Treatment target $CO_2$ level.

losses between the three MA's tested were not significant. However, out of the three storage conditions tested (RA vs. HRA vs. MA) only the spears kept under MA were suitable for the fresh market. At the end of the experiment, the asparagus stored under MA was still firm with good color and overall appearance. Those stored under RA and HRA exhibited various levels of limpness, wrinkling and their tips were usually more opened.

## CONCLUSIONS

In this study, the effects of storage conditions as well as precooling on the keeping quality of green asparagus were investigated and the results led to the following conclusions:

(1) Precooling the asparagus prior to storage reduced the losses by more than 20%; however, no significant differences were observed among the 3 precooling techniques studied.

(2) The use of low temperature, high relative humidity (RH) and modified atmosphere (MA) for storage of domestically produced green asparagus did improve the keeping quality over refrigeration (RA) alone and refrigeration with high relative humidity. MA stored asparagus had better color, firmer texture, fresher appearance and significantly lower mass losses (10% under MA) than those stored under RA with 80% RH (mass loss of 69% and with >95% RH (mass loss of 41%).

(3) The silicone membrane windows utilized to maintain the MA composition were successful in controlling the $CO_2$ levels but unable to provide enough $O_2$ for the product respiration. Regular injections of $O_2$ were required to maintain the concentration around acceptable levels.

(4) It was also observed that the $O_2$ concentration under which the respiration process became anaerobic was around 3%. MA storage of asparagus should therefore be performed in an atmosphere containing no less than 3% $O_2$.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the research grant received from the Natural Science and Engineering Research Council of Canada.

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# THE TEMPERATURE DIFFERENCE BETWEEN A MICROORGANISM AND A LIQUID MEDIUM DURING MICROWAVE HEATING[1]

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Accepted for Publication March 6, 1991

## ABSTRACT

*One current explanation for research findings of enhanced microbial kill under microwave heating is that of selective absorption of microwave energy within the organism. In the present study, the temperature difference between a microorganism and its surrounding liquid medium were studied using well-established heat transfer principles. Results of the analysis show that because of the microscopic size of bacteria, the surface area to volume ratios of these organisms are extremely high, resulting in rapid heat loss to the surrounding environment. Thus the microorganisms have to possess extremely high dielectric loss factors to maintain even small temperature differences. The energy absorption difference required for a 1°C difference between microorganism and medium is presented for a range of conditions.*

## INTRODUCTION

Recent studies (Dreyfuss and Chipley 1980; Khalil and Villota 1988) have reported that microwave heating appears to enhance bacterial kill in comparison to conventional heating. One explanation that has been advanced for such results is that the microorganisms selectively absorb microwave energy, resulting in a bacterial temperature that is higher than that of the surrounding medium. The IFT Expert Panel on Food Safety and Nutrition have referred to this explanation

[1]Salaries and research support provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Approved as Journal Article No. 42-91. References to commercial products and trade names is made with the understanding that no discrimination and no endorsement by The Ohio State University is implied.
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Journal of Food Processing and Preservation **15** (1991) 225–230. *All Rights Reserved.*

in their Scientific Status Summary (IFT 1989), stating in addition, that ". . . it is not possible to prove or disprove such claims, since the internal temperature of a bacterial cell cannot be measured."

Although it is not possible with present day technology to measure temperatures within bacterial cells, it is certainly possible to examine the situation analytically, since the basic physics of heat transfer to microscopic bodies, such as bacteria, are well understood. The thermal behavior of bacteria can easily be analyzed using these principles. It is then possible (using basic information on physical properties and parameters related to biological materials) to gain further insight into the actual temperatures and behavior of bacterial cells under microwave heating. Accordingly, the objective of the present study is to develop simple mathematical relationships for estimation of the temperature difference between a microorganism and the surrounding liquid medium under conditions of microwave heating, to determine those conditions under which the temperature difference may be significant. Notably, the present study is not an attempt to argue for or against the existence of significant temperature differences, but rather to examine the situation in light of the available heat transfer information.

## MATERIALS AND METHODS

It is assumed that the microorganism is of spherical shape, as with cocci, and that it is located within a small sample of liquid within a microwave oven. The liquid sample and the microorganism are assumed to heat at *different but uniform* rates of energy generation. Due to the microscopic size of the organism, a low Biot number situation can be assumed, and a lumped analysis is appropriate. The rate of temperature rise of the organism can then be represented by (see list of symbols for details):

$$\rho_m C_{pm} V_m (dT/dt) = \dot{u}_m V_m - hA_m[T - T_\infty(t)] \quad (1)$$

where $T_\infty$ is the temperature of the liquid medium, which can be determined from the following simple energy analysis, assuming negligible heat losses.

$$\rho_l C_{pl} (dT_\infty/dt) = \dot{u}_l \quad (2)$$

Solving Eq. (2), $T_\infty$ is given by:

$$T_\infty = (\dot{u}_l/\rho_l C_{pl})t + T_i \quad (3)$$

where $T_i$ is the initial temperature of the fluid. Substituting Eq. (3) into Eq. (1), the following relation is obtained.

$$dT/dt = ( \dot{u}_m/\rho_m C_{pm}) - (hA_m/\rho_m C_{pm} V_m)[T - \dot{u}_l t/\rho_l C_{pl}) - T_i] \quad (4)$$

Rearranging terms,

$$dT/dt + (hA_m/\rho_m C_{pm} V_m)T = (\dot{u}_m/\rho_m C_{pm}) + (hA_m/\rho_m C_{pm} V_m)[(\dot{u}_l t/\rho_l C_{pl}) + T_i] \quad (5)$$

Equation (5) is of a form easily solvable by means of integrating factors (Ross 1964). Using the condition that the initial temperature of the microorganism is the same as that of the fluid [i.e., $T(0) = T_i$], the following relation is obtained.

$$T = (\dot{u}_l/\rho_l C_{pl})t + (V_m/hA_m)[\dot{u}_m - \dot{u}_l(\rho_m C_{pm}/\rho_l C_{pl})] + T_i + (V_m/hA_m)[\dot{u}_l(\rho_m C_{pm}/\rho_l C_{pl}) - \dot{u}_m] \exp\{-(hA_m/\rho_m C_{pm} V_m)t\} \quad (6)$$

Using the fact that for a sphere, the ratio, $V_m/A_m = r/3$; and subtracting Eq. (3) from Eq. (6), the temperature difference between the microorganism and the surrounding medium may be obtained as:

$$T - T_\infty = (r/3h)[\dot{u}_m - \dot{u}_l(\rho_m C_{pm}/\rho_l C_{pl})] \{1 - \exp(-3ht/r\rho_m C_{pm})\} \quad (7)$$

The properties of the microorganism, $\rho_m$ and $C_{pm}$, and of the liquid medium, $\rho_l$ and $C_{pl}$, are composition-dependent. The density would be expected to increase with decreasing moisture content (since most biological constituents with the exception of lipids are denser than water), while the specific heat would increase with increasing water content. Whatever the nature of these changes, the ratio $(\rho_m C_{pm}/\rho_l C_{pl})$ can be approximately set equal to unity in Eq. (7), yielding

$$T - T_\infty = (r/3h)[\dot{u}_m - \dot{u}_l]\{1 - \exp(-3ht/r\rho_m C_{pm})\} \quad (8)$$

The value of h depends on the flow conditions about the microorganism, and will be used as a variable in simulation. Using a realistic microorganism diameter of 1 micron, or $10^{-6}$ m (Burdon and Williams 1968), and reasonable values of

$\rho_m$ ($\approx$ 1000 kg/m$^3$) and $C_{pm}$ ($\approx$ 4000 J/kg°C) we can calculate the difference in energy generation rate [$\dot{u}_m - \dot{u}_l$] required to obtain a 1°C difference in temperature between the microorganism and the liquid. Further, if various values of fluid energy generation rate ($\dot{u}_l$) and convective heat transfer coefficient (h) are assumed, it is possible to calculate the ratio of energy generation rates ($\dot{u}_m/\dot{u}_l$) required for a 1°C temperature difference between microbe and medium. Such calculations were conducted for a range of values of $\dot{u}_l$ and h, for various values of elapsed time, t.

## RESULTS AND DISCUSSION

Results of calculations are presented in Table 1. In all cases, time effects resulting from the exponential term in Eq. (8) were found negligible. The calculations show that for most reasonable values of h (100–500 w/m$^2$°C; Alhamdan *et al.* 1990), the energy generation rate within the microorganism must be *from 60 to 1000 times* that of the medium, in order for a 1°C difference to exist. Even for the exceedingly low heat transfer coefficient of 10 w/m$^2$°C, the energy generation ratio must be approximately 7 to 21 for such a difference to exist.

The energy generation rate within a material due to microwave heating has been shown to be proportional to the dielectric loss factor (Goldblith 1967), according to the relation:

$$\dot{u} = \omega \epsilon_o E^2 \kappa'' \tag{9}$$

TABLE 1.
RATIOS OF ENERGY GENERATION RATE ($\dot{u}_m/\dot{u}_l$) REQUIRED TO MAINTAIN A 1°C TEMPERATURE DIFFERENCE BETWEEN A MICROORGANISM AND THE SURROUNDING MEDIUM; AS CALCULATED FOR VARIOUS VALUES OF $\dot{u}_l$ AND CONVECTIVE HEAT TRANSFER COEFFICIENT, h.

| Convective heat transfer coefft, h (w/m$^2$°C) | Energy generation ratio ($\dot{u}_m/\dot{u}_l$) | | |
|---|---|---|---|
| | Rate of energy generation in liquid ($\dot{u}_l$, w/m$^3$) | | |
| | $3 \times 10^6$ | $5 \times 10^6$ | $10^7$ |
| 10 | 21 | 13 | 7 |
| 100 | 201 | 121 | 61 |
| 500 | 1001 | 601 | 301 |

Mudgett (1986) indicates that the dielectric loss factor ($\kappa''$) can be calculated from the volume fractions of the phases of a mixture, with the aqueous phase properties being predictable using the Hasted-Debye models for aqueous ionic solutions, involving a dipole and an ionic loss component. Thus, the energy generation rate from Eq. (9) can be considered directly proportional to the contributions of these two components.

Since microorganisms are composed of materials that are of biological origin, their dielectric properties would be expected to fall within reasonable ranges for biological materials. The above analysis therefore shows that if a microorganism undergoing microwave heating is to maintain even a 1°C temperature difference with the surrounding liquid, its energy generation rate, and consequently its dielectric loss factor has to be many times greater than that of the liquid. While these results do not necessarily prove that temperature differences between microbe and liquid are low, they do indicate the range of dielectric properties that must occur in the internal contents of the microbe for significant temperature differences to exist.

Finally, it may also be noted that the above argument is based on thermal considerations alone, and does not take into account impedance mismatches between the microorganism and its environment, which might result in reflection of waves from the surface, and reduce the energy generation rate within the microorganism. In addition, the size of microorganisms is far smaller than the wavelength of microwaves (ranging from 1 mm to 1 m; corresponding respectively to frequencies of 300000 to 300 MHz), resulting in a low interaction between the microwave field and the microorganism (Ohlsson 1989). These points tend to argue against the existence of high energy generation rates within individual microorganisms.

## CONCLUSIONS

If a microorganism within a liquid medium undergoing microwave heating is to maintain a temperature 1°C higher than the medium, the rate of energy generation within the microorganism would have to be many times higher than that within the liquid. Since the rate of energy generation depends on the dielectric properties of the microbial contents, inpedance matching, and size in relation to microwave wavelength, a number of arguments exist against the possibility of such large energy generation rates within individual microbes.

## LIST OF SYMBOLS

A Surface area ($m^2$)
$C_p$ Specific heat (J/kg°C)
E Electric field strength (volts/m)

h Convective heat transfer coefficient ($w/m^2°C$)
r Radius of microorganism (m)
t Time (s)
T Temperature (°C)
$T_\infty$ Fluid temperature (°C)
$\dot{u}$ Energy generation rate ($w/m^3$)
V Volume ($m^3$)
$\epsilon_o$ Permittivity of free space ($8.854 \times 10^{-12}$ farad/m)
$\kappa''$ Dielectric loss factor of material
$\rho$ Density ($kg/m^3$)
$\omega$ Angular frequency (radians/s)

**Subscripts**

i Initial
l Liquid
m Microorganism
$\infty$ Liquid

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# GUIDE FOR AUTHORS

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

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

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

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

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

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

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

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

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

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

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

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

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

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

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

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JOURNAL OF FOOD PROCESSING AND PRESERVATION VOL. 15, NO. 3

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