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CONTENTS

CHARACTERISTICS OF PURE CULTURE CANOLA SAUCE FERMENTATION

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ABSTRACT

The compositional and organoleptic quality of canola sauce, a condiment similar to soy sauce, was improved by inoculating Pediococcus halophilus, Saccharomyces rouxii *and* Torulopsis versatilis *into canola mash during the moromi stage fermentation. Before inoculation, these microorganisms were cultivated in media containing 18% (w/v NaCl until cell counts of 10^7–10^8 CFU/mL were reached. The three organisms were inoculated either simultaneously or sequentially. Total fermentation time for koji and moromi stages was 31 days. Both sequential and simultaneous inoculations of canola mashes produced sauces which had chemical qualities quite similar to that of Kikkoman soy sauce. Chemical analyses showed the sauces to contain 1.2% (w/v) total soluble nitrogen, 0.4–0.42% (w/v) amino nitrogen, with average nitrogen yield of 67.2%, 1.60–1.88% (w/v) lactic acid, 1.5–1.6% (w/v) ethanol, 0.75–1.56% (w/v) glucose, and 17.46–17.52% NaCl. However, an excessive amount of lactic acid and the absence of some characteristic soy sauce aromatic components gave the canola sauce a distinct "sharp taste" and "raw flavor".*

INTRODUCTION

Canola sauce fermentation, like soy sauce, involves a two-stage process. The first stage involved a 72 h growth of *Aspergillus oryzae* and/or *Aspergillus sojae* on a mixture of a residue of hydrolyzed canola meal and roasted ground wheat, to form the koji. The koji was mixed with the previously extracted enzyme hydrolysate, containing 18% (w/v) NaCl, and was allowed to undergo the second stage (moromi) fermentation (Ma and Ooraikul 1986). Yong and Wood (1976) showed that moromi fermentation was initiated by lactic acid bacteria, which

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created favorable conditions for yeasts to complete the fermentation. *P. halophilus* has been associated with the production of lactic acid (Yokoatuska 1981 and Fukushima 1985). In addition to *S. rouxii,* which is responsible for the principal alcoholic fermentation in soy mash, another yeast, *T. versatilis,* is also involved in moromi fermentation. Establishing itself significantly at the latter stage of the fermentation, *T. versatilis* produces the alkyl phenols and the aromatic alcohols which are characteristic of mature aroma, associated with good quality soy sauce (Yokotsuka 1981, 1985; Fukushima 1985).

The canola sauce developed thus far (Ooraikul *et al.* 1980; Ma and Ooraikul 1986), lacked the aromatic components produced by these microorganisms, since moromi fermentation was only four weeks long and relied entirely on natural inocula. This paper describes further attempts to improve the sauce quality by deliberately inoculating the canola mash with pure cultures to accelerate production of desired aromatic compounds.

MATERIALS AND METHODS

Microorganisms and Culturing Procedures

Stock mold cultures, *A. oryzae* and *A. sojae,* were revitalized and cultured on Potato Dextose Agar (PDA) in roux bottles at 37°C for 48 h.

P. halophilus ATCC 21786 was revitalized in both Sodium Acetate Medium 1 (SAM1) broth and agar at 30°C for 4 days, after which the inoculum was cultured in a similar medium containing 18% (w/v) NaCl at pH 6.9–7.0. The culture was incubated at 30°C for 7 days. Both salt-free and salt-containing inocula were cultured statically. *S. rouxii* ATCC 13356 and *T. versatilis* ATCC 20191 were revitalized in YM broths and agars at 26°C for 4 days. They were then cultured in YM media containing 18% (w/v) NaCl, at pH 4.5 and 30°C, for 4 days in a shaking incubator. The media used were obtained from Difco Laboratories (Detroit, MN), and the formula for the preparation of SAM1 is described in the ATCC catalog (ATCC, Rockville, MD).

Determination of Microbial Growth

P. halophilus, S. rouxii and *T. versatilis,* growing in their respective media containing 18% (w/v) NaCl were enumerated on agar plates with similar media compositions. A 0.1 mL aliquot of serially diluted culture was used for viable cell enumeration. The growth of the organisms in saline media was also recorded by optical density (OD) measurements at 550 nm. A 10% inoculum in 50mL of the appropriate salt medium was used for OD determinations.

The viable cells of the microorganisms in the canola mash during moromi fermentation were enumerated on agar plates containing 0.25% sodium pro-

pionate, which prevented the molds from overgrowing the plates (Yong and Wood 1976). The agar media were prepared with and without NaCl.

Preparation of Koji and Moromi

The procedure for koji preparation was adapted from Ma (1985). A meal to solvent ratio (M/S) of 1:6.3 was used in the prehydrolysis of canola meal with enzyme Alcalase 0.6L (Novo Industri A/S, Dagsvaerd, Denmark), i.e., 15.4 mL Alcalase 0.6L was added to 50 g canola meal and 315 mL water. About 66% of enzyme hydrosylate was pressed out of the hydrolyzed meal and 50 g of roasted ground wheat was added to the residue, bringing the moisture content of the mixture to 40–45%.

About 0.2% (w/w) of a mixture of *A. oryzae* and *A. sojae* was added to the canola-wheat mixture and incubated at 30°C for 72 h. The mixture was stirred 20 and 40 h after incubation. An 18% brine solution, made from 140 mL of the enzyme hydrolysate, was mixed with the 72 h-old koji to produce moromi. The pH of the moromi was adjusted with HCl to about 6.5–7.0 before the start of the second stage fermentation.

Moromi Fermentation

Three sterile 500 mL erlenmeyer flasks, containing the pH-adjusted moromi mash, were aseptically inoculated with 7 mL each of cultures of *P. halophilus* (7 days old), and *S. rouxii* and *T. versatilis* (3 days old). Flask CS1 was first inoculated with *P. halophilus,* incubated at 30°C until pH of the mash dropped to 5.0, then inoculated with *S. rouxii* and *T. versatilis*. Flask CS3 was inoculated with all three cultures from the beginning, while flask CS2 was not inoculated (control). The mash was stirred, and the flasks stoppered with sterile dispo plugs before incubation at 30°C for a total of 31 days. The moromi was stirred before being sampled aseptically, every 2–4 days, throughout the period of fermentation. The samples taken were analyzed for their composition.

At the end of fermentation the raw sauce was pressed out, heated to 80°C and held for 30 min. The heated sauce was cooled to room temperature, centrifuged at 10,000 rpm for 30 min and filtered through Whatman #1 filter paper to obtain a clear and refined canola sauce.

Kikkoman shoyu, a Japanese soy sauce naturally fermented for at least six months, purchased from a local store, was used for quality comparison.

Chemical Analyses

The total soluble nitrogen (TSN), amino nitrogen (AN) and pH of the samples were determined using procedures described by the A.O.A.C. (1980). The total soluble solids (TSS) of the mashes, sampled during the fermentation, were

evaluated with an Abbe refractometer. The glucose, sucrose, lactic acid, ethanol and glycerol contents were determined by a UV method, adapted from Methods of Enzymatic Food Analysis (Boehringer Mannheim 1984). The phenylethanol content of the sauce was analyzed using a slightly modified GC method (Kahn and Conner 1972). The analysis was performed on a Varian Aerograph Model 3700 (Varian Associates, Palo Alto, CA), equipped with a column packed with Tenax-GC (Applied Sci. Labs., Inc., State College, PA), 60–80 mesh, operated at temperatures of 210°C (column), 250°C (injector), and 270°C (detector).

Sensory Evaluation

Semi-trained panelists, consisting of seven Oriental students in the Department of Food Science, University of Alberta, were asked to rate canola sauces, in comparison with Kikkoman sauce, for acceptance on a 9-point hedonic scale. The scores were analyzed using ANOVA and Duncan's Multiple Range Test programs.

RESULTS AND DISCUSSION

Growth of Pure Cultures

The saline adaptation method described by Yong and Wood (1976), whereby the microorganisms were subcultured into media of increasing salinity until they reached desired cell counts, was omitted when it was observed that the salt-free cultures could survive and grow equally well when subcultured directly into media containing 18% NaCl.

Yong and Wood (1976) adjusted the pH of the yeast medium to 4.50 with 1M HCl. However, Yong *et al.* (1978) observed better growth when yeast medium was acidified with lactic acid. In the present experiment, HCl was used since lactic acid content in the final sauce was analyzed to determine the efficiency of *P. halophilus* in the fermentation.

Figure 1 shows the growth of the three salt-tolerant microorganisms enumerated on agar plates. OD could also be used satisfactorily to monitor the growth, since correlation coefficients between the OD's and viable cell counts were 0.83 (df 5), 0.89 (df 4), both significant at $p = 0.05$, and 0.96 (df 9), significant at $p = 0.01$, for *T. versatilis*, *S. rouxii* and *P. halophilus*, respectively. Therefore, for routine propagation of the microorganisms, OD would be a quicker and cheaper method to monitor growth, as compared to enumeration on agar plates.

S. rouxii and *T. versatilis* grew quickly to their maximum counts of 7×10^7 and 1.5×10^8 CFU/mL, respectively, after 3 days in the salt broth (Fig. 1). This corresponded to OD values of 1.50 and 1.78, respectively. Further incubation of the organisms resulted in a slight decrease in the viable cell counts. However, *P. halophilis* exhibited a lag phase with a slight decrease in viable counts during

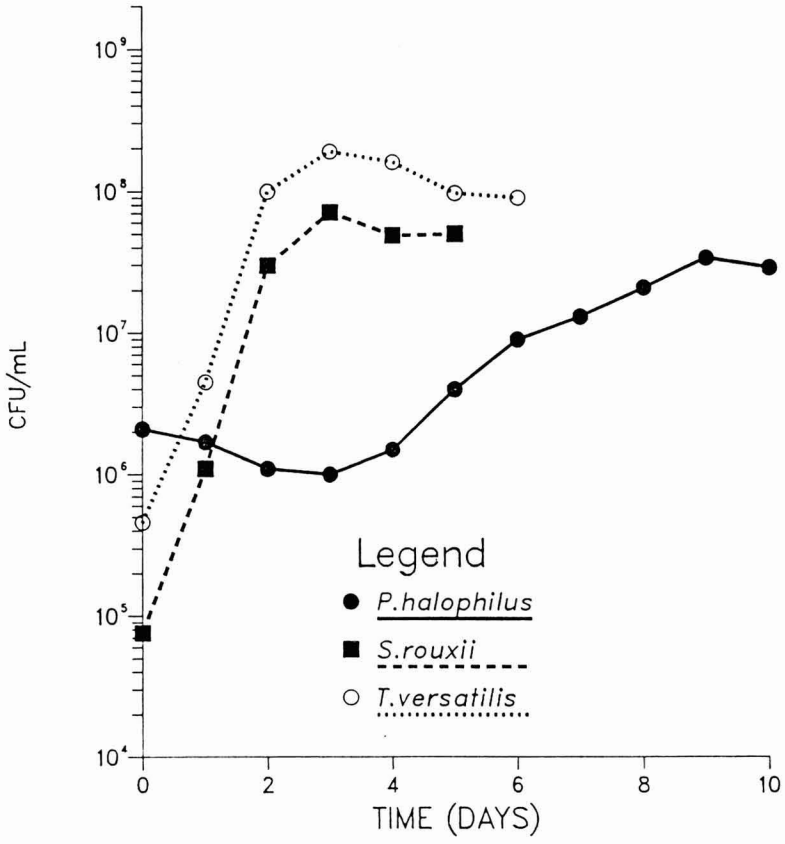

FIG. 1. GROWTH OF SALT-TOLERANT MICROORGANISMS (average of duplicate plate counts)

the first 3 days, and did not reach the maximum count of 3×10^7 CFU/mL (OD 0.33) until 9 days. Therefore, the yeasts were used for moromi inoculation after 3 days growth, and the bacteria after 7 days.

Koji and Moromi Fermentations

Total soluble nitrogen (TSN) has been suggested as an index of soy sauce quality (Hesseltine and Wang 1972). It is important to maximize the yield of TSN during the koji stage, where most of the protein hydrolysis takes place due to the growth of *A. oryzae* and/or *A. sojae*. During the moromi stage, further protein hydrolysis occurs, but the majority of the fermentation products are aroma and taste components. In the present experiment, canola meal was prehydrolyzed with Alcalase 0.6L, using a meal to solvent (M/S) ratio of 1:6.3. This gave a TSN yield of 0.92% (w/v), which was higher than the 0.85% obtained by Ma

and Ooraikul (1986). The meal was further hydrolyzed when it was mixed with ground, roasted wheat and mold cultures, after the hydrolysate had been pressed out, to form koji in the usual manner. After 72 h incubation at 30°C, the koji was remixed with the hydrolysate and salt to produce moromi.

Growth of Microorganisms During Moromi Fermentation

In CS1, where the bacterium and yeasts were inoculated sequentially, *P. halophilus* grew to a maximum viable count of 2.5×10^8 CFU/g in about 5 days, then dropped to about 1×10^7 in 10 days and remained at this level to the end of fermentation (Fig. 2). Yeasts which were inoculated after 4 days, when pH of the moromi had dropped to 5.0, increased to a maximum of 4.5×10^7

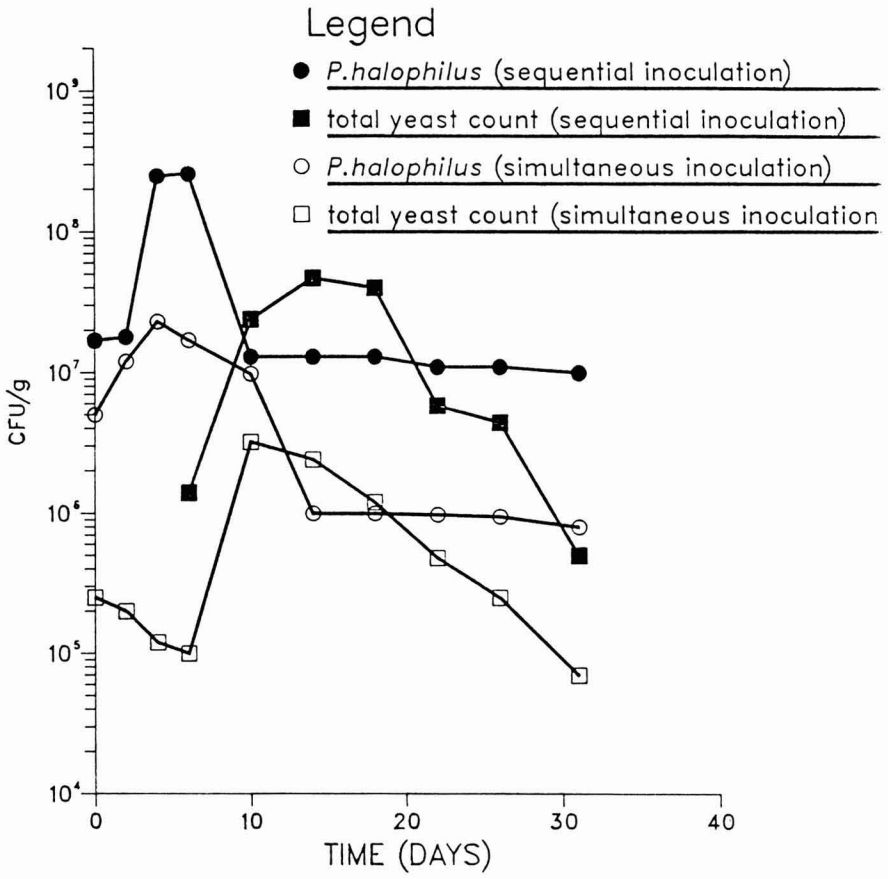

FIG. 2. VIABLE COUNTS OF MICROORGANISMS IN MOROMI (average of duplicate plate counts)

after 14 days, then gradually dropped to 5×10^5 by the 31st day. Microbial growth in CS3, on the other hand, was much lower, with the bacterium growing to a maximum of 2.5×10^7 in 4 days then decreasing to 1×10^6 after 14 days, while the yeasts had an 8 day lag phase before growing to 3×10^6 after 10 days and dropping to 8×10^5 by the end of fermentation. This suggested nutritive competition among the bacteria and yeasts in CS3, where all three organisms were inoculated simultaneously. Yokotsuka (1986) reported that salty conditions, high initial pH and the presence of lactic bacteria also inhibited yeast growth. The yeasts have been shown to grow better in salty medium when the pH is between 4.5–5.0 (Onishi and Shiromaru 1984; Yong and Wood 1976). None of the three microorganisms were detected in CS2, where no inoculation was made.

Total Soluble Solids and pH Changes During Moromi Fermentation

Total soluble solids (TSS) in CS1 and CS3 increased rapidly from 27.5 and 26.3% to 31.8 and 31.2% respectively, in 4–6 days, then decreased slightly for the next 2–4 days before rising again to 34.2 and 35.5%, respectively, by the end of fermentation (Fig. 3). In CS2, TSS increased less rapidly, and without

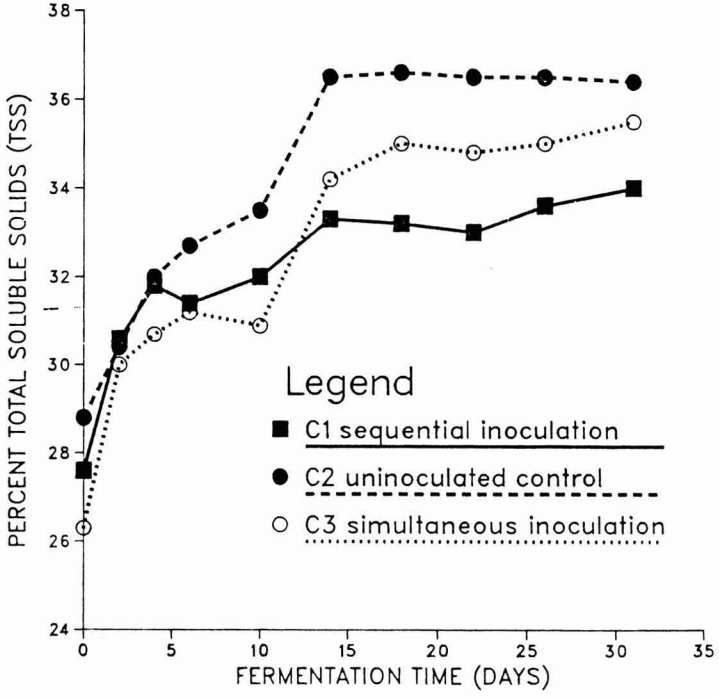

FIG. 3. CHANGES IN TOTAL SOLUBLE SOLIDS IN MOROMI (average of duplicate determinations)

any temporary decrease, to 36.5% after 14 days, where it remained until the end of fermentation. The presence of bacteria and yeasts in CS1 and CS3 caused somewhat faster degradation of substrates. The TSS increase in CS2 indicated the presence of enzymes, and possibly some adventitious microorganisms carried over from the koji stage.

The initial TSS rise in CS1 and CS3 corresponded to a sharp decrease in pH (Fig. 4) as carbohydrates in the mashes were metabolized to lactic acid by *P. halophilus*. Yeasts in CS3 did not contribute significantly to the initial TSS increase since there was virtually no growth in the first 8–10 days (Fig. 2). The second TSS rise in both CS1 and CS3 was due largely to the activity of *S. rouxii* since its vigorous growth around pH 5.0 caused the breakdown of larger sugar molecules into simple sugars and alcohols.

The slight TSS rise in CS1 and CS3 after day 22 (Fig. 3) was attributed to *T. versatilis*, which produced other alcoholic and phenolic compounds. *T. versatilis* has been reported to dominate the latter part of the fermentation after the growth and activity of *S. rouxii* has subsided (Fukushima 1985).

The gradual pH decrease in CS2 from 7.0 to 5.4 (Fig. 4) indicated production of some acids. Even without inoculation, production of some lactic acid was

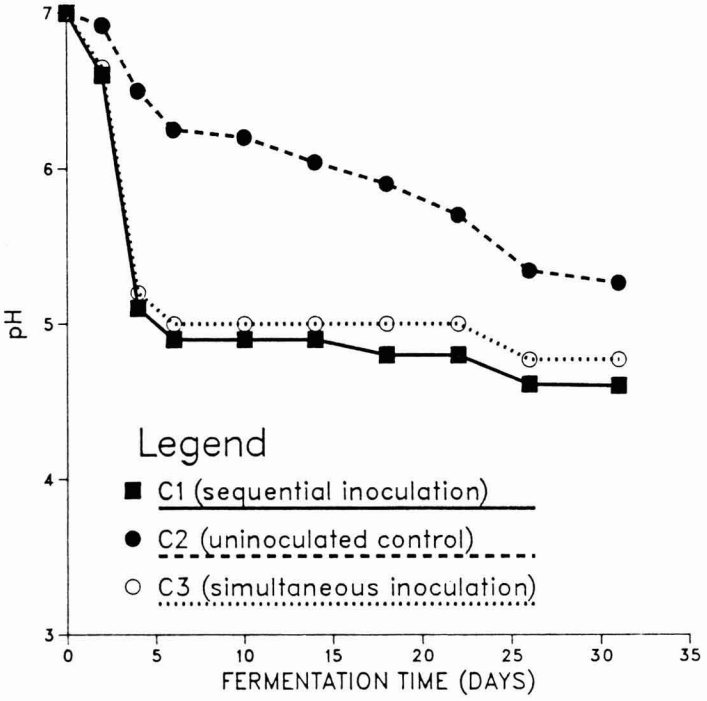

FIG. 4. CHANGES IN pH IN MOROMI (average of duplicate determinations)

detected in CS2. Yong and Wood (1976) also observed a pH decrease in an uninoculated mash, even when koji had been produced under strict aseptic conditions. Therefore, the substrates in such cases must be hydrolyzed, during moromi fermentation, by the molds or enzymes carried over from the koji stage. The presence of *P. halophilus* in CS1 and CS3 not only produced lactic acid more rapidly, and in greater quantities, but also accelerated the pH drop required for yeast fermentation to begin.

Lactic Acid Production During Moromi Fermentation

As much as 2.03 and 1.85% (w/v) lactic acid was produced after 31 days in CS1 and CS3, respectively. However, after refining, lactic acid in the sauces was reduced to 1.6–1.8% (w/v), which was still almost four-times more than the amount found in Kikkoman sauce (Table 1). The greater amount of acid in CS1 and CS3 may have accounted for the perception of sharp taste by the panelists in sensory evaluation. Inoculation of a smaller quantity of *P. halophilus* inoculum into the moromi may alleviate this problem.

Production of Alcohols During Moromi Fermentation

S. rouxii produced ethanol and glycerol during brine fermentation. Wood (1982) observed that the typical soy sauce aroma did not develop without alcoholic fermentation. Most of the ethanol production in CS1 and CS3 coincided with the period when the pH of the mashes was reduced to 4.6–5.0. The final ethanol contents of the refined sauces of 1.50–1.60% (w/v) compared favorably with the amount found in Kikkoman sauce (Table 1).

Glycerol, a by-product of yeast fermentation and also a hydrolytic product of the lipids in the mash (Yokotsuka 1960), was found in all sauce samples. The concentrations of 0.53 and 0.51% (w/v) in CS1 and CS3, respectively, were

TABLE 1.
LACTIC ACID[a] (% W/V), ETHANOL[a] (% W/V), GLYCEROL[a] (% W/V) AND 2-PHENYLETHANOL[b] (PPM) CONTENTS IN REFINED CANOLA AND KIKKOMAN SAUCES.

Samples	Lactic Acid	Ethanol	Glycerol	Phenylethanol
CS1	1.88	1.60	0.53	56
CS2	0.67	-	0.38	32
CS3	1.60	1.50	0.51	48
KS	0.50	1.62	1.59	156

[a]: Average of two determinations.
[b]: Average of four determinations.
-: Not detected.

considerably higher than the 0.38% in CS2 (Table 1), indicating a substantial contribution of yeast to glycerol production. The low glycerol contents in canola sauces were comparable to the 0.4–0.5% (w/v) reported by Yokotsuka (1960) for soy sauces produced from defatted soybean meal. The 1.5% (w/v) glycerol in Kikkoman sauce was suggestive of either whole soybeans, large yeast inoculum or a mixture of whole and defatted soybeans being used to produce the sauce.

The aroma constituent, phenethyl alcohol (2-phenylethanol or PEA) was detected in all the sauce samples (Table 1). As indicated previously, PEA, alkyl phenols and other aromatic alcohols were produced by *T. versatilis*, which dominated the latter part of moromi fermentation. The small quantity present in CS2 implied that some production of PEA was possible without the yeast. The concentrations of PEA in the canola sauces were about three-times lower than that detected in Kikkoman sauce, which may partly explain the lower sensory scores given to the canola sauces. Longer fermentation time or a greater quantity of *T. versatilis* inoculum used may improve this quality parameter.

Other Quality Parameters

The total soluble nitrogen (TSN) in CS1 and CS3 was 1.20% (w/v). This was slightly lower than the 1.33% (w/v) of Kikkoman sauce. The amino nitrogen (AN) of 0.40–0.42% (w/v), nitrogen yield of 67.2–67.8%, and AN/TSN ratio of 0.33–0.35, all indices for assessment of sauce quality (Hesseltine and Wang 1972), were lower than those in the commercial sauce, which were 0.70%, 73.7% and 0.53, respectively. The glucose content in CS1 of 0.70% (w/v), was lower than the 1.17% (w/v) found in the commercial sauce, and 1.56% (w/v) in CS3. The sucrose contents of 0.13% and 0.20% (w/v) in CS1 and CS3, respectively, were lower than the 0.39% (w/v) found in the commercial sauce. These indicated inadequate protein hydrolysis, and excessive sugar fermentation in canola sauces, especially in CS1.

Amino acid concentrations in canola sauces were generally lower than those in Kikkoman sauce, though some, e.g., glutamic acid, alanine and tyrosine, were considerably higher. The organic acid profile of the canola sauces was similar to that of Kikkoman sauce, but individual concentrations were generally higher in the former. The organic acids detected included acetic, citric, formic, lactic, malic, propionic, pyroglutamic, pyruvic and succinic acids.

The salt contents of 17.29–17.52% (NaCl) of canola sauces were comparable to the 17.64% of the commercial sauce. Also, the color of the canola sauces was similar to that of Kikkoman sauce, except the latter had a reddish tinge, whereas canola sauces were yellowish.

A semi-trained sensory panel gave average overall acceptance scores of 5.3, 5.9, 6.0 and 7.6 to CS1, CS2, CS3 and Kikkoman sauce, respectively. Though the scores are not statistically different from one another, the Japanese sauce

was consistently rated higher than the canola sauces. Among the canola sauces, CS3, where all three microbial cultures were inoculated simultaneously into the moromi, was most preferred. Panelists' remarks revealed that canola sauces were rated lower than Kikkoman sauce mainly because of their excessive acidity, giving the sauces a "sharp taste". A "raw" flavor in canola sauces, which may be due to inadequate heat treatment and deficiency in some aromatic components, was also mentioned.

Duplicate batches of canola sauce showed the major characteristics such as pH, color, TSS and alcohol contents to be similar, within experimental errors, to those in the first batches. Therefore, detailed chemical and sensory analyses were not performed on these samples.

This study has shown that it is possible to produce canola sauce of acceptable quality with the introduction of appropriate microorganisms during moromi stage fermentation. However, it is very important to use optimum quantities of the inocula at appropriate times in the fermentation to produce optimum concentrations of the desirable sensory components. Acidity, especially lactic acid content, in canola sauce must be reduced by limiting the extent of lactic fermentation. More vigorous yeast fermentation, especially by *T. versatilis,* must be promoted to increase the contents of alkyl phenols and alcohols such as PEA. Simultaneous inoculation of *P. halophilus, S. rouxii* and *T. versatilis* is recommended, but the quantity of the bacterial inoculum must be reduced and that of the yeasts increased.

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BIOLOGICAL EVALUATION OF A HEAT TRANSFER SIMULATION FOR STERILIZING LOW-ACID LARGE PARTICULATE FOODS FOR ASEPTIC PACKAGING

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ABSTRACT

A biological procedure was devised to evaluate de Ruyter and Brunet's heat transfer computer simulation for sterilizing particulate foods in a swept surface heat exchanger (SSHE). Turkey cubes measuring 12.7 mm were inoculated with spores of Clostridium sporogenes *PA3679, using a so-called "knotted string" method. Inoculated cubes were introduced into a viscous starch medium and thermally processed in a SSHE to a fractional survivor endpoint. Processed product was packaged aseptically, incubated and examined for survivors. Biological sterilization values calculated from the inoculated packs support the validity of the simulation. Also, a novel method was devised to measure particle residence times in hold tubes using electro-magnetic induction.*

INTRODUCTION

Thermal sterilization processes for low-acid foods for conventional static retorts are ordinarily derived by heat transfer simulation in those instances where reliable heat penetration data are available. Under those circumstances, simulation methodology is well defined and accepted throughout the canning industry (NCA 1968). However, simulation of thermal processes for large particulate foods for aseptic packaging has received little attention.

de Ruyter and Brunet (1973) proposed a simulation for sterilizing large particulates in a swept surface heat exchanger (SSHE). A computer program was written to handle the complex calculations. At about that same time, Manson and Cullen (1974) also developed a mathematical model for sterilizing large particulates in a SSHE by assuming an infinite heat transfer coefficient between the liquid medium and the surface of the particles. By using Stumbo's integrated sterilization concept, the latter authors showed the importance of particle residence time distributions on process lethality.

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Dial (1985) advocated use of Ball's Formula Method (Ball and Olson 1957) to calculate sterilization processes for large particulates for aseptic packaging by considering only the lethality in the hold tube of a SSHE system. This approach is interesting because of its simplicity, but it does not allow calculation of lethality in the heating and cooling zones of the system.

Sastry (1986) used finite element analysis for evaluating thermal sterilization processes for large particulate foods for aseptic packaging. By calculating temperature distributions within large particles, his simulation showed that particle size, residence time distributions within the heat exchanger and the hold tube and convective heat transfer coefficients between fluid and particle were critical processing factors.

Evaluating the published reports, the de Ruyter and Brunet simulation for sterlizing large particulate foods in a SSHE for aseptic packaging seemed to represent a rational mathematical approach, and one based on sound and well established thermal processing principles. The de Ruyter and Brunet simulation permits the use of varying input parameters such as steam temperature, steam flow and product flow to obtain a given process lethality. The original program was modified by us to evaluate the lethality achieved for a given set of input parameters. A user interface for input data was also created to facilitate the entry of processing information into the modified de Ruyter and Brunet program.

The primary objective of the study described herein was to determine the validity of the de Ruyter and Brunet heat transfer simulation for calculating sterilization processes for large particulate foods for SSHE sterilizers. We believe that the only conclusive way of determining the validity of a simulation was to undertake a microbiological or inoculated pack study. Inoculated packs have often been used with conventional low-acid canned foods for process verification in instances of questionable heat penetration data. Some modifications in the usual inoculated pack protocol were devised to accommodate the use of a commercial size SSHE.

MATERIALS AND METHODS

Preparation and Standardization of Spore Suspension

A stock spore suspension of *Clostridium sporogenes* PA3679 was prepared using Egg Meat Medium (Difco Laboratories, Detroit, Michigan) as the sporulation medium. Four 200 mL capacity screwcap bottles, each containing 150 mL distilled water and 10 g EMM were prepared and sterilized at 121°C for 15 min. After cooling, each bottle of medium was inoculated with a recently sporulated PA3679 culture. The inoculum was preheated for 15 min in flowing steam.

Inoculated bottles were incubated at 30°C, and sporulation was followed by microscopic examination.

Peak sporulation was reached after about 72 h. The spore crop was harvested using appropriate aseptic techniques. The liquid portion of the culture was filtered through sterilized cheese cloth and nonabsorbent cotton filters. The final suspension was stored unwashed over glass beads at about 1°C.

A refractile spore count of the stock spore suspension was made with a bacterial counting chamber. A viable heated count was made at dilutions predicated from the refractile count. The viable count was based on heating the final dilutions in flowing steam for 15 min and on using a deep-tube counting procedure with 10 replicates per dilution. The counting medium consisted of 5% Trypticase (Baltimore Biological Laboratory, Baltimore, Maryland), 0.5% Peptone (Difco) and 1.5% Noble Agar (Difco) as adapted from Wagenaar and Dack (1958). Colony counts were made after approximately 40 h of incubation at 30°C.

The counting medium was prepared and dispensed in 200 mL quantities into 250 mL capacity screwcap bottles. The medium was sterilized at 121°C for 15 min. Heat sterilized sodium thioglycollate (20%) and filter sterilized sodium bicarbonate (10%) were pipetted into the melted medium just before use to give respective concentrations of 0.1% and 0.14%.

Particulate Product for Inoculated Packs

Packs were conducted using 12.7 mm (½ in.) turkey cubes in a starch gravy. Cubes were made from mechanically deboned turkey without added sodium nitrite. The product consisted of turkey, water, salt, sodium phosphate, brown sugar and flavoring. The cubes were cut to size, individually quick frozen and placed in 9.1 kg plastic bags in corrugated cartons by the supplier for shipment to us. The product was held frozen until used.

The cubes were quite uniform in size and showed little distortion during thermal processing. From 570 to 730 kg of turkey and gravy were prepared for each inoculated pack. The product had the following composition: 69.5% tap water, 25.3% turkey cubes (Land 'O Frost, Lansing, IL), 5.1% starch (Rezista, A. E. Staley, Decatur, IL) and 0.1% sucrose. The pH of each formulated batch was about 6.8.

Gravy was made by the addition of the starch and sugar to cold tap water in a 750 l steam-jacketed mixing tank. The mixture was heated to about 82°C with continuous agitation. Frozen turkey cubes were added to the heated gravy and the product temperature was brought back to 82°C. Product viscosity was adjusted as close as possible to 8,000 centipoise by minor additions of either water or a concentrated starch slurry as required. Viscosity was measured at 65°C with a Brookfield Dial Instrument (Brookfield, Stoughton, MA) equipped with a No. 3 spindle and set at 12 rpm.

Inoculation of Turkey Cubes

Exactly 200 turkey cubes were inoculated with spores of PA 3679 for each pack. A so-called "knotted string" method was employed. The string method of inoculation provided a means of placing the inoculum as near as possible to the geometric center of each cube and of identifying inoculated from uninoculated cubes. Strings were prepared from six-strand Floss (J. and P. Coats) in lengths of about 18 cm. Each string was knotted about one-third of its length from one end of the string.

Three duplicate rows of double faced tape 12.7 mm were attached to a small piece of plywood. Duplicate rows of tape were spaced 5 cm apart; they were 60 cm long. Two parallel ink lines exactly 6.35 mm apart were drawn on the plywood between each duplicate row of tape for the inoculation of 12.7 mm turkey cubes. The knotted strings were stretched between the tape so that the knot from the short end of the string was directly over the first ink line. Then, each string was inoculated with 0.01 mL (about 10^5 spores) at a position on the string as close as possible over the second ink line from the knot.

A one mL pipette (Stepper, Tridak Division, Indicon, Brookfield Center, CT) equipped with a 25.4 mm, 25-gauge cannula was used to inoculate the strings. Each set of inoculated strings was allowed to air dry for about 2 h before being threaded through the turkey cubes. Thin diameter eye needles were used to thread the strings through the center of each cube. Each string was drawn through the cube and secured by tying a square knot. The free ends of each string were cut off and discarded. Care was taken during inoculation to keep the cubes at 0°C (wet ice temperature). Before starting the packs, viable heated counts were made on three inoculated strings using the deep-tube counting procedure and the counting medium described above. After drying, each inoculated string was placed in a sterile chilled blender jar along with 100 mL of sterile chilled distilled water and blended for 2 min at high speed. Ten mL of blended diluent were transferred to a sterile 15×125 mm screwcap tube and heated for 15 min in flowing steam. Based on a heated viable count, each string should have had a theoretical count of 1.2×10^5 spores of PA3679 per string. The average count of the three inoculated strings equaled 1.4×10^5, thus there was no significant change in count during drying of the spores on the strings.

Aseptic Packaging Line and Pack Protocol

The aseptic packaging line for large particles consisted of a 750 l stainless steel mixing tank for preparing the turkey and gravy, a Waukesha positive displacement pump, a Marlen pump (Marlen Research Corp., Overland Park, KS, Model 629), a commercial size Contherm (Alfa-Laval) horizontal SSHE system having three separate heating sections, a hold tube, and five separate cooling sections, a Waukesha aseptic positive displacement pump, and stainless

steel piping to convey sterilized product either to the aseptic packaging machine or divert it back to the mixing tank. A Conoffast (Continental Can Company, Norwalk, CT) machine capable of handling up to about 20 mm size particles aseptically was used to package the turkey cubes and gravy product. At start-up, the "sterile side" of the line was sterilized by recirculating water at 121°C for at least 30 min. The aseptic packaging machine was sterilized by dry heat from a predetermined computer controlled sterilization program.

In preparation for each inoculated pack, about 50 L of turkey and gravy from the mixing tank were distributed between three plastic pails. The inoculated cubes were divided about equally between the pails of product and mixed into the product with a stainless steel paddle. The pails were covered with plastic covers to minimize product heat loss, however, there was only a short delay between adding the inoculated particles to the pails of product and dumping the product into the hopper of the Marlen pump.

Nineteen thermocouples were located in the product sterilization line to monitor temperatures of the liquid carrier medium during heating and cooling, and steam, condensate, and cooling water temperatures throughout the SSHE sections. The Type J thermocouple probes were made of stainless steel. Each thermocouple probe was prechecked for accuracy against a mercury thermometer (Palmer Instruments, Inc., Cincinnati, OH) certified for accuracy by the manufacturer. The thermocouples were connected to a Kaye Digi III Recorder (Kaye Instruments, New Bedford, MA). An RTD was located at the inlet of the hold tube. The RTD was wired directly to the Taylor Instrument Control panel and served as a temperature recorder-controller for the third heater with the system operating in the "automatic" mode. The RTD was also monitored by the Digi Strip recorder.

Temperature readings from the recorder were transferred and stored in either a Texas Instruments Portable Memory Terminal, Silent 700 or a Hewlett-Packard Portable Plus. At the completion of a product run, the stored time-temperature data were transferred into a Hewlett-Packard 1000 Mini-Computer for analysis.

Product processing temperature was allowed to stabilize in the SSHE system over a period of about 30–45 min before the inoculated turkey cubes were incorporated into the product. Once product temperature had stabilized, the inoculated particles were introduced into the hopper of the Marlen pump. Just before this, product flow from the mixing tank to the Marlen was momentarily stopped so that only enough product was present in the hopper of the Marlen to just cover the piston chambers. The details of the Marlen pump are described by Wagner (1984). Immediately after adding the inoculated particles, uninoculated product in the mixing tank was restarted to follow the inoculated particles through the line.

The aseptic positive displacement pump on the "sterile side" served to control product back pressure 5.6 kg/cm^2 (about 80 psi) for thermal processing and to

meter the processed and cooled product to the aseptic particulate filler. Processed product was filled into 170 g (6-oz) cups which were heat-sealed and separated into two-pack units. The filled cups were packed as they were run in prenumbered corrugated cartons. The cups were incubated at 35°C. Examinations for swells were made at frequent intervals during incubation. Swells were removed from the incubator as they were detected and refrigerated. Incubation of the nonswelled cups was continued for at least one month after recording the last swelled cup. Swelled and nonswelled cups were opened and examined for the presence of inoculated cubes at the end of incubation.

Thermal Resistance of Spores in Buffer and Product

A standardized PA3679 aqueous spore suspension was prepared to contain 10^7 spores per mL. Thermal resistance of spores of the standardized suspension was determined in neutral 0.067 M phosphate buffer before the inoculated packs were started. Ten-tube replicate sets were prepared for each selected heating time. Each tube (16 × 125 mm) was inoculated with 0.1 mL standardized suspension plus 0.9 mL buffer. Survivors after heating were determined with Pork-Pea Medium (Andersen 1951) to which sodium thiogylcollate and sodium bicarbonate were added at respective concentrations of 0.10% and 0.14%. Each set of tubes was vaspar sealed, incubated at 35°C and examined for growth (turbidity and gas) over a period of 3 months.

Thermal resistance of spores of the standardized suspension was determined in finely ground turkey and gravy product after each inoculated pack. Conventional TDT cans (American National Can Company, Barrington, IL) were filled with 17.0 g/can. Ten-can replicate sets were prepared and inoculated for each selected heating time. Survivors were determined by direct product incubation at 35°C. Examinations for growth (swells) were made over a period of one month after recording the last swelled can.

Analysis of TDT and inoculated pack data

D-values (time in min to reduce a spore population by 90%) were calculated from fractional survivor endpoint data for spores heated in phosphate buffer and ground turkey in gravy. D-value (t/LOG "M"-LOG "S") was calculated for each fractional survivor endpoint (Stumbo 1965), where "M" is the total spore load per replicate sample times the number of replicates run. Term "S" is the number of replicates exhibiting growth at each survivor endpoint. At a fractional survivor endpoint, each positive replicate is presumed to have originated from a single surviving spore. Term "t" is the corrected heating time in min. Heating time was corrected for lethality of the come-up time or that time for product to reach retort temperature. The lag correction factor for phosphate buffer in 16 ×

125 mm screwcap tubes was 1.4 min; the corresponding factor for the ground turkey and gravy product in TDT cans was 2.4 min. Inoculated pack results were evaluated in terms of a biological sterilization value [Bio-F_o = D (LOG "M"-LOG "S")].

Theoretical Residence Times of Product in Hold Tubes

The hold tubes of our SSHE system consisted of stainless steel pipe with an internal diameter of 4.7 cm. In this discussion, the hold tubes are designated as either "short" or "long". The "short" hold tube had a length of 6.9 m as measured along the center axis of the tube. The "long" hold tube could be divided into 5 individual and equal sections with an overall length of 58.2 m. The hold tubes were insulated with 3.8 cm of dense fiber glass to minimize radiant product heat loss.

The theoretical average residence time in seconds was calculated by dividing holding tube volume by flow rate. The theoretical hold time for turbulent flow was calculated by multiplying the average flow time by 0.83 and for laminar flow by multiplying the average flow time by 0.5 (Gavin 1985). Thus, for the turkey and gravy product at a flow rate of 22.7 L/min, a hold tube diameter of 4.7 cm and a length of 6.9 m, the theoretical average hold time was 32.2 s. On this basis, the theoretical hold times for turbulent flow and for laminar flow were 26.8 and 16.1 s, respectively.

Particle Residence Time Measurements

To measure particle residence time distributions, a copper coil of about 80 turns was wound around the entrance and the exit end of the hold tube. The coils were wired to a small amplifier and then connected to a double pen potentiometer (Oscillographic Recorder, Model 7402A, Hewlett-Packard). Small magnets (about 3mm × 3 mm × 6 mm or smaller) were centered in turkey cubes. The magnets were made from 3 mm thick flexible magnetic fabric (Industrial Magnets, Boyne City, MI). Hence, there was little chance of damaging the rotors of the positive displacement pumps. Also, the small magnets did not markedly increase the weight of the turkey cubes. Based on 10 randomly selected turkey cubes, the average weight was 2.30 g with a standard deviation of 0.24 g. The magnets imbedded in the turkey cubes were no heavier than 0.2 g. Magnets as small as 0.1 g were used successfully.

As a magnetic particle was introduced into the starch gravy medium and as it passed through the first copper coil around the hold tube, the pen recorder detected a change in electromotive force. A similar response was detected as the particle passed through the second coil. The elapsed time between the two

blips on the recording chart of the potentiometer was timed with a stopwatch as particle residence time in the hold tube.

By circumventing the magnetic SSHE units, cubes with magnets were introduced into the starch medium and pumped through either the "short" or "long" hold tube section by the nonaseptic positive displacement pump. Normal back pressure in the hold tube was maintained by pumping against the aseptic positive displacement pump. The medium was recirculated back to the steam jacketed mixing tank where product temperature was held at about 70°C. Each particle was collected in a small strainer and recirculated unless the particle had broken-up which was uncommon. Over the course of several hours of running, the viscosity of the starch medium did not change appreciably. Particle residence time distributions as affected by hold tube length and product flow rate were determined in this manner.

TABLE 1.
HEAT RESISTANCE OF SPORES OF PA3679 SUSPENSION IN 0.067 M PHOSPHATE BUFFER, pH 7.0 AT 121°C (250°F)

Heating Time (min) [1]	Fractional Survivor Endpoint [2]	D-value [3]
10.0	10/10	--
11.0	3/10	1.67
12.0	1/10	1.69
13.0	0/10	
14.0	0/10	
	AVG	1.7

[1]Lag correction factor for lethality of retort come-up time = 1.4 min.

[2]Number of replicate tubes showing growth/number of replicates run.

[3]Time in min. to reduce the inoculum level tenfold. Results are based on the inoculum level of 1.2×10^6 viable spores/replicate.

RESULTS AND DISCUSSION

Inoculated Packs

Prior to starting the inoculated packs, the heat resistance of spores of the PA3679 suspension to be used was determined in neutral 0.067M phosphate buffer at 121°C (250°F). Parenthetically, all temperature references in this section will be cited in degrees Fahrenheit, since this format is conventionally used in thermal process calculations. As shown in Table 1, the average D-value of spores in buffer equaled 1.7 min. This value compares closely to those of similar spore suspensions of PA3679 which have been prepared in our laboratory.

Dimensions and operating characteristics of the SSHE used in the inoculated packs are summarized in Table 2. Mutator rotor speeds were set to minimize

TABLE 2.
DIMENSIONS AND OPERATING CHARACTERISTICS OF SSHE FOR INOCULATED PACKS

Parameter	Simulation Input Value
A. SSHE Units	
. No. Heating Units	3
. No. Cooling Units	5
. Diameter of Units:	
I.D.	6.075 in (15.4 cm)
O.D.	6.485 in (16.5 cm)
. Length	6.5 ft (2.0 m)
. Mutator Shaft Diameter	2.990 in (7.6 cm)
. No. Scrapper Blades/Unit	6 (2 Rows of 3)
. Mutator Speed:	
Heaters 1 and 2	125 to 220 rpm
Heater 3	150 rpm
Coolers	150 rpm
. Heat Transfer Coefficient	
Heater	210 BTU/(hr ft.^{2} ^{o}F)
Cooler	130 BTU/(hr ft.^{2} ^{o}F)
B. Hold Tube Sections	
. Diameter I/S	1.86 in (4.7 cm)
. Length (Center line)	
"Short" Section	22.8 ft (6.9 m)
"Long" Section	191.1 ft (58.2 m)
. Heat Transfer Coefficient	1.2 BTU/(hr /ft ^{2} ^{o}F)
C. Product Flow Rate (Marlen Pump)	6.0 gal/min (22.7 l/min)
D. Refrigerant Flow (Water)	2000 lb/hr (4,400 kg/hr)

product burn-on on the walls of the SSHE heating units and yet avoid extensive particle damage which would likely occur at high speeds (> 300 rpm). Product flow rate was adjusted according to the filling speed of the Conoffast filler. Flow rate was about 7% greater than the maximal filling speed of the filler. Thus, inoculated particles were expected to by-pass the filler to some extent. Also, unscheduled stoppage of the packaging machine for whatever reason during the early part of the pack obviously increased the number of inoculated particles that by-passed the filler.

Table 3 summarizes the characteristics of the starch carrier medium and the turkey cubes used in the packs and simulation calculations. The de Ruyter and Brunet heat transfer simulation applies to spherical particles. Particle size input values into the simulation are based on the radius of the largest particle. Because the heating characteristics of a sphere of 12.7 mm size and a cube of 12.7 mm size would be somewhat different, the diagonal dimension of the cube was used for the input value. Thus, for a ½ in. turkey cube (12.7 mm), the actual input value was 0.354 in (9 mm) rather than 0.25 in (6.4 mm). This represents a very conservative approach for handling cube shaped particles in the de Ruyter and Brunet heat transfer simulation model.

The heat resistance of spores of PA3679 in the turkey and starch gravy was determined after each inoculated pack. Table 4 presents D-values of spores in product from one pack at heating temperatures ranging from 113° (235°F) to

TABLE 3.
CHARACTERISTICS OF TURKEY CUBES AND LIQUID STARCH CARRIER MEDIUM FOR THE INOCULATED PACKS

Parameter	Simulation input value
A. Carrier Medium Phase	
. Density	63.7 lb/ft^3 (1.022 g/cm^3)
. Specific Heat	0.9 BTU/(lb, °F)
. Initial Temperature	About 65.6°C (150°F)
. Viscosity	8,000 centipoise
B. Particle Phase	
. Density	65.5 lb/ft^3 (1.050 g/cm^3)
. Specific Heat	0.75 BTU/(lb, °F)
. Initial Temperature	About 65.6°C (150°F)
. Thermal Conductivity	0.23 BTU/(hr ft °F)
. Particle Dimension	0.354 in (12.7 mm cube)
. Final Center Temperature	About 35°C (95°F)

TABLE 4.
HEAT RESISTANCE OF SPORES OF PA3679 IN ONE LOT OF TURKEY AND GRAVY

Heating Temp. °C[1]	Heating Time, min[2]	Fractional Survivor Endpoint[3]		D-Value[4]
12.1	4.0	10/10		
(250)	5.0	8/10		0.81
	6.0	2/10		0.88
	7.0	2/10		1.03
	8.0	0/10		
			AVG	0.91
118	12.0	10/10		
(245)	13.0	7/10		2.08
	14.0	4/10		2.16
	15.0	0/10		
			AVG	2.1
116	26.0	10/10		
(240)	28.0	6/10		4.44
	30.0	5/10		4.70
	32.0	0/10		
			AVG	4.6
113	50.0	10/10		
(235)	55.0	8/10		8.91
	60.0	6/10		9.52
	65.0	0/10		
			AVG	9.2

[1]Values in parenthesis are °F; these represent the actual temperatures run.

[2]Corrected heating time; lag correction factor = 2.4 min.

[3]No. TDT cans showing survivors/No. of cans inoculated

[4]Based on an inoculum level of 1.2×10^6 spores per container and on incubation at 35°C for at least one month after the appearance of the last swell.

121°C (250°F). A plot of these data on semi-logarithmic graph paper gives a thermal resistance curve with a slope or z-value equal to 8.5°C (15.2°F).

Results of three repetitive inoculated packs with the "short" hold tube and those of one pack with the "long" hold tube section of the SSHE are given in Table 5. Product processing temperatures were controlled ± 0.3°C (± 0.5°F) of set temperature. Figure 1 illustrates product temperatures at the beginning of the hold tube (end of the third heater) and at the end of the hold tube (beginning of the first cooling section) from run number one. As shown in Table 5, good agreement was obtained between Bio-F_o values from the four inoculated packs.

TABLE 5.
INOCULATED PACK DATA FOR 12.7 mm TURKEY CUBES IN GRAVY PROCESSED IN "SHORT" AND "LONG" HOLD TUBES

Parameter		Observed or computed values			
		"Short" Hold Tube			"Long" Hold Tube
		Run 1	Run 2	Run 3	Run 4
A.	Starch Medium, °C (°F)				
	Initial Temp	66 (150)	66 (150)	69 (156)	66 (150)
	End, Heater 1	105 (221)	109 (228)	102 (216)	102 (215)
	End, Heater 2	129 (265)	128 (263)	128 (263)	113 (236)
	End, Heater 3	133 (273)	134 (273)	134 (273)	124 (255)
	End, Hold Tube	133 (272)	134 (273)	133 (272)	122 (253)
B.	Hold Time (Sec)				
	Average Flow	32.2	32.2	32.2	270
	Turbulent Flow	26.8	26.8	26.8	225
	Laminar Flow	16.1	16.1	16.1	135
C.	Flat Cups with "Strings"	107	83	60	11
D.	Swelled Cups,[1]				
	Total Swells	28	5	12	140
	Swells with "Strings"	21	5	3	118
E.	"Strings" at Strainer[2]	41	63	77[3]	37
F.	Lost "Strings"[4]	31	49	60	34
G.	Log "M"[5]	7.253	7.090	6.945	7.110
H.	Log "S"	1.447	0.699	1.079	2.146
I.	D_{250}	0.52	0.48	0.68	0.87
J.	Bio-F_o	3.0	3.1	4.0	4.3

[1]Containers showing growth of putrefactive anaerobe.

[2]Particles that by-passed the filler were collected in a large strainer.

[3]Temporary lid sealing problem at aseptic packaging machine.

[4]Inoculated particles which were not recovered.

[5]No. "Strings" in flat cups plus No. "Strings" in swelled cups times inoculum per string. For runs 1 - 3, inoculum = 1.4×10^5/string; for run 4, inoculum = 1.0×10^5/string.

Inoculated pack experience has shown that within this lethality range a Bio-F_o value may deviate at least one unit from the F_o determined from heat penetration data. The fact that the number of swells in Table 5 usually exceeded the number

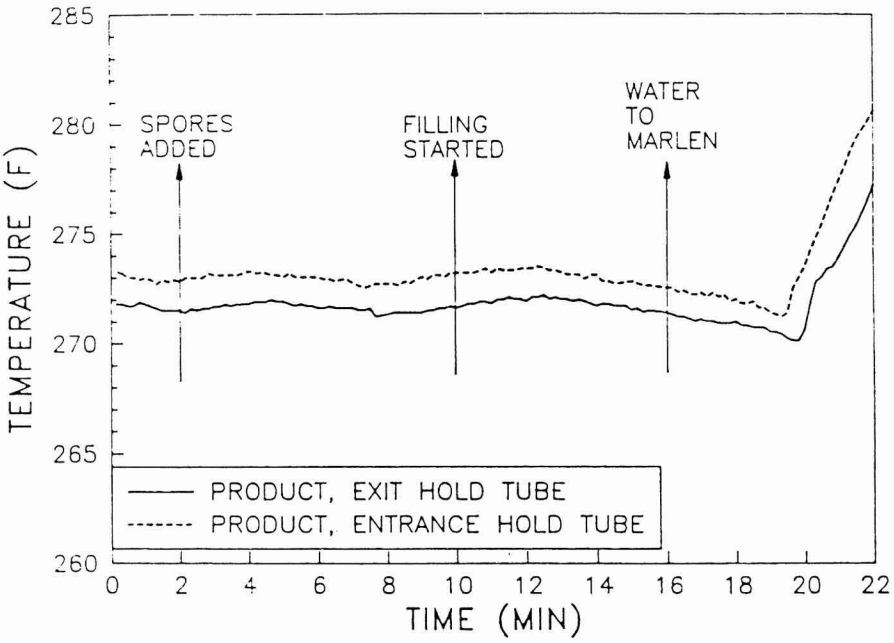

FIG. 1. CARRIER MEDIUM TEMPERATURE PROFILES AT ENTRANCE AND EXIT ENDS OF "SHORT" HOLD TUBE FOR INOCULATED PACK NUMBER ONE

of containers with inoculated particles was attributed to occasional break-up of inoculated particle(s) during the early part of the cooling phase.

Table 6 shows individual F_o values computed from the de Ruyter and Brunet simulation for the 12.7 mm turkey cubes and gravy from run number one with the "short" hold tube and the run with the "long" hold tube as derived from average, turbulent and laminar product flow assumptions. The values shown were calculated on the basis of $z = 10°C$ (18°F). Substitution of $z = 8.9°C$ (15.2°F) into the de Ruyter and Brunet program as actually found for spores of PA3679 in the turkey and gravy rather than $z = 10°C$ (18°F) did not change the sterilizing values shown. The Bio-F_o's for the two runs are also shown in Table 6.

In analyzing the data in Table 6, it should be stressed that the inoculated pack results, of course, reflect the cumulative lethality of the heating, hold tube and cooling sections of the SSHE. On the other hand, the simulation is capable of calculating theoretical product F_o values for each section of the SSHE for varying processing parameters. In terms of average flow, the "total lethality" values for turkey cubes calculated from the data for the "short" and "long" hold tubes show good agreement to the corresponding Bio-F_o values when the sensitivity of the inoculated pack procedure is considered. However, in terms of laminar flow, the simulation underestimates the "total lethality" by a considerable

TABLE 6.
SIMULATION STERILIZATION VALUES FOR 12.7 mm TURKEY CUBES AND GRAVY BASED ON EITHER AVERAGE, TURBULENT, OR LAMINAR FLOW CHARACTERISTICS VERSUS THE CORRESPONDING BIO-F_0 VALUES FROM INOCULATED PACK DATA[1]

Parameter	"Short" Hold Tube (Run 1)		"Long" Hold Tube (Run 4)	
	Turkey Cubes	Gravy	Turkey Cubes	Gravy
A. F_0, Simulation Model				
. Average Flow				
End, Heater 3	<0.01	4.67	<0.01	<0.01
End, Hold Tube	<0.01	9.03	2.80	8.02
End, Cool	1.63	5.20	1.90	0.47
Total Lethality	1.6	18.9	4.7	8.5
. Turbulent Flow				
End, Heater 3	<0.01	4.15	<0.01	0.47
End, Heater Tube	<0.01	7.75	1.60	6.19
End, Cool	0.86	4.50	1.70	0.41
Total Lethality	0.9	16.4	3.3	7.1
. Laminar Flow				
End, Heater 3	<0.01	2.53	<0.01	0.28
End, Hold Tube	<0.01	4.80	0.10	3.68
End, Cool	0.10	3.17	0.70	0.55
Total Lethality	0.1	10.5	0.8	4.5
B. Bio-F_0	3.0	--	4.3	--

[1]See Table 5 for run processing temperature data.

amount. In other words, a presumption of laminar particle flow is quite conservative in calculating thermal processes for the sterilization of large particulate foods in a SSHE system.

Calculations show that all of the process lethality in the turkey cubes occurred in the cooling phase, when the "short" hold tube was used. In contrast, when the "long" hold tube was used, a significant portion of the total lethality was produced in the hold tube section of the sterilizer.

The de Ruyter and Brunet simulation does not take into account SSHE rotor speed in determining particle lethality. In a practical sense, this omission appears to have minor importance because little lethality is generated within large particles in the heating section(s) of SSHE systems as illustrated by the data presented in Table 6. Because of the probability of excessive particle damage, it is doubtful that rotor speeds under commercial processing conditions will vary greatly from those used here.

Another observation from the data in Table 6 concerns the lethality generated in the carrier medium versus that generated in the particles with respect to hold

tube length. With the "short" hold tube and corresponding high processing temperature and short processing time, the ratio of "total lethality" of the gravy to the turkey cubes is 11.8. With the "long" hold tube and corresponding lower processing temperature and longer processing time, the comparative ratio is 1.8. These results suggest that severe overprocessing of the carrier medium will occur when attempting to sterilize the centers of large particulates in SSHE systems. On the other hand, the use of lower temperatures and longer processing times to sterilize large particles will result in less overprocessing of the carrier medium, which may result in higher quality products.

Residence Time

In applying the de Ruyter and Brunet heat transfer simulation in commercial situations for sterilizing large particulate foods, several factors must be considered. In the absence of definitive particle residence time data, it seems probable that particle flow for most particulates will be presumed to be laminar. Further, because of the likely possibility that some particles will break up in the cooling phase, lethality in cooling will be ignored. With these presumptions, sterilization processes for large particulates in general, may require processing temperatures

TABLE 7.
CALCULATED VERSUS MEASURED RESIDENCE TIMES OF 12.7 mm TURKEY CUBES IN GRAVY FOR "SHORT" HOLD TUBE AT VARYING PRODUCT FLOW RATES

Parameter	Residence time (sec) at varying flow rates (gpm)[1] 12.6	9.6	7.4
A. Calculated Time[2]			
Average Flow	15.3	20.1	26.1
Turbulent	12.8	16.8	21.7
Laminar	7.7	10.1	13.1
B. Measured Time[3]			
Fastest Particle	11.7	16.2	21.6
Slowest	16.6	23.4	25.4
Avg.	13.1	17.7	23.5
Std. Dev.	1.0	1.4	1.2

[1]Flow rates in gpm correspond to 47.7, 36.3 and 28.0 lpm.

[2]See Methods for calculations. Hold tube length = 6.9 m (22.8 ft).

[3]Based on measurements of single magnetic turkey cubes through the hold tube. Measured values based on 20 replicates for flow rates of 12.6 and 9.6 gpm and 10 replicates for 7.4 gpm.

of 138°C to 143°C (280°F to 290°F) with holding times of 4 to 6 min. Attempts to reduce processing hold times will require reliable methodology to measure the shortest particle residence time under expected commercial processing conditions. With this in mind, a method was devised to measure particle residence time distributions using magnetic particles as described earlier. Unfortunately, the magnetic particle idea did not evolve until the end of the inoculated pack work.

Tables 7 and 8 summarize residence time distributions on individual magnetic turkey cubes which were conducted in the "short" and "long" hold tube sections with respect to varying product flow rates. Also shown are the calculated hold times as determined from average, turbulent, and laminar flow criteria. These data illustrated at least two important points. First, calculated hold times based on laminar flow assumptions gave conservative estimates of particle flow rate through both hold tubes. In all cases, the fastest magnetic particles were much slower than those based on presumed laminar flow for each product flow rate variable. Second, as hold tube length increased and as flow rate decreased, variations in residence times from particle to particle increased dramatically.

TABLE 8.
CALCULATED VERSUS MEASURED RESIDENCE TIMES OF 12.7 mm TURKEY CUBES IN GRAVY FOR "LONG" HOLD TUBE AT VARYING PRODUCT FLOW RATES

Parameter	Residence time (sec) at varying flow rates (gpm)[1] 17.1	12.4	6.4
A. Calculated time:[2]			
Average Flow	95	130	253
Turbulent	79	109	211
Laminar	47	65	126
B. Measured time:[3]			
Fastest Particle	89	106	176
Slowest	94	122	265
Avg.	90	117	228
Std. Dev.	1	4	35

[1]Flow rates in gpm correspond to 64.7, 46.9 and 24.2 lpm.

[2]See Methods for calculations. Hold tube length = 58.2 m (191.1 ft).

[3]Based on measurements of single particles with magnets timed through the hold tube. Measured values based on 10 replicates per flow rate.

The individual magnetic particle residence time data for the "long" hold tube and a flow rate of 6.4 gpm (24.2 lpm) showed two interesting flow patterns as depicted in Fig. 2. One group of particles appeared to travel through the hold tube rather rapidly, whereas the other group of particles appeared to travel in a much slower pattern. Note that none of the particles showed an intermediate residence time. It seemed that an individual particle once starting either laminar or turbulent flow continued this pattern throughout the length of the hold tube. Of course, this phenomenon may not occur with a product containing many particles as would occur in almost all commercial production situations, hence this observation may have little, if any, practical consequence. On the other hand, further exploratory work in this area appears to be warranted.

In conclusion, inoculated pack data lend credence to the de Ruyter and Brunet simulation for determining thermal processes for sterilizing large particulate foods for aseptic packaging. The simulation appears to be appropriate for setting thermal processing parameters for sterilizing large particulate foods in SSHE systems. However, in general, inoculated pack-type studies undoubtedly will be required to confirm the adequacy of any thermal processes submitted to regulatory agencies in the United States. Finally, there is a need to better understand the nature of

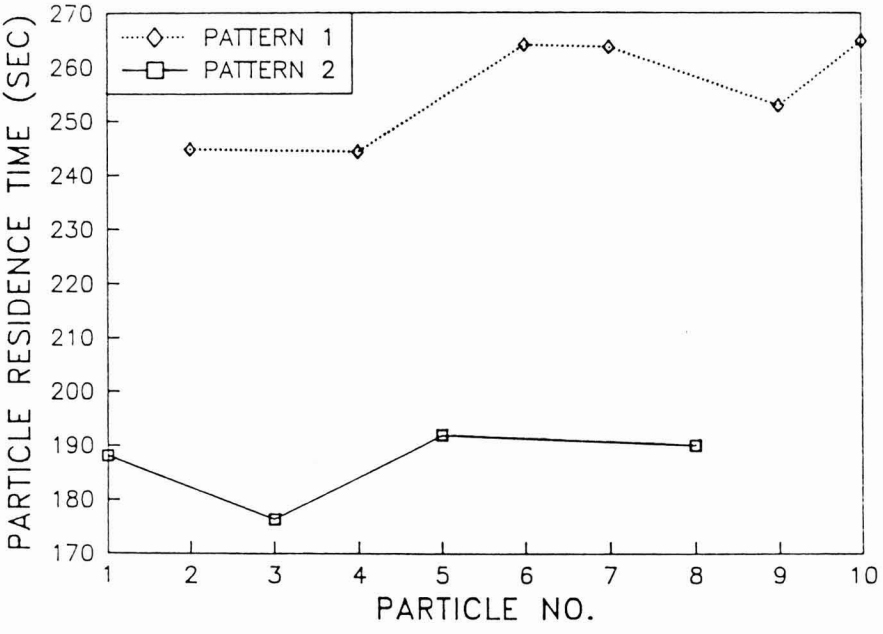

FIG. 2. RESIDENCE TIMES FOR 12.7 MM PARTICLES FROM TEN INDIVIDUAL RUNS THROUGH THE "LONG" HOLD TUBE AT 6.4 GPM (24.2 LPM) AS DETERMINED BY THE MAGNETIC PARTICLE METHOD: pattern 1 represents the slowest moving particles; pattern 2 represents the fastest

large particle flow and particle residence times in SSHE systems under variable experimental conditions. The magnetic particle idea may be one means of attacking this difficult problem.

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GLUCOSINOLATE CHANGES IN BLANCHED BROCCOLI AND BRUSSELS SPROUTS

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ABSTRACT

Three major glucosinolates in broccoli, i.e., glucoiberin, glucoraphanin, and glucobrassicin were greatly reduced by both water and steam blanching. Water blanching produced the most significant glucosinolate loss. Blanched brussels sprouts did not exhibit this significant reduction of glucosinolates. This inhibition of glucosinolate loss probably is due to the physical configuration of brussels sprouts, i.e., a tight, compact vegetable that is more resistant to the leaching effects of blanching compared to the loose structure of broccoli. Thus, flavor and the numerous physiological changes and attributes associated with glucosinolates are retained to a much greater degree in blanched brussels sprouts than in blanched broccoli.

The sulfur containing glucosinolates are secondary plant metabolites present as glucosides in *Brassica* vegetables, condiments, oil seeds and forage crops such as rape (Fenwick *et al.* 1982). Glucosinolate breakdown products possess important sensory properties, e.g., odor and flavor (MacLeod 1976), as well as inducing numerous physiological changes including carcinogenesis inhibition (Wattenberg 1974), goiter formation, and hepatic fibrosis with mitochondrial swelling (Nishie and Daxenbichler 1982).

Glucosinolates are readily hydrolyzed under moist conditions by the coexisting endogenous enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1). The products of enzyme decomposition of glucosinolates are ß-D-glucose, sulfate and an organic aglucon moiety. Depending on conditions, such as the proteins present, pH, trace elements, etc., the aglucon can undergo intramolecular rearrangement and/or fragmentation yielding products including thiocyanates, iso-

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thiocyanates, nitriles, cyanides and oxazolidine-2-thiones (Van Etten and Daxenbichler 1977; Tookey *et al.* 1980). About 100 different glucosinolates have been isolated from various plants, but *Brassica* vegetables usually contain less than ten. Trivial names are commonly used for the glucosinolates but use of the generic name glucosinolate with an appropriate prefix added to identify the R group provides a nomenclature that defines the chemical structure of each.

Reports of glucosinolate losses during cooking (Sones *et al.* 1984; McMillan *et al.* 1986) prompted this investigation into the effect of blanching on putative glucosinolate changes in two common *Brassica* vegetables, broccoli and brussels sprouts. Volatile isothiocyanates will be lost during heat treatments such as blanching, but blanching will also inactivate myrosinase, inhibiting glucosinolate hydrolysis and the formation of breakdown products (MacLeod 1976).

MATERIALS AND METHODS

Broccoli (*Brassica oleracea*; "Early One") and brussels sprouts (*Brassica oleracea*, "Jade Cross E") were field transplanted 30 in. apart surrounded by black plastic sheets.

The vegetables were harvested and replicate 500 g samples of broccoli or brussels sprouts were bagged in cheesecloth prior to processing. An open steam kettle with water temperature maintained at 99°C was used for water blanching. Each vegetable was water blanched 4 min. Vegetables blanched by steam utilized a pilot plant steam chamber with the steam temperature held at 99°C–102°C. Each vegetable was blanched 5.5 min. Both water and steam blanching durations and temperatures were similar to a typical commercial operation (Dietrich *et al.* 1977). Immediately after blanching, the vegetables were quenched in cold water for 3.5 min and freeze-dried (Virtis Co., Gardiner, NY). Since Miller (1982) reported that reduction of glucosinolates occur at drying temperatures in excess of 15°C, the freeze-drier shelf temperature did not exceed 5°C. Blanched, dried, ground vegetables were stored at −2°C until analysis. Unblanched vegetables were dried, ground and stored in the same manner.

Water or steam distillates remaining after blanching were collected and concentrated approximately 10-fold by rotary evaporation at 45°C. Concentrates were kept at −2°C until analysis.

Glucosinolates were analyzed in the dried vegetables using high performance liquid chromatography (HPLC) (Goodrich *et al.* 1988) following on-column desulphation by the method of Truscott *et al.* (1983).

Analysis of variance of all data was accomplished according to procedures described in Steel and Torrie (1960).

RESULTS AND DISCUSSION

The HPLC analyses of glucosinolates from unblanched, water blanched, and steam blanched broccoli are presented in Table 1 and those of brussels sprouts are presented in Table 2. Unblanched broccoli containing the greatest concentrations of specific glucosinolates, i.e. glucoiberin, glucoraphanin and glucobrassicin had significant losses when steam blanched with further losses after water blanching. Increased leaching of total glucosinolates during water blanching was observed as the amount of glucosinolates present in the blanch water and steam distillate (Table 3). Total solids of vegetables are more readily leached during water blanching when compared to steam blanching (Dietrich *et al.* 1977). Two of the three glucosinolates present in large concentrations in unblanched broccoli, with concomitant high losses post blanching, contain a methylsulphinyl side chain.

Glucosinolates were present in unblanched brussels sprouts at much greater concentrations than in broccoli. Although glucobrassicin was present in large concentrations in both vegetables, it was about 4-fold greater in unblanched brussels sprouts than in unblanched broccoli. In addition, a much greater loss

TABLE 1.
BLANCHING OF BROCCOLI

Glucosinolate [2]	Unblanched (mg/g)	Water Blanched (mg/g)	Steam Blanched (mg/g)
Glucoiberin [3]	1.27±.01^{a1}	0.25±.01^{c}	0.70±.12^{b}
Progoitrin [4]	0.06±.00^{a}	0.09±.01^{a}	0.36±.08^{a}
Sinigrin [5]	0.05±.00^{a}	0.0^{b}	0.0^{b}
Glucoraphanin [6]	4.45±.07^{a}	0.91±.08^{c}	2.68±.21^{b}
Glucosinalbin [7]	0.0^{b}	0.08±.01^{b}	0.38±.09^{a}
Gluconapin [8]	0.0^{a}	0.0^{a}	0.17±.17^{a}
Glucobrassicanapin [9]	0.11±.00^{b}	0.0^{b}	0.0^{b}
Glucobrassicin [10]	4.96±.07^{a}	0.60±.34^{c}	2.43±.50^{b}
Neoglucobrassicin [11]	0.70±.04^{a}	0.05±.01^{b}	0.24±.07^{b}
Total	11.59±.12^{a}	1.97±.30^{c}	6.97±1.25^{b}

[1]Mean values are on a freeze-dried basis. Different letter superscripts indicate significant ($P \leq 0.05$) differences of each glucosinolate between treatments.
[2]Trivial names. Glucosinolate side chains are: [3]3-methylsulphinylpropyl; [4]2-hydroxybut-3-enyl; [5]prop-2-enyl; [6]4-methylsulphinylbutyl; [7]p-hydroxybenzyl; [8]but-3-enyl; [9]pent-4-enyl; [10]3-indolylmethyl; [11] 1-methoxy-3-indolylmethyl.

TABLE 2.
BLANCHING OF BRUSSELS SPROUTS

Glucosinolate [2]	Unblanched (mg/g)	Water Blanched (mg/g)	Steam Blanched (mg/g)
Glucoiberin	1.52±.33[a1]	1.65±.24[a]	1.14±.04[a]
Progoitrin	2.94±.25[a]	2.89±.10[a]	2.23±.26[a]
Sinigrin	1.58±.20[a]	1.51±.08[a]	1.24±.13[a]
Glucoraphanin	2.37±.43[a]	2.83±.34[a]	1.64±.65[a]
4-Hydroxyglucobrassicin	0.62±.04[a]	0.57±.00[ab]	0.43±.07[b]
Glucobrassicin	17.81±.70[a]	15.49±.25a	14.38±2.68[a]
4-Methoxyglucobrassicin	2.86±.21[a]	2.73±.17[ab]	2.10±.28[b]
Total	29.70±2.15[a]	27.67±1.17[a]	23.16±3.50[a]

[1]Mean values are on a freeze-dried basis. Different letter superscripts indicate significant ($P \leq 0.05$) differences of each glucosinolate between treatments.
[2]Trivial names. See Table 1 for individual glucosinolate side chains.

of glucobrassicin occurred in the water blanched broccoli than in the water blanched brussels sprouts. Although the actual mean glucobrassicin loss in steam blanched brussels sprouts appeared greater than the mean glucobrassicin loss in steam blanched broccoli, the large standard error of the mean steam blanched brussels sprouts value (Table 2) contributed to this difference. Heaney and Fenwick (1980) observed relatively large concentrations of glucosinolates in specific cultivars of brussels sprouts indicating a genetic control of these compounds. Moisture and nitrogen availability during growth also effect the glucosinolate content of brussels sprouts (Heaney *et al.* 1983). In contrast to the dramatic glucosinolate reduction in blanched broccoli, blanching the brussels sprouts did not significantly reduce the major glucosinolates. Only the indole containing 4-hydroxy and 4-methoxyglucobrassicins showed significant decreases in blanched brussels sprouts. As seen in Table 3, blanching brussels sprouts by water enhanced glucosinolate losses as compared to steam. However, there was some escape of steam before condensation occurred in the steam chamber. This steam loss would tend to cause some underestimation of glucosinolates in the steam residue.

The results of this study indicate that large glucosinolate losses occur in blanched broccoli, but not in blanched brussels sprouts. The physical configuration of these vegetables could be the cause of this effect. The tight, compact sprout appears to be more resistant to the leaching effects of water or steam

TABLE 3.
TOTAL GLUCOSINOLATES OF LIQUID RESIDUES AFTER BLANCHING

	μg lost / g vegetable blanched [1]	
	Water Blanched	Steam Blanched
Broccoli	630.9 ±211.5[a2]	14.0 ±1.4[b]
Brussels Sprouts	501.6 ± 39.0[a]	4.8 ±2.6[b]

[1]Fresh Weight.
[2]Mean ± S. E. Different superscripts indicate significant ($P \leq 0.05$) differences.

blanching as compared to the loose structure of the broccoli stalk and flower head. Thus, flavor quality, the desirable indole glucosinolates and perhaps other natural compounds with respect to their anti-carcinogenic attributes (Stoewsand *et al.* 1988), are retained to a great degree in blanched brussels sprouts.

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STORAGE STABILITY OF MINIMALLY PROCESSED FRUIT

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ABSTRACT

Fresh peeled and halved apricots, peaches and pears were dipped in various solutions to retard product browning and texture loss during refrigerated storage. Calcium and zinc compounds were effective in reducing enzymatic browning. The addition of an oxygen scavenger into the packaged product significantly reduced texture loss from structural polymer breakdown for apricot and peach halves but not for pear halves. Oxygen reduction in packaged pears resulted in only slightly retarded texture loss.

INTRODUCTION

Consumers are currently indicating an increased demand for fresh fruits and vegetables, over their processed counterparts (Rice 1987). Fresh fruits and vegetables have firmer texture and different flavor than processed items. There is also an additional demand for this fresh product to be in a convenient, 100% edible form. This is illustrated by the increase production of products such as shredded fresh lettuce, which has more than doubled in production, from less than 102 million lb in 1985 to almost 250 million lb in 1987 (Bradshaw 1988). The production of shredded lettuce has potential for increased growth, along with other minimally processed fresh fruits and vegetables, if their storage lives can be extended; opening markets in areas where they are now unavailable. For this to be accomplished the products must undergo some form of light processing.

Light or minimal processing usually describes a fresh product in a convenient peeled, cored or sliced, 100% edible form, contrasting to regular processing, which includes frozen, canned, dried, etc. Fruits and vegetables lose their typical fresh appearance and characteristic texture after being held in cold storage for only a short time. This change is accelerated when the plant cells have been bruised or also when they have been ruptured, which occurs in the process of

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peeling, slicing, etc. Physical rupturing of plant cells, whether by cutting or crushing, leads to an acceleration in respiration of the plant tissues, and physical decay, through rapid increase in oxidative reactions. Reaction rates may increase up to 25 fold over the normal physiological rate, (School and Holt 1986). Because of this, the injured cut surface must be treated to minimize degradative chemical reactions.

Texture loss is one of the most obvious changes that occurs, resulting largely from enzyme initiated physiologically degrading reactions. Enzymes such as ß-galactosidase, pectin methyl esterase and polygalacturonase affect the products storage stability (Lidster *et al.* 1986; Steele and Yang 1960). Various methods have been used to control these reactions. Buick and Damoglou (1987) used vacuum packaging of fresh sliced carrots with limited success. Dick *et al.* (1985) found that compounds such as chlorogenic acid and quercetin could, under certain conditions, inhibit the cell wall degrading activity of the enzyme ß-galactosidase. Rai (1980) determined that vanillin was effective against polygalacturonase, which can depolymerize galactose in cell-wall materials. Compounds such as cysteine and carbon monoxide have been shown to have limited effectiveness against the oxidative enzyme polyphenyloxidase (Friedman *et al.* 1986). Dipping fresh strawberries in calcium lactate and also acidified calcium chloride solution before canning increased firmness in the final product (Morris *et al.* 1985).

One way of extending the storage life of fresh fruits or vegetables is to develop a minimum processing procedure that reduces undesired physiochemical reactions and does not produce the severe textural changes associated with freezing or dehydration. Such a product would retain more of the characteristics of the original fresh product than a processed one.

This study was initiated to develop treatment procedures that would be effective in extending the storage life of minimally processed fruits.

MATERIALS AND METHODS

Five varieties of freestone peaches (Riponette, Fairmont, Suncrest, Flamecrest, Fay-Elberta), three of apricots (Perfection, Tilton, Royal), and Bartlet pears were used in this study. The fruit was obtained both directly from orchards and from commercial produce distribution centers. The apricots were halved and the peaches and pears were both halved and peeled with knives. All fruit halves were first dipped for one minute in a solution of 2% ascorbic and 2% citric acids, to retard surface enzymatic browning and to remove the cellular exudate, followed by draining. Following this preliminary dip, various chemical treatments were tested that either had been shown to retard some types of product degrading reactions, or because of their chemical structure were ascertained to be possibly effective in these areas. These treatments consisted of 2 min dips in one of the

following, 5% calcium phosphate, 5% calcium lactate, 5% ammonium phosphate, 1–6% $CaCl_2$, 1% $ZnSO_4$, 1% $ZnCl_2$, 1% $ZnCl_2$ + 2% $CaCl_2$, 2% vanillin, 0.01% rutin, 0.01% quercetin, and 1% alky aryl sulfonate (Nacconol). Replacated samples (4–6 halves each) were placed in 0.05 mm polyethylene-Saran-polyester laminate pouches and heat sealed. Three different packaging procedures were used; (1) aerobic, pouches containing a few small holes; (2) anaerobic (ANA), a 10 cm square commercial oxygen scavenger envelope (Mitsubishi International Corp., 50 California St., San Francisco, CA), containing activated iron oxide (added to each pouch before sealing); and (3) modified atmosphere (MA): atmospheric sealing with the product consuming oxygen and giving off carbon dioxide. The packaged fruit was stored at 2°C.

Moisture reduction was accomplished in a forced air dehydrator operating at 70°C.

Fruit color was monitored using a Minolta Chroma Meter CR-100 with a 1 cm diameter sensing head to obtain L*, a* and b*. A DP-100 Data Processor was used to record and evaluate the readings of 4–8 halves per sample. The L* readings are a measure of the products lightness, going from white (L* = 100) to black (L* = 0), and the a* and b* indicate hue, measuring +a* (red), −a* (green), +b* (yellow) and −b* (blue). The rational of using reflectance measurements to follow product quality changes is discussed by Hunter (1975). For quantifying textural changes, Bourne (1974) indicated that any method for measuring deformation force resistance in a fruit is adequate. An Instron texturemeter, equipped with a 2kg load cell was used to determine the maximum force required to deflect the cup-down sample 5mm, by depressing a 12 mm diameter horizontal rod against it at the rate of 1.3 cm/min. Calcium and zinc were determined by atomic absorption spectroscopy (Powell and Tease 1982).

Sensory panel evaluation was by Duo-trio (ASTM 1968), simultaneous presentation of the product to 20 judges in the morning followed by 20 judges in the afternoon. Statistical significance was determined using a one-tailed test.

RESULTS AND DISCUSSION

Aerobic Packaging

When oxygen is available, an enzyme in the product, such as polyphenyl oxidase, can readily react with its substrate, such as chlorogenic acid, to produce an undesired brown pigmented material. Ascorbic acid has been the most effective compound in general use to retard this type of browning.

In studies on peaches, only the calcium and zinc chloride treatments proved any more beneficial in retarding surface darkening than the standard ascorbic acid dip. In studies with both Riponette and Fay Elberta peach varieties the average final reflectance L* value of the stored halves for the Ca and Ca/Zn

TABLE 1.
RIPONETTE PEACH ANALYSIS (PPM)

Dip solution	Calcium	Zinc
Control	37	4
2% $CaCl_2$	440	3
2% $CaCl_2$/1%$ZnCl_2$	360	180

treated fruit was 9.4% and 12.4% greater than the untreated controls, indicating a visually discernible difference. This same effect was not observed in the apricot studies, with the exception of zinc sulfate treated halves, which were consistently lighter than any of the other samples.

Dipping peach halves in the calcium chloride and calcium chloride/zinc chloride solutions increased the level of the divalent ions in treated halves (Table 1).

Calcium addition to peach halves resulted in their maintaining a firmer texture longer (Table 2) than the control. In studies using the Faye Elberta as well as the Riponette varieties, there was also an apparent firming of the cell structure in samples with added calcium chloride, between the 2 week and 5 weeks storage time, as compared to the control. However, the difference was not statistically significant. With apricots, zinc addition did not have any effect on texture (Table 3).

Calcium chloride imparted a taste to the product at higher treatment levels. In a taste panel evaluation, Castlebright apricot halves dipped in a 3% calcium chloride solution, (200 ppm calcium in tissue) did not differ in taste from the

TABLE 2.
EFFECT OF CALCIUM AND ZINC TREATMENT ON FAY ELBERTA PEACH HALVES TEXTURE DURING 2°C STORAGE

Storage Time (Wks)	Force (Newtons)			
	Control	$CaCl_2$	$ZnCl_2$	$CaCl_2$/$ZnCl_2$
0	12.0	12.0	12.0	12.0
2	4.5	3.0	4.4	3.3
5	1.8a	3.2b	3.2b	4.4c

Numbers with different letters are significantly different at the 5% level.

TABLE 3.
TEXTURE OF APRICOT HALVES STORED AT 2°C FOR 4–5 WEEKS

Variety	Texture (N)		
	Control	$CaCl_2$	$CaCl_2/ZnCl_2$
Castlebright	4.02	5.10	4.49
Royal	7.03	13.62	9.36

control. However, those dipped in a 5% solution, which resulted in a 480 ppm calcium level, had a flavor difference which was significant at the 0.1% level. Panelists rated the calcium treated product as tasting slightly sweeter than the control, but did not indicate presence of any off-flavor.

Modified Atmosphere and Anaerobic Packaging

By reducing the availability of oxygen, the undesired oxidative chemical and enzymatic reactions are minimized or eliminated. In MA storage the fruit respiration that occurs during the initial storage of the product in sealed pouches results in a depletion in the headspace of oxygen and a production of carbon dioxide.

Modified atmosphere packaging has been reported as a storage method for decreasing texture breakdown during storage. Smith *et al.* (1987) in a report on cold storage of whole apples, indicated that the fruit remained firm longer during storage when it was sealed inside a pouch made from low gas permeability film. A general hypothesis that the mode of action for retarding texture breakdown in MA is apparently related to the alleviation of water stress in the packaged fruit has been proposed by Ben-Yehoshua *et al.* (1983).

In this study scavenging the headspace oxygen from packaged, partially processed, peach halves retarded textural breakdown to an even greater extent than MA. Different varieties of peaches from different sources all showed this reduction in texture loss (Table 4).

Dipping Fairmont freestone peaches in vanillin, a substance reported to retard cellular breakdown by Rai (1980), did not significantly affect texture changes during modified atmosphere refrigerated storage. However, when these peaches were sealed in a bag with an oxygen absorber there was a statistically significant ($P<0.05$) decrease in texture loss (Fig. 1). By the thirteenth week of storage samples stored anaerobically had dropped 30% in texture force compared to a 63% drop for those held in modified atmosphere. When another peach cultivar (Suncrest) with a much softer texture was used, an increase in fruit firmness was observed during the first few weeks of anaerobic storage (Fig. 2). Evidently,

TABLE 4.
TEXTURE LOSS (+INCREASE) IN FRESH, PEELED PEACH HALVES STORED AT 2 DEGREE C FOR 7 WEEKS

Variety	Brix	Initial Texture (N)	Packaging Medium			
			Modified		Anaerobic	
			Texture	Rate*	Texture	Rate*
Fairmont	11	>21	8.0	1.7^{+}	17.0	0.5^{+}
Suncrest	9	7	4.0	0.5^{++}	10.5	+0.5^{++}
Flamecrest	9	21	3.9	2.4	7.6	1.9

*N/wk
Significantly different at: + 0.1000 level, ++ 0.0100 level.

when an available oxygen source is eliminated a pectin degrading reaction is retarded. Reduced oxygen and increased carbon dioxide levels were reported by Burton (1974) to affect plant cell breakdown. He reported that in whole fresh apples a CO_2 level greater than 12% and an 0_2 level less than 2% retarded the rate of texture loss during storage, and also, that secondary reactions associated with carbohydrate and acid metabolism were also effected.

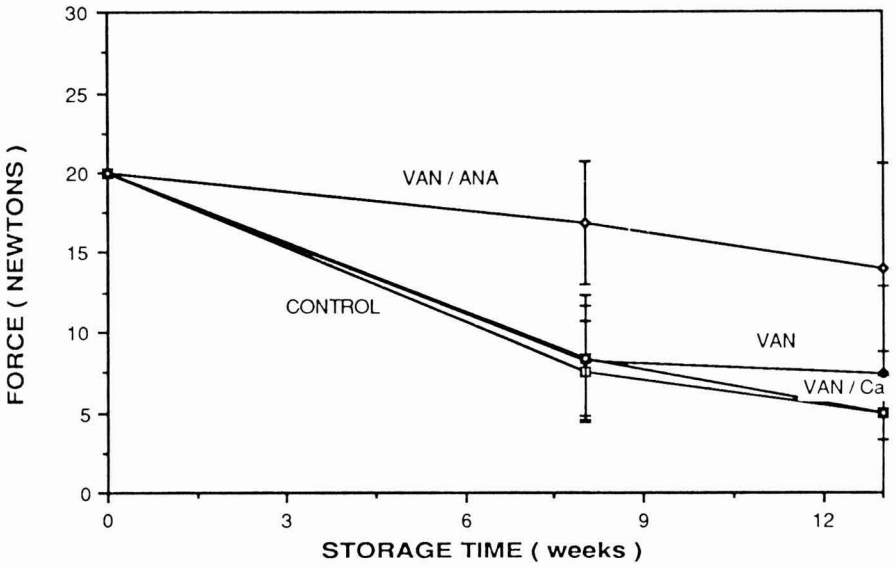

FIG. 1. RELATIONSHIP OF MODIFIED ATMOSPHERE AND ANAEROBIC PACKAGING OF FRESH PEELED FAIRMONT PEACH HALVES TREATED WITH VANILLIN AND CALCIUM CHLORIDE ON TEXTURE CHANGE; VERTICAL LINES INDICATE ONE STANDARD DEVIATION

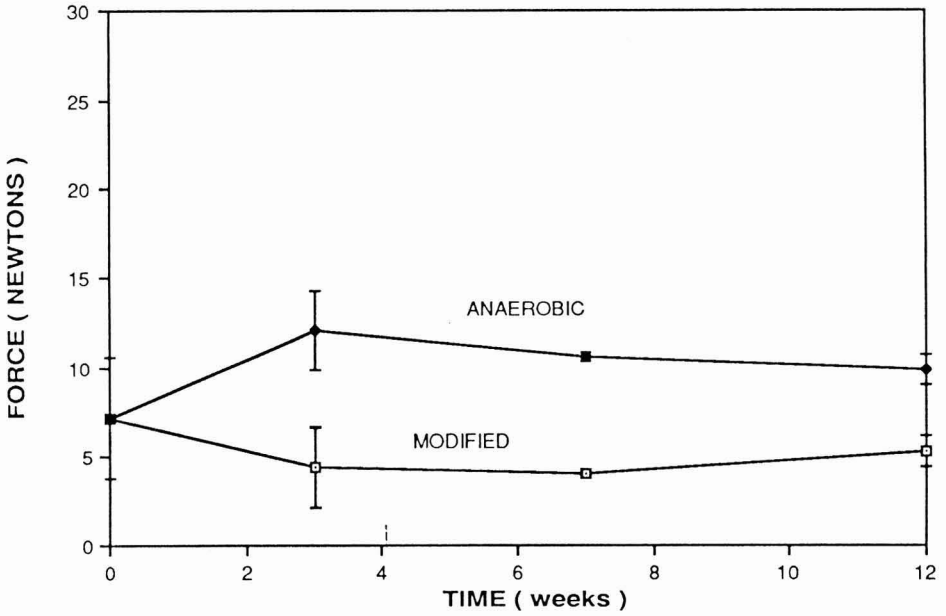

FIG. 2. RELATIONSHIP OF MODIFIED ATMOSPHERE AND ANAEROBIC PACKAGING OF FRESH PEELED SUNCREST PEACH HALVES ON TEXTURE CHANGE DURING STORAGE AT 2°C

Reducing the oxygen level by absorbing only a portion of the oxygen headspace retarded texture loss (Table 5). Sealed pouches of peach halves contained about 250mL of air and 450 g of fruit per bag. Since 3g of activated iron oxide should absorb the oxygen in 500mL of air (Mitsubshi International Corp.), this should be adequate to provide the desired anaerobic conditions. However, in actual tests it was found necessary to provide an excess of the oxygen absorber. This could be due to oxygen permeation of the film during storage, or from occluded oxygen migrating slowly from the fruit pieces. This latter effect was demonstrated by Bolin and Steele (1987) in dried apples.

Taste panel evaluation between aerobically and anaerobically stored peaches showed no detectable flavor differences until the samples had been stored 2 weeks. At two weeks storage there was a significant difference at the 1% level, with most of the panel members indicating a preference for the anaerobic stored peaches. The firmer texture of the anaerobically stored halves was probably a factor in the panels choice.

All the peeled peach halves retained their light yellow color during storage when held in both anaerobic and modified atmosphere packages. The only consistent color differences were shifts in green tints ($-a^*$), observed in all the anaerobic samples. These samples also exhibited an increase in the saturation

TABLE 5.
RELATIONSHIP OF THE AMOUNT OF OXYGEN ABSORBED TO TEXTURE CHANGE IN FRESH FLAMECREST PEACH HALVES STORED FOR 7 WEEKS AT 2°C

Oxygen Absorber (g)	Force (N)
0	3.9
1	4.2
3	6.6
9	7.5

index $\sqrt{a^{*2} + b^{*2}}$ indicating an increase in overall pigmentation. Pigmentation increase was not observed in modified atmosphere samples.

No observable microbial growth occurred on any of the fruit tested under the 2°C storage condition.

Partial drying was considered a possible method for firming cells to decrease turgor loss rate during storage. This treatment did not prove advantageous for lightly processed fruit. Drying Suncrest fresh peeled peach halves to 10–50% weight loss (WL) resulted in an initial texture reduction, which persisted during storage (Table 6). The initial lightness increase in the partially dried samples is probably because of densification of the fruit surface, which would result in an increased reflectance.

Pears lost texture readily during storage, independent of packaging atmosphere. Pear halves packaged in MA underwent a reduction in texture force readings of 99% after four weeks storage; the ANA fruit dropped 95% in the same time period. In addition, pears darkened during storage, with both MA and ANA packaged samples showing a decrease in the saturation index. Pears that were quartered but not peeled maintained a lighter flesh color than peeled fruit (Table 7).

TABLE 6.
EFFECT OF PARTIAL DRYING ON STORAGE OF SUNCREST MA STORED PEACHES

Storage (days)	Control		10% WL		20% WL		50% WL	
	L*	Force (N)	L*	Force (N)	L*	Force (N)	L*	Force (N)
0	67.3	13.3a	67.6	11.2a	68.0	5.2b	73.1	4.4b
9	61.5	9.0a	49.8	3.8c	43.5	1.4c	47.5	2.4c
56	54.3	2.3c	53.4	2.5c	54.5	2.2c	--	--

Numbers with different letters are different at 0.1000 level.

TABLE 7.
CHANGE IN COLOR OF PEELED AND UNPEELED PEARS HELD IN MODIFIED ATMOSPHERE AT 2°C

Storage Time (wks)	L*		a*		b*	
	Unpeeled	Peeled	Unpeeled	Peeled	Unpeeled	Peeled
0	77.80	77.80	-3.67	-3.67	18.93	18.93
2.5	73.13	67.65	-2.35	-1.87	18.18	20.36
6	68.68	63.36	-2.84	-3.27	18.59	14.39

Standard errors: L* 1.55, a* 0.168, b* 0.612

Unpeeled anaerobic packaged Castlebright apricot halves maintained a statistically firmer texture ($P<0.05$) during refrigerated storage than fruit held in modified atmosphere (Fig. 3). A larger variability occurred in texture readings between the halves of individual apricots than in peaches or pears, due to the large textural variation within a given individual fruit. Texture readings on one side of an apricot could be 30–40% less than on the other side. In the case of

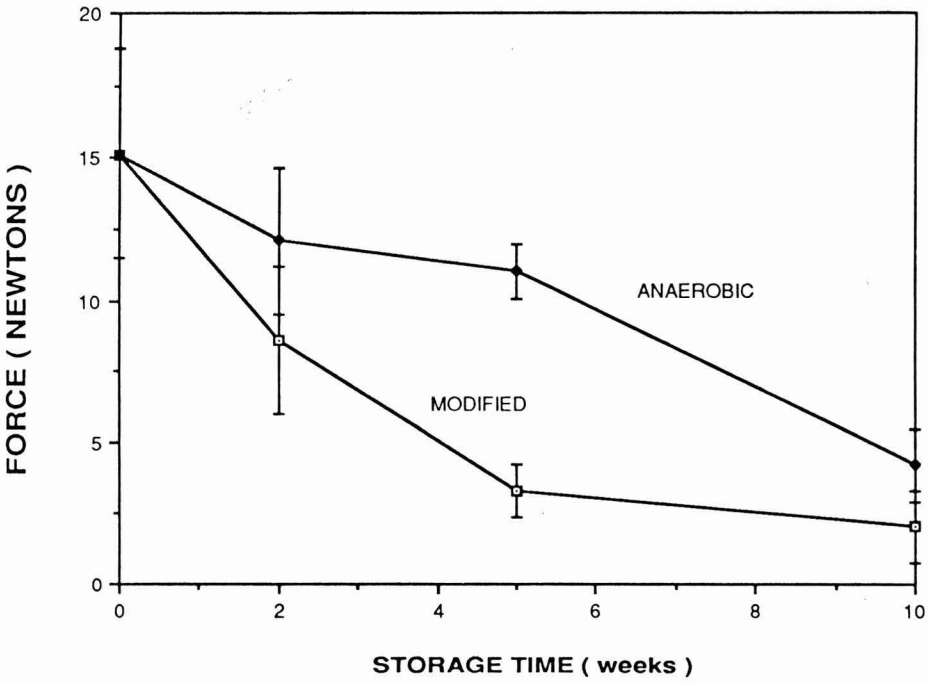

FIG. 3. TEXTURE CHANGE IN CASTLEBRIGHT APRICOT HALVES PACKAGED UNDER DIFFERENT ATMOSPHERES

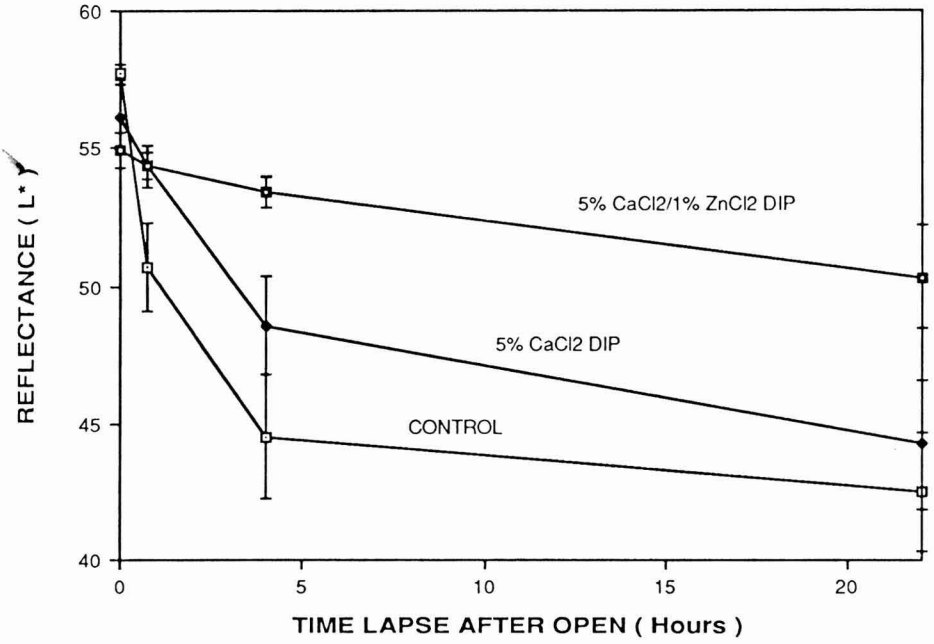

FIG. 4. DARKENING OF OPENED POUCHES OF APRICOT HALVES STORED AT 2°C for 10 WEEKS

apricot halves, packaging medium did not influence the rate of product darkening which, was slight (L* value reduction of 4–7 units). Even though the halves did not darken excessively during storage, as soon as the packages were opened oxidative browning occurred. The rate of browning varied with the treatment the fruit received (Fig. 4). A combination 1% zinc chloride/5% calcium chloride dip reduced darkening to the greater degree. Neither calcium chloride or zinc chloride was as effective as the combination of the two salts. The phosphates, lactate, rutin, quercetin and nacconol dips were all ineffective in significantly retarding darkening and texture loss in anaerobic stored apricot halves during five weeks cold storage. However, calcium lactate and quercetin treated samples did maintain a slightly firmer texture. Dick, *et al.* (1985) found that quercetin was effective on retarding apple softening.

These studies demonstrate that peeled and halved peaches and apricot halves can be processed to retain their light color and firm texture during cold storage. The optimum treatment condition consisted of a 2% calcium chloride/1% zinc chloride dip followed by anaerobic packaging and storage at 0–2°C. Similar treatment does not retard degradation of pears.

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THE INFLUENCE OF THE SUCROSE POLYESTER, SEMPERFRESH™, ON THE STORAGE OF MICHIGAN GROWN "MCINTOSH" AND "GOLDEN DELICIOUS" APPLES[1]

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ABSTRACT

"Golden Delicious" and "McIntosh" apples were treated with the sucrose polyester, Semperfresh™, to determine its influence on fruit maturity parameters during cold storage. The objective of this experiment was to determine if Semperfresh application could delay ripening and improve fruit storage stability.

Semperfresh treatment reduced apple ripening rate as observed by several parameters including color and texture. Treated apples had delayed color development (internal and external Hunter color reflectance measurements) during 4 months of storage (39°F; 5°C). Semperfresh increased fruit penetrometer (firmness) readings of both varieties during storage. Measurements for pH, total acidity, and soluble solids were not affected by Semperfresh treatment. Sensory evaluation using a triangle test, indicated that flavor and textural changes were not detected when apples treated with 1.2% Semperfresh were compared to untreated apples after two months storage.

INTRODUCTION

Apples are stored in controlled atmosphere (CA) environments to slow ripening and extend shelf-life. A typical CA regimen obtains low oxygen (3%) and ethylene with elevated carbon dioxide (10–20%) concentrations to reduce the respiration rate while avoiding anaerobic respiration and death. In addition to retarding fruit ripening, CA storage has been shown to reduce flavor changes,

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pigment and nutrient losses of stored fruits (Kader 1986). CA storage units are expensive to build and maintain and once a chamber has been opened, the fruit must be used in a relatively short time.

Recently, sucrose polyesters (SPE) have been used successfully for post-storage preservation. Smith and Stow (1984) applied SPE to "Cox's Orange Pippin" apples following storage and found increased internal CO_2 levels, reduced yellow color development and increased firmness. However, they found that a 1.25% SPE formulation did not affect texture, color or weight loss of apples stored at 3.5°C for up to 5 months. Chu (1986) reported that SPE application following 'low-oxygen' storage of "McIntosh" and CA storage of "Delicious" apples, reduced softening during 21 days of additional storage at 15°C and 90–95% RH. Banks (1984) observed that SPE coating to "Cox's Orange Pippin" apples, decreased the internal O_2 and increased the internal CO_2 contents when apples were held at 4°C. Drake *et al.* (1987) applied the SPE, Semperfresh, to "Golden Delicious" apples following CA and refrigerated storage. Refrigerated, treated apples had delayed color development, greater firmness and higher acid content than control apples. CA stored, treated apples had delayed color development and higher acid content but the texture was not altered. These results indicate that Semperfresh is effective in delaying ripening and extending the post-storage life of apples.

The purpose of this experiment was to determine if treatment with the sucrose polyester, Semperfresh, prior to noncontrolled atmosphere cold storage of Michigan apple varieties "Golden Delicious" and "McIntosh" would improve shelf-life and reduce the need for CA storage. Extending the shelf-life of these cultivars will reduce the costs associated with CA storage for apples stored less than 6 months.

MATERIALS AND METHODS

"McIntosh" and "Golden Delicious" apples were obtained from a commercial orchard in central Michigan early in November, 1987. "McIntosh" apples had some bruising and were previously waxed at the orchard. Subjective color and flavor observations indicated that both varieties had ripened slightly beyond the point at which Semperfresh would normally be applied. Using commercially harvested slightly ripened fruit will demonstrate whether Semperfresh is acceptable for apples harvested by standard practices. The fruit was held for 4 days at 39 F (5°C) until treatments were applied. Prior to treatment with Semperfresh, apples were removed from cold storage, washed in cold soapy water to remove wax coating, rinsed and air dried. "Golden Delicious" fruit was subjectively sorted by color and the more yellow apples were removed.

Semperfresh Preparation and Treatment

A 3.6% Semperfresh stock solution was prepared by mixing 144 g of Semperfresh (Inotek International Co., Painesville, OH) powder with 4 L of distilled/deionized H_2O in a large Waring Blendor. Mixture was blended for 1 min (at low speed), held for 1 h, then blended for 1 min and held for 1 h prior to use. Final dilutions were prepared by diluting this 3.6% Semperfresh solution to the desired concentration. Apple treatments included (1) 0%; (2) 0.6%; (3) 0.9%; and (4) 1.2% Semperfresh dips.

Whole apples were randomly separated into four groups of 60 apples, dipped in a treatment solution for approximately 3 s, air dried, placed in plastic bag-lined containers, twist-tied, and put into cold storage at 39 F (5°C). At 2 months of storage the plastic bags were opened to reduce possible changes caused by ethylene accumulation or O_2 and CO_2 changes. Three replications of each treatment were removed at 30 day intervals throughout the 4 month storage interval and analyzed for color (internal and external), firmness, pH, total acidity, soluble solids, and degree of brown core.

Quality Evaluations

Skin Color was measured using the Hunter Color Difference Meter (Model D25-2) standardized with a yellow tile ($L_L = 78.4$; $a_L = -3.0$; $b_L = 22.7$) for ''Golden Delicious'' apples and a pink tile ($L_L = 68.8$; $a_L = 23.2$; $b_L = 9.4$) for ''McIntosh'' apples. Reflectance measurements were made on the surface and internal flesh from two apples from each variety.

Firmness (lb) was determined by using an Effegi Penetrometer (Effegi Pressure Tester model FT327, Alfonsine, Italy) with a 7/16 in. (11mm) diameter, flat cylinder plunger. Values were taken by slicing away skin and inserting penetrometer at three different locations along an equatorial plane of 2 apples per treatment for each cultivar. **Soluble Solids** (Brix) content was determined by manually squeezing juice from longitudinal slices from 3 apples per treatment onto an Abbe Refractometer. **Total Acidity** (% Malic Acid) was determined by mixing 50 g of apple tissue (from 3 apples) with 50 mL of distilled/deionized water in a blender. Homogenate (25 mL) was filtered through Whatman #5 filter paper and titrated with 0.1N NaOH to a pH of 8.0. **Brown Core** was evaluated by slicing apples through the center and examining visually. Apples were scored either with or without brown core.

Sensory Evaluation was conducted using a triangle test, taste panel after 2 months storage to determine if significant differences could be detected in apple slices between the 0% or 1.2% Semperfresh treatments. Sensory evaluations were performed in the Michigan State University, Sensory Evaluation Facility from 10–12 PM. No information was provided to panelists regarding apple slice

treatments. Apples were sliced into wedges and a single wedge was presented for each sample in three small cups identified by 3 digit random codes. The panels consisted of 20+ untrained, college age, men and women for the "McIntosh" and "Golden Delicious" samples. Crackers, rinse water and a rinse cup were also provided. Panelists were requested to choose the odd sample based on flavor and/or texture and then give comments. They were also requested to note if the flavor and texture differences detected were objectionable. Statistical analysis of taste panel data was done by the method of Roessler *et al.* (1978).

The experimental design was a 2 factor (Semperfresh Concentration and Storage Time), Randomized Complete Block with replicates. Analysis of Variance (AOV) was used to determine statistical relationships. Significance was determined at the $p=0.05$ level for all analyses. Treatment means were compared using a Protected-Least Significant Differences (LSD) test.

RESULTS AND DISCUSSION

Color Measurements

Internal color of **"McIntosh"** apples was not significantly affected by Semperfresh application. There was a shift in color during the 4 months of storage which can be attributed to fruit maturation of apples from all treatments (Fig. 1). Flesh color changed from $L_L = 66.6$ to $L_L = 70.1$, indicating a lighter color and from $a_L = -3.2$ to $a_L = -1.7$, indicating a reduction in green color. Hunter b_L values increased from 16.0 to 17.2 during 3 months of storage and then decreased to 16.0 by 4 months. The influence of storage on the hue angle and chroma of internal and external color for each variety is shown in Table 1. There is no trend for hue angle and chroma for the internal color of "McIntosh" apples during storage.

External skin Hunter a_L values were significantly influenced by an interaction between Semperfresh concentration and storage interval. Apples for all treatments had an increase in red color until two months of storage followed by a decrease in red color (Fig. 2). This may be due to changes in ethylene, O_2 and/or CO_2 concentrations when the plastic bags were opened after two months of storage. External L_L changed very little during the 4 month storage interval (Fig. 3). External tissue b_L values decreased from 12.5 to 9.2 at 2 months and then increased to 12.7 at 4 months of storage (Fig. 3). There was marked variation of the hue angle and chroma during storage (Table 1).

Semperfresh treatment did not influence internal flesh L_L and a_L values of **"Golden Delicious"** apples. However, an influence was observed for treatment concentration on b_L values. Semperfresh caused lower b_L values (less yellow), suggesting that ripening was delayed (Fig. 4). Storage duration influenced in-

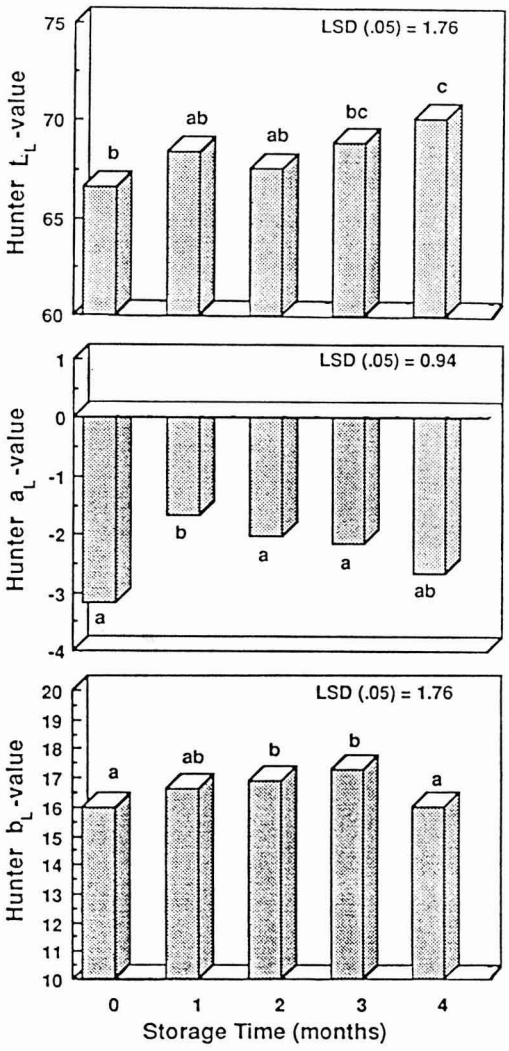

FIG. 1. INFLUENCE OF STORAGE INTERVAL ON INTERNAL FLESH COLOR OF "MCINTOSH" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

ternal color measurements of "Golden Delicious" apples (Fig. 5). Internal flesh L_L readings increased as storage progressed and a lighter color was observed. Internal a_L values increased from -6.9 to -2.6 at 4 months of storage, indicating a reduction in green color. An increase in b_L values 25.4 to 27.5 was observed during the 4 months of storage, showing an increase in yellow color. The hue

TABLE 1.
COLOR OF MCINTOSH AND GOLDEN DELICIOUS APPLES

Storage	McIntosh				Golden Delicious			
	Internal Color		External Color		Internal Color		External Color	
Time (Months)	Hue*	Chroma**	Hue*	Chroma**	Hue*	Chroma**	Hue*	Chroma**
0	-78.7	16.3	56.3	15.0	-74.8	26.3	-72.6	28.2
1	-84.2	16.8	42.0	17.3	-81.0	28.1	-81.0	30.0
2	-82.9	17.0	35.0	16.8	-81.6	28.3	-84.7	30.0
3	-82.7	17.3	52.4	16.4	-85.9	28.0	-89.4	28.9
4	-80.4	16.2	52.4	16.1	-84.6	27.6	+88.7	31.2

* hue angle = $\tan^{-1}(b_L/a_L)$; ** chroma = $(a_L^2 + b_L^2)^{1/2}$

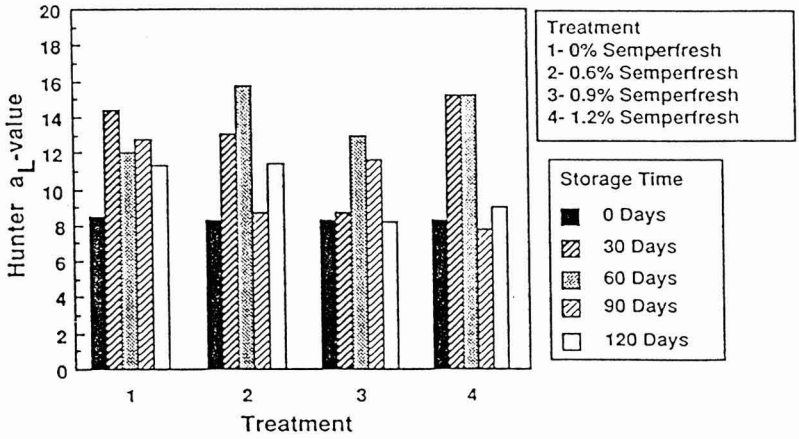

FIG. 2. INFLUENCE OF SEMPERFRESH CONCENTRATION AND STORAGE INTERVAL ON THE EXTERNAL COLOR OF "MCINTOSH" APPLES

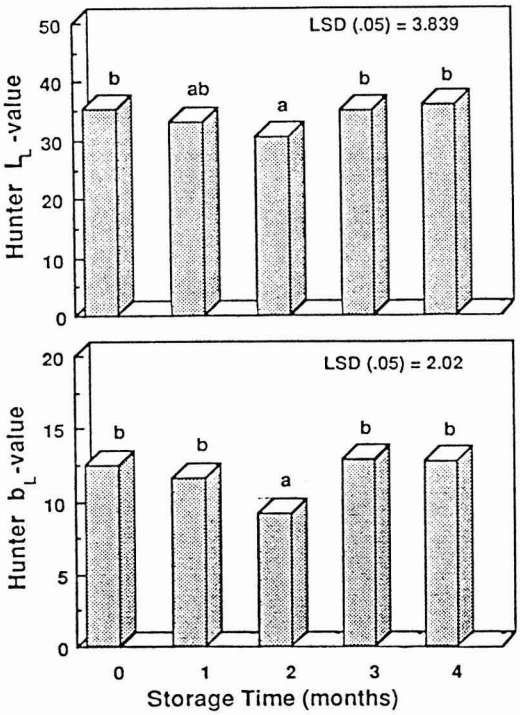

FIG. 3. INFLUENCE OF STORAGE INTERVAL ON EXTERNAL COLOR OF "MCINTOSH" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

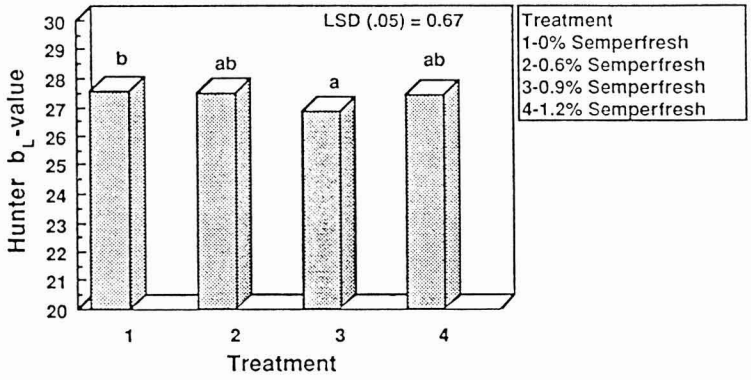

FIG. 4. INFLUENCE OF TREATMENT CONCENTRATION ON THE INTERNAL FLESH COLOR OF "GOLDEN DELICIOUS" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

angle appears to shift toward blue during the storage of "Golden Delicious" apples (Table 1).

External Hunter color of "Golden Delicious" apples was not influenced by Semperfresh concentration. External color did change during the 4 month storage interval. External Hunter L_L, a_L and b_L values increased during storage, indicating an increase in lightness and yellowness (Fig. 6). This may be due to the fact that Semperfresh was applied after the ethylene rise had begun. In practice, Semperfresh would be applied when the apples are immature but fully sized. The hue angle for the external color of apples shifted from redness (Table 1). This may indicate that Golden Delicious apples did not ripen during the 4 month storage interval.

Fruit Pressure Test

Semperfresh treatment maintained the firmness during storage of both "McIntosh" and "Golden Delicious" varieties. Increasing Semperfresh concentration resulted in decreased softening during storage (Fig. 7 & 8). The increase in firmness following Semperfresh treatment contrasts to the findings of Smith and Stow (1984). They applied the SPE, 'Prolong' to "Cox's Orange Pippin" apples prior to a 5 month storage interval at 3.5°C. They reported that a 1.25% formulation had no effect on the firmness, color or weight loss of stored apples. The difference in our results may be due to chemical differences between 'Prolong' and Semperfresh. The use of only six measurements with the Effegi penetrometer may have contributed to these findings.

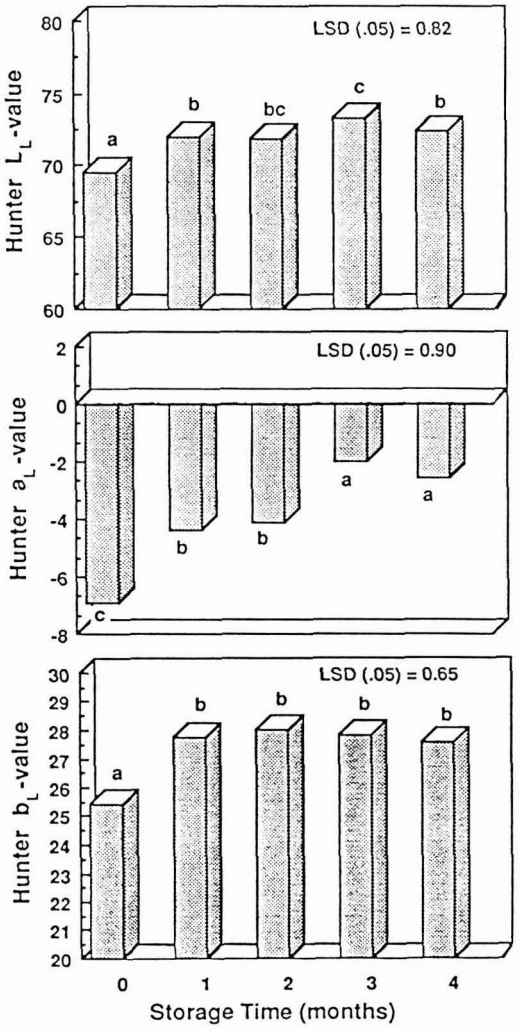

FIG. 5. INFLUENCE OF STORAGE INTERVAL ON THE INTERNAL FLESH COLOR OF "GOLDEN DELICIOUS" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

Soluble Solids

Soluble Solids were not affected by Semperfresh treatment for either "McIntosh" or "Golden Delicious". Soluble solids were affected by storage and decreased from 12.5% to 11.2% at 4 months, for "McIntosh" apples. Soluble solids of "Golden Delicious" apples increased from 12.6% to 13.4%

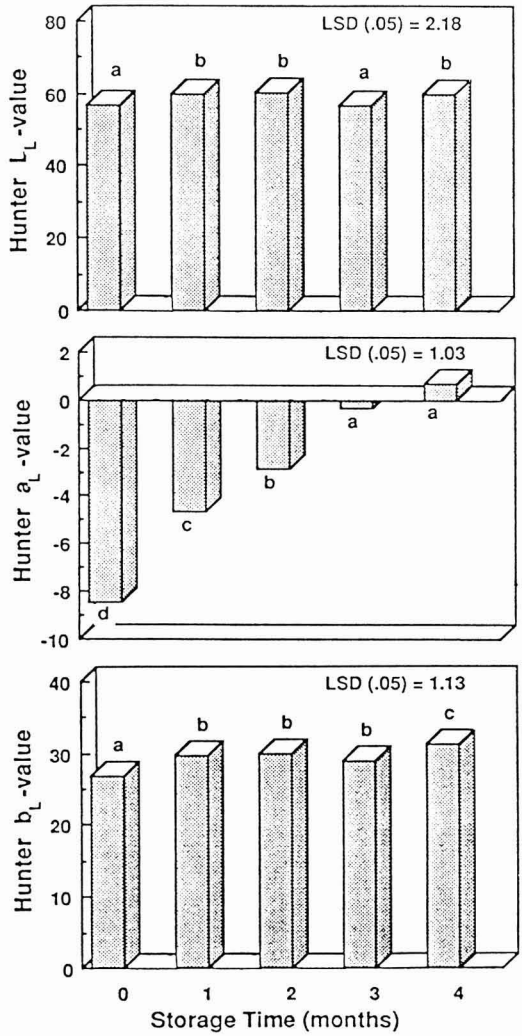

FIG. 6. INFLUENCE OF STORAGE INTERVAL ON THE EXTERNAL COLOR OF "GOLDEN DELICIOUS" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

after 2 months then decreased to 11.5% after 4 months. A decrease in soluble sugars can be attributed to respiration of carbohydrates as an energy source.

Total Acidity & pH

Semperfresh treatment did not influence pH or total acidity (TA) in either cultivar. During storage the TA increased for "Golden Delicious" apples from

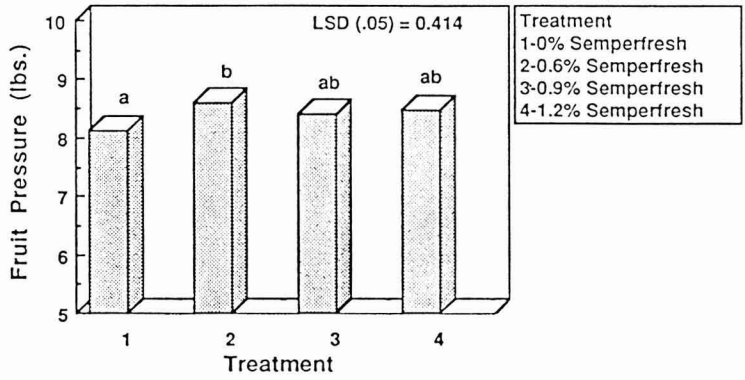

FIG. 7. INFLUENCE OF TREATMENT CONCENTRATION ON THE PRESSURE MEASUREMENTS OF "MCINTOSH" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

0.27% (0 months, pH = 3.3) to 0.30% (2 months, pH = 3.4). This was followed by a decrease to 0.19% (4 months, pH = 4.0). TA for "McIntosh" apples followed a similar pattern increasing from 0.38% (0 months, pH = 3.1) to 0.41% (2 months, pH = 4.1) then decreasing to 0.35% (4 months, pH = 3.7). These findings contrast with the results of Drake *et al.* (1987), who reported an increase in the acid content of Semperfresh treated apples. If Semperfresh delays ripening, then an increase in the malic acid content of treated apples is expected. The apples used in this study may have matured past the point where Semperfresh is normally applied and a significant change in acid content was not observed. Brown core was not detected in any apples during the study.

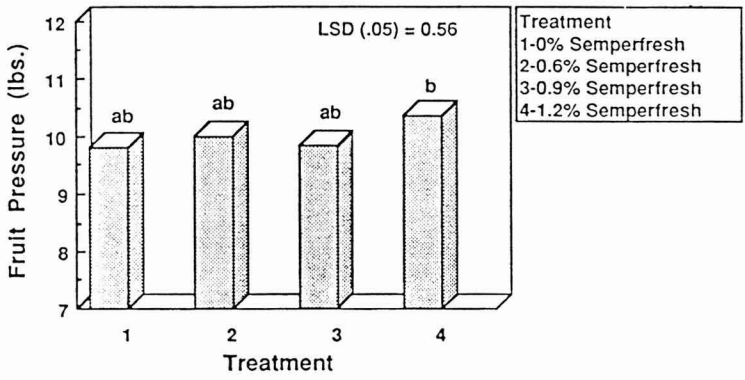

FIG. 8. INFLUENCE OF TREATMENT CONCENTRATION ON THE PRESSURE MEASUREMENTS OF "GOLDEN DELICIOUS" APPLES (Bars with the same letter (i.e., a, b, or c), are not significantly different at the $p = 0.05$ level.)

Sensory Evaluation

The triangle taste panel results indicated that the panel could not detect differences in flavor and texture resulting from Semperfresh treatment for either variety.

(a) **"McIntosh"**: An insignificant number (10 of 21 panelists) were able to pick the odd sample in the taste test. Three of the ten mentioned an objectionable flavor and one of the ten mentioned an objectionable texture.

(b) **"Golden Delicious"**: An insignificant number (11 of 22 panelists) were able to pick the odd sample. Three of the eleven mentioned an objectionable flavor with one of the eleven noting an objectionable texture.

Semperfresh is applied to the skin of apples and is not expected to be absorbed into the apple flesh. Treated apples which are properly washed will likely have very little Semperfresh residue following processing which would contribute to the final flavor of apple products. The main reason for using sensory evaluation in this study was to determine if Semperfresh treatment would cause anaerobic respiration in stored apples. Anaerobic respiration would greatly affect the flavor of stored apples and reduce the final product quality (Kader 1986). Our results indicate that Semperfresh applied at less than 1.2% will not cause significant flavor changes of "McIntosh" or "Golden Delicious" apples stored for 4 months in regular cold storage.

CONCLUSIONS

Beginning the study with ripened apples, Semperfresh treatment of "McIntosh" and "Golden Delicious" apples had significant effects on fruit quality during storage. Both cultivars had comparable texture to untreated apples following Semperfresh treatment. Semperfresh treated "McIntosh" apples exhibited slower ripening after bags were opened at 60 days. No significant influence by Semperfresh treatment on external color of "Golden Delicious" apples was noted, however, internal color measurement indicated that ripening was slowed by chemical treatment. Internal quality measurements for pH, soluble solids and TA were not affected by Semperfresh treatment. The dramatic influence of Semperfresh on these parameters, reported by other researchers (Drake *et al* 1987), may have been reduced by the advanced maturity of apples prior to treatment. Results of the triangle taste panel conducted on both varieties showed that no significant flavor or textural differences were detected between control apples and apples treated with 1.2% Semperfresh when both were stored for two months.

ACKNOWLEDGMENTS

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EFFECTIVENESS OF ORGANIC CHELATORS IN SOLUBILIZING CALCIUM AND ZINC IN FORTIFIED CEREALS UNDER SIMULATED GASTROINTESTINAL pH CONDITIONS

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ABSTRACT

The effectiveness of various chelating agents on the solubilization of zinc and calcium, in a calcium-fortified wheat flake cereal, has been evaluated using an in vitro method. This method is based on solubility during a sequential pH treatment simulating gastrointestinal conditions (endogenous pH of cereal, to pH 2, to pH 7.5). Zinc oxide was added to the cereal at 100% of the U. S. RDA for zinc, alone or in combination with the following organic chelators: citrate, malate, a combination of citrate and malate, lactate and malate, citrate and lactate, glucose, histidine, cysteine, and a combination of histidine and cysteine. The complexation of zinc by citric acid in a 10:1 or 25:1 ligand:zinc molar ratio increased the solubility of both zinc and calcium as compared to the calcium-fortified cereal where zinc was added without ligand. Histidine, cysteine, and the combination of histidine and cysteine enhanced zinc solubility, but did not affect calcium solubility. The other ligands (malate, citrate and malate, lactate and malate, citrate and lactate, glucose) did not have a significant effect on zinc and calcium solubilization.

INTRODUCTION

Continuing health recommendations for fewer calories are leading to the necessity of increased nutrient density in food. If this is to be achieved through

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fortification, it is essential that the nutrients added have the potential for absorption. Fortified cereals have been major contributors of iron and vitamins for some time, and it is expected that zinc might also be added (Food and Nutrition Board 1974). However, it has been reported that minerals ingested simultaneously in a fiber containing meal may interact with one or more other compounds to form insoluble complexes (Platt and Clydesdale 1987). In order to prevent this occurrence, compounds might be added to the fortified food to complex the mineral in question (Clydesdale 1988). Complexation of metal ions by chelation also plays an important role in the absorption of minerals by maintaining their solubility under conditions where they would otherwise be insoluble. Several ligands have been found to increase the availability of minerals via this effect such as: citric, lactic, tartaric acids, amino acids, peptides, and simple sugars (glucose, fructose), EDTA, and other synthetic chelates (Kratzer and Vohra 1986).

Kojima *et al.* (1981) observed that the combination of ascorbic and citric acids lead to the solubilization of 70% of the iron content of cooked pinto beans. The ligand:iron molar ratio used in that study was rather high (290:1). Nadeau and Clydesdale (1986) found that a 10:1 citrate:iron molar ratio promoted in vitro solubilization of iron. Gillooly *et al.* (1983) noted an improvement of the geometric mean of iron absorption by human subjects from a basic rice meal following the addition of citric, ascorbic, malic, and tartaric acids. Lyon (1984), from in vitro studies, found that citric acid was very efficient at solubilizing zinc and iron, but was somewhat poorer at solubilizing calcium and magnesium. However, when the citrate:calcium ratio was estimated, it was found to be about 300:1, an impractical amount due to flavor problems. Lyon (1984) also reported that histidine partially solubilized zinc, but had no effect on iron, calcium, and magnesium, while Scholmerich *et al.* (1987) have shown histidine to improve zinc absorption in animals at a histidine:zinc molar ratio of 0.08:1 or 0.5:1.

The reducing potential of cysteine, along with its role as a ligand, generated substantial amounts of ferrous ion (pH 2) at the apparent expense of complexed iron in a wheat flake cereal subjected to a sequential gastrointestinal pH treatment from endogenous pH, to 2, to 6 (Nadeau and Clydesdale 1987). By using an in vivo procedure on ileal segments of adult rats, Wapnir *et al.* (1983) showed that ligand:zinc ratios equal to or less than 3:1 of L-glutamate, glycine, L-histidine, L-tryptophane, and glycylglycine were otpimal for zinc absorption. An excess of ligand reduced zinc absorption. Using the triple-lumen intenstinal perfusion technique in human subjects, Bei *et al.* (1986) obtained a fourfold net increase in calcium absorption when a solution containing 4 mM glucose polymer was perfused in the jejunum over that found when no glucose polymer was present. In addition, the administration of glucose polymer doubled net zinc absorption.

With the advent of multi-mineral fortification in food and the potential for formation of unavailable (poly)mineral-(poly)ligand complexes it is essential to

establish conditions which might counteract such effects. Therefore, this study was initiated to evaluate the feasibility of using various chelating agents, such as citrate, malate, lactate, glucose, histidine, and cysteine, alone or in combination, to prevent the formation of insoluble Ca-Zn ligand complexes which have been reported in systems, such as cereals, containing phytate, proteins, and fiber (Mills 1985; Platt and Clydesdale 1987).

MATERIALS AND METHODS

Cereal Sample

An experimental pilot plant batch of wheat flake cereal was obtained, courtesy of General Mills, Inc. It was fortified during the manufacturing process with calcium (tricalcium and dicalcium phosphate) at 20% of the U.S. RDA/28 g cereal, and iron at 100% U.S. RDA/28 g cereal. As well, it contained whole wheat, sugar, salt, cereal malt syrup, vitamin C, vitamin E, trisodium phosphate, niacinamide, iron, annatto extract, color, vitamin A, B_6, B_2, B_1, B_{12}, and folic acid. Prior to analysis, the wheat flakes were ground in a food blender (Osterizer, Galaxie), and those particles passing through a sieve of 500 μm analyzed.

Reagents

All reagents were of analytical grade, and solutions were prepared with double distilled, deionized (DDD) water. All glassware was acid-washed with concentrated HCl and thoroughly rinsed with distilled water.

Zinc oxide (certified ACS, dry process) was obtained from Fisher Scientific Co. (Medford, MA). Citric acid (free acid anhydrous), L−(+)−lactic acid (30% aqueous solution, grade L-111), L-histidine (hydrochloride, monohydrate), and L-cysteine (hydrochloride, monohydrate) were obtained from Sigma Chemical Co. (St. Louis, MO), all were stored in a dessicator, with the exception of L−(+)−lactic acid which was refrigerated. Dextrose (Certified ACS, anhydrous) and malic acid (purified) were obtained from Fisher Scientific Co. (Medford, MA).

Atomic Absorption Standards

Standards of 1.00 ppm zinc and 5.00 and 10.00 ppm calcium were prepared from 1,000 ppm zinc and calcium Fisher Certified Atomic Absorption Standard Reference Solutions (Fisher Scientific Co., Medford, MA). A 0.5% lanthanum chloride solution ($LaCl_3.7H_2O$, Fisher Certified) was included in all calcium analyses to correct for possible phosphate interference.

Ligand:Zinc Molar Ratios

Zinc was added to cereals at 100% of the U.S. RDA per serving (15 mg Zn/28 g cereal). The following ligand:zinc molar ratios used were based on previous reports in the literature (Wapnir *et al.* 1983; Bei *et al.* 1986; Nadeau and Clydesdale 1987):

—Control: Zn at 100% of the U.S. RDA, no ligand added;
—Citrate:Zn (3:1), (10:1), (25:1);
—Malate:Zn (10:1);
—Citrate:malate:Zn (2:3:4);
—Citrate:lactate:Zn (2:3:4);
—Lactate:malate:Zn (2:3:4);
—Glucose:Zn (20:1);
—Histidine:Zn (3:1);
—Cysteine:Zn (3:1);
—Histidine:cysteine:Zn (1.5:1.5:1).

Preparation of Ligand Complexes

Aqueous solutions of zinc oxide were prepared with each of the ligands or combination of ligands. The amount of zinc oxide added was calculated to provide 2.5 mg Zn/4.7 g cereal (100% U.S. RDA/28 g cereal) when 1 mL of the ligand:zinc solution was added to the cereal. After thorough stirring (30 min), the pH of the ligand:zinc solutions was brought to 2.0 by dropwise addition of 6N and 1N HCl to ensure complete solubilization of zinc oxide.

Sequential pH Adjustment

To a solution of zinc oxide alone, or zinc oxide with ligand(s), was added a slurry containing 4.7 g cereal in 50 mL water. The mixture was stirred for 10 min, and to simulate gastrointestinal pH conditions, the pH was adjusted to 2.0 by dropwise addition of 6N and 1N HCl, under magnetic stirring. The slurries were then incubated at 37°C in an agitating water bath for 30 min. The pH was then raised to 7.5 by dropwise addition of 6N and 1N NaOH, under magnetic stirring, and the samples reincubated. The slurry was then transferred to a 100 mL volumetric flask and made to volume with water.

Total Zinc and Calcium

For total mineral analyses, duplicate 10 mL aliquots of the 100 mL sample slurry were pipetted, while stirring, into separate 100 mL digestion flasks containing 20 mL of concentrated HCl and 3 boiling beads, and left overnight. The following day, they were digested in boiling concentrated HCl for 30 min,

allowed to cool, filtered through ashless filter paper (Whatman No. 40) into 100 mL volumetric flasks, and made to volume with water.

Soluble Zinc and Calcium

Duplicate 30 mL aliquots of sample slurry were centrifuged at 3335 Xg for 20 min (International Equipment Co., IEC model K, Damon, Needham Hts, MA) and 10 mL samples of the supernatant were treated as described above.

Atomic Absorption Procedure

Samples designed for total and soluble zinc analyses were aspirated directly into the Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer, model 372) after calibration with 1.00 ppm standard zinc solution, using a zinc specific hollow cathode lamp at a wavelength of 213.9 nm. For total and soluble calcium determinations, 5 mL aliquots were pipetted into 25 mL volumetric flasks, containing 2.5 mL of a 5% stock solution of $LaCl_3$, and made to volume with water. Calcium determinations were performed after calibration with 5.00 and 10.00 ppm standard calcium solutions containing $LaCl_3$, using a calcium specific hollow cathode lamp at a wavelength of 422.7 nm.

Statistics

Data were analyzed by the Student's t-test (Steel and Torrie 1980). Significance was noted at the 95 and 99% levels.

RESULTS AND DISCUSSION

There are numerous reports in the literature which define the potential negative effects which calcium and zinc have on their respective bioavailability when added to food together. The reasons for such effects are often related to the formation of an insoluble zinc-calcium ligand complex (Clydesdale 1988). In order to be efficiently absorbed, a mineral must come in contact with all parts of the intestinal mucosa (Ashmead *et al.* 1985) which requires that it be soluble. Calcium is absorbed by active transport and passive diffusion. It is absorbed only when present in a water-soluble form in the intestine and not precipitated by another dietary constituent (Czajka-Narins, 1984). Zinc appears to be associated with a low molecular weight ligand such as citrate. In this form, it is translocated across the cell membrane by a carrier-mediated system. When given as an aqueous solution, mean absorption ranges from 43 to 69%, whereas it is only 14 to 42% when consumed with foods (Czajka-Narins 1984). Enhancing factors for calcium and zinc absorption may act through solubilization and ion-

ization of the mineral, which facilitate passive diffusion, formation of mineral-ligand complexes, which prevent the precipitation of insoluble calcium or zinc complexes, or slowing down of transit in the gastrointestinal tract, allowing more time for mineral absorption (Czajka-Narins 1984). Solubility, in reference to the bioavailability of a mineral, not only refers to the solubility of an ion, salt, hydrate or complex, but also to the type and strength of chemical bonds involved with those species (Clydesdale 1988). Also, as food moves throughout the gastrointestinal tract, environmental conditions of pH, concentration of the reaction species, and nature and concentration of digestive enzymes may affect the bioavailability of minerals. Although the solubilization of minerals is not the sole factor it certainly has a role to play in the potential availability of minerals from a food.

Citric acid is a known chelator of divalent cations. Recent research has suggested that citric acid, in its capacity as a low molecular weight zinc-binding ligand, could be responsible for the efficient absorption of zinc from human milk (Casey *et al.* 1981; Martin *et al.* 1981; Fransson and Lonnerdal 1982; Hurley and Lonnerdal 1982). In this study, where citric acid was complexed with zinc prior to addition to calcium supplemented wheat cereals, the solubility of both zinc and calcium was enhanced ($P < 0.05$) at citric acid to zinc molar ratios of 10:1 or greater at the end of a simulated gastrointestinal sequential pH adjustment (Fig. 1). This dependency on the relative concentration of citrate:mineral has also been noted with iron in several other studies as noted by Clydesdale (1988). In the case of iron, it has been suggested that the increased concentration of citrate causes a non-polymerizable citrate complex to form, rather than one which polymerizes and precipitates (Spiro *et al.* 1967). However, the addition of 1 g of citric acid in a simple Latin American-type of meal reduced iron absorption by human subjects to about one-third of the control although no explanation could be given (Hallberg and Rossander 1984).

Citric acid caused a relatively greater solubilization of zinc (4-fold increase) than of calcium (2.5-fold increase) (Fig. 1). Similarly, in vitro studies carried out on cereals have shown that citric acid was very efficient at solubilizing zinc, but was poorer at solubilizing calcium (Lyon 1984). In that case, however, the molar ratio of citric acid to zinc was estimated to be greater than 300:1. Citric acid seems to have a stronger affinity for zinc than for calcium, probably as a result of the ability of transition elements to form coordination compounds with ligands (Kratzer and Vohra 1986). The effectiveness of citric acid to chelate zinc and calcium, even after keeping the pH at 7.5 for 1h, is probably due to ionization of the carboxyl groups at that pH value (Kojima *et al.* 1981; Leigh and Miller 1983). Those results, however, should not be directly extrapolated to humans. Rumenapf and Schwille (1987) obtained a decrease in intestinal calcium absorption of healthy men when citrate was administered orally at a citrate:Ca molar ratio of 4:1, the Ca-citrate complex being less absorbable than ionized calcium.

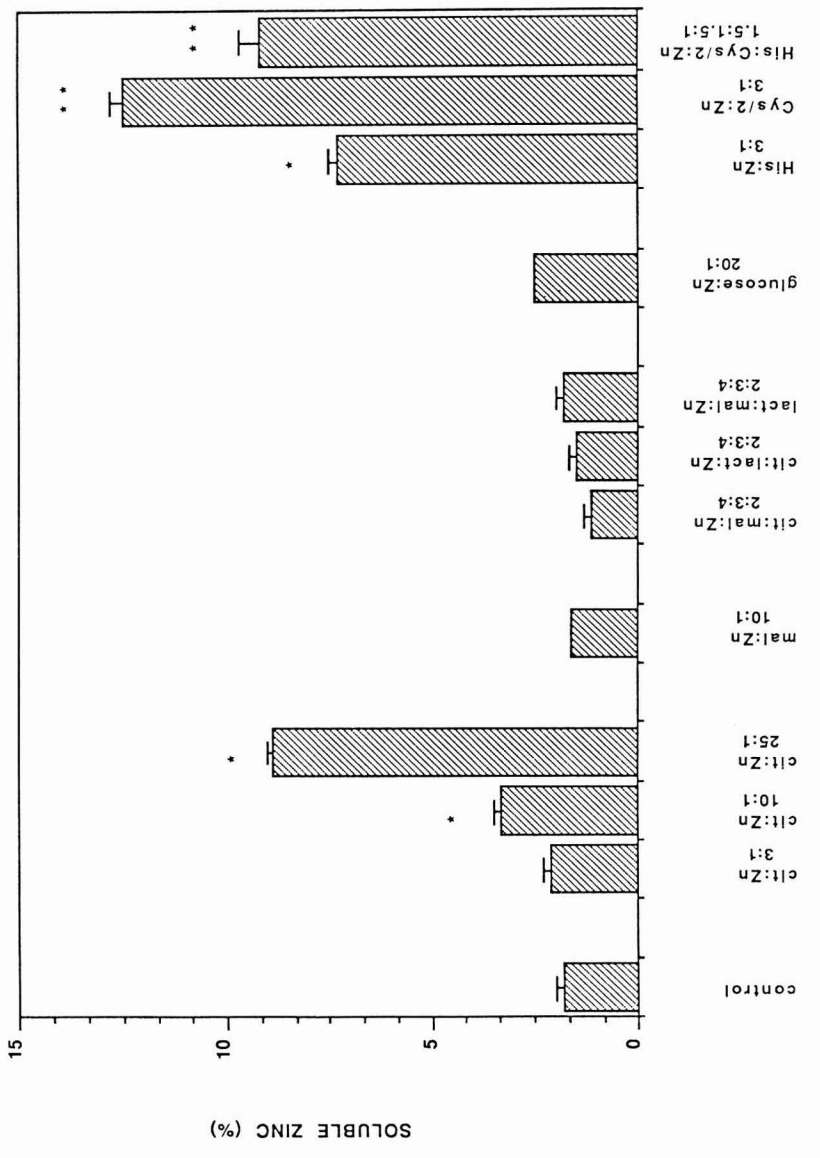

FIG. 1. EFFECT OF VARIOUS LIGANDS ON THE PERCENTAGE OF SOLUBLE ZINC IN A FORTIFIED WHEAT FLAKE CEREAL (± standard error)
* Significantly different from control ($P < 0.05$).
** Significantly different from control ($P < 0.01$).

Malic acid has been shown to complex iron (molar ratio 10:1) in cereals during an acid incubation period of 1h, in a form which was stable through neutralization (pH 6) (Nadeau and Clydesdale 1986). A molar ratio of 10:1 (malate:zinc) was also chosen in this study because it might be found in food fortification programs, and because it was effective with citrate in promoting calcium and zinc solubilization. Unfortunately, malic acid, unlike citrate, was not effective at this ratio (Fig. 1 and 2). This could be due to a difference in the chemical structure of the ligands, with citric acid having three ionizable groups and malic, only two, and/or to differences in pK values, and/or to differences in bond strengths.

Gorman and Clydesdale (1984) have shown that the stability constant for citrate and malate was higher than lactate for ferric and ferrous ascorbate complexes, indicating that the strength of association between iron and citrate or malate was greater than that between iron and lactate. Therefore, citrate and malate would be more potent than lactate in maintaining the complex in soluble form. It was postulated that zinc and calcium solubility might be enhanced by the combined use of chelating agents to complex zinc. However, the combination of citric and malic acid in our study did not significantly improve the solubilization of zinc or calcium, nor did the combined use of citrate and lactate, or malate and lactate with zinc in 2:3:4 molar ratio, have any beneficial effect on zinc and calcium solubilization (Fig. 1 and 2). It could be that the molar ratio of ligands to zinc chosen was not appropriate to facilitate the formation of a stable soluble complex of zinc to ligands.

Bei *et al.* (1986) demonstrated that the presence of glucose polymers in the jejunum enhanced calcium, magnesium, and zinc absorption in humans. Wood *et al.* (1987) have also shown that glucose polymers improved the intestinal absorption of calcium in humans, and attributed that effect to the hydrolysis of polymers to glucose by intestinal enzymes. In the present work, glucose complexed with zinc (20:1 molar ratio) caused an increase in zinc solubility (Fig. 1), although that effect was significant only at $P < 0.1$ Glucose:zinc complex did not significantly modify calcium solubility (Fig. 2). Although it is always very hazardous to compare in vivo results with in vitro, we may suggest that the differences observed could be due to the use of an inappropriate ratio or to a mechanism which is unrelated to complexation. It is obvious that in vitro studies will never reproduce the in vivo situation in all its complexity.

There is no question that amino acids play an important physiological role in zinc solubility. A small percentage of zinc in the body is bound to amino acids, more specifically histidine and cysteine, which are involved in the transport of this cation (Freeman and Taylor 1977). Wapnir *et al.* (1983) examined the role of certain amino acids as ligands to facilitate the intestinal absorption of zinc by ileal segments of adult rats. They found that at a 130:1 histidine:zinc molar ratio, the absorption of zinc was less than one quarter that obtained at a 3:1 ratio. The

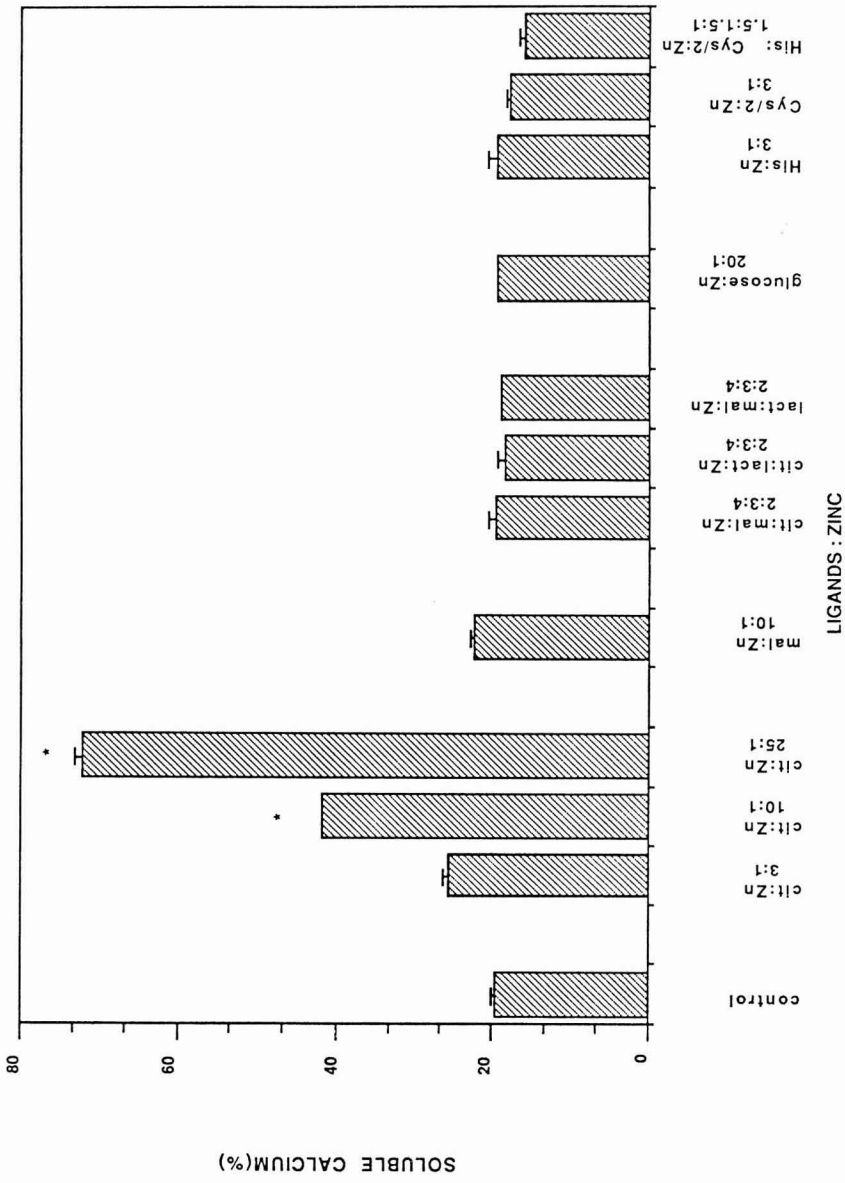

FIG. 2. EFFECT OF VARIOUS LIGANDS ON THE PERCENTAGE OF SOLUBLE CALCIUM IN A FORTIFIED WHEAT FLAKE CEREAL (± standard error)
* Significantly different from control ($P < 0.05$).

absorption of zinc in the presence of L-histidine was maximal at near physiological pH (7.5). At lower pH, there was no significant association with the chelator, since most of the zinc remained free and unionized.

The results of this study show that a 3:1 histidine:zinc molar ratio significantly increased ($P < 0.05$) zinc solubility after a sequential pH adjustment to 2 and to 7.5 (Fig. 1). However, the solubility of calcium was not improved by adding histidine (Fig. 2).

Snedeker and Greger (1983) have observed a greater utilization of zinc by rats when fed a diet low in protein and supplemented with cysteine, or with a combination of cysteine and histidine, rather than when fed a low protein diet without added ligand, or a diet low in protein with added histidine. The results obtained in our study go in the same direction as those obtained in vivo by Snedeker and Greger (1983). Cysteine was found to be the most efficient ligand in increasing zinc solubility, although, like histidine, this amino acid did not affect clacium solubility. The combined use of cysteine and histidine at a 1.5:1.5:1 molar ratio with zinc caused a greater increase ($P < 0.05$) in the solubilization of zinc as compared to the single use of histidine (Fig. 1), but did not significantly affect the solubility of calcium as compared to the control (Fig. 2).

Cysteine constitutes a particularly stable ligand as it forms a five- or six-member chelating agent with zinc. Also, binding of zinc can occur either at the S or O sites, and according to Ashmead *et al.* (1985), the greater the number of rings, the greater the stability of the complex. Flynn *et al.* (1984), calculating stability constants in various forms of iron, demonstrated that cysteine stabilized iron in solution until approximately pH 7.4. It was suggested by Nadeau and Clydesdale (1987), in an in vitro study, that cysteine could hinder the oxidative hydroxide cross-linking process through the reducing properties of the thiol group, and by its sulfur-mediated iron complexation ability.

CONCLUSION

Among the various chelating agents tested, citric acid, when complexed with zinc in a 10:1 or 25:1 molar ratio, and added to a fortified wheat flake cereal, was found to enhance the solubilization of both zinc and calcium. Histidine, cysteine, and a combination of histidine and cysteine, increased the solubility of zinc, but not of calcium. The in vitro method used in this study, which monitored the solubility of zinc and calcium, should be considered only as an indicator of the proportion of those minerals potentially available for transport, and does not reproduce physiological conditions. It seems only logical to predict that as increased and necessary fortification proceeds in the future, it will be essential to use specific enhancing agents to prevent occurrences in the food

which would decrease bioavailability. In vitro screening procedures, such as those used in this study, will have to be developed to assess the efficacy of fortification and any intervention imposed by the food processor.

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

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