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EFFECT OF PROCESSING TEMPERATURES ON MICROBIOLOGICAL AND CHEMICAL QUALITY OF SOY MILK PRODUCED BY RAPID HYDRATION HYDROTHERMAL COOKING

PRAVATE TUITEMWONG[1], LARRY E. ERICKSON[1,3]
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ABSTRACT

Soy milk samples produced from 11% soybean slurry cooked by the rapid hydration hydrothermal cooking (RHHTC) process at temperatures from 105–157C for 24–90 s were studied for their sterility, microbiological quality, flatulent sugar reduction, and physical characteristics. Soy milk produced at the temperature of 132C, or higher, for 29 s or longer was sterile. All of the bacteria (Pseudomonas aeruginosa, Serratia lequefaciens, S. rubidaea, *and* Enterobacter cloacae *including the bacilli cells and spores) were destroyed completely. At lower temperatures, 105–127C, the Gram negative bacteria were destroyed; however, the spores of* Bacillus licheniformis *and* B. cereus, *Gram positive bacteria survived the heat. Temperatures of 143–157C with a retention time of soy slurry solids in the holding tube of 30 s or longer are recommended to sterilize the product. The mean residence time of soy solids processed by the RHHTC unit was 30 s.*

INTRODUCTION

Heat treatment is important for the utilization of soybeans as human foods. Heat destroys antinutrients such as trysin inhibitors, goitrogenic substances, phytohemeagglutinins, and phytate. Thermal processing plays a major role in convert-

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ing soy protein to more easily digestible forms (Liener 1958; Hong 1989) and destroying the contaminating organisms present in the beans (Buono 1988). This enables soybean products, such as soy milk and tofu, to be sterile and have a longer shelf-life. The microbiological safety of the soy products is established by following an appropriate heating process.

Heating modifies soy proteins, permitting more complete digestibility and utilization of the desirable sulfur-containing amino acids (Liener 1958; Hong 1989; Longenecker *et al.* 1964). Severe heat treatment, however, causes less availability of proteins and amino acids (Liener 1958; Angeles and Marth 1971).

There have been many attempts to exploit heat treatment of soybeans to create more acceptable products, increase yields, and reduce beany flavors and flatulence associated with raffinose and stachyose (Johnson 1978; Hung 1984; Hong 1989). Wilkens *et al.* (1967) proposed a high temperature, rapid hydration grinding for dehulled soybeans. The method is presently known as "hot grind" method. Temperatures of 80–100C were used with a holding time of at least 10 min to complete the destruction of lipoxygenase, the enzyme responsible for beany off-flavors.

Johnson and Snyder (1978) soaked soybeans for 15–20 s and blanched them for 30 min with 0.5% sodium bicarbonate at 95–100C before grinding. Soy milk samples were prepared from the heat beans and compared in quality and yield to those produced from soybeans soaked in tap water and 0.5% sodium bicarbonate solution. The blanched soybeans were "cold" ground at 20–25C, while the cold soaked soybeans were "hot" ground with boiling water. To recover the protein loss due to heating (Bourne 1970), a homogenizer was used. Blanching the soybeans caused considerable loss of solids even though homogenization was applied. Hot grinding gave higher solid yields than that of the blanched sample, but less than the unheated control. This was also true with the proportion of protein and lipid. The treated samples gave higher carbohydrate content than the control.

Soy milk can be made from whole grain soybeans. Nelson *et al.* (1976) has made use of the heating of undisrupted soybeans in the "Illinois Process". By this method, soybeans were soaked in tap water and then blanched in 0.5% sodium bicarbonate solution for 30 min. The beans were ground and forced through 0.25 and 0.028 in. screens before being heated to 94C. The slurry was homogenized at 3500 and 500 psig to reduce the particle size. The product was formulated with sucrose, salt, and flavor. It was reheated and rehomogenized before it was packaged. Heating the whole grain causes excessive loss of protein (Johnson and Snyder 1978; Bourne 1970). However, the authors reported proper homogenization after bean disruption ensures good quality and yield of the product (Johnson and Snyder 1978).

Soaking or blanching soybeans in 0.5% sodium bicarbonate, for 5–10 min, readily destroyed trypsin inhibitors in the product (Nelson *et al.* 1976). In collaboration with the inventing group, the Illinois process was modified (Priepke *et al.* 1980). After being soaked, soybeans were blanched in 0.25% sodium bicarbonate solution instead of 0.5% solution. The grinding processes were similar except the screen pore size was 0.023 in. The homogenization is a one-step homogenization at 3500 psig. A more stable product was claimed.

Soy milk can be made either from soaked or dry soybean grains. Grinding the beans with boiling water to eliminate the hydration step, which is believed to be responsible for the formation of beany flavors, was recommended (Steinkraus and Hackler 1966). "Rapid Hydration Hydrothermal Cooking", RHHTC, was used to produce soy milk from soy flour (Johnson 1978; Johnson *et al.* 1981). The authors claimed that more than 90% of trypsin inhibitors was destroyed. Soy flour was mixed with water to obtain the desired solid content before being fed to the direct steam injection tube and held in the holding tube for 20–120 s at temperatures ranging from 121–157C. The soy milk product was cooled in a heat exchanger. All of the solid and protein were incorporated into the soy milk product. The yields (per unit volume) were as high as 90% for both solids and protein. The maximum yield of solids and protein were obtained from the sample treated at 154C for 40 s. The high yields of soy milk were associated with several chemical and physical phenomena. Optimum heat treatment and the extreme shear force associated with steam injection and flashing are probably the causes (Hong 1989; Johnson 1978).

Beany flavor has been the major obstacle to the acceptance of soybean products by many western consumers. Heating at 80C for 30 min can destroy the enzyme lipoxygenase, which generates hydroperoxides leading to the formation of beany flavors (Blase 1990). There have been numerous reports claiming that heat can reduce the extent of beany flavors (Alsoe and Adler-Nissen 1988; del Rosario *et al.* 1984). Hence, reduction or elimination of beany flavors is possible. Further, steaming is used as a part of deodorization in soybean oil refinement (Shyder and Kwon 1987). With the abrupt increase of the temperature, the enzyme has less time for its reaction. Steam injection was introduced to fulfill the requirement of fast heating, which rapidly destroys the lipoxygenase enzyme. The RHHTC process increases the temperature of the soy slurry up to the steam temperature in a few seconds. The mix of slurry and steam is held at the specified temperature for a certain time, ranging from 20–200 s, before it is flash evaporated to reduce pressure, and, hence, the temperature to less than 100C. Further cooling can be added in order to reduce the risk of nonenzymatic browning. A high quality soy milk can be obtained (Johnson 1978).

Flatulence is a concern of bean consumers. It has been reported that raffinose

and stachyose may cause flatus gas formation. The concentrations of the sugars are higher in soybean than other legumes. The details are mentioned elsewhere (Hong 1989; Rackis 1978). Human enzyme systems in the small intestine do not contain α-galactosidase, which can cleave the α-1,6 linkage of raffinose and stachyose. Thus, the sugars pass through the small intestine to the large intestine, where the bacterial fermentation of these sugars occurs and the flatus gases are produced. The effects of soybean oligosaccharides on human digestion have been reviewed (Rackis 1978).

The bacterial spores present in soybeans and soybean flour are heat resistant (Buono *et al.* 1990). Heating at 100C for 5 min or 110C for 1 min is apparently not sufficient to completely destroy the bacterial spores. Autoclaving soy milk at 121C for 15 min in order to obtain a sterile soy milk has been recommended (Buono *et al.* 1990). Tuitemwong and Fung (1991) reported the short shelf-life of tofu products might be caused by bacteria originally present in the soybeans.

High shear and heating have a high potential to be beneficial in the destruction of both bacteria and undesirable oligosaccharides. Therefore, the objectives of this study were to observe the effects of heating in the RHHTC process on microbiological and chemical qualities and the reduction of flatulent sugars of soy milk. The isolates were identified as to genera and species in order to determine the effective temperature and time for the production of soy milk. The concentration of sugars in soy slurry and soy milk such as galactose, fructose, melibiose, sucrose, raffinose, and stachyose were analyzed by the HPLC method. Total solids and physical properties of the soy milk and soy milk products were monitored. The effective operating conditions were identified.

MATERIALS AND METHODS

Soybeans and Dehulled Soybean Flour

Certified seed grade soybeans, Williams, grown in Kansas from the crop of 1989 were used throughout the study. They were purchased from the Department of Agronomy, Kansas State University, and stored in a clean dry place at room temperature. The beans were passed through a rough, corrugated roller, which had a speed differential of 2.5. The distance between the rollers was set at about ½ to ⅔ of the seed diameters. The cracked beans were collected and separated from the hulls by passing them through a Kice separator (Kice Metal Products Co., Wichita, KS). The vacuum pressure of 0.8 psig was used to separate the light weight hull from the higher density bean flakes. The partially dehulled soybean flakes were fast ground using a Fitz hammermill equipped with a 0.51 mm (0.02 in.) screen. Soybean flour was distributed and packed into small plastic bags and stored in a freezer (−10C) until use.

Rapid Hydration Hydrothermal Cooking (RHHTC) Process

The rapid hydration hydrothermal cooking process (RHHTC), Fig. 1, was developed at Kansas State University (Johnson 1978). The steam injection cooker is comprised of a screw feeder with a Moyno pump adjusted to the speed of 40 rpm for feeding soy slurry to mix with the steam at the hydroheater valve with the pin valve fully opened (Fig. 2). The mixture of the steam and soy slurry is passed through the holding tube, which is a 37 foot-long, standard 1 in. diameter stainless steel tube with ID 1.049 in. It is well-insulated to ensure the efficiency of the process and a steady temperature of the mixture in the tube. The heated solution exits through a flash evaporator/cooler where the pressure is reduced from 40–70 psig to atmospheric pressure. Consequently, the temperature is reduced from the two phase equilibrium processing temperature to less than 100C. The mean holding time is dependent on the flow rates, back pressure, and desired temperature of the mix. It ranged from 20–200 s. For starting-up, the system was allowed to reach steady-state under continuous operation using tap water in place of the soy slurry. The temperature and pressure are controlled by the back pressure valve at the outlet of the holding tube. After the desired steady state was achieved, the process was left running with water as the medium for at least 15 min to presterilize the equipment before soy milk was made (Tuitemwong 1992).

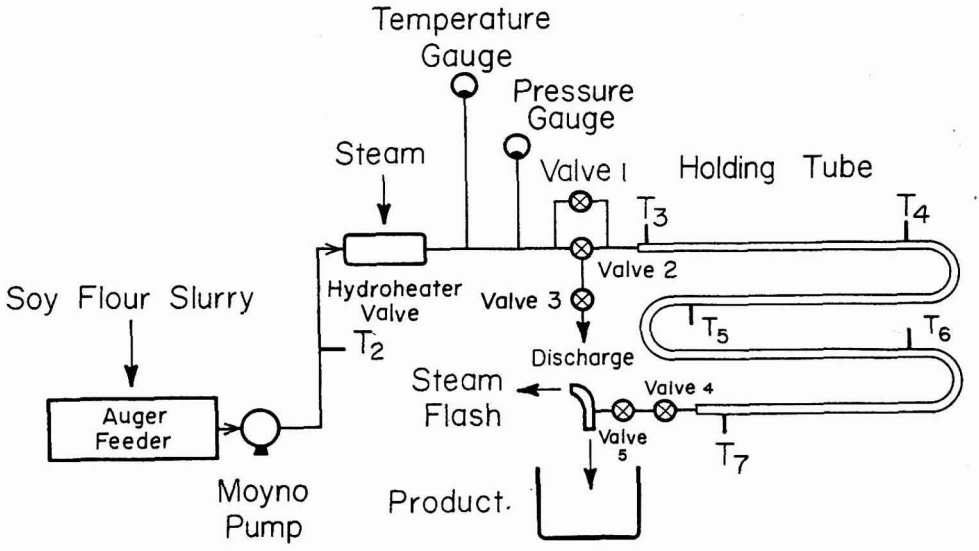

FIG. 1. RAPID HYDRATION HYDROTHERMAL JET COOKER AND FLASH COOLER (Johnson 1978).

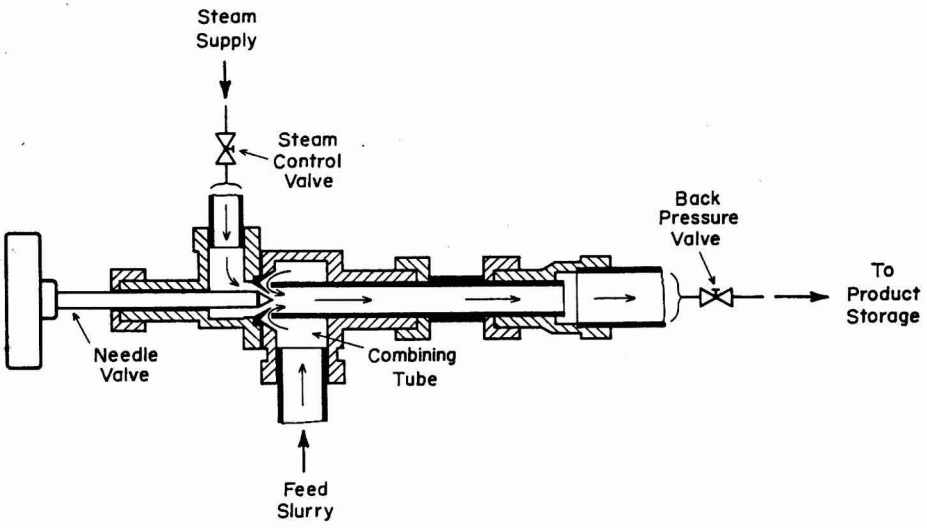

FIG. 2. DESIGN OF THE HYDROHEATING VALVE
(Johnson 1978).

Flow Rate Measurement

The volumetric flow rate of the RHHTC process was determined by measuring the amount of soy milk product produced per unit time, ml/s. The system was operated at 152C for 10 min to reach its steady state. The, 1.5 L of soy milk was collected and the elapsed time was recorded. The measurement was done in triplicate.

The flow rate of the liquid driven by the Moyno pump alone was also measured. Initially, both the Moyno pump and steam were used to set up the operation at 152C; then, the pump was stopped to let the steam clear the liquid inside the holding tube. Then the steam supply was closed and the pump was used to feed the liquid to the holding tube. Time of the breakthrough of the liquid was recorded. The system was run for 10 min to obtain a steady state operating condition; then, the volumetric flow rate of the liquid was measured. Soy slurry was fed to the pump intermittently as a step tracer to observe the breakthrough time of the soy solids. The time for transferring the soy slurry solids from the pump to the flash cooler was determined as the solid retention time (without steam) of the process.

Residence Time Distribution

The RHHTC process was run at 152C. Soy slurry (11% solids) was used as tracer: it was fed to the system using a step change in concentration from water

to soy slurry. The hot soy milk coming out at the flash cooler was collected 10 s after the slurry was fed to the pump and every 10 s later for a total of 140 s. The liquid samples were analyzed for their solid content. The distribution of the soy slurry solids was observed by plotting total solids of each sample against elapsed time following the step change.

RHHTC Soy Milk Preparation

Soybean flour, 11%, was mixed with hot water, 95–98C, in a blender at high speed for 30 s. The system was operating at the desired conditions using tap water as a medium. The slurry samples were heated at 105–157C by the high pressure steam (max. = 70 psig). The mixture was held in the tube for 20–120 s, depending on the operating conditions. Soy milk products were collected hot at the outlet with a sterile container. The soy milk was cooled to room temperature before use.

Total Solids Analysis

Approximately 10 ml of soy slurry or soy milk were placed into preweighed aluminum cups and weighed. The cups were placed in an oven at 105C for 24–48 h (AOAC 1984). The samples were removed from the oven and placed in a desiccator to cool them to room temperature (25C). Then they were weighed on the same four-digit balance. The analysis was done in triplicate.

Viscosity

Two hundred ml of each soy milk sample were equilibrated to room temperature, 25C. They were analyzed for their viscosity by using a Brookfield synchrolectric viscometer model VS 4 (Brookfield Engineering Laboratories, Stoughton, MA). The measurement was made with spindle numbers 2 and 3 depending on the range of the viscosity. The spindle was allowed to rotate for 10 rounds before the reading was made.

Carbohydrate Analysis

Carbohydrates in the soy slurry and soy milk were extracted from the samples and prepared for analysis by the method described elsewhere (Buono 1988; Kwak and Jeon 1986; Tuitemwong 1992). Ten grams of a sample were weighed into a 25 ml volumetric flask. Isopropanol was added to make a final volume of 25 ml. The mixture was mixed well and allowed to stand at room temperature for 20 min. Then the soy slurry or soy milk-alcohol mixture was transferred into a 70 ml centrifuge tube. The precipitated protein was separated by centrifugation

(Domon/IEC Division B-20A) at 5,000 rpm for 5 min at room temperature. The supernatant was transferred into a Buchner filter equipped with a 5.5 cm diameter Whatman # 50 filter paper. The filter unit was used along with the aid of a suction pump (Kenberger Inc. model N726 TTP SN 3465) to generate the vacuum condition in the flask and draw the liquid through the filter paper into a receiving tube in the flask. The filtrate was passed through a syringe connected with a sep pak C-18 cartridge to remove fat and pigment compounds. Each sep-pak cartridge was prewashed sequentially with 2 ml of methanol and 5 ml of distilled water. Subsequently, the filtrate was passed through the C-18 column. The first 2 ml of the sample filtered through the cartridge was used to wash the column, and was discarded. The next 5 ml of the filtrate was collected and, again, filtered through 0.45 μm, 3.5 cm diameter, Whatman filter paper to remove fine particles in the sample. An aliquot of 20 μl was injected into the Varian LC model 5000 high pressure liquid chromatograph (HPLC) using a loop injector connected with an autoinjector. The HPLC unit was equipped with a main column, Brownlee Amino Spheri 5 (Biosystem Co,), connected with 3 cm Bio-system guard column and 1 cm anion exchange column to filter the sample before passing to the main column. A mixture of 75% acetonitrile in aqueous solution was used as a mobile phase with a flow rate of 1 ml min. The refractive index detector was set at 8X attenuation. The reference cell of the refractive index detector was filled with the mobile phase, 75% acetonitrile, prior to the analysis. The calibration and zero adjustment of the detector were carried out. The strip chart recorder (Varian 9176) was set at a chart speed of 0.5 cm/min and span of 1 mV. To quantify the peaks, standard curves of galactose, glucose, fructose, sucrose, melibiose, raffinose, and stachyose (in an aqueous solution containing 60% ethanol) with the concentrations of 0.05, 0.1, 0.2, 0.5, and 1.0 g/100ml were established to permit the back calculation to the sugar concentrations in the samples using peak height comparison. The standard preparation was treated similarly to that of the samples. Each standard was injected at least 3 times to get an average of its peak height. The standard sugars were also analyzed at the beginning and in between the analysis of the samples to monitor the presence of any deviation of retention time and peak height. Peak height of each peak was manually measured in millimeters. The data from the standard sugars were plotted and used to generate a regression line or calibration curve for each sugar. Each peak height from the sample was measured to obtain the sugar concentration in the sample. The results were multiplied by a dilution factor of 2.5, which was calculated from 10 g of sample in total volume of isopropanol/soy milk solution of 25 ml.

Bacterial Enumeration, Isolation, and Identification

Soy slurry and RHHTC soy milk from each replication were diluted from 1:10 to $1:10^5$ by phosphate buffer pH 7.0. The serial dilutions were spiral plated us-

ing a Spiral Plating System (Spiral Systems, Inc., Bethesda, MD). The plates were incubated at 32C for 48 h before the enumeration was made. All plates were counted by using both a manual count and a laser counter to prevent errors that might occur due to the enumeration of the laser counter on plates containing mixed cultures. About 300 distinctive colonies from countable plates were randomly selected, reisolated and identified to assure purity of the cultures. All isolates were restreaked on plate count agar, PCA. Gram staining and catalase test were performed on every isolate. Gram negative bacteria were restreaked on TSA (Tryptic soy agar) and EMB (Eosin methylene blue) agar. The colonies grown on TSA and EMB agar were selected. All isolates were checked for the oxidase reaction. Oxidase negative colonies were identified by using Micro ID test kits (Organon Technica Co., Durham, NC), while the oxidase positive isolates were identified by Oxi/Ferm tube test kits (Hoffman-LaRoche Inc., Nutley, NJ). The identifications were obtained by comparing the results with those in the Micro ID manual and Oxi/Ferm manual, respectively.

Gram positive isolates were identified by using conventional biochemical testing media (Remel Co., Kansas City, KS); morphological tests were also employed. Colonies were identified by genus and species.

RESULTS AND DISCUSSION

Rapid Hydration Hydrothermal Cooking Process

Tables 1 and 2 show the temperatures and retention times of the RHHTC unit that was used to produce soy milk. The temperatures were in the range from 105–157C, while the mean retention times were in the range of 24–34 s. However, heating at 157C resulted in a longer mean residence time because it required a pressure of 70 psig, which is about the same as the maximum pressure of the steam source. Therefore, the back pressure valve was only slightly opened at this temperature causing a high resistance to flow and a longer holding time. At other temperatures, however, the flow rate could be controlled to achieve the desirable range of heat treatment. The temperature and pressure were highly correlated because of the two fluid phase mixture. However, the pressures of soy slurry-steam mixture read from the exit gauge were slightly higher than those from the steam table for the measured temperature.

Total volume of the holding tube was 6083 ml. With the slurry flow rate of 25 ml/s (operated at 152C), the mean residence time of the soy slurry solids in the holding tube should be in the range of 238 s (Table 1). However, the values from the tracer input experiment with steam injection showed that the breakthrough time was approximately 30 s after the slurry was fed to the pump. This observation shows that for two phase flow in the holding tube, more gas phase (steam)

TABLE 1.
OPERATING CONDITIONS OF THE RHHTC PROCESS AT 152C[a]

Total volume (pump to flash cooler), ft^3 (ml)	0.24 (6800)
Holding tube inside diameter, in (cm)	1.049 (2.66)
Cross sectional area, ft^2 (cm^2)	0.006 (5.4)
Flow rate (with steam), ml /s	25.60
Flow rate (without steam), ml /s	26.20
Solid retention time (without steam), s	260.00
Mean Residence time (with steam), s	30-40

[a] Values are the means of 3 determinations.

is present than liquid phase (slurry). When the steam line was closed leaving the Moyno pump to feed the slurry to the holding tube, the pressure reading was 8 psig at the outlet, and it took 196 s (3:16 min) to observe the breakthrough at the flash cooler. The measured flow rates of the liquid with and without steam injection were approximately the same with the values of 25.6 and 26.2 ml/s, respectively. The steam did not have a significant effect on the flow rate of the liquid. The flow of the steam created rapid mixing and rapid heat and mass transfer; thus, uniform conditions (or equilibrium) were observed after a short distance into the tube. Rapid steam condensation and fast heating of the slurry occur (Burton 1988).

Figure 3 provides information on the soy slurry residence time for the RHHTC process operating at 152C. The graph shows the concentration of the soy slurry solids as a function of elapsed time from the start of solids feeding. Some of the solids found their way out of the process with a duration of 25–30 s. The major portion of the solids came out of the flash cooler between 30 and 40 s after they were fed into the process. The soy slurry particles coming out of the system with the shortest residence time were considered important to the microbiological quality of the RHHTC soy milk because they received the least thermal processing time.

TABLE 2.
RETENTION TIME OF THE RHHTC UNIT AT TEMPERATURES OF 220–315F COMPARED TO THOSE REQUIRED FOR THERMAL DESTRUCTION[a]

Temperature F (C)	Time s	Pressure psig	Equivalent Time s [b]
220 (104.4)	24.0	0-1	1949.5
230 (110.0)	25.0	5.0	542.5
240 (116.0)	31.3	8.0	150.9
250 (121.1)	31.4	18.0	42.0
260 (126.7)	29.0	20.0	11.7
270 (132.2)	29.0	28.0	3.3
280 (137.8)	27.0	34.0	0.9
290 (143.3)	28.3	46.0	0.3
300 (148.9)	30.5	51.0	0.1
305 (151.7)	32.0	59.0	0.04
310 (154.4)	33.5	62.5	0.02
315 (157.2)	90-190	70.0	0.01

[a] Values are the means of 3 determinations.
[b] Time required to have the same equivalent of destruction effect as that of F=42 s at 250 F on *B. thermoaciduran* (Cheftel and Thomas, 1965); A value of z=18 F was used.

Microbiological Quality or RHHTC Soy Milk

The sterilization of soy milk using the RHHTC process was achieved at temperatures above 132C for retention times of 29 s or longer (Tables 2 and 3). The initial microbial numbers in the soy slurry were about 10^4–10^5 cells/ml. The RHHTC soy milk treated at 143 to 157C was sterile and could be kept for

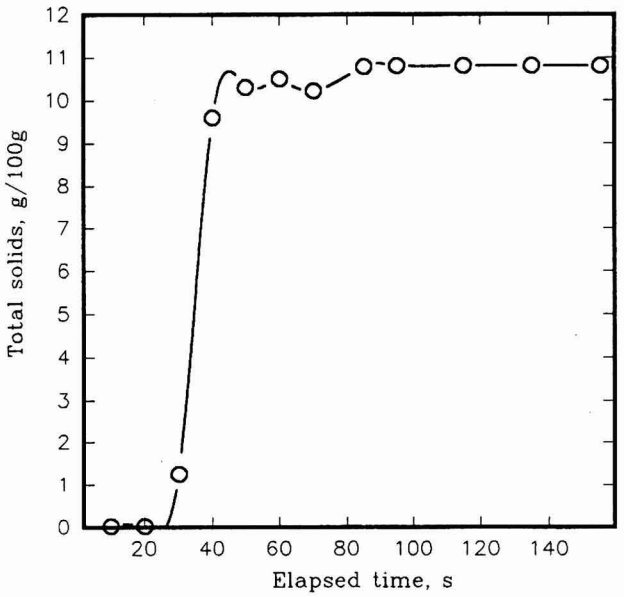

FIG. 3. RESIDENCE TIME DISTRIBUTION OF THE RHHTC PROCESS OPERATING AT 152C
Results are for a step change in total solids.

months without precipitation or spoilage. At temperatures lower than 132C (105–127C) with the mean retention times of 24–31.1 s, bacterial cells and spores originally presented in soybean flour survived the heating and grew well on plate count agar (PCA). The predominant Gram negative bacteria in soy slurry were *Pseudomonas aeruginosa, Serratia liquefaciens, S. rebidaea,* and *Enterobacter cloacae,* while the Gram positive bacteria are *Bacillus licheniformis* and *B. cerus.*

At 105C for 24 s, the survivors were only 0.29% of the original number. The actual numbers of microorganisms surviving the heating process are reported in Table 3. Increasing the temperature reduced the surviving cell number sharply. The reductions were more than 99% in all cases. The temperatures and times used in this study can be compared to known thermal inactivation requirements at 250F or 121.1C (Table 2). Experimental heating times, therefore, are capable of being compared to predicted values based on results reported elsewhere. Table 2 shows the estimated holding times required to sterilize the slurry. For example, the soy milk treated at 105C needed to have a residence time of about 2,000 s to have an equivalent effect as that of the soy milk produced at 121.1C (250F) based on F = 0.7 min (or 42 s) and z = 18 °*F* for *Bacillus thermoaciduran*, the thermal resistant bacilli (Cheftel and Thomas 1965). At the temperatures of 110, 116, and 121C, the corresponding holding times to completely destroy the bacilli

TABLE 3.
SURVIVAL BACTERIAL NUMBERS OF BACILLUS SP. (CFU/G) IN SOY MILK HEAT TREATED BY THE RHHTC UNIT[a]

Temperature F (C)	Initial Bacterial Number	Final Bacterial Number
220 (104.4)	$6.2x10^4$	185
230 (110.0)	$6.2x10^4$	131
240 (116.0)	$2.7x10^4$	50
250 (121.1)	$8.1x10^4$	26
260 (126.7)	$3.4x10^4$	3
270 (132.2)	$1.9x10^4$	0
280 (137.8)	$1.8x10^4$	0
290 (143.3)	$3.0x10^4$	0
300 (148.9)	$3.7x10^4$	0
305 (151.7)	$3.5x10^5$	0
310 (154.4)	$1.3x10^5$	0
315 (157.2)	$3.8x10^4$	0

[a] Values are the means of 3 determinations.

cells and spores are 542.45, 150.94, and 42 s, respectively. At the temperature of 127C the required holding time of 11.7 s was shorter than the mean retention time of 29 s. However, at 127C, *Bacillus licheniformis* and *B. cerus* were found to survive. The solids, protein, and fat in soybean flour might provide some protection to the bacteria (Yasair *et al.* 1946). There is a well-known protective effect of fat on bacterial cells in particulates (Locin and Merson 1979). High temperature increases the solubility of water in the fat and the rate of heat transfer, but the heating time is very short. Therefore, when the time required to bring the cells to the steady state temperature and the residence time distribution are considered, a longer mean retention time than the theoretical thermal destruction time is needed. When the soy slurry was heated at temperatures higher than 132C,

all of the organisms were killed. From Tables 2 and 3, it is evident that the RHHTC unit can be operated at several combinations of time and temperature to produce sterile soy milk. Consequently, temperatures of 143–157C were chosen to produce the sterile RHHTC soy milk for making soy yogurt and frozen soy yogurt (Tuitemwong 1992).

Physical Properties of RHHTC Soy Milk

The characteristics of soy milk are shown in Table 4. The neutral pH of 6.8 was observed for all soy milk samples. Soy milk produced at 143C had the lowest viscosity (14.5 cp) while that produced at 152C had the highest viscosity (142.3 cp); see Table 4. Higher processing temperatures resulted in higher viscosity soy milk samples at temperatures up to 152C. The viscosity of soy protein-water mixtures is highly dependent on solid concentrations and processing temperature (Hung 1984; Kim *et al.* 1984); generally, others (Hong 1989; Kim *et al.* 1984; Hung 1984) reported that the viscosity of soy milk samples increased with temperature. However, Hong (1989) found a sharp decline of the viscosity of the soy milk produced at 163C (325F), which needs a steam pressure of 95 psig, the maxi-

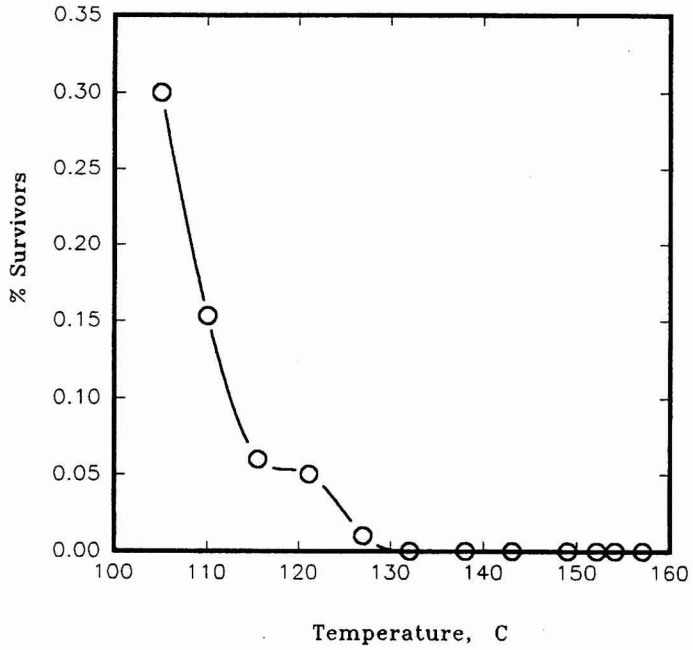

FIG. 4. SURVIVING BACTERIA (%) IN RHHTC SOY MILK AFTER THE STEAM INJECTION HEAT TREATMENT FOR 28–35 S

TABLE 4.
THE CHARACTERISTICS OF RHHTC MILK PRODUCED AT DIFFERENT TEMPERATURE BY THE RHHTC PROCESS[x]

Temp. C	pH	Slurry solids g/100ml	Soy milk solids g/100ml	Viscosity[y] cp	Concentration, mg/100ml (mg/g)[z]		
					Sucrose	Raffinose	Stachyose
143	6.85	10.40	9.64	14.5[a]	377[d] (39.1)	127[e] (13.2)	484[f] (50.2)
149	6.80	10.11	8.88	71.6[c]	365[d] (41.1)	104[e] (11.7)	440[f] (49.5)
152	6.81	10.15	9.08	142.3[d]	367[d] (40.4)	116[e] (12.8)	462[f] (50.9)
154	6.80	10.00	8.77	66.5[c]	334[d] (38.1)	127[e] (14.5)	440[f] (50.2)
157	6.80	9.23	8.31	26.5[b]	334[d] (40.2)	116[e] (14.0)	386[f] (46.5)

[a-f] Different letters in the same column indicate significant difference.
[x] Values are the means of 3 determinations.
[y] Viscosity of soy slurry is 12.5 cp.
[z] mg sugar/g dry solids.

mum steam pressure used. The 15% soy slurry treated and shear force of the RHHTC process break the cell walls of the soybeans releasing carbohydrate and lipid to the solution. The protein-protein and protein-carbohydrate interactions play major roles in the viscosity of the RHHTC soy milk (Hong 1989). In this study, however, soy milk made from 11% soy slurry and produced at 152C had the highest viscosity, which is a good characteristic for yogurt production (Hong 1989; Johnson 1978) (see also Table 7).

The soy milk treated at 143C had the highest final solids content, and the solids decreased as the treating temperatures increased (Table 4). Higher temperatures required more steam which diluted the final products. Johnson (1978) and Hong (1989) employed a 90 psig steam to produce soy milk; they also found that at the higher temperature, the soy milk obtained has lower solid content compared to those treated at lower temperatures. Table 4 shows the observed final solids concentrations; based on a starting slurry with 11% soy flour, the final solid concentration was 8–19% lower than the original value.

The soy milk samples, however, were quite stable. They showed no precipitation though they had high solids (9%) and were stored at a refrigeration temperature for 2–3 months. Soybean proteins were partially denatured, but the 7S and 11S protein subunits were still intact (Hung 1984; Hong 1989). Large particles (2–10 μm in diameter) in raw soy milk constituted about 40% of the total proteins (Table 5) and contained mainly 11S subunits. These particles were not found in the presence of mercaptoethanol suggesting disulfide, S-S, bonds between the protein subunits. The disulfide bonds are originally present in soybean protein (Ono *et al.* 1991). Soybean 11S subunits have a sulfur-rich salt soluble group. Hydrophobic and disulfide exchange interaction were thought to be the forces between the two protein molecules (Sathe 1990). Heating also caused more medium size particle formation due to the agglomeration of small particles, mainly 7S proteins. However, some medium size particles were in turn formed from large particles because of the breaking of disulfide bonds by heating. Dissociation and reassociation of soy protein were observed and considered important to the stability of the gel structure (Cheny 1981). Peptide interactions are needed for gel formation of soy proteins. Heating soy protein isolate at 95C for 30 min was optimal for the stimulation of gel formation (Hartnett and Satterlee 1990); it causes irreversible disruption of the quaternary structure of 11S globulin with subsequent dissociation into subunits (Wolf and Tamura 1969). Thus, heating has a great effect on gel formation and the stability of soy protein in an aqueous solution (Chacon *et al.* 1990). It leads to a high viscosity of the soy milk (Table 4). A certain amount of heat is required to dissociate protein bodies. Heat insolubilization of soy protein is not always predictable because partial denaturation (dissociation of subunits) may increase solubility and emulsification (Johnson

TABLE 5.
PROXIMATE ANALYSIS OF RHHTC SOY MILK PRODUCED AT 149C (290F)[a]

Soybean variety	Williams
Carbohydrate[b], %	22.0
Free lipid (hexane extract), %	12.10
Total lipid (acid hydrolysis), %	23.46
Protein, %	40.80
Ash, %	5.22

[a] Hong, 1989.
[b] Calorimetric method; the per cent by difference is 30.16.

TABLE 6.
THE COMPARISON OF TOTAL SOLIDS AND SUGARS CONCENTRATIONS IN SOYBEAN SLURRY AND RHHTC SOY MILK[y]

Source	Solids	Mean values of concentrations, mg/100ml (mg/g solids)		
		Sucrose	Raffinose	Stachyose
Soybean slurry	10.2[a]	485[c] (47.5)[i]	118[e] (11.6)[m]	541[g] (53.0)[n]
RHHTC Soy milk	9.0[b]	345[d] (38.3)[k]	103[f] (11.4)[m]	461[h] (51.2)[n]

[a-n] Different letters in the same column indicate significant difference.
[y] Values are the means of 12 determinations.

1978). However, the RHHTC unit provides excellent heat treatment, and the extreme shear encountered in steam injection and flashing enhance the solubility of soy protein in RHHTC soy milk (Johnson 1978; Hung 1984; Hong 1989).

Chemical Quality of RHHTC Soy Milk

Sugar concentrations were reduced due to the steam injection heat treatment by the RHHTC process. However, based on the solids present, raffinose and stachyose concentrations were not significantly different ($\alpha = 0.05$) from those found in soy slurry (Table 6). The average values of the final concentrations were 345, 103, and 461 mg/100ml for sucrose, raffinose, and stachyose, respectively. The reduction appears to be the effect of dilution for raffinose and stachyose. The reduction of sucrose may be because of browning reactions (Hong 1989). No significant differences of the stachyose concentrations among the soy milk samples produced at 143, 149, and 157C were found ($\alpha = 0.05$) (Table 4).

Soy milk solids were significantly different from those found in soybean slurry ($P < 0.05$) (Table 4). Soy milk produced at 143 and 149C had 11% solids reduction, which was at the same level as that of the stachyose. This is expected because of the condensation of steam (Burton 1988). The soy milk treated at 152C and 154C had a reduction of sucrose concentration of 17 and 25%, respectively.

Table 7 shows the comparison of physical characteristics of RHHTC soy milk produced from fast grind (0.51 mm opening screen) dehulled soybean flour at

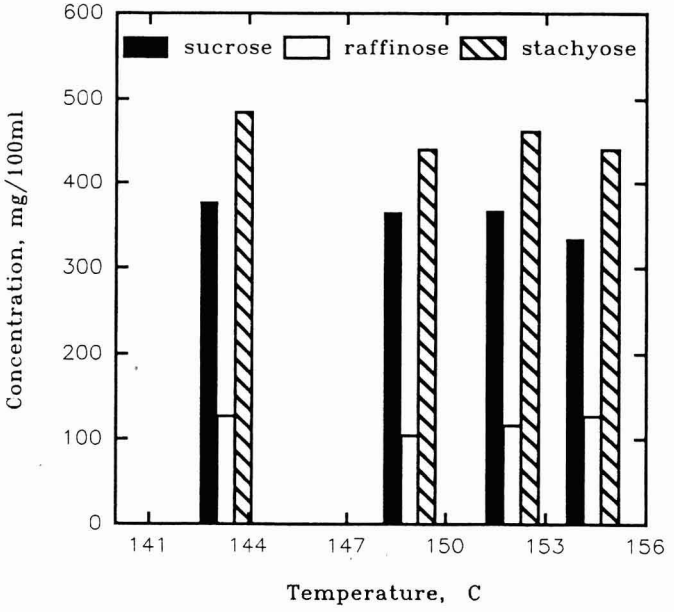

FIG. 5. CONCENTRATIONS OF SUGARS IN RHHTC SOY MILK PRODUCED AT 143, 149, 152, AND 154C FOR 30–40 S

different solids concentrations and temperatures found in this study and those from the previous studies (Hung 1984; Hong 1989). Soy protein produced at 152C may have protein structure changes that enable it to form gel better than the other treatments (Adams 1991). This evidence was supported by the observation of high viscosity of the samples (Fiora *et al.* 1990). The structure may also have a protective effect on the destruction of sugars in soybean slurry. The viscosity of the soy milk produced at 152C in this study was higher than that found by Hong (Hong 1989). Recovery of the solids in this study was also higher than that of the previous work. This may be due to the mechanical mixing of soy slurry used in this study that provided more homogeneous slurry; thus, the solids were heated evenly and thoroughly. The previous studies employed hand mixing. The color of the soy milk was in the acceptable range of 83–85 Agtron color units; higher numbers indicate lighter colors (Hong 1989). It was apparent that solids content and processing temperature had a great effect on the viscosity of the soy milk.

RHHTC soy milk contained higher concentrations of sugars than the hot grind soy milk because it was produced at slightly higher solids concentrations (Table 8). The RHHTC soy milk had the highest viscosity of those compared in Table 8. The pH of all soy milk samples was neutral (6.4–6.8).

TABLE 7.
PHYSICAL PROPERTIES OF RHHTC SOY MILK PRODUCED FROM FAST GRIND DEHULLED SOY FLOUR

Temp. C	% TS Slurry	% TS Soy milk	Color	Viscosity
157[x]	10	7.25	84[a]	13[c]
152[y]	11	9.0	-	142
157[x]	12	8.7	85[a]	23[c]
157[x]	15	10.24	83[a]	520[c]
152[x]	18	14.8	0.39[a]	23[c]

[x] Hong, 1989.
[y] This work.
[a] Agtron color value; higher values indicate lighter colors (Hong, 1989).
[a] Simon color value; higher values indicate darker colors (Hong, 1989).
[c] Viscosity at 9% solids at 25 C (Hong, 1989).

TABLE 8.
CONCENTRATIONS OF SUGARS AND OTHER CHARACTERISTICS OF HOT GRIND SOY MILK AND RHHTC SOY MILK

	Hot grind[a]	Heat exchanger[b]	RHHTC[c]
Sucrose, mg/100ml	373	-	345
Raffinose, mg/100ml	77	-	103
Stachyose, mg/100ml	332	-	467
pH	6.4	-	6.8
Solids, %	7.3	10	9.0
Viscosity, cp	22.5	22.8	142

[a] Buono, 1988.
[b] Hung, 1984.
[c] This work.

CONCLUSIONS

Soy milk produced by the RHHTC process is sterile. Its microbiological, chemical, and physical qualities are superior to those produced by hot grind and heat exchanger methods. The processing is rather simple yet effective. The hydro-heating valve provides fast mixing of the feed slurry leaving a very short time for the hydration and heating of the soy flour, which reduces the reaction time for lipoxygenase. Thus, less beany flavors are found in the soy milk. Because of the capability of continuous operation of the RHHTC process, it is feasible to produce commercial quantities of soy milk for direct consumption or for further processing into products such as soy yogurt and frozen soy yogurt.

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CONTROLLED ATMOSPHERE STORAGE OF 'DELICIOUS' APPLES IN HIGH AND VARIABLE CARBON DIOXIDE[1]

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ABSTRACT

Two strains of 'Delicious' apples were held in controlled atmosphere (CA) storage at various carbon dioxide levels for nine months before removal and evaluation. Oxygen in all atmospheres was 1%, and carbon dioxide levels were 1, 3, or 5% with an additional carbon dioxide treatment that was increased every six weeks from 1 to 5% over the storage period. Storage temperature for all treatments was 1C. Little quality difference was noted for the 'Delicious' apples immediately after storage or after an eight day ripening period regardless of CO_2 level in storage. Mean values for firmness, external and internal color, soluble solids content, titratable acidity and amount of scald did not differ between the

[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.
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apples from the different storage treatments. Total carbohydrates and fructose were higher in apples stored at CO_2 levels above 1%. Sensory panelists found no flavor differences in apple juice after long term CA storage regardless of CO_2 stroage level.

INTRODUCTION

Removal of CO_2 during long term controlled atmosphere (CA) storage of 'Delicious' apples is a costly operation. Not only is the cost of CO_2 removal a concern, but also one must consider the additional cost of purchase and installation of this type of equipment (Blanpied 1988; Waelti and Cavalieri 1990). Recommendations for CA in Washington State suggest O_2 and CO_2 levels be maintained at 1 to 2%, respectively (Olsen 1990). Presently commercial CA storage atmospheres are usually 1–3% O_2 and CO_2 (Meheriuk 1991). Most of the CA atmosphere recommendations are based on a static or recirculating-type system. Many of the new CA systems being placed in operation are purge- or flow-through-type systems. Atmosphere recommendation for the long term storage of 'Delicious' apples in a static system may not be valid for a purge system. Reports concerning apple injury as a result of high CO_2 in CA storage are numerous (Chen 1985; Meheriuk 1985; Olsen 1985). Superficial scald is minimized with reduced CO_2 or when the CO_2 level is held below the O_2 level in CA storage (Chen *et al.* 1985; Dilley *et al.* 1989). Patterson and Workman (1962), however reported decreased scald on apples with the use of increased CO_2 in storage. Discoloration of the internal flesh of apples has been linked to high CO_2 (Little and Peggie 1987; Liu and Pan 1989). In 'Delicious' apples (Meheriuk *et al.* 1984) this discoloration due to high CO_2 was also found to be storage temperature dependent. Meheriuk and Porritt (1973) found no difference in the internal flesh color of 'Delicious' apples in CO_2 atmospheres up to 2.5%.

Blanpied and Smock (1981) reported external and internal discoloration of 'Delicious' apples in high CO_2 storage along with external problems of mealiness and internal cavities. Increased firmness has also been reported in apples stored in high CO_2 atmospheres (Bramlage *et al.* 1977; Lau 1985). Mattheis and Olsen (1989) found that 'Delicious' apples could be stored for long periods of time in an atmosphere of 1% O_2 and CO_2 up to 3%, and fruit were of better quality when the storage temperature was 1.1C compared to 0C. Dilley (1989) proposed an atmosphere of 1.5% O_2 and 3% CO_2 for best general storage of apples.

Treatment of apples with a high concentration rate of CO_2 prior to long-term storage has met with good success. Couey and Olsen (1975) found exposure of 'Golden Delicious' apples to CO_2 levels of 20% delayed softening and loss of titratable acidity. 'McIntosh' apples were found to respond unfavorably to CO_2

treatment prior to storage, but sensory panelist preferred the CO_2 treated fruit (Bramlage *et al.* 1977).

There is some confusion about the best atmosphere for the long term storage of 'Delicious' apples. Mixed reviews have been reported on the best CO_2 concentration for maintaining good apple quality. No doubt exists about the cost of CO_2 removal. Most of the previous research has been conducted with a constant or static-type CA system. Many of the CA storage facilities now have a flow-through or purge-type CA capabilities. This research was conducted to determine quality differences of 'Delicious' apples under various CO_2 atmospheres (above present recommended amounts) in a purge-type facility. Increased CO_2 in the storage atmosphere, for long term storage of 'Delicious' apples, would result in reduced storage cost.

MATERIALS AND METHODS

Eighty 'Delicious' apples ('Red Spur' and 'Oregon Spur') were harvested at commercial maturity (WAMP 1986) for CA storage on September 27, 1989, and October 2, 1990, from four replicate trees of each strain. Within 3 h of harvest all apples were placed in CA chambers (Ca. 0.14 M^3) and the atmosphere established in 24 h or less at 1C. Oxygen in all atmospheres was established and maintained throughout the storage period at 1 $\pm$ 0.1%. Four individual carbon dioxide atmospheres (1, 3, 5 and variable %) were established and maintained at $\pm$ 0.1%. Carbon dioxide in the variable chamber was first established at 1% and increased by 1% every six weeks. Atmospheres were established and maintained with a computer control system (Technical Consulting Services, Chelan, WA 98816). Nitrogen was supplied by a Generon Membrane Separation System (Generon, Houston, TX). After nine months in the four atmospheres (1% O_2, 1% CO_2; 1% O_2, 3% CO_2; 1% O_2, 5% CO_2; and 1% O_2, variable CO_2) apples were removed from CA for quality analysis.

Twenty apples from each 'Delicious' strain, harvest, atmosphere, and tree were used for quality analysis. Eight apples from all treatment combinations were examined immediately upon removal from storage. An additional eight apples from each treatment combination were examined after an 8-day ripening period at 20C. Four apples from each treatment and replications were used for sensory analysis. Quality analysis consisted of evaluation for respiration, external and internal color, firmness, soluble solids content, titratable acidity, carbohydrates, and visual observations for defects.

External and internal fruit color was determined with The Color Machine (Pacific Scientific, Silver Springs, MD) using the Hunter L, a, b system and calculated hue values (Hunter and Harold 1987). Firmness was determined using

an LC Pressure Tester Model EP-1 equipped with a 1.11-cm probe and values reported in Newtons. Titratable acidity was determined with a Radiometer titrator, Model TTT85 (Radiometer, Copenhagen). Acids were titrated to pH 8.2 with 0.1N NaOH and expressed as percent malic acid. An Abbe-type refractometer with a sucrose scale calibrated at 20C was used to determine soluble solids concentrations. Carbohydrates were determined by the high performance liquid chromatography method described by Bio-Rad (Bio-Rad, Richmond, CA). Scald was determined by visual assessment.

Sensory flavor was evaluated on external juice by a panel of seven judges (Tree Top, Inc.) that had previously been trained in apple juice flavor attributes. The original selection and training of the panelists was completed according to the Spectrum procedures described by Meilgaard *et al.* (1978a). Flavor intensity of each attribute was based on the Spectrum universal intensity scale, where 0 = absent and 15 = extremely intense, using reference samples as described by Meilgaard *et al.* (1978b). Extensive preliminary trials were conducted to minimize panel fatigue and variation among panelists.

For each treatment, four random apples were quartered and juiced through a Champion Juicer (Plastaket Mfg. Co., Lodi, CA) at ambient temperature. The juice from each treatment was evaluated immediately by all panelists in a laboratory environment. Each panelist received the sample in a 3-digit coded glass cup covered with a glass watch glass. Panelists were instructed to evaluate prior to taste. Samples were presented at 15 min intervals. One treatment was presented to the panel at a time. The order in which the treatments were presented was randomized at each session. Each evaluation session was limited to four samples.

Statistical Analysis

Flavor attribute data from the two 'Delicious' strains were combined to produce one profile per carbon dioxide storage treatment level. Each point within the various profiles shown in Fig. 1, and Table 4 represents an average value for four determinations by each of the seven panelists. The data was analyzed by analysis of variance (ANOVA) using Statgraphics (STSC, Inc., Rockville, Maryland).

Analysis of variance (ANOVA) for quality data was determined by MSTAT (1988). Based on significant F-test, means were separated by Tukey's Honestly Significant Difference Test (HSDT).

RESULTS AND DISCUSSION

The amount of carbon dioxide (CO_2) up to 5% in the storage atmosphere of 'Delicious' apples had no influence on the firmness of the fruit even after a 9-month

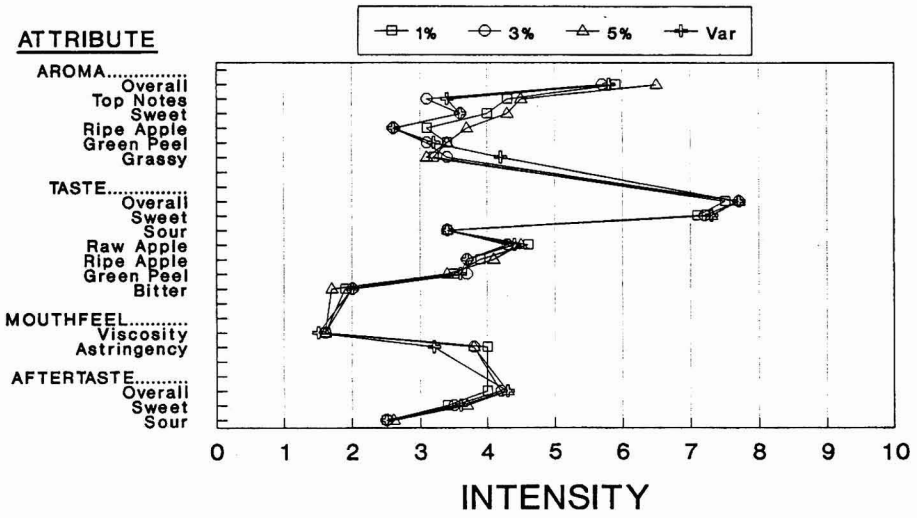

FIG. 1. FLAVOR PROFILE OF APPLE JUICES FROM 'DELICIOUS' APPLES STORED AT 1% O_2 AND VARIOUS CARBON DIOXIDE LEVELS

storage period (Table 1). Firmness level was identical also for all levels of CO_2 after eight days of ripening except where CO_2 was increased (variable) to simulate loss of scrubbing capacity over the storage period. This change or drop in firmness for the apples from the variable CO_2 treatment was almost 3N and would be considered a serious loss if an extended period of time was required to market the fruit. There were differences in the firmness level between the strains of 'Delicious', but both strains reacted the same under similar storage conditions.

No firmness problems for 'Delicious' apples were apparent in this study with steady CO_2 levels of either 1, 3, or 5%. This lack of textural problems is contrary to previous reports. Blanpied and Smock (1981) reported mealiness and cavities in the flesh of 'Delicious' apples when CO_2 levels exceeded 3%. Other reports (Bramlage *et al.* 1977; Couey and Olsen 1975) have dealt with high CO_2 as a short term treatment for apples prior to a normal CA of 3% CO_2 or less and have shown incresaed fruit firmness retention with CO_2 treatment. Most of the previous studies were conducted using a static-type CA system. With a purge-type system, as used in this study, CO_2 levels in CA can be increased with no loss in firmness.

External Hunter color (L, a, b, YID) of 'Delicious' apples was not influenced by different CO_2 levels in long term storage (Table 1). But Hunter b or yellow color and YID, a browning indicator, did change when an 8-day ripening time was considered. Regardless of CO_2 level, Hunter b values or yellow color did increase from zero to eight days of ripening time. This increase in yellowness was identical over all CO_2 levels and amounted to an average incrase of 13%.

TABLE 1.
QUALITY ATTRIBUTES OF 'DELICIOUS' APPLES AFTER LONG TERM CA STORAGE AT 1% O_2 AND VARIOUS CARBON DIOXIDE LEVELS

	Firmness (N)	SSC (%)	TA (% Malic)	Hunter Color (external) L	a	b	YID	Scald (%)	Visual Rate (1-5)[z]
Strain									
'Red Spur'	69.6 a[y]	12.2 NS	0.19 NS	41.4 a	18.0 NS	12.2 a	85.8 NS	26.8 NS	1.5 NS
'Oregon Spur'	68.0 b	12.7	0.18	38.2 b	18.5	10.1 b	85.3	30.8	1.5 NS
Atmosphere X Ripe (days)									
1% 0	69.0 a	12.3 NS	0.19 NS	38.9 NS	18.4 NS	10.4 c	84.4 bc	12.5 b	1.2 b
1% 8	68.7 a	12.4	0.19	39.3	18.1	12.3 a	91.4 a	28.9 a	1.4 ab
3% 0	70.0 a	12.4	0.19	39.0	18.8	10.9 bc	86.8 ab	38.0 a	1.7 a
3% 8	68.5 a	12.6	0.18	40.3	17.8	11.6 ab	85.3 bc	31.5 a	1.5 a
5% 0	70.3 a	12.5	0.19	39.7	18.0	10.2 c	80.7 c	31.8 a	1.5 a
5% 8	68.4 ab	12.5	0.19	40.9	18.5	11.8 ab	85.5 bc	30.6 a	1.6 a
Var 0	69.2 a	12.4	0.18	39.2	18.5	10.4 c	84.5 bc	33.2 a	1.5 a
Var 8	66.5 b	12.4	0.18	40.8	18.2	11.7 ab	85.6 bc	24.0 ab	1.6 a

[z]Visual scald on a scale of 1 to 4 (1 = no scald; 2.5 = 50% surface with scald; 5 = 100% surface area with scald.

[y]Mean separation within strain, or atmosphere by ripe using Tukey's HSD, ($P \leq 0.05$).

YID also increased as ripening time passed from zero to eight days, but this increase in YID was only significant at the 1% CO_2 treatment. No change in YID was evident for all other CO_2 treatments over the 8-day ripening period.

Superficial scald, a cosmetic color problem associated with apples, in long term storage was not inhibited by levels of CO_2 (Table 1). Immediately after removal from storage apples stored in a 1% CO_2 atmosphere displayed less scald than apples held in higher CO_2, but after eight days of ambient temperature ripening there was no difference in the amount of scald present regardless of the amount of CO_2 in the storage atmosphere. This lack of difference in the amount of visual scald present regardless of CO_2 storage atmosphere was distinguishable as either a percentage of the total number of apples with scald symptoms or as a visual observation where the apples were rated on the amount of apple skin area affected.

Both visual assessment and objective color determinations agreed with the amount of scald present (Table 1). Apples, when visually rated higher for scald, also displayed increased YID color or a browner appearance. Blanpied (1990) reported increased scald with decreased CO_2, but this decrease was dependent on the O_2 level. We found no difference in the amount of scald present in atmospheres containing 1, 3 or 5% CO_2, all at 1% O_2.

Internal Hunter color (Table 2) was influenced by the level of CO_2 in the storage atmosphere and by ambient temperature storage. Apples from (3 or variable %) CO_2 storage were distinctly lighter (higher L values) in color than apples from a 1% CO_2 storage. This internal color difference was not present when Hunter color a and b, and YID values were considered. Regardless of the amount of CO_2 in the storage atmosphere after eight days ripening time Hunter L, a, b and YID values were similar.

No incidence of core browning were apparent in this study over all CO_2 levels. Meheriuk (1985) observed reduced flesh browning when apples were held at −0.5C coupled with low CO_2 levels, but no reduction at a higher storage temperature. Liu and Pan (1989) reported increased flesh browning with high CO_2 in the storage atmosphere. In this study storage temperature was maintained at 1C and regardless of CO_2 level no flesh browning problems were evident.

Total carbohydrates were 5–9% higher in apples held in 3, 5 or a variable % CO_2 atmosphere than apples held in a 1% CO_2 atmosphere (Table 3). Total carbohydrates of apples held in either 3, 5 or a variable % CO_2 atmosphere did not differ, but contained a higher amount of fructose than apples from a 1% CO_2 atmosphere. Differences in sucrose and sorbitol in apples from the different CO_2 storages were similar, except the apples held in a variable CO_2 storage, which had a higher amount of these two sugars, compared to apples from a 1% CO_2 storage atmosphere. Glucose content was higher in apples from 5 and variable % CO_2 than apples stored at 1% CO_2. Apples stored in 3% CO_2 had glucose values similar to the other CO_2 treatments. Retention of total carbohydrates and

TABLE 2.
INTERNAL COLOR OF 'DELICIOUS' APPLES AFTER LONG TERM CA STORAGE AT 1% O_2 AND VARIOUS CARBON DIOXIDE LEVELS

	Hunter Color (internal)			
	L	a	b	YID
Strain				
'Red Spur'	71.7 NS[z]	-3.0	21.3 NS	47.9 NS
'Oregon Spur'	72.4	-3.0	21.3	47.6
Atmosphere X Ripe (days)				
1% 0	70.1 c	-3.2 NS	20.4 d	46.9 b
1% 8	71.4 bc	-2.6	21.9 ab	50.1 a
3% 0	72.9 ab	-3.3	21.1 bcd	46.5 b
3% 8	71.7 bc	-2.8	21.4 abc	48.6 a
5% 0	71.8 abc	-3.4	20.5 d	45.6 b
5% 8	72.1 ab	-2.8	21.9 a	49.4 a
Var 0	73.5 a	-3.3	20.9 cd	45.7 b
Var 8	72.7 ab	-2.8	21.9 a	49.1 a

[z]Mean separation within strain, or atmosphere by ripe using Tukey's HSD. ($P \leq 0.05$).

fructose sugars was best at CO_2 levels above 1%. Regardless of the difference in total carbohydrates or the individual sugars, taste panelists could not distinguish a taste difference and any difference in sugars due to different CO_2 storage levels would be considered inconsequential.

The average flavor profile of the four apple juices made from 'Delicious' apples stored at the various carbon dioxide levels is shown in Fig. 1. There appears to be little, if any, difference between the profiles except for a slight increase in the overall aroma impact and fresh apple top notes of the juice from apples stored at the 5% carbon dioxide level compared to the juice from apples stored at the 3% carbon dioxide level (Table 4). In addition, the grassy aroma was slightly

TABLE 3.
CARBOHYDRATE CONTENT OF 'DELICIOUS' APPLES AFTER LONG TERM CA STORAGE AT 1% O_2 AND VARIOUS CARBON DIOXIDE LEVELS

Treatment	Carbohydrates ($mg \cdot g^{-1}$)				
Atmosphere %	SUC	GLU	FRUC	SOR	Total CHO
1 CO_2	1.46 b[z]	3.41 b	6.23 b	0.37 b	11.46 b
3	1.51 ab	3.56 ab	6.60 a	0.41 ab	12.07 a
5	1.55 ab	3.71 a	6.79 a	0.41 ab	12.45 a
Var	1.67 a	3.70 a	6.78 a	0.44 a	12.58 a
Cultivar					
'Oregon Spur'	1.52 NS	3.61 NS	6.66 NS	0.41	12.19 a
'Red Spur'	1.57	3.58	6.54	0.40	12.09 b

[z]Mean separation within atmosphere or strain by Tukey's HSD. ($P \leq 0.05$).

higher in intensity in the juice from 'Delicious' apples stored under variable CO_2 level compared to the juice from apples stored at the 5% CO_2 level. Although there were some minor differences in the aroma intensity attributes between the treatments, there were no differences in taste and aftertaste attributes. However, there was a slight decrease in the juice astringency from apples stored under the variable CO_2 level compared to juice from apples stored at the 1% carbon dioxide level.

In general, the profile data suggests that there are some minor differences in flavor attribute intensities between the juices from the apples stored under the four carbon dioxide atmospheres. However, this is data obtained from a trained panel; and as such, the average consumer may not be able to detect these minor differences.

There is good economic benefit to storing 'Delicious' apples for long term under higher CO_2 than is presently recommended (Waelti and Cavalieri 1990). Significant cost savings for CO_2 scrubbing can be obtained by increasing the CO_2 set-points in CA storage. Cost reductions are possible because scrubbing systems operate more efficiently when processing storage air with high CO_2 concentrations than with low concentrations. For example, a typical carbon scrubber that removes 79 Kg of CO_2 in 24 h at a 2.0% CO_2 concentration will remove 177

TABLE 4.
AVERAGE INTENSITY OF THE VARIOUS FLAVOR ATTRIBUTES FROM JUICE PRODUCED FROM 'DELICIOUS' APPLES STORED AT 1% O_2 AND VARIOUS CARBON DIOXIDE LEVELS

	Carbon Dioxide Levels			
Flavor Attributes	1%	3%	5%	Variable
Aroma				
Overall Impact	5.9±0.9	5.7±0.8[b]	6.5±1.0[a]	5.8±0.9
Fresh Apple Top Notes	4.2±1.7	3.0±1.7[b]	4.5±1.6[a]	3.4±0.8
Sweet Aroma	3.9±1.2	3.5±1.5	4.3±1.1	3.6±1.2
Ripe Apple	3.1±1.7	2.5±1.7	3.7±1.3	2.6±1.0
Green Peel	3.4±0.9	3.1±1.2	3.4±0.8	3.1±0.7
Grassy	3.1±1.1	3.4±1.1	3.1±0.9[b]	4.2±1.2[a]
Taste				
Overall Impact	7.5±0.4	7.7±0.3	7.7±0.4	7.7±0.5
Sweet	7.1±0.4	7.2±0.4	73.±0.5	7.2±0.5
Sour	3.4±0.7	3.3±0.6	3.4±0.7	3.4±0.7
Raw Apple	4.6±1.0	4.3±1.0	4.5±0.9	4.3±0.7
Ripe Apple	3.9±1.4	3.7±1.1	4.1±0.8	3.7±1.1
Green Peel	3.5±0.7	3.7±0.9	3.4±0.7	3.6±0.5
Bitter	1.9±0.5	2.0±0.5	1.7±0.8	2.0±0.5
Mouth Feel				
Viscosity	1.6±0.4	1.6±0.4	1.6±0.4	1.5±0.3
Astringency	4.0±0.9[a]	3.8±0.8	3.7±0.7	3.2±0.8[b]
After Taste				
Overall Impact	4.0±0.7	4.0±0.7	4.3±0.8	4.2±0.7
Sweet	3.4±0.7	3.3±0.6	3.6±0.8	3.5±0.7
Sour	2.5±0.7	2.4±0.7	2.6±0.5	2.5±0.5

Values are means ± standard deviation.

[a, b]Means with different superscript within each row significantly differ ($P \leq 0.05$).

Kg per 24 h at 3%, and 236 Kg of CO_2 per 24 h at a 4% CO_2 concentration. Thus, the amount of CO_2 removed is directly proportional to the CO_2 concentration of the storage air. Similar efficiencies are possible when scrubbing CO_2 by purging the CA rooms with nitrogen gas, such as is generated with pressure sensing adsorption (PSA) or membrane air separators.

Previous research has noted numerous problems with apples stored at various CO_2 levels in a static-type CA system. In this study, using a purge-type CA system, no quality problems were evident with 'Delicious' apples stored for nine months in 1% O_2 and 5% CO_2 at 1C. If one considers both the substantial cost savings that are possible with increased CO_2 in the storage system coupled with good quality apples, there is a possible economic advantage to increasing the CO_2 storage level of 'Delicious' apples.

CO_2 injury is generally present when the fruit is wet with condensation from defrost cycles or drenches. Although atmosphere gases were humidified in the experimental CA chambers, the fruit in these chambers never stayed wet, and it is possible that the required conditions for CO_2 injury were not present.

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STABILITY OF FROZEN STARCH PASTES: EFFECT OF FREEZING, STORAGE AND XANTHAN GUM ADDITION

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ABSTRACT

Starch pastes act as protective systems of the solid elements in precooked frozen foods, minimizing their dehydration and chemical changes during storage. The effect of xanthan gum (0.3 % w/w) on corn starch and wheat flour pastes (10 % w/w), frozen at different freezing rates and stored between −5 and −20C, was analyzed. Freezing modified the quality attributes of the starch pastes increasing exudate production, structure deterioration and rheological changes compared to the unfrozen samples. Starch pastes showed a pseudoplastic behavior and apparent viscosities decreased in frozen samples. High freezing rates led to smaller ice crystals (indirect microscopic observation) and the absence of starch retrogradation. Starch retrogradation and ice recrystallization, both contributed to the deterioration of the frozen paste during storage (spongy structure, marked decrease of apparent viscosity and increase of syneresis). The addition of xanthan gum minimized spongy structure formation, exudate production and rheological changes; however, no protective effect was observed on ice crystal sizes and on amylopectin retrogradation by Differential Scanning Calorimetry.

INTRODUCTION

Sauces and gravies, relevant components of precooked frozen foods, may exhibit textural changes and water release (syneresis) after thawing, decreasing

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consumers' acceptability. Hydrocolloids become an aid to overcoming these problems. These components, mainly used as texturizers, also have effects on emulsion stability, suspension of particulates, crystallization control, syneresis inhibition, etc. (Shuman 1960; Igoe 1982; Glicksman 1982; Budiaman and Fennema 1987a,b).

Starch gels are metastable and nonequilibrium systems (Slade and Levine 1987; Biliaderis and Zawistowski 1990), and therefore undergo structure transformations during storage and processing. Retrogradation is the crystallization of both starch polysaccharides, amylose and amylopectin; the chain aggregation involves molecular association favored by hydrogen bonding. Syneresis, low water holding capacity and textural changes occurring upon freezing have been mainly attributed to starch retrogradation (Hanson *et al.* 1951; Osman and Cummisford 1959; Schoch 1968; Brunnek and Koptelova 1970; Chan and Toledo 1976). However, other works have emphasized the importance of ice formation on the stability of these systems (Chan and Toledo 1976; Przybyl *et al.* 1983).

In order to relate macroscopic modification (syneresis and textural changes) with structural parameters (retrogradation and ice crystals formation), the influence of xanthan gum on the stability of a simplified model of gelatinized starch pastes was analyzed with the following objectives: (1) to determine the influence of freezing rate and frozen storage temperature of gelatinized pastes (corn starch and wheat flour) on exudate production, rheological behavior, ice crystal sizes and starch retrogradation and (2) to analyze the effect of xanthan gum addition on the behavior of these pastes.

MATERIALS AND METHODS

Materials

Commercial corn starch (Refinerías de Maíz, Argentina) was used in the experiments with the following composition (% w/w wet basis): water content 11.3, proteins 0.3, lipids 0.6, ash 0.3, ratio amylose/amylopectin 25/75.

Wheat flour (Molinos Río de La Plata S.A., Argentina) with the following composition (% w/w wet basis): water content 12.5–13, proteins 10, lipids 1.5, ash 0.6, crude fibers 0.6, ratio amylose/amylopectin 25/75.

Xanthan gum (Saporitti Hnos., SACIF, Argentina) with a water content of 11% (w/w), ash 9% (w/w); maximum values of arsenic 3 ppm and lead with other heavy metals 10 ppm. The viscosity of a 1% solution containing 1% NaCl was 1400 cp.

Sample Preparation

Starch pastes (10% w/w wet basis) were prepared by heating 400 ml batches in a thermostatic bath at 90C. The mix was continuously stirred; it reached 80C in approximately 6 min and remained at an average temperature of 83C for 4 min (Ferrero *et al.* 1992). Half of the samples contained 0.3% w/w (wet basis) of xanthan gum.

The gelatinization process was followed with a polarizing microscope (Ortholux II, Leitz, Germany) to verify that all the starch granules had lost their Maltese Crosses. At the final point, the swollen granules still kept their identity. Dry matter content of the gelatinized pastes was determined on each batch at 120C until constant weight with a Mettler LP16 infrared drying unit, on a PE 300 balance with a GA 44 printer (Mettler Instrumente AG, Switzerland).

Obtained pastes were cooled in a constant temperature room at 20 ± 1C for 2 h and placed in small cylindrical holders. The samples were then frozen in cold chambers at −20 and −80C to a final center temperature of −20C with different freezing rates. Heat flux from both sides was unidirectional and parallel to the axis of the cylindrical samples. When necessary, lateral insulation was provided by acrylic and expanded polystyrene to get different freezing rates. Insulation was removed after freezing. The highest velocity was obtained by dipping the samples in liquid nitrogen. Thermal histories during freezing were recorded with Cu-Constantan thermocouples.

Freezing rates were determined according to the IIR (International Institute of Refrigeration 1972) as the minimum distance from the surface to the thermal center divided by the time elapsed between the moment the surface reaches 0C and the moment the thermal center reaches a temperature 10C colder than the temperature of initial ice formation in the system. The measured initial freezing point of the starch paste was −0.6C. Assayed freezing rates ranged between 0.1 cm/h to values higher than 100 cm/h. In commercial practice 0.2–0.5 cm/h correspond to slow freezing (cold rooms), 0.5–3 cm/h to quick freezing in air blast or plate freezers, 5–10 cm/h to rapid freezing and 10–100 cm/h to ultra-rapid freezing (IIR 1972). Frozen samples in their molds were stored in cold chambers at −5, −10 and −20C for three months. Chambers' temperature stability was ±0.5C; thermal histories of the chambers were controlled with Cu-constantan thermocouples. Samples were thawed under controlled conditions: (a) in a constant temperature room at 20C ± 0.5C for exudate production and retrogradation measurements; (b) at 60C ± 0.1C for rheological tests.

Exudate Production

Samples weighing 3 g, were placed in cylindrical plastic molds, 2.5 cm in diameter and 1.5 cm high with removable bottoms. Each sample was individually frozen with the following rates: 0.3 cm/h, 0.6 cm/h and 270 cm/h until reaching a temperature of −20C, and then stored 12 h at this temperature before being placed in the cold storage chambers. Water holding capacity was determined by capillary suction of a porous material (filter paper). The bottom of the molds were removed to put the thawed samples in contact with the filter paper; the lateral wall of the cylinders were also removed afterwards. The geometry of the samples was maintained along the contact time (1 min). The increase of the wet area was measured under controlled conditions. The squared filter paper, Whatman No. 1 (side = 15 cm), was previously dried in an oven until reaching constant weight.

Syneresis indexes were expressed according to:

$$\% \text{ exudate} = \frac{d - d_i}{d_o} \times 100 \quad (1)$$

where d = equivalent diameter (cm) of the wet area produced by the thawed sample; d_i = equivalent diameter (cm) of the wet area produced by the unfrozen sample and d_o = equivalent diameter of the thawed sample at the initial contact time. All runs were made in duplicate. The equivalent diameters of the wet areas were measured with an Image Analyzer (Morphomat 30, Zeiss, Germany). The equivalent diameter was defined as the diameter of a circle that has the same surface area as the measured figure.

Rheological Measurements

A rotational viscometer Haake Rotovisco RV2 (Germany) with a sensor MVIP of concentric cylinders with profiled surfaces and a thermostatic system at 60C was used. The shear rate (D) ranged from 0–1024 s^{-1}, with one increasing sequence of 3 min followed by 1 min at the maximum value and the corresponding decreasing sequence in 3 min.

Samples of 50 g weight were frozen in plastic cylinders 4.5 cm in diameter. The freezing rates assayed were 0.3 cm/h, 0.8 cm/h and ultrarapid freezing (> 100 cm/h). Frozen samples were thawed in a thermostatic bath at 60 ± 0.1C. Thawed samples were poured from their molds into the viscometer cup trying to minimize shear forces due to manipulation.

The apparent viscosities (η_{ap}) at D = 512 s^{-1} were used to compare the effect of freezing rate and storage temperature.

Ice Crystal Size Determinations

Cylindrical starch paste samples 0.8 cm in diameter and 0.5 cm high, located in sample holders of aluminum foil, were frozen to a final center temperature of −20C with freezing rates of 0.1 cm/h, 2 cm/h, 15 cm/h, and ultrarapid freezing in liquid nitrogen (> 100 cm/h). At different storage times samples in triplicate were selected randomly, dipped in the fixative solution (absolute ethanol: formaldehyde 40%: water, 45:10:45), which was precooled at the corresponding storage temperature, and processed according to the freeze-fixation method (Ferrero *et al.* 1992). Obtained samples were micrographed in a Leitz Ortholux II microscope with a photographic camera Leitz Vario Orthomat (Leitz, Germany) Ice crystal sizes were obtained from the micrographs by the measurement of the holes left in the system. At least 150 crystals were measured with the Image Analyzer to determine equivalent diameter distributions. Histograms of the relative frequencies of crystal diameters as a function of equivalent diameter were obtained for each freezing rate and storage condition. Ice recrystallization rate during storage, kinetic parameters and activation energy were evaluated.

Differential Scanning Calorimetry

A DuPont 910 DSC system and Series 99 Thermal Analyzer with a temperature control was used to measure the retrogradation enthalpies (ΔH) associated with starch retrogradation. The heat transitions involved in "melting" the amount of retrograded starch were calculated in joules per gram of dry mass.

Gelatinized paste samples (5 g) were frozen in cylindrical plastic holders of 2.5 cm in diameter and 2 cm high. Freezing rates assayed were 0.3 cm/h, 1 cm/h, 2.3 cm/h, 4.3 cm/h and 12.0 cm/h. Storage temperatures were −1, −5, −10 and −20C. Aliquots of the homogenized thawed samples were placed in aluminum pans and then hermetically sealed. In all cases samples were run in duplicate. Total sample weight was 15 mg, measured in a ± 0.1 mg precision balance Mettler AE240 (Mettler Instrumente AG, Switzerland). Run temperatures ranged from 20 to 120C at a rate of 10C/min with an instrument sensitivity of 0.1×10^{-3} Joule/s cm. A heavier empty pan (double pan) was used as reference to balance the heat capacity of the sample.

The calibration coefficient of the instrument was determined from thermograms of Indium, which has a known transition enthalpy. The heats of transition (ΔH) were calculated from the peak areas measured with the Image Analyzer.

After each test, the pans were punctured and the mass of the samples were determined by drying at 120C during 12 h in the infrared drying unit.

RESULTS AND DISCUSSION

Exudate Production

Freezing rate has an important effect on exudate production in corn starch (just frozen) and wheat flour gels (just frozen) (Fig. 1). Higher velocities led to lower exudate values, as reported by Suzuki *et al.* (1977) in white sauces.

Wheat flour gels showed lower values due to a higher water holding capacity related to the presence of flour proteins, mainly gliadin and glutenin. Similar results were reported by Hanson *et al.* (1951) and Osman and Cummisford (1959) for frozen white sauces. As stressed by Pomeranz (1985) proteins have a particular ability to bind water, evidenced also in texture properties.

Xanthan gum addition decreased exudate production. This effect was more important in wheat flour pastes (just frozen), which showed exudate values lower than 5% (Fig. 1).

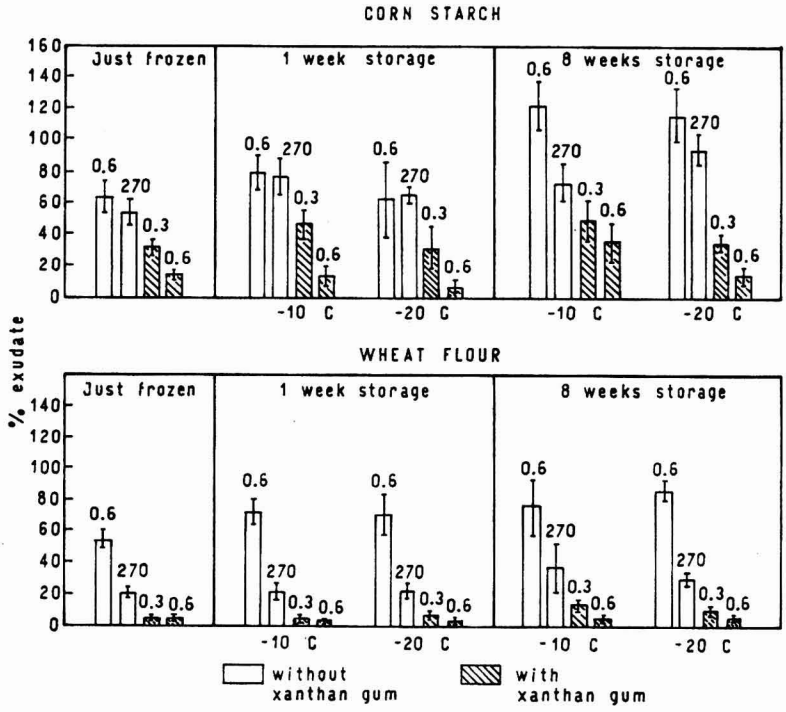

FIG. 1. EFFECT OF FREEZING RATE, TEMPERATURE AND STORAGE TIME ON EXUDATE PRODUCTION OF CORN STARCH AND WHEAT FLOUR PASTES WITH AND WITHOUT XANTHAN GUM

Numbers indicate freezing rates (cm/h). Bars indicate confidence limits ($P < 0.05$).

During frozen storage, an increase of exudate production of corn starch (8 weeks storage) pastes without xanthan gum was observed. Exudate percentages of samples stored at −10 and −20C did not differ significantly ($P \geqslant 0.05$). Exudate production remained at initial levels up to 8 weeks of storage when xanthan gum was added (Fig. 1).

Wheat flour pastes (8 weeks storage) showed a lower degree of phase separation than the corn starch pastes. In this case, exudate was higher at −10C than at −20C during storage time (Fig. 1).

In both pastes a spongy matrix was formed at −5C. This structure was not observed when samples were frozen in liquid nitrogen. The addition of xanthan gum also avoided the development of a spongy structure, even at high storage temperatures; in this case, exudate levels were higher at −5C than at −10 and −20C.

Rheological Measurements

Effect of freezing rate on rheological curves of frozen corn starch pastes is shown in Fig. 2 (solid line). Nonsignificant differences were observed when comparing the pseudoplastic curves of unfrozen (UF) and frozen samples in liquid nitrogen (N) (Fig. 2a,b); both of them exhibited a smooth texture. Rapid (R) and slow (S) freezing rates produced "grainy" and "spongy" structures, respectively. Figures 2a, b and c showed a thixotropic behavior, however lower velocities (Fig.

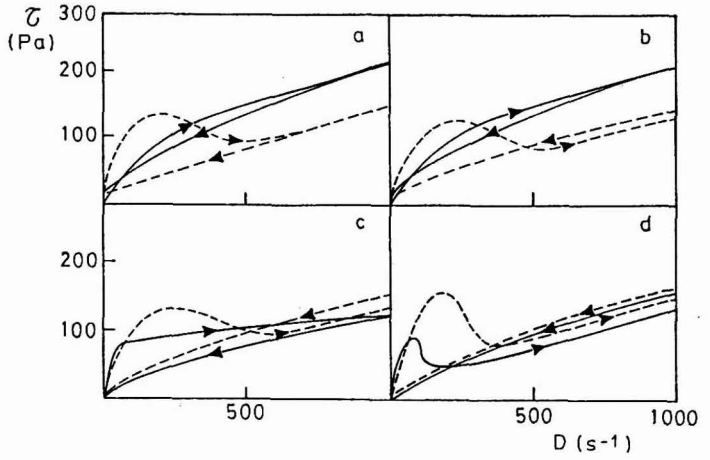

FIG. 2. INFLUENCE OF FREEZING RATE ON SHEAR STRESS (τ) VS SHEAR RATE (D) CURVES OF CORN STARCH PASTES
a) UF: unfrozen, b) N: 216 cm/h, c) R: 0.8 cm/h and d) S:0.3 cm/h.
—— paste without xanthan gum, - - with xanthan gum

2d) led to a characteristic viscoelastic behavior with a rheodestruction peak at D values lower than 500 s^{-1}. At high D values the decreasing sequence curve lies over the increasing one, which can be attributed to a major separation of the aqueous phase that increased the concentration of the solid phase and its viscosity.

The addition of xanthan gum to the starch paste modified the shape of the τ vs D curves (Fig. 2, dotted line) compared with the results corresponding to corn starch pastes without hydrocolloid. A marked viscoelastic behavior was observed in frozen and unfrozen samples for $D < 500\ s^{-1}$. Freezing rate had no significant effect on the rheological curves in accordance with the smooth and homogeneous appearance observed in all frozen and unfrozen samples.

Wheat flour pastes showed a rheological behavior (Fig. 3, solid line) similar to corn starch samples. Rheopexy was not observed in slow frozen samples; this fact can be related to a lower liquid separation and an underdeveloped spongy structure. Wheat proteins might account for the higher water retention that minimized solvent separation during freezing. Syneresis enhances the formation of a thick fibrillar network, which turns into a spongy structure.

Xanthan gum, similarly, minimized freezing rate effect on wheat flour pastes. Rheological curves of frozen samples did not differ significantly from the unfrozen one (Fig. 3, dotted line).

During frozen storage, samples frozen in liquid N_2 did not change their viscoelastic behavior. Starch pastes frozen at 0.3 cm/h maintained the rheological

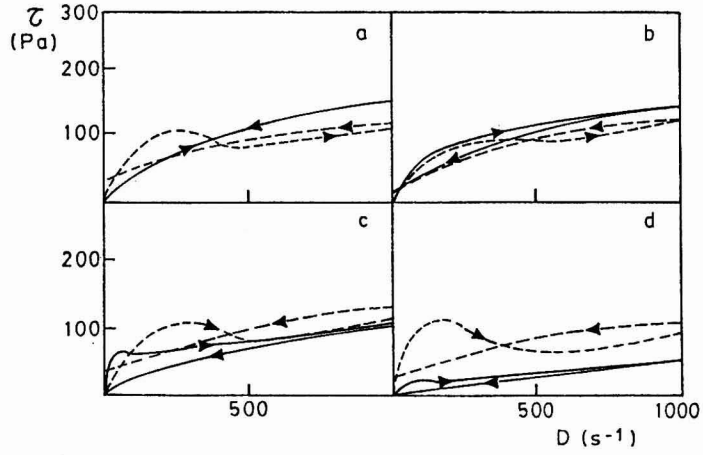

FIG. 3. EFFECT OF FREEZING RATE ON SHEAR STRESS (τ) VS SHEAR RATE (D) CURVES OF WHEAT FLOUR PASTES
a) UF: unfrozen, b) N: 216 cm/h, c) R: 0.8 cm/h, d) S: 0.3 cm/h.
—— paste without xantham gum, - - with xanthan gum

TABLE 1.
APPARENT VISCOSITIES AT 512 s^{-1} OF CORN STARCH AND WHEAT FLOUR PASTES WITH AND WITHOUT XANTHAN GUM

	S.time (weeks)	F.rate (cm/h)	Storage Temperature(C)					
			-5	-10	-20	-5	-10	-20
			without xanthan gum			with xanthan gum		
corn starch	1	216.0	309	211	233	157	201	205
		0.8	94	131	123	144	157	166
		0.3	55	83	87	226	176	163
	12	216.0	416	311	302	216	*	141
		0.8	123	126	192	135	143	186
		0.3	68	70	66	209	189	195
wheat flour	1	216.0	251	206	198	145	286	*
		0.8	151	122	145	132	185	150
		0.3	43	103	148	161	222	94
	12	216.0	163	241	191	142	247	265
		0.8	110	146	168	155	147	113
		0.3	47	45	84	101	176	186

* not measured values corresponded to the viscoelastic zone
standard error: 12 %

behavior already described with the characteristic overshoot related to the destruction of the spongy structure. Samples frozen at 0.8 cm/h showed an increasing rheodestruction peak during storage at −5C.

The development of the characteristic overshoot in the rheological curves was less evident at −5C in wheat flour pastes than in corn starch ones. The addition of xanthan gum in both pastes minimized the effect of storage temperatures. Apparent viscosities were evaluated at $D = 512\ s^{-1}$ (where power law is valid) in order to analyze the effects of freezing rate and frozen storage conditions. Results are shown in Table 1. Analysis of variance (ANOVA) with significance level of 95% was performed on samples stored for 1, 2, 4, 8 and 12 weeks at different constant temperatures. ANOVA showed that freezing rate was the most important factor in corn starch pastes deterioration while storage time and temperature did not have a relevant effect, in both systems. Xanthan gum hindered the adverse effects caused by freezing; the other factors: freezing rate, storage time and temperature did not have a significant effect.

Modeling of the Rheological Curves

Ostwald-De Waele model (power law) was applied (Eq. 2) to the results for $D > 500\ s^{-1}$.

$$\tau = mD^n \tag{2}$$

where m is the consistency index and n the flow behavior index. Parameters m and n were obtained by nonlinear regression analysis (SYSTAT, SYSTAT Inc., USA) for $D > 500\ s^{-1}$.

Table 2 shows the effect of freezing rate on both parameters n and m, respectively, for all the tested formulations. Differences were analyzed with the Student's t test for means comparison with a 95% confidence level. Nonsignificant differences were found in the n values corresponding to UF samples (corn starch and wheat flour unfrozen pastes), N samples (frozen at 216 cm/h) and R samples (0.8 cm/h); however, this index increased significantly for the lower freezing rate, S samples (0.3 cm/h). Consistency index (m), in corn starch pastes, for R and S conditions, differed significantly from N values; values of m at UF and N conditions were nonsignificantly different.

In wheat flour pastes only consistency index for the lower freezing rate (S) was significantly different.

When xanthan gum was added, nonsignificant differences were found for both indexes n and m in samples frozen at different velocities.

Indexes m and n did not exhibit significant modifications ($P \geq 0.05$) during frozen storage. Thus, xanthan gum addition suppressed the deleterious effect of freezing and storage.

TABLE 2.
EFFECT OF FREEZING RATE AND XANTHAN GUM ADDITION ON THE FLOW BEHAVIOR INDEX (n) AND THE CONSISTENCY INDEX (m)

paste composition		unfrozen (UF)	Freezing rate (cm/h)		
			216 (N)	0.8 (R)	0.3 (S)
corn starch	n	0.337	0.309	0.363	0.754
	m	22.21	23.59	9.30	0.58
corn starch with x. gum	n	0.342	0.408	0.339	0.378
	m	14.09	8.22	10.39	10.16
wheat flour	n	0.373	0.430	0.380	0.506
	m	8.35	8.06	6.22	1.90
wheat flour with x.gum	n	0.534	0.501	0.549	0.526
	m	2.63	5.77	2.48	3.35

n and m values are mean of 16 replicates, standard error for n values: 6% and for m values: 13%

Ice Crystal Formation and Ice Recrystallization Measurements

Ice crystal size distributions were analyzed from the micrographs by goodness of fit test X^2 (chi-square) with a significance level of 95%, which allowed the use of the normal distribution in order to calculate mean values and standard deviations.

As freezing rate decreased, mean ice crystal size increased, leading to a microstructure deterioration. Crystal size distributions obtained with the rapid freezing rate of 15 cm/h had a narrower diameter range, a lower mean equivalent diameter ($D = 30.89 \pm 1.81$ μm), and a different shape, compared to the slow freezing rate of 2 cm/h ($D = 37.34 \pm 2.50$ μm). Wheat flour proteins did not show a particular protective effect against freezing, since mean equivalent diameter of corn starch and wheat flour pastes did not differ significantly ($P \geqslant 0.05$).

The addition of xanthan gum to corn starch pastes did not show a significant effect on ice crystal size at different freezing rates ($D = 34.49 \pm 1.66$ μm and 29.69 ± 0.84 μm for 2 and 15 cm/h, respectively) compared to the pastes without the gum. A similar tendency was observed in the case of wheat flour pastes.

Recrystallization of ice during frozen storage can be visualized as an enlargement of the ice crystal sizes due to storage time, higher temperature levels and temperature fluctuations (Martino and Zaritzky 1988). In our case, experiments were designed to evaluate recrystallization at constant temperatures; fluctuations in temperature were minimized. Figure 4 shows micrographs of corn starch pastes frozen at 2 cm/h (a), stored at −20C during 60 days (b) and stored at −5C during 8 days (c) and 60 days (d); the white zones correspond to the voids left by the ice crystals.

Similar results were obtained with flour pastes samples. At higher temperatures and longer storage times, mean equivalent diameter of ice crystals increased.

Frequencies of occurrence of ice crystals with different equivalent diameters, corresponding to the micrographs shown in Fig. 4 are represented in Fig. 5. Histograms showed an increase of the larger crystals at the expense of the smaller ones. Pastes with xanthan gum also showed ice recrystallization; histograms shifted to larger equivalent diameters with increasing storage time and temperature (Fig. 6). Thus, xanthan gum addition did not avoid ice recrystallization.

Curves of equivalent ice crystal diameter versus storage time (Fig. 7) showed a tendency to reach different limit equivalent diameters (D_1) depending on storage temperature.

A mathematical model that fits the experimental data needs to consider that as ice crystal size increases, the driving force of recrystallization declines and stops when a stable crystalline size is reached. Thus, the driving force should be the difference between the instantaneous curvature of the system and the limit curvature (Martino and Zaritzky 1989), i.e.:

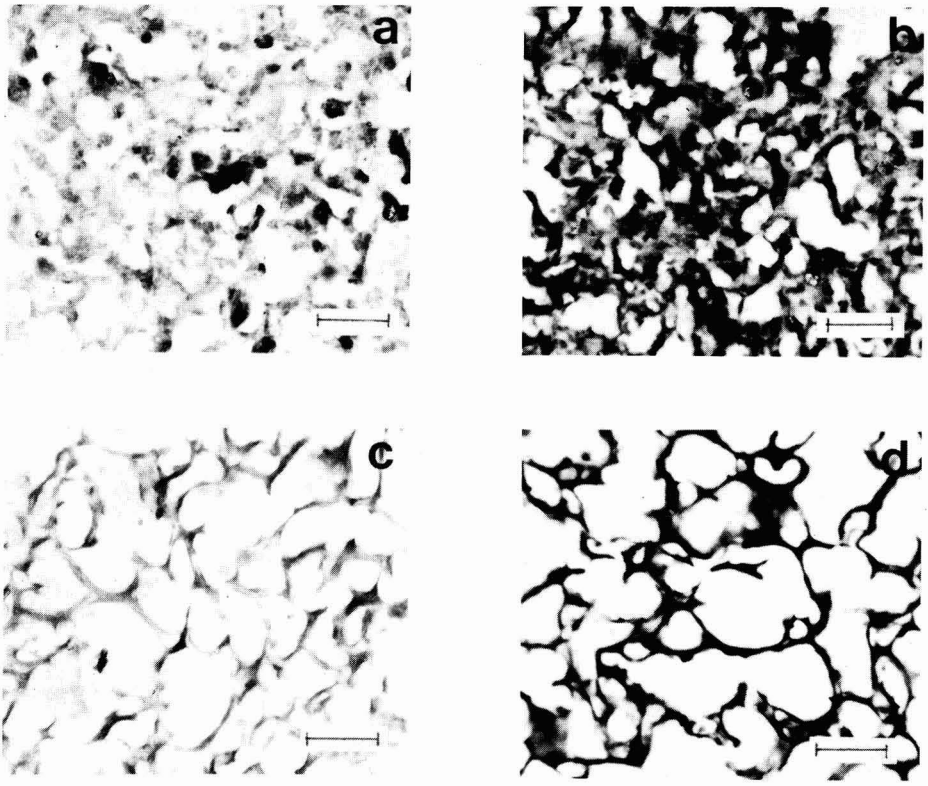

FIG. 4. EFFECT OF STORAGE ON CORN STARCH SAMPLES FROZEN AT 2 cm/h
a) Just frozen, b) stored 60 days at −20C, c) stored 8 days at −5C, d) stored 60 days at −5C. Bar = 100 μm.

$$\frac{dD}{dt} = k\left(\frac{1}{D} - \frac{1}{D_1}\right) \tag{3}$$

where D is the mean equivalent ice crystal diameter at time t, D_1 the limit equivalent diameter and k the kinetic constant. Integration of Eq. (4) led to the following expression:

$$\ln\left(\frac{D_1 - D_o}{D_1 - D}\right) + \frac{(D_o - D)}{D_1} = \frac{k\,t}{D_1^2} \tag{4}$$

with D_0 = initial equivalent diameter.

Kinetic constants of this model at each tested temperature and for each paste were obtained by nonlinear regression analysis of the experimental D vs time

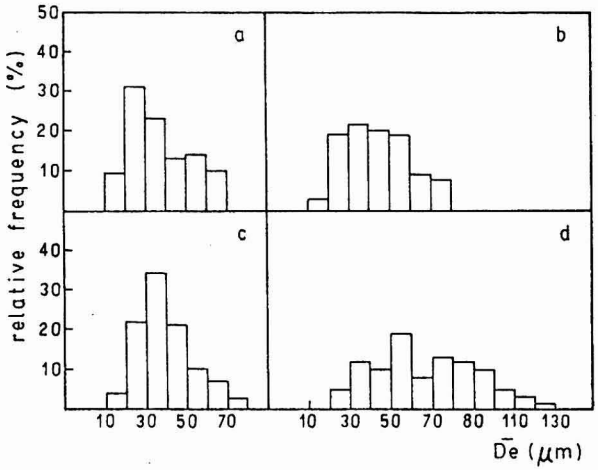

FIG. 5. HISTOGRAMS OF RELATIVE FREQUENCY (%) VS EQUIVALENT ICE CRYSTAL DIAMETER OF CORN STARCH PASTES WITHOUT XANTHAN GUM FROZEN AT 2 cm/h
a) Just frozen, b) stored 60 days at −20C, c) stored 8 days at −5C, d) stored 60 days at −5C.

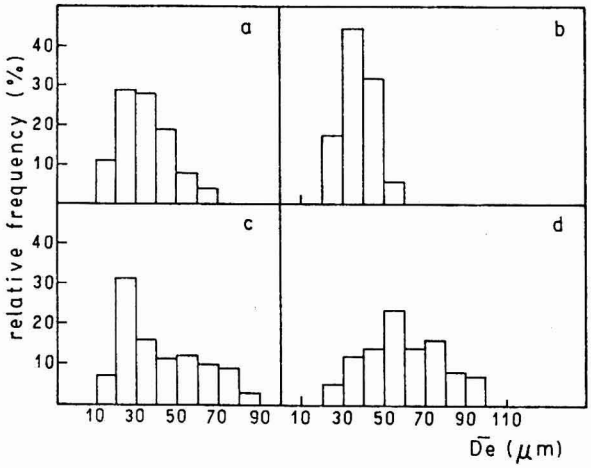

FIG. 6. HISTOGRAMS OF RELATIVE FREQUENCY (%) VS EQUIVALENT ICE CRYSTAL DIAMETER OF CORN STARCH PASTES WITH XANTHAN GUM FROZEN AT 2 cm/h
a) Just frozen, b) stored 60 days at −20C, c) stored 8 days at −5C, d) stored 60 days at −5C.

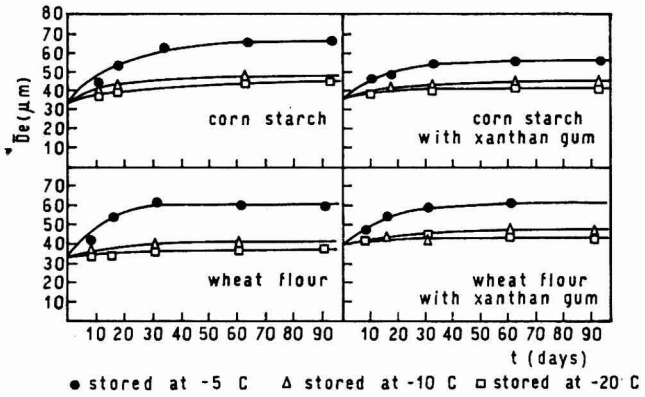

FIG. 7. MEAN EQUIVALENT ICE CRYSTAL DIAMETER (De) VS STORAGE TIME OF CORN STARCH AND WHEAT FLOUR PASTES WITH AND WITHOUT XANTHAN GUM FROZEN AT 2 cm/h

data with the Systat program (Table 3). Experimental values of D_0 and D_1 were used in each case. Nonsignificant differences were found between recrystallization kinetic constants corresponding to pastes frozen at different velocities, which means that the model was adequately chosen and kinetic constants only depend on storage temperature.

Arrhenius Law was applied to calculate the activation energies (E_a) of the recrystallization process for each system. Table 3 shows E_a values obtained by linear regression ($\ln k = \ln k_0 - E_a/RT$); nonsignificant differences ($P \geqslant 0.05$) were found between these values.

Thus, we can conclude that xanthan gum addition did not alter ice recrystallization rate, nor its activation energy. Commonly, hydrocolloids are recommended as ice crystal growth inhibitors (Shuman 1960), but this study together with some others (Reid *et al.* 1987; Budiaman and Fennema 1987a,b) showed that the stabilization character of hydrocolloids should be explained, at least in some cases, on another basis like high water retention after thawing or their possible capability to undergo molecular entanglement in the freeze concentrated matrix surrounding the ice crystals (Slade and Levine 1986).

Starch Retrogradation

Starch retrogradation can be considered as a two step process (Miles *et al.* 1985), a short term one related to amylose crystallization (thermically irreversible below 100C) and a long term one that involves amylopectin crystallization (thermically reversible). Differential Scanning Calorimetry (DSC) only allowed the quantification of the reversible process (Roulet *et al.* 1990).

TABLE 3.
ICE RECRYSTALLIZATION KINETIC CONSTANTS AND ACTIVATION ENERGY FOR PASTE SYSTEMS FROZEN AT 2 CM/H

	T (C)	k ($\mu m^2\ d^{-1}$)	Ea (KJ/mol)
corn starch paste without xanthan gum	-5	232.66 (37.32)	55.43 (13.69)
	-10	93.90 (3.26)	
	-20	56.93 (4.62)	
corn starch paste with xanthan gum	-5	227.71 (70.17)	57.90 (12.98)
	-10	96.81 (18.21)	
	-20	55.94 (24.51)	
wheat flour paste without xanthan gum	-5	218.46 (36.24)	46.47 (10.02)
	-10	93.43 (32.45)	
	-20	54.26 (13.19)	
wheat flour paste with xanthan gum	-5	219.54 (23.87)	56.65 (9.02)
	-10	72.21 (13.34)	
	-20	40.50 (6.74)	

standard deviation between parentheses
Ea values did not differ significantly ($P \geq 0.05$)

Retrogradation enthalpies were only detected for corn starch pastes frozen at rates lower than 1 cm/h, although a marked macroscopic modification was observed in the physical appearance of most of the pastes. For corn starch pastes, high freezing rates did not produce a detectable retrogradation peak; lower freezing rates (1 cm/h and 0.3 cm/h) led to measurable peaks (2.05 ± 0.35 J/g and 2.97 ± 0.20 J/g, respectively) although both values did not differ significantly ($P \geq 0.05$). The presence of xanthan gum did not modify the effect of freezing rate on amylopectin retrogradation. The values of enthalpy were similar to those of corn starch pastes without xanthan gum (2.38 ± 0.26 J/g and 2.76 ± 0.36 J/g for freezing rates of 1 cm/h and 0.3 cm/h, respectively).

A different result was obtained for pastes of wheat flour with and without hydrocolloid; retrogradation peaks were not detected for any of the used freezing rates.

DSC thermograms of samples stored at -1, -5, -10 and -20C were obtained at different storage times. The samples stored at -1C and -5C showed an increase of the transition enthalpy and a shift of the peak temperature to lower values with increasing storage times. Results corresponding to samples stored at -5C are shown in Fig. 8. This transition was calculated according to:

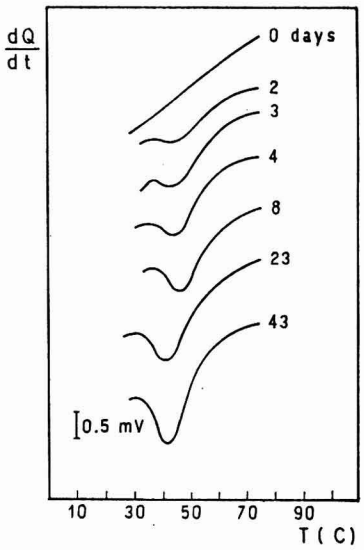

FIG. 8. DSC THERMOGRAMS OF CORN STARCH PASTES FROZEN AT 12 cm/h AND STORED AT −5C.

$$\Delta H = \frac{A(60\ B\ E\ \Delta qs)}{m} \tag{5}$$

where A is peak area of the DSC thermogram (cm^2), m is the starch mass (mg, dry basis), B is the time base (min/cm), E is the cell calibration coefficient (mW/mV), Δqs is the y axis sensibility (mV/cm) and ΔH is the enthalpy change (J/g).

Starch retrogradation kinetic was determined with the ΔH values obtained at different storage times. Avrami equation was fitted to the experimental values of ΔH as follows:

$$\theta = \frac{\Delta H_t - \Delta H_o}{\Delta H_\infty - \Delta H_o} = 1 - e^{(-kt^n)} \tag{6}$$

where ΔH_∞, k and n were coefficients determined by nonlinear regression analysis and θ is the retrograded fraction. ΔH_∞ is the maximum enthalpy (limit value) and ΔH_0 is the enthalpy of the just frozen sample.

Obtained values of ΔH_∞ and k for two different storage temperatures are shown in Table 4. Amylopectin retrograded fraction (θ) at −1 and −5C as a function of storage time is shown in Fig. 9. At storage temperatures of −10 and −20C, DSC retrogradation peaks were not detected during the tested period.

Textural characteristic of "sponginess," observed when low freezing rates or high storage temperatures were used and attributed to amylose crystallization, should not be related to the enthalpic change measured by DSC and produced by amylopectin retrogradation. Amylose retrogradation was described by Morris (1990) as the coarsing of the fibrillar network (Kikuchi and Izutsu 1981). Low freezing rates or high storage temperatures enhanced gel desolvation and the formation of high amylose concentration zones with the thickening of the fibrillar structure that leads in turn to the spongy matrix.

In order to understand the effect of freezing rate and storage temperature on starch retrogradation, basic concepts of polymer science can be considered. According to the Slade and Levine (1987) definition, starch is a "water compatible polymer which exhibits nonequilibrium melting, annealing and crystallization behavior, characteristic of a kinetic metaestable, water plasticized, partially crystalline polymer system with a small extent of crystallinity." Glass transition as a physicochemical phenomenum can govern starch properties, processing and stability. Below the glass transition temperature (Tg), polymer material becomes "glassy," the molecular motion is so slow that crystallization never occurs in a finite period of time. For temperatures above Tg and below the crystal melting temperature (Tm), the material is "rubbery" and sufficient motion of the polymer occurs allowing crystallization. Rapid cooling (quenching) of a hot polymer melt, increases Tg value; thus, crystal formation may not be observable at Tm, even

TABLE 4.
RETROGRADATION KINETIC PARAMETERS OF CORN STARCH AND WHEAT FLOUR PASTES DURING FROZEN STORAGE

	Storage temperature			
	-1 C		-5 C	
	$k(days^{-1})$	$\Delta H_\infty(J/g)$	$k(days^{-1})$	$\Delta H_\infty(J/g)$
corn starch	0.309 (0.064)	8.883 (0.330)	0.114 (0.033)	9.288 (0.836)
corn starch with xanthan gum	0.369 (0.227)	6.730 (0.372)	0.216 (0.063)	6.132 (0.271)
wheat flour	0.289 (0.079)	2.897 (0.142)	0.130 (0.024)	2.575 (0.150)
wheat flour with xanthan gum	0.248 (0.023)	3.285 (0.071)	0.235 (0.034)	2.383 (0.084)

r = correlation coefficient of the nonlinear regression, with r maximum = 0.994 and r minimum = 0.970
standard deviation between parentheses.

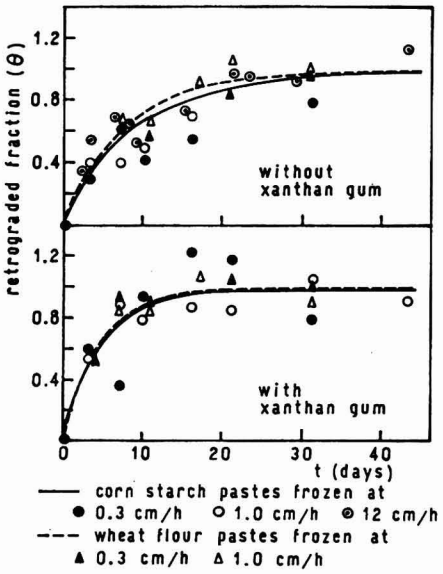

FIG. 9. RETROGRADED FRACTION (θ) VS STORAGE TIME OF CORN STARCH AND WHEAT FLOUR PASTES WITH AND WITHOUT XANTHAN GUM

Full and dashed lines correspond to Avrami model.

for a crystallizable polymer (Hiemenz 1984). In the case of starch pastes, rapid freezing avoided crystallization of both amylose and amylopectin, producing an homogeneous structure without a spongy network and the absence of amylopectin retrogradation peaks.

In comparison with starch pastes, only a slightly spongy structure was observed with the wheat flour pastes for the lower freezing rate; interaction between proteins and amylose would be responsible for this behavior.

Xanthan gum addition did not avoid amylopectin retrogradation, but it inhibited the development of the characteristic spongy matrix that is related to amylose retrogradation. It had no effect on ice crystal formation or recrystallization. Thus, the low exudate values observed in these samples can be attributed to the high water holding capacity of the hydrophilic gum.

Amylose and amylopectin structures may explain differences in retrogradation behavior when xanthan gum is present. Amylose chains are linear and longer than amylopectin ones; during gelatinization amylose is released outside the granule and forms an external matrix. Amylose has then a higher exposure to other components of the paste like hydrocolloids. Amylose-hydrocolloid interaction competes with amylose-amylose aggregation decreasing the probability of retrograda-

tion occurrence. Similarly Biliaderis and Zawistowski (1990) discussed the network properties of starch gels when starch hydrolysis products of low molecular weight were added.

Lack of detectable retrogradation during frozen storage at −10 and −20C can be explained, considering that at temperatures lower than −5C [glass transition temperature of the maximally freeze-concentrated unfrozen matrix of the gelatinized paste according to Slade and Levine (1987)] amylose and amylopectin chains have a reduced mobility that limits the molecular association responsible for the retrogradation phenomena.

Xanthan gum is a natural stabilizer with a good consumers' acceptability and, as demonstrated in the present study, proved to maintain textural characteristics and low exudate levels even at slower freezing rates. Thus, the use of xanthan gum could be considered a valid alternative to high freezing rates, with the consequent decreasing equipment investment.

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PRODUCTION OF FLAVOR BY A MUTANT OF YEAST[1]

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ABSTRACT

Strain CCU-N_{16}-18143 was derived with N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment three times from a wild strain of yeast CCU-16 as a mutant to produce more delicious and intense flavors for nutrient beverage from glucose medium. This mutant was identified by computer system as Saccharomyces *sp. The optimal culture medium for the production of flavor is one liter of medium containing 100 g glucose, 12 g ammonium nitrate, 3 g yeast extract, 1 g magnesium sulfate, 1 g ammonium sulfate, 2 g potassium dihydrogen phosphate, pH: 3.5. The optimal culture conditions are: temperature: 30C; agitation: 150 rpm., 50 ml medium in 500-ml Hinton flask; incubation time: 96 h. The volatile flavor compounds in the culture medium were analyzed by capillary gas chromatography and capillary gas chromatography-mass spectrometry. It was found that more volatile compounds were produced by mutant strain than wild strain. The content of isoamyl alcohol increased from 7.76 to 61.64%, whereas ethyl alcohol decreased from 85.25 to 5.77%.*

INTRODUCTION

Biotechnology has been applied in food processing for several thousand years. It includes microbial technology, enzyme technology, plant cell technology and animal cell technology (Knorr and Sinskey 1985; Fleet 1986). The application of biotechnology in the flavor industry has only been developed since the 1980s (Gatfield 1988; Dziezak 1986; Bedoukian 1985; Gocho 1984).

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Today the value of flavor products is US$ 1 billion worldwide. These are produced mostly by chemical synthesis, but high-value flavor compounds are still extracted from plants. The production of flavor compounds from agriculture is affected by weather, season, geography and even politics. Flavor compounds produced by chemical synthesis lack optical selectivity. They must be separated before use. Those produced from microorganisms do not have this disadvantage, and they can fill the requirements for natural products (Bedoukian 1985; Gocho 1984; Kemper 1983; Chyou 1989).

The production method for flavor using biotechnology includes cultivation of plant cells, tissue cultures and modification of natural substrates with enzymes and fermentation (Seitz 1984). At present, tissue culture is expensive. Modification with enzymes is widely used in the manufacture of cheese, but only on a small scale (Guo 1983; Langard *et al.* 1988). Production of flavor by fermentation was widely used in dairy fermentation, soy sauce and wine industries since the late 19th century.

For the production of more delicious and intense flavors, mutation of microorganisms is often used. In 1980 Kielland and Brandt found that yeast mutants can produce more 2,3-pentanedione, D-amylalcohol and α-aceto-α-hydroxybutyrate (Rowlavds 1984). Molzahn used mutants of yeast derived from NTG-treatment to improve the flavor of beer (Rowlavds 1984). With ultraviolet (UV) irradiation and NTG-treatment Poland improved the production of baker's yeast (Rowlavds 1984). Another paper reported that a yeast mutant treated with ethyl methanesulfonate (EMS) can produce more flavor compounds (e.g., diacetyl, 2,3-pentanedione and acetoin) (Rolavds 1984).

In this study, we treated yeast strains from Paolyta Company, one of the famous nutritive liquor producers in Taiwan, for the purpose of deriving mutants that can produce more delicious and intense flavor compounds.

MATERIAL AND METHODS

Composition of Media

YM medium. YM medium was used for slant culture. It is composed of 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone and 10.0 g glucose in 1 L of medium. The pH value is 6.5.

Synthetic Medium. Synthetic medium was used for the screening of mutants and for the investigation of culture conditions. The composition is 3.0 g yeast extract, 3.5 g peptone, 2.0 g potassium dihydrogen phosphate, 1.0 g ammonium sulfate, 1.0 g magnesium sulfate hydrate and 100 g glucose in 1 L of medium. The pH value is 5.5.

Complete and Minimal Media. These two media were used for the screening of mutants. Complete medium is composed of 10.0 g yeast extract, 20.0 g each of peptone, glucose and agar in 1 L of medium. Minimal medium is composed of 7.0 g yeast nitrogen base without amino acid, 20.0 g glucose and 20.0 g agar in 1 L of medium. The pH value of the broth is 5.5.

Reagents and Substrate

Yeast extract, malt extract and yeast nitrogen without base amino acid were purchased from Difco Company, USA, EMS from Sigma Company, USA, NTG from Kasei Chemical Company, Japan, and peptone from Wako Pure Chemical Industries Company, Japan. The other chemicals were all reagent grade products of Wako Pure Chemical Industries Company, Japan.

Experimental Methods

Induction and Screening of Mutants. *Wild Strain.* Wild strains (CCU-4, CCU-8, CCU-16) were provided by Paolyta Company. Those strains were all yeast.

Mutation Treatment. UV-irradiation (wave length 253 nm, distance 40 cm, irradiation time 30 min), NTG (1 mg/ml, 20 min) and EMS (3%, 10 min) treatments were as described by Pan and Wang (1989).

Screening of Flavor-Producing Mutants. After UV-irradiation, NTG and EMS treatment, cells were diluted and spread on agar plates. After 72 h incubation, single colonies were transferred into complete and minimal media. The strains which could grow in complete medium but not in minimal medium (auxotrophs) were selected and then cultivated in synthetic medium. Culture broth was then screened for flavor-producing strains.

Sensory Evaluation Method. *Selection of Evaluator.* To assure accuracy, the evaluators were assessed by triangular test (Henry 1977; Harries 1973; Moskswitz 1974; Copez *et al.* 1990).

Sensory Evaluation. Sensory evaluations were done following the methods of Larmond (Wang 1980; Masschelein and Jeunehomme 1976). The results are expressed on a nine-point hedonic scale. Ten panelists participated in sensory evaluation. The more pleasant the panelists' ratings, the higher the scores were.

Analytical Methods. *Extraction of Flavor Compounds.* Fermentation broth was centrifuged to remove cells (7,000 × g, 15 min) and then extracted with a mixture of pentane and ethyl ether (1:1) for 2 h in a Likens-Nickerson extractor. Solvent with flavor compounds was dehydrated using anhydrous copper sulfate

and then filtered. The filtrate was concentrated by microdistiller (Ficher 255) to about 1 ml. The concentrate was transferred to capillary tubes (8 cm × 3 mm i.d.), and concentrated again in a water bath (40C) to 1.5 cm in height. The concentrated flavor compounds then underwent the analysis below.

Instrumental analysis of volatile compounds. Gas Chromatography (GC). GC-8A system of Shimadzu Co., Japan was used. The conditions were as follows: column: capillary column of Chrompack fused silica Cp-Wax 52 CB; 50 m × 0.22 mm (i.d.); carrier gas: hydrogen (1.2 ml/min); detector: FID; temperature of detector and injector: 250C; temperature of column: initial temp.: 50C (0 min), final temp.: 200C (60 min), temp. gradient: 2C/min; split ratio: 50:1.

Gas Chromatography-Mass Spectrometry (GC-MS). 5985 B GC-MS system of Hewlett-Packard Co., U.S.A., was used for the analysis. The conditions were as follows: column: capillary column of Chrompack fused silica Cp-Was 52 CB; 50 m × 0.32 mm (i.d.); carrier gas: helium (1.5 ml/min); temperature of injector: 250C, temp. of interface: 200C, temp. of column: initial temp.: 50C, final temp.: 200C, temp. gradient: 2C/min; temp. of ion source: 200C; electron current: 70 eV; electron multiplier voltage: 2600 V.

Retention Index (R.I.) of Gas Chromatography (Majlat *et al.* 1974). From the retention time of sample compound and standard compounds, retention index can be derived using the following equation:

$$\text{R.I.} = 100\ i\ \left(\frac{t_x - tM_{(n)}}{tM_{(n+i)} - tM}\right) + 100\ n$$

where t = retention time; x = unknown compound; M = standard compound (n-alkane); n, n+1 = carbon number of n-alkane; i = difference of carbon number of n-alkane; and 100 n = R.I. of n-alkane with carbon number of n.

Investigation of Optimal Culture Conditions and Medium Composition for Flavor Production. One loop of cells grown on slant was transferred to a 500-ml Hinton flask with 50 ml medium. The optimal culture conditions and medium composition under 30C, 150 rpm for 24-h cultivation were then investigated.

Micrograph and Strain Identification of Microorganisms. Cells of mutant grown on YM medium at 30C for 2 days were transferred to a glass slide. After fixation, a micrograph was taken. Barnett's computer system was followed for strain identification (Barnett and Pankhurst 1974; Barnett *et al.* 1979, 1983).

RESULTS AND DISCUSSION

Induction and Screening of Flavor-Producing Mutants

The total number of mutants and the number of mutants that can produce flavor for nutrient beverage are shown in Table 1. Although 314 strains of mutants were derived using UV-irradiation, there were only three strains that could produce flavor. NTG-treatment produced the best results. Out of 446 mutant strains, 48 of them could produce flavor. 135 strains of mutants derived from EMS-treatment, only 1 strain could produce flavor. Rowlavds (1984) indicated that auxotrophic mutants (valine$^-$) derived from NTG-treatment could produce large amounts of diacetyl and that those derived from EMS-treatment (isoleucine$^-$, valine$^-$) could improve the flavor of beer by producing diacetyl and 2,3-pentanedione.

TABLE 1.
MUTANTS INDUCED BY VARIOUS MUTAGENS FOR THE PRODUCTION OF FLAVOR

Wild strain	Mutagen[1]	No. of mutants	No. of mutants with flavor[2]
CCU-4	UV	103	0
	NTG	63	3
	EMS	35	1
CCU-8	UV	117	1
	NTG	109	8
	EMS	48	0
CCU-16	UV	94	2
	NTG	274	37
	EMS	52	0

[1] UV : Ultraviolet-irradiation
NTG: N-methyl-N-nitro-N'-nitrosoguanidine
EMS: Ethyl methanesulfonate
[2] Flavor for nutrient beverage

Comparison of Flavor-Producing Ability of the Mutants

Out of 52 strains of mutants derived, four stains treated with MTG (CCU-N_8-24, CCU-N_{16}-18126 and CCU-N_{16}-18143) were selected, as they could produce more delicious flavor (greater sensory score).

After diluting the fermentation broth three times with distilled water, the flavor sensory evaluation was performed. Diluent of strains CCU-N_8-24 and CCU-N_{16}-18143 have stronger flavor than wild strains (Fig. 1). Strain CCU-N_{16}-18143 was used throughout the study.

Micrograph and Strain Identification of Strain CCU-N_{16}-18143

Micrographs of CCU-N_{16}-18143 showed that the cell is spherical to oval, buds in multiple form, can form a round or oval ascospore. The strain cannot assimilate lactose or nitrate.

The results of computer identification are shown in Table 2. These indicate that the strain has a 95% probability of being *Saccharomyces cerevisiae*.

Optimal Culture Conditions and Medium Composition for Flavor Production

Effect of pH Value. Although strain CCU-N_{16}-18143 grew best at pH 6.0, the flavor produced at pH 3.5 (fruity-green flavor) is more suitable for nutrient beverage than that produced at higher pH value (winey flavor).

Spencer (1983) reported that *Arthrobacter globiformis* and *Pseudomonas* sp. could produce flavor at high pH values (9.5 and 8.0, respectively) but that flavor produced at pH 3.5–4.4 was more delicious.

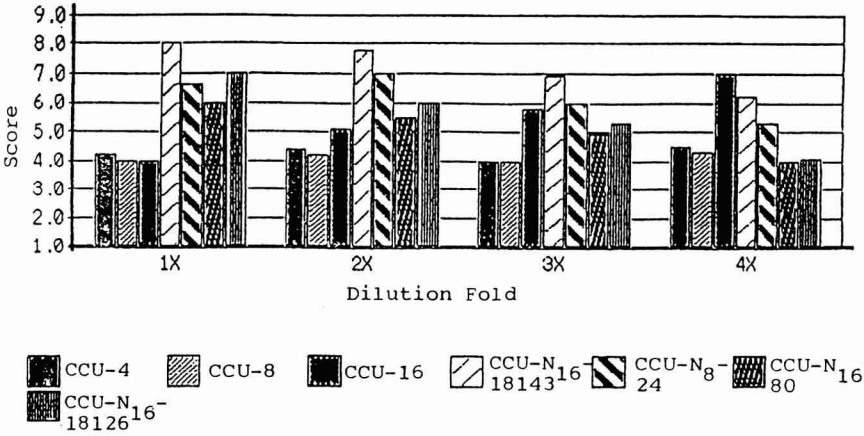

FIG. 1. SENSORY EVALUATION OF FLAVOR STRENGTH

TABLE 2.
COMPUTER IDENTIFICATION OF THE STRAIN CCU-N_{16}-18143[1]

Type: Yeast Biochemical Card
Final Elapsed: 24 h

GAL -	LAC -	SUC +	MLT -	CEL -	AMG +
XYL -	ARA -	TRE -	MLZ -	RAF +	NAG -
XLT -	DUL -	ADO -	PAL -	CLY -	SOR -
ERY -	MEL -	CYC -	GLU +	INO -	NIT -
2KD -	URE -	48H -			

95 % Saccharomyces cerevisiae
4 % Unidentified organism

[1] According to Barnett, J. A., Payne, R. W. and Yarrow, D. (1983).

Effect of Cultivation Time. Flavor produced after 96-h cultivation was best for nutrient beverage. On the seventh day, the fruity-green odor changes to winey odor. After the tenth day, the flavor becomes unacceptable.

Effect of Carbon Source. Different kinds of carbon sources (glucose, lactose, maltose, xylose, galactose, sucrose, sorbitol, fructose and soluble starch) are used to produce flavor. Glucose is most suitable for flavor production. Tony *et al.* (1989) reported that the affinity of yeast to glucose is better than for other carbohydrates. The utilization efficiency of glucose is twice than that of fructose (Tony *et al.* 1989). The flavor formed by strain CCU-N_{16}-18143 with sucrose or maltose is winey.

The effect of glucose concentration on flavor production was studied. With 100 g glucose in 1 L of medium, cell growth and flavor production were best. Nsaium and Averbach (1967) found that high concentrations of glucose would inhibit the formation of mitochondria. $NADH_2$ accumulation depresses the activity of pyruvate dehydrogenase. The TCA cycle is then blocked. At the same time, the induction of the formation of pyruvate decarboxylase favors the transition of acetaldehyde to ethyl alcohol.

Effect of Nitrogen Source. With ammonium nitrate as the nitrogen source, strain CCU-N_{16}-18143 can produce more flavor than the others (urea, peptone, casein, sodium nitrate, ammonium sulfate, ammonium dihydrogen phosphate).

If urea or casein are used as nitrogen sources, an offensive smell is formed. The reason may be that they cannot permeate the cell wall and so cannot form precursors of flavor compounds (Nasium and Averbach 1967).

The optimal concentration of ammonium nitrate for the production of flavor was determined to be 12 g in 1 L of medium.

Effect of Yeast Extract Concentration. There are different kinds of amino acids, carbohydrates, water-soluble vitamins and metal ions in yeast extract. It is an important constituent of the medium. The effect of the concentration of yeast extract was studied. Three grams in one liter was the most suitable for cell growth and flavor production.

Effect of Incubation Temperature. Because the flavor compounds are all volatile, the incubation temperature cannot be too high. Pereira and Morgan (1958) found that yeast can produce more acids at lower temperatures. Strain CCU-

TABLE 3.
COMPARISON OF VOLATILE COMPOUNDS PRODUCED BY WILD STRAIN CCU-16 AND MUTANT CCU-N_{16}-18143

Peak No.	Compound	Percentage (%) CCU-16	CCU-N_{16}-18143
1	Acetal	0.17	4.76
2	1,3-Dioxone	-	1.48
3	Ethyl alcohol	85.26	5.77
4	1-Propanol	0.34	1.06
5	2-Methyl propanal	1.42	7.40
6	1-Hexanol	0.05	0.70
7	Pentane	-	0.68
8	Isoamyl alcohol	7.78	61.64
9	Acetoin	0.04	0.50
10	Acetic acid	-	0.30
11	Ethyl n-octanoate	-	0.93
12	Propanoic acid	-	1.40
13	Phenethyl acetate	-	1.27
14	2-Phenyl ethanol	0.50	16.97
15	Nonanoic acid	-	2.07

TABLE 4.
IDENTIFICATION OF VOLATILE COMPOUNDS PRODUCED BY MUTANT CCU-N_{16}-18143

Peak No.	Compound	I (CW-20M)	M.W.	ID	Characteristic MS data m/e (relative intensity)
1	Acetal	896	118	MS,GC	45(100) 73(52) 43(20) 29(19) 47(19) 103(19) 89(7) 104(1)
2	Ethyl alcohol	902	46	MS,GC	31(100) 45(75) 29(33) 46(27)
3	1,3-Dioxone	930	320	MS,GC	101(100) 43(71) 78(68) 55(48) 41(27) 71(25) 54(22) 58(17)
4	1-Propanol	1083	60	MS,GC	31(100) 27(39) 29(26) 42(24) 60(24) 59(10) 57(3) 45(1)
5	2-Methyl propanal	1139	86	MS,GC	43(100) 41(94) 27(52) 42(23) 39(16) 74(13) 73(5) 75(1)
6	Pentane	1243	72	MS,GC	73(100) 45(66) 43(48) 71(25) 29(26) 45(23) 41(21)
7	1-Hexanol	1284	102	MS,GC	56(100) 43(72) 41(52) 55(51) 42(41) 69(30) 87(27) 29(23)
8	Isoamyl alcohol	1434	88	MS,GC	41(100) 55(94) 42(65) 43(61) 70(45) 71(5) 69(4.6) 73(0.6)
9	Acetoin	1558	88	MS,GC	45(100) 43(61) 88(21) 42(9) 73(4.6) 46(3.3) 55(1)
10	Acetic acid	1669	60	MS,GC	43(100) 45(95) 60(91) 42(18) 44(6.5)
11	Ethyl n-octanoate	1835	172	MS,GC	88(100) 29(37) 41(33) 43(28) 101(27) 127(23) 70(18) 115(12)
12	Propanoic acid	1854	74	MS,GC	43(100) 41(71) 73(52) 27(42) 39(20) 45(17) 42(7)
13	Phenethyl acetate	1919	164	MS	104(100) 43(82) 105(71) 91(23) 73(22) 103(16)
14	2-Phenyl ethanol	2074	122	MS,GC	91(100) 92(55) 122(26) 65(16) 39(15) 51(11) 31(8) 63(7)
15	Nonanoic acid	2166	158	MS,GC	60(100) 73(77) 57(59) 43(54) 41(38) 55(36) 40(21) 115(20)

N_{16}-18143 produced a pleasant odor at 25C. At higher temperatures (30 and 35C), the odor formed was unpleasant.

Effect of Medium Volume. Cell growth requires sufficient oxygen. Cells were cultivated with different volumes of medium in 500-ml Hinton's flasks. A medium volume of 50 ml was most suitable for cell growth and flavor production.

The Identification and Comparison of Flavor Components Produced by Wild Strain and Mutant

Both cultivation broths of wild strain (CCU-16) and mutant (CCU-N_{16}-18143) were extracted using a Liken-Nickerson extractor. The volatile compounds were identified with GC-MS. The wild strain produced 8 kinds of compounds, and 15 kinds of compounds were produced by the mutant (Table 3).

Alcohols are the most important kind of flavor compounds produced by the wild strain. Ethyl alcohol and isoamyl alcohol are the compounds produced in higher concentrations.

Flavor compounds produced by mutants were identified by GC-MS (Table 4). Alcohols such as ethyl alcohol, 1-propanol, isoamyl alcohol, and 2-phenyl alcohol compose the greatest proportion of flavor compounds. Odor description of volatile

TABLE 5.
ODOR DESCRIPTION OF VOLATILE COMPOUNDS PRODUCED BY MUTANT CCU-N_{16}-18143

Peak No.	Compound	Odor description[1]
1	Acetal	Agreeable, refreshing, fruity-green odor
2	Ethyl alcohol	Sweet-ethereal, mild odor
3	1,3-Dioxone	Very sweet, mild-ethereal
4	1-Propanol	Alcoholic-nauseating sweet, winey odor
5	2-Methyl propanal	Masking, fuels, acrylic odor
6	1-Hexanol	
7	Pentane	Somewhat chemical-winey, slightly fatty and fruity odor
8	Isoamyl alcohol	Choking, disagreeable cough-provoking, somewhat alcoholic odor, fruity-winey
9	Acetoin	Tensely creamy-fatty-buttery
10	Acetic acid	Pungent, stinging sour odor
11	Ethyl n-octanoate	Fruity-winey, sweet odor
12	Propanoic acid	Pungent sour odor reminscent of sour milk, cheese or butter
13	Phenethyl acetate	Very sweet, rosey-fruity honey-like odor
14	2-Phenyl ethanol	Mild and warm, rosey-honey-like odor
15	Nonanoic acid	Mild nut-like odor

[1] According to Arctander, S. (1969).

compounds produced by strain CCU-N_{16}-18143 is shown in Table 5 (Arctander 1969). Vester *et al.* (1967) discovered large amounts of fatty acids in the fermentation broth of *Clostridium* sp. Lennart *et al.* identified flavor compounds such as alcohols and fatty acids from the fermentation broth of *Bacteroides fraqilis*, *Clostridium perfringens* and *Propionibacterium acnes* (Chuch 1978), showing the importance of the compounds' contribution to the flavor.

It has been shown that mutants can produce more delicious and intense flavors than wild strains (Rowlavds 1984; Spencer 1983). In this study, the wild strain and mutant CCU-N_{16}-18143 produced about the same kinds but different amounts of flavor compounds. The production of isoamyl alcohol by mutant increased from 7.78 to 61.64% and that of 2-phenyl ethanol from 0.5 to 16.96%, whereas the production of ethyl alcohol decreased from 85.26 to 5.77%. At the same time, the content of fatty acids (acetic acid, propanoic acid, nonanoic acid), esters (phenyl acetate, ethyl n-octanoate), pentane and 1.3-dioxone all increased.

Results of this study indicate that mutant CCU-N_{16}-18143 produces stronger flavor and better quality flavor than wild strains and is potentially useful for improving the flavor of nutrient beverage.

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BOOK REVIEWS

FOOD PACKAGING: PRINCIPLES AND PRACTICE. Gordon L. Robertson. Marcel Dekker, Inc., 270 Madison Ave., New York, NY 10016. 676 pages. $165.

This book of twenty chapters presents a comprehensive background on the principles and practice of food packaging, including the various aspects of packaging that are relevant to processing, food deterioration, preservation, and distribution.

Chapter 1 introduces the basic functions of food packaging. Chapters 2 through 5 deal with the chemical and physical structures of food packaging polymers, and their relationships with mechanical (tensile, burst, impact, tear, and so on), barrier, optical, and thermal properties. Other topics included in these chapters are theory of permeation, techniques for measuring permeability, and technologies for extrusion, lamination, blow molding, thermoforming, heat sealing, vacuum metallization, printing, etc. Chapters 6 through 9 deal with the manufacturing, converting, and properties of paper, glass, and metal packaging materials. Also discussed are metal container making process, aerosol containers, container design, and corrosion of metal packaging materials. Chapters 10 through 12 deal with the deteriorative reactions in foods, preservation and processing technologies, and shelf-life of foods. The influences due to intrinsic factors, environmental factors, and package parameters on shelf-life of foods are elucidated using kinetics models, Arrehenius equation, Fick's law, Henry's law, moisture sorption isotherms, etc. The principles of accelerated shelf-life testing and time-temperature indicators are also reviewed. Chapter 13 deals with the principles of aseptic processing, aseptic packaging systems, and integrity testing for aseptic packages. Chapter 14 deals with the principles of microwave heating and the different types of susceptors and shielding devices used in microwaveable packages. Chapters 15 through 19 deal with packaging of meats, fruits and vegetables, dairy products, cereal and snack foods, and beverages. The principles of modified atmosphere packaging of meats and mathematical modeling for controlled atmosphere packaging for fresh fruits and vegetables are discussed. The final chapter deals with the safety and legislative aspects of food packaging in the United States and in Europe, including a review on principles of food and package interactions.

Compared to other food packaging books in the market, the strength of this book is its well-researched contents. Professor Robertson has done a remarkable job of condensing and integrating his many years of experience and the information from hundreds of published articles. The book has a good balance between

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theories and practice. Each chapter is written clearly and provides sufficient breadth and depth for the topic. The reference sections, which contain hundreds of well-selected citations, are big time-savers for those who desire more information. Many of the latest developments in food packaging are included in the book, such as those relating to microwaveable packaging and modeling of controlled atmosphere packaging for fresh fruits and vegetables.

Overall, this is the best food packaging book I have reviewed. It is a useful reference for food scientists, packaging engineers, and libraries. However, as a professor teaching food packaging courses, I wish Professor Robertson would include more examples and problems in the next edition and the publisher would make the book more affordable, so that it can also be used as a textbook.

KIT L. YAM

SENSORY EVALUATION PRACTICES. Herbert Stone and Joel L. Sidel. Academic Press, 1250 Sixth Ave., San Diego, CA 92101. 388 pages.

The focus of this book is on the operation of a sensory evaluation program in a business environment. Chapter 1 reviews the principles of sensory evaluation and provides an historical overview of the field. Chapter 2 describes the mechanics of organizing and implementing a sensory program, including guidelines for developing a professional staff, establishing adequate testing facilities, selecting panelists and handling work requests.

The next six chapters cover the basic sensory testing methodologies. As the authors introduce the various test methods, they pose problems frequently encountered by the sensory specialist along with alternative courses of action to handle them. For example, Chapter 3 discusses selected scaling techniques and suggests various options for analyzing "just-right" responses and other categorical data. Test design and statistical considerations are presented in Chapter 4. Since sensory testing often involves many products, the emphasis is on multiple factor test designs and analyses. Two and 3-factor analysis of variance models are discussed in clear detail. Discriminative testing is presented in Chapter 5. Although the methods for difference testing are well-developed and the tests are easy to execute, there are various pitfalls associated with their use. Many of these issues are discussed, including identifying individual response patterns that can diminish the quality of the data, minimizing test bias, and the merits of collecting replicate responses as opposed to testing additional subjects. Descriptive analysis techniques and their applications are reviewed in Chapter 6. The majority of the space is devoted to a comparison of the QDA method, which the authors clearly favor, to the other established techniques. Unfortunately, the concentration on a single

method makes it difficult for the reader to gain a balanced perspective of the field. However, the strength of this chapter is its handling of issues surrounding panelist performance. Guidance for monitoring individual panelist's performance and specific statistical approaches for doing so are clearly described. Chapter 7 describes the major classes of affective tests including laboratory panels, central location and home use tests. Questions specific to this area of testing that are addressed include: should preference questions be asked after difference and when is a small yet statistically significant difference meaningful. Chapter 8 is entitled, "Special Problems" and discusses a variety of topics, such as establishing sensory-instrumental relationships, quality assurance and stability testing, substantiating advertising claims and the role of sensory evaluation in the product development cycle. This chapter is somewhat of a mixed bag but illustrates the various ways in which strong interactive relationships between sensory professionals and other branches of the company make good business sense. In the epilogue (Chapter 9) the authors echo a commonly held opinion among sensory professionals, that sensory evaluation is one of the least understood and most misused functions in the research and development process. By recognizing this barrier, the authors help build awareness of sensory evaluation and reinforce its standing within the business and academic communities.

As a whole, the book is directed to the more experienced sensory practitioner who already has a basic understanding of test methodology, experimental design, statistics and human perception. However, technical managers, research directors and marketing personnel can benefit from reading selected chapters. The book is a valuable tool both at the bench and in the classroom. I highly recommend it as a teaching tool but only for more advanced students. Its problem solving approach sets it apart from other current texts, and because of this, it fills a very important niche in the sensory evaluation literature. Its only drawback is in its meager coverage of multivariate statistical techniques. Such techniques are becoming commonplace in sensory evaluation and advanced readers would benefit from greater exposure to these procedures.

BEVERLY J. TEPPER

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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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