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EFFECT OF HEAT TREATMENT ON FIRMNESS OF APPLES AND APPLE SLICES

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Accepted for Publication August 3, 1993

ABSTRACT

Effects of heat treatment on firmness of apples (Golden Delicious, McIntosh and Delicious) and apple slices were studied. Heat treatment (45C, for 1.75 h) of Golden Delicious and Delicious apples significantly increased their firmness, but no significant increase was observed for the McIntosh cultivar. After storage for 7 days at 2C, the treated apples were firmer than those stored at 10, 18, and 25C. Apple slices prepared from the heat treated apples were also firmer at 21 days storage than those prepared from nontreated apples: differences ranging from 12% for McIntosh to 48% for Delicious. This beneficial firming effect of heat treatment might have application in producing high quality minimally processed slices from Golden Delicious and Delicious apple cultivars.

INTRODUCTION

Consumption trends of fruits and vegetables have changed greatly during the last decade. There is currently an increased demand for fresh fruits and vegetables at the consumer level. At the same time, consumers desire products that are of premium quality and easy to prepare and serve. Minimally processed fruits and vegetables are products that have the attributes of convenience and fresh-like quality. The forms of such products vary widely, depending upon characteristics of the raw materials and consuming patterns. Most minimally processed products are raw with living tissues. In processing such products, the raw fruits and vegetables are faced with mechanical injury or wounding that, in turn, set into action complex physiological changes. Hence, in most cases, the end products are more perishable than the unprocessed raw materials.

Tissue softening is one of the major problems confronted in the shelf-life extension of minimally processed products because enzymes related to cell wall degradation are not inactivated. Various treatments have been investigated for

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retardation of the softening of sliced fruits. Some studies have concentrated on the application of an active packaging system with modified atmosphere packaging and calcium infiltration as possible measures to retain firmness of processed products (Rosen and Kader 1989; Ponting *et al.* 1972; Poovaiah 1986; Myers 1989; Roll and Chism III 1987; Labuza and Breene 1989). Recently, studies on quality extension of fresh intact fruits using heat treatment have been carried out by several researchers. Exposure of fresh commodities to heat shock temperature (higher than normal ambient temperature) results in the modification of physiology and in many cases, interruption of synthesis of normal cellular protein including the enzymes, and inactivation of the enzymes related to quality loss in fruits (Brodl 1989; Klein and Lurie 1990). Liu (1978) and Porritt and Lidster (1978), among others, have studied the inhibition of ripening and retardation of softening of apples during storage by heat treatments immediately after harvest. In order to extend their shelf-life, apples were treated with 38–40C air for several days. This particular treatment of whole apples might be considered impractical in several aspects, such as the time for the treatment, growth of microorganisms in mechanically damaged tissue during the treatment, large space requirement for the treatment and the need to maintain high humidity in the room to prevent surface drying during treatment (Liu 1978). Such treatments retarded ripening in some apple cultivars. Similar studies on chopped or sliced fruits and vegetables were carried out using heat treatment at 50–80C as means of preblanching for a short time to improve firmness (McFeeters 1985). Recently, it has been reported that heat treatment of apples produced some beneficial effects on color and firmness of apples (Kim *et al.* 1993).

In order to reduce softening of whole apples and apple slices during storage and marketing, heat treatments were carried out on three apple cultivars and the effects of heating and storage conditions on firmness of the apple slices were studied.

MATERIALS AND METHODS

Apples

Three apple cultivars, (Golden Delicious, McIntosh and Delicious) grown during the 1991 season at the New York State Agricultural Experiment Station orchard were used for the study. The apples were harvested based on normal harvest dates established for cultivation of the apples in the region. After harvest, the apples were placed in wooden crates and stored in a cold room (2C and 90% RH) for less than two months before the heat treatments were applied.

Heat Treatment

For each cultivar, 120 apples of uniform size and color were placed into two large perforated plastic buckets with lids (0.27 m I.D. x 0.28 m depth), immersed in a steam injected water bath at 45±0.5C for 1.75 h and then kept overnight at 2C. All of the cultivars were heat treated in one batch. Twenty treated apples of each cultivar were put into perforated, unsealed polyethylene bags and stored for seven days at 2C, 10C, 18C, and 25C. Stored apples were peeled, cut into 16 slices and measured for firmness.

To study the effect of longer term storage on firmness, 50 treated apples of each cultivar were stored for 21 days at 2C. Apple samples were taken weekly and immediately peeled, sliced and measured for firmness.

To investigate the effect of heat treatment on firmness of apple slices during storage, the heat treated three apple cultivars were peeled and each apple cut into 16 slices. Two hundred grams of slices were transferred into polyethylene film bags, left unsealed and stored at 2C. Firmness of apple slices was measured at intervals during storage.

Measurement of Firmness

Firmness of the apple slices was measured with the Lloyd Texturemeter (M5K) using a back extrusion test cell (Shannon and Bourne 1971) with dimensions of 7.3 cm I.D. x 8.5 cm internal height . One hundred grams of the slices were loosely filled in the test cell for each measurement. The plunger (6.5 cm diameter) speed for extrusion was 200 mm/min and downward movement of the plunger was automatically reversed at 9 mm from the bottom of the cell. The extrusion forces were taken as the maximum peak on the chart paper and expressed as a mean value of Newton (N) force from 6 replicate measurements per sample.

RESULTS AND DISCUSSION

Effect of Different Storage Temperature on Firmness of Apples

In our previous study, we found that heating at 45C for 1.75 h produced firmer apple slices compared to the nonheated control apples and that Golden Delicious and Delicious apples responded best to the heat treatments among 11 apple cultivars studied in terms of color and texture (Kim *et al.* 1993). To investigate the effect of storage temperature on firmness of the heat treated apples, the treated apples were kept at 2C, 10C 18C and 25C for 7 days, sliced and measured for firmness by back-extrusion. Figure 1 shows the firmness of

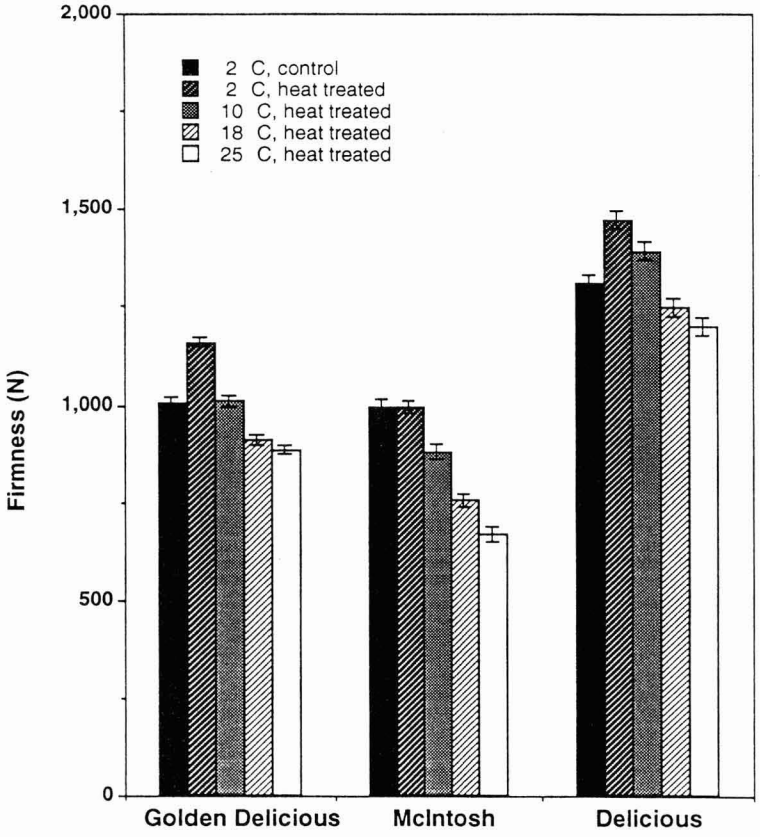

FIG. 1. EFFECT OF VARIOUS STORAGE TEMPERATURES ON FIRMNESS OF HEAT TREATED THREE APPLE CULTIVARS AFTER 7 DAYS
The bars indicate standard deviations.

the treated apples after 7 days storage at various temperatures. Firmness of Golden Delicious apples at 2C was significantly greater than at the other temperatures. Firmness of the apples decreased with increased storage temperature. Firmness decreased more rapidly with increasing storage temperature in McIntosh compared to Golden Delicious apples. Delicious apples were firmer overall than the other two cultivars.

Changes in Firmness During Storage

Figure 2 shows the influence of storage at 2C on firmness of Delicious and Golden Delicious apples. Firmness was significantly higher in the heat treated apples at 0 day than in the controls. Over 21 days storage, there was an additional increase in firmness of the heat treated apples. Initially, the treated

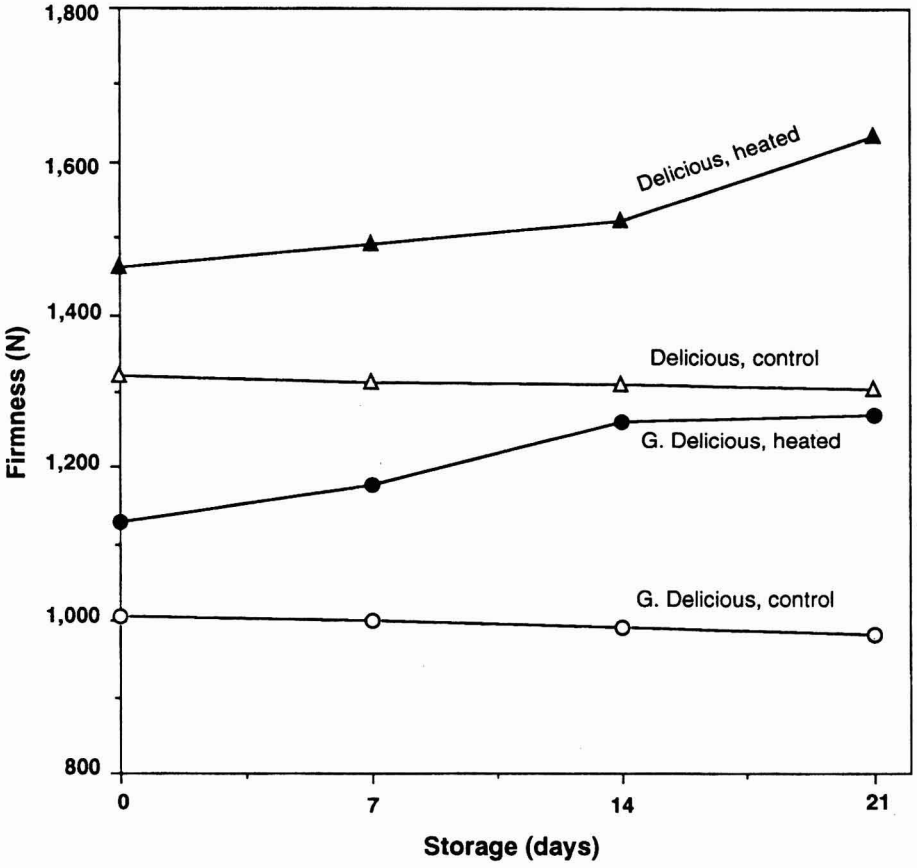

FIG. 2. CHANGES IN FIRMNESS OF HEAT TREATED AND NONTREATED (CONTROL) DELICIOUS AND GOLDEN DELICIOUS APPLES DURING STORAGE AT 2C

apples were approximately 11–12% firmer than the nontreated apples; after 3 weeks storage these differences had increased to 25–29%. McIntosh apples showed no significant response to the heat treatment (data not shown). Klein and Lurie (1990) reported that the Granny Smith apples heated at 38C for 4 days were appreciably firmer than the nontreated apples during 3–6 months storage at 0C.

Changes in Firmness of Apple Slices

A large difference in firmness of the apple slices was also observed during storage at 2C between the heat treated and the nonheated (control) apples (Fig.

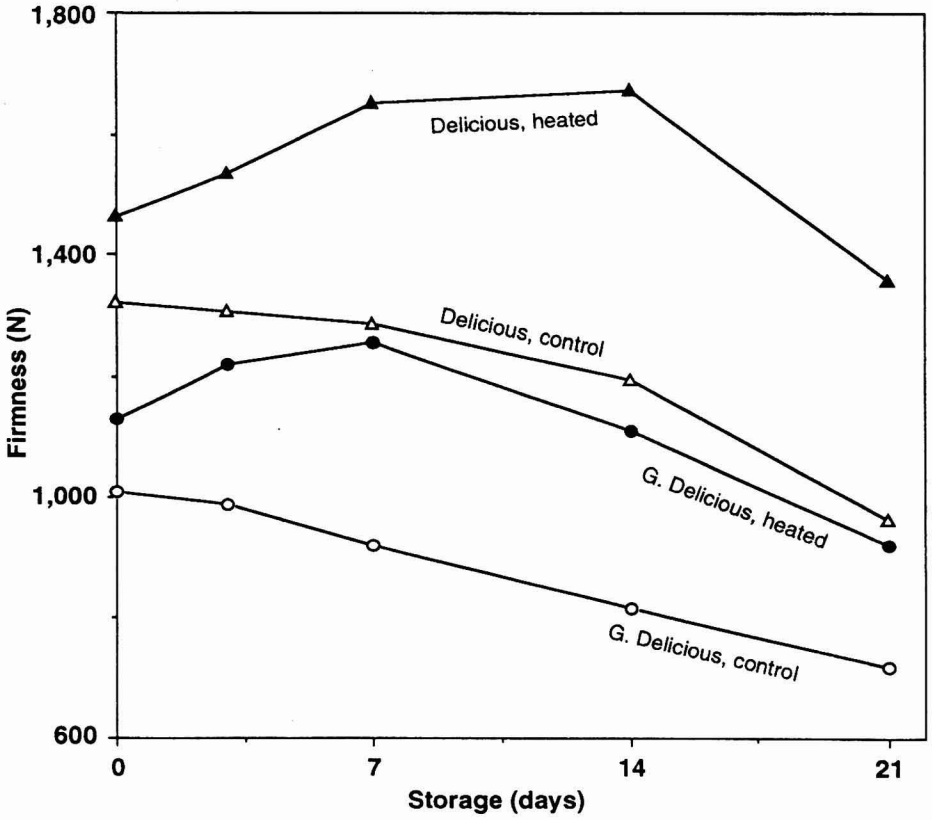

FIG. 3. CHANGES IN FIRMNESS OF SLICES PREPARED FROM HEAT TREATED AND NONTREATED (CONTROL) DELICIOUS AND GOLDEN DELICIOUS APPLES DURING STORAGE AT 2C

3). Firmness of slices prepared from heated apples first increased during storage and then decreased, while firmness of slices prepared with nontreated apples exhibited a steady decrease over time. Firmness increased in heated Golden Delicious and Delicious apple slices up to 7 and 14 days storage, respectively. Firmness of slices prepared from heated Golden Delicious apples was approximately 34% higher at 7 days storage and that of Delicious apples was 48% higher at 14 days storage than the initial values. McIntosh showed only 12% difference between the heat treated and the nontreated at 7 days storage (data not shown). However, for all three apple cultivars, the firmness difference between the heated and the nonheated controls lessened with storage time.

In general, fruit tissue softened when heated at high temperatures partly due to the loss of turgor, but also due to a variety of chemical changes in the

cell wall matrix polysaccharide, especially in pectin. This polysaccharide modification can be influenced by a number of factors, principally pH, enzymes and the amounts and types of salts that are present (Van Buren 1979). In our study, the firmness of the apple slices prepared from apples heated at 45C for 1.75 h was improved and it increased during storage. A possible explanation is that such heating activates pectin methylesterase, which then demethylates pectic substances that allow cross-linking of the freed carboxyl groups by internal calcium ions. Lee *et al.* (1979) found similar trends in low temperature blanching of canned carrots. The firmness of Anna apples heated for 4 days at 38C in air and then stored for 6 weeks at 0C was about 8% higher than the control (Lurie and Klein 1992). Porritt and Lidster (1978) reported that firmness of Spartan apples was unaffected immediately after heat treatment at 38C in air for 2–6 days, but became 36.7% greater than in the control after storage at −1C for 4 months.

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INFLUENCE OF REACTION CONDITIONS ON THE FORMATION OF NONDIALYZABLE MELANOIDINES FROM D-FRUCTOSE AND GLYCINE

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and

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ABSTRACT

The influence of the water content, molar ratio of reagents, time and temperature on the formation of nondialyzable melanoidines of the model system D-fructose-glycine has been studied.

The rate constants of the reaction for molar ratios 2.5:1, 1:1, 1:2.5 have been calculated to be $(2.38 \pm 0.17) \times 10^{-5}\ s^{-1}$, $(3.94 \pm 0.28) \times 10^{-5}\ s^{-1}$ *and* $(5.11 \pm 0.36) \times 10^{-5}\ s^{-1}$, *respectively.*

The activation energy has been determined (105.0 ± 3.6) kJ/mol and (74.6 ± 7.1) kJ/mol for molar ratios 2.5:1 and 1:2.5, respectively.

INTRODUCTION

In the past eight decades the Maillard reaction (Maillard 1912) has managed to reveal its ample opportunities for research under model conditions and food products. The influence of a number of factors on the reaction mechanism as a whole or on its separate stages has been studied. The results obtained so far provided an opportunity to outline the common regularities about the influence of temperature, reaction time, water content, type of reagents, molar ratio, etc. (Reynolds 1963; Eichner and Karel 1972; Eriksson 1982; Feather and Nelson 1984; Chun *et al.* 1986; Huang Ru Duo and Feather 1988; Ames 1990). At the same time these studies revealed a number of specific sides in the flow of the reaction as well as the necessity for even more systematic and detailed

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experiments to be carried out under comparable conditions. Each of the kinetic studies known to us (Song *et al.* 1966a,b, 1967; Labuza *et al.* 1970; Jokinen *et al.* 1976; Warmbier *et al.* 1976; Chun *et al.* 1986) is interesting and necessary for understanding the interactions taking place, but few of the available investigations provide an opportunity for making convincing comparisons and drawing definite conclusions. The kinetics of carbohydrate-amine transformations in food products (Herrmann and Nour 1977; Mizrahi *et al.* 1977; Chun *et al.* 1982; Kim *et al.* 1988; Sun Kang *et al.* 1989; Ibarz *et al.* 1989; Ibarz and Bermejo 1991) is not sufficiently well studied. Traditionally, the development of the reaction is measured through the absorption in the UV and Vis spectrum ranges. Several new approaches have been applied lately (Milic 1987; N'Soukpoe-Kossi *et al.* 1988) in kinetic investigations.

In our previous publications (Obretenov *et al.* 1986, 1990) we had begun an investigation into the influence of certain reaction factors on the synthesis of nondialyzable melanoidines under model conditions by preparative quantitative isolation of these products. These studies revealed well the specific character of each of the investigated models. The rate constants and the activation energy of the processes had been determined. To draw convincing conclusions, however, much more experimental data are needed. With this goal in mind, we offer a discussion in this work of the results obtained from the interaction of fructose with glycine under different reaction conditions.

MATERIALS AND METHODS

D-fructose and glycine used were trade mark products with a p.a. qualification. The following amounts of reacting substances were used depending on the molar ratios:

Fructose:glycine:

5:1 — 18.750g + 1.560g = 20.310g
2.5:1 — 17.320g + 2.880g = 20.200g
1:1 — 14.400g + 6.00g = 20.400g
1:2.5 — 9.800g + 10.210g = 20.010g
1:5 — 6.430g + 13.510g = 19.940g

At molar ratios used, the chosen amounts provided about the same total mass (20 g) of the reaction mixture, against which the amount of added water was determined, creating in this way the prerequisites for comparability of the obtained yields of nondialyzable melanoidines expressed in grams. This approach was predetermined by the impossibility of calculating the theoretical yield because the molecular mass of the products was not known.

The experiments were carried out in a round-bottom flask in a glycerol bath with refluxing and intense stirring. The reaction mixture was dialyzed for 96 h through a cellophane membrane against distilled water. The nondialyzable part was concentrated using a rotational vacuum evaporator at a temperature lower than 30C, dried under vacuum and stored in a dark place in an inert atmosphere.

The pH of the reaction mixture was not corrected.

The amount of water soluble nondialyzable melanoidines was determined by full extraction with water at 50C. The water extract was evaporated to dryness under the described conditions. The insoluble residue was dried and weighed.

The UV and IR analyses were performed with a Specord UV-Vis apparatus, Karl Zeiss, Jena (in water solutions) and UR-20, Karl Zeiss, Jena (in tablets of potassium bromide) at similar weight concentrations.

The rate constants were calculated using the NONLIN program, which implements the Newton-Raphson method (Johnson 1980) and was adapted for the CM-4 computer. The multi-factor regression dependence was found using the MULTR computer program for multiple regression by the method of least squares (IBM 1974).

RESULTS AND DISCUSSION

A number of experiments were carried out to study the influence of the reaction conditions on the synthesis of nondialyzable melanoidines in the model system fructose-glycine with different water content, molar ratios and reaction time.

Experiments with Different Amounts of Water and Molar Ratios

The synthesis of nondialyzable melanoidines was carried out with fructose:glycine molar ratios of 5:1; 2.5:1; 1:1; 1:2.5; 1:5 and water content from 15 to 100% against the dry matter for a duration of interaction of 7 h at a temperature of 100C. The choice of the last two factors was made on the basis of our previous investigations (Obretenov *et al.* 1986, 1990) and provide for the comparability of our results.

It is interesting to note that the yield of nondialyzable melanoidines was influenced considerably both by the ratio of reacting components and by the water content (Fig. 1). Along with certain common regularities in this change, there are also certain specific features. Highest yield of melanoidines was obtained at molar ratio of 1:1, and lowest at molar ratio 5:1 and 15% water content. The result was about 3.5 times higher for the first ratio. Although smaller, the differences in the yields from all experiments amounted to several times. The yields gradually decreased with increasing the water content. When

the water was 100% the yield was lowest for molar ratio of 5:1 and highest for molar ratio 1:2.5. The amount of melanoidines at molar ratio of 1:1 had an intermediate value.

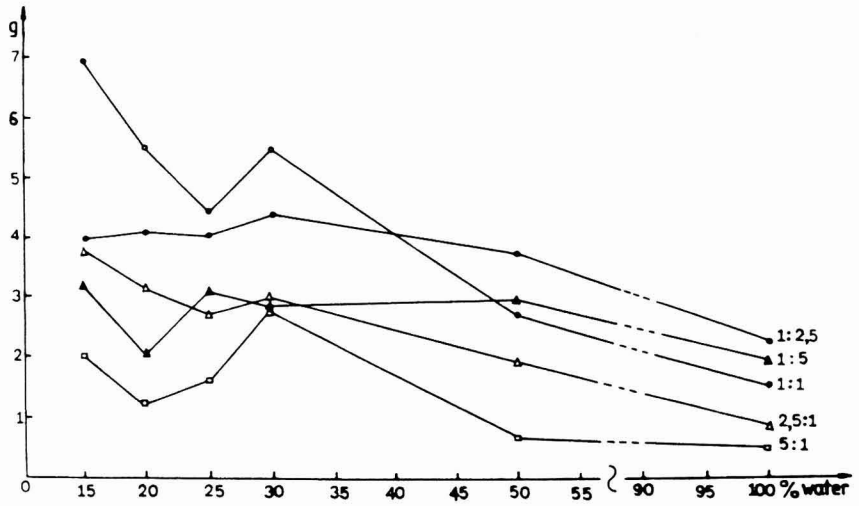

FIG. 1. INFLUENCE OF THE MOLAR RATIO AND WATER CONTENT ON THE FORMATION OF NONDIALYZABLE MELANOIDINES FROM THE FRUCTOSE-GLYCINE MODEL SYSTEM
(temperature 100C; time 7 h)

The increase in water content had a specific influence on the change in melanoidine yield for the separate molar ratios. If we compare the yields at 15% and 100%, there was a decrease of several times. However, within the interval 20–30% an increase was observed. Some of the yields were higher or commensurate with the case of 15% water content (molar ratios 5:1; 1:2.05; 1:5) and lower for the remaining molar ratios.

The lowest yields at 15% water content and molar ratios 1:5 and 5:1 could be explained by the greater viscosity of the reaction medium not providing the necessary contact between the reacting molecules as a result of which there was a shortage of the component with the lower content and the reaction was slowed down. Under the same conditions this shortage was less strongly expressed at ratios 2.5: 1 and 1:2.5, the yields were higher and very similar to one another, while for equal amounts of fructose and glycine (ratio 1:1), there was no shortage of component for the interaction and the yield was highest.

The increase in water content to about 30% for all molar ratios resulted in increasing the melanoidine yield probably as a result of improving the contact

between the reacting molecules after which the reaction was slowed down because of the effect of dilution.

Experiments with Different Reaction Time

These experiments were carried out at fructose:glycine molar ratios of 2.5:1; 1:1; 1:2.5, water content of 15 and 20%, temperature of 100C and duration of interaction of up to 50 h.

The yields of nondialyzable melanoidines at a fructose:glycine ratio of 1:1 increased with increasing the reaction time more significantly until about the 21st hour, after which their change was smaller. There was no essential difference between the amounts of reaction products at water contents of 15 and 20% (Fig. 2 and 3). More precisely, until the first hour the reaction took place at a faster rate for 15% water content, until about the 24th hour the yields were higher than those from the experiments with 20% water, after which the yields from the experiments with 15% water content were again prevalent (Fig. 2 and 3).

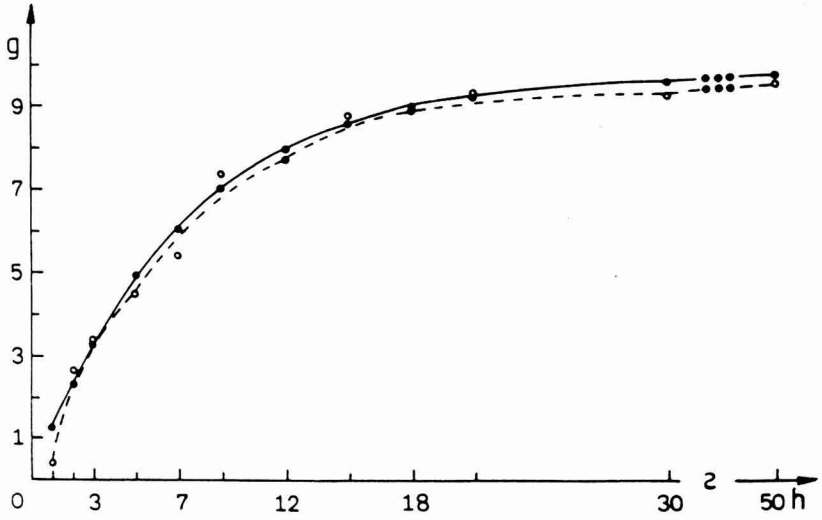

FIG. 2. DEPENDENCE OF THE NONDIALYZABLE MELANOIDINE YIELD ON TIME FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM
(molar ratio 1:1; temperature 100C, 20% water content)

----- experimental values; _____ theoretical values

The pH of the reaction medium changed fast until the first hour, decreasing from 6.20 to 3.50 and to 3.20 until the third hour, after which it remained practically constant.

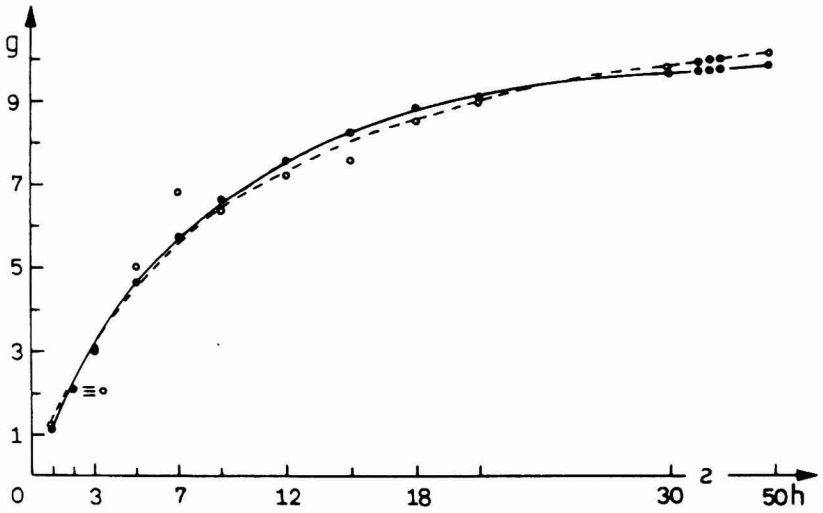

FIG. 3. DEPENDENCE OF THE NONDIALYZABLE MELANOIDINE YIELD ON TIME FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM
(molar ratio 1:1; temperature 100C, 15% water content)

----- experimental values; _____ theoretical values

All melanoidines isolated up to here were characterized by UV- and IR-spectra. They absorbed intensely in the UV-range within the interval of 270 to 310 nm. The curve was plateau-like without a clear maximum or essential differences between the curves. The IR-spectra were analogical in their information value. The absorption was more strongly expressed at 1050–1150, 1200–1250, 1350–1470, 1600–1750 and 3000–3500 cm^{-1}. These spectral ratios are specific for melanoidines.

The yield of nondialyzable melanoidines depending on time is described by Eq. (1)

$$y = A_o(1 - e^{-kt}) \tag{1}$$

where: k is the rate constant, s^{-1}
A_o is the maximum yield for a sufficiently long time, g

The kinetic data (Table 1, Fig. 2, 3, 4, 5) indicate that the interaction between fructose and glycine until nondialyzable melanoidines were obtained took place at a 2.2 times faster rate for molar ratio of 1:2.5 in comparison with molar ratio 2.5:1 (5.11×10^{-5} s^{-1} and 2.38×10^{-5} s^{-1}). With an excess of fructose the maximum yield was higher (8.620 g compared to 6.970 g). The rate

TABLE 1.
KINETIC DATA ON THE FORMATION OF NONDIALYZABLE MELANOIDINES FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM[1]

Molar ratio	A_o, g	$k \times 10^{-5}$ s^{-1}	Determination coefficient R^2, %	Fischer's criteria $\alpha = 0.05$			
				$F_{exp.}$	$F_{theor.}$	$\nu 1$	$\nu 2$
2.5:1	8.62	2.38 ±0.17	96.90	343.85	4.84	1	11
1:1	9.70	3.94 ±0.28	96.29	259.55	4.96	1	10
1:1[2]	9.79	3.50 ±0.25	97.68	463.10	4.84	1	11
1:2.5	6.97	5.11 ±0.36	98.10	394.37	5.32	1	8

[1]Reaction conditions: temperature 100C, 20% water content
[2]The experiment was carried out at water content of 15%

constant of the reaction for a molar ratio of 1:1 occupied an intermediate position (3.94×10^{-5} s^{-1}) at a higher maximum melanoidine yield (9.700 g). The comparison of these data obtained at 20% water content and the same molar ratio with the results of the model with 15% water content did not show essential differences (rate constant 3.50×10^{-5} s^{-1} and maximum yield 9.790 g). The graphically presented data (Fig. 2-5) indicate a good correspondence between the experimental and values calculated according to the accepted kinetic model from Eq. (1).

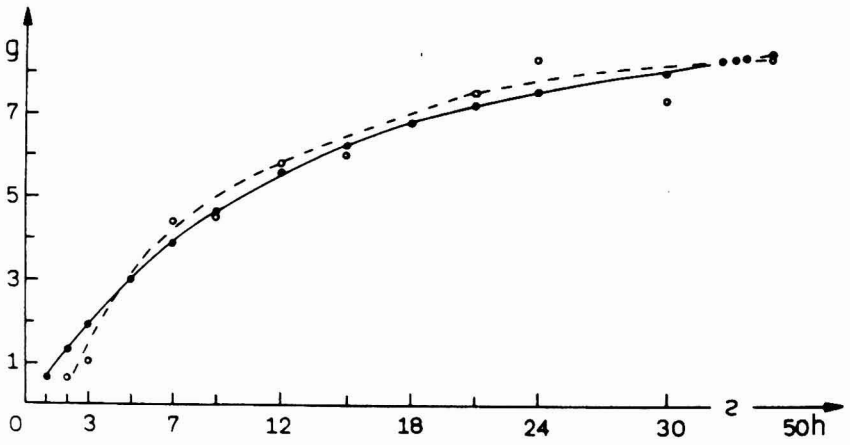

FIG 4. DEPENDENCE OF THE NONDIALYZABLE MELANOIDINE YIELD ON TIME FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM
(molar ratio 2,5:1, temperature 100C, 20% water content)

----- experimental values; _____ theoretical values

Here we can afford to make a comparison between the model systems glucose-glycine (Obretenov *et al.* 1986) and fructose-glycine. The kinetic data obtained under the same reaction conditions indicate that melanoidine formation with glucose takes place almost twice as fast compared to that of fructose. The rate constant for molar ratio 2.5:1 was 4.55×10^{-5} s^{-1} and 9.40×10^{-5} s^{-1} for molar ratio 1:2.5. It is known that the interaction of fructose with glycine takes place faster in the early stages of the reaction (up to the fifth hour) while for a longer period of time glucose shows a higher reactivity (Kato *et al.* 1969; Sapronov and Kolcheva 1975). The probable reason for this difference could be found in the higher instability of fructose in an acid medium, which rapidly turned into reaction-capable intermediates, resulting in the browning of the reaction mixture. After these intermediates were consumed the rate of interaction decreased compared to that of glucose. This assumption ties up both with the

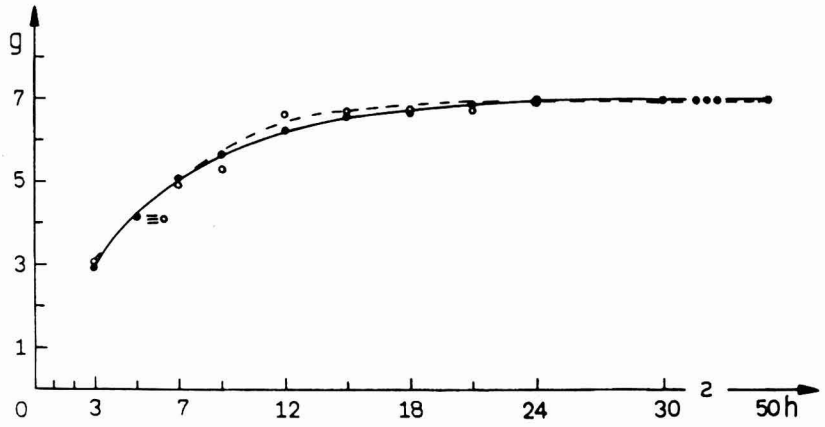

FIG. 5. DEPENDENCE OF THE NONDIALYZABLE MELANOIDINE YIELD ON TIME FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM
(molar ratio 1:2.5, temperature 100C, 20% water content)

----- experimental values; _____ theoretical values

with the availability of a well-expressed absorption around 300 nm of the melanoidines obtained from fructose and glycine, characteristic, in our opinion of lower molecular melanoidines, and with the earlier, compared with glucose, accumulation of water insoluble melanoidines amounting to 67% up to the 3rd hour and reaching 93% until the 30th hour.

Further on it was of interest, using the multi-factor analysis, to study the influence of the amount of fructose (X_1), glycine (X_2) and reaction time (X_3) on the yield of nondialyzble melanoidines from the model system fructose-glycine. The following regression equation was obtained as a result of the mathematical and statistical analysis:

$$Y = 16.750 - 0.985\ X_1 - 1.870\ X_2 + 0.451\ X_3 + 0.130\ X_1 X_2 - 0.0065\ X_3^2 \qquad (2)$$

$R_M = 0.9212, R_M^2\ 100 = 84.86\%,$

$F_k^e = 32.53 > F_k^t\ (5.29;\ \alpha = 0.05) = 2.54$

The comparison of the experimentally found values of F_k^e and F_k^t for a confidence level of $\alpha = 0.05$ and degrees of freedom ν_1 and ν_2 indicates that Eq. (2) describes adequately our experimental data, explaining 84.86% of the dispersions of Y.

Equation (2) was optimized using the modified simplex method with a computer program (Himmelblau 1972). As a result, the optimal conditions for

the synthesis of nondialyzable melanoidines from this model system were found, as well as the optimal yield: $X^*_1 = 14.390g, X^*_2 = 7.560g, X^*_3 = 35h$, yield $Y^* = 10.410$ g. The experiments carried out under the optimal conditions resulted in obtaining a yield of 10.220 g. The similar values of the estimated and experimentally obtained yield confirm the adequacy of Eq. (2).

TABLE 2.
KINETIC DATA AND THERMODYNAMIC PARAMETERS ABOUT THE FORMATION OF NONDIALYZABLE MELANOIDINES FOR THE FRUCTOSE-GLYCINE MODEL SYSTEM
Reaction conditions: time up to 50 h; water content, 20%

Molar ratio	$k \times 10^{-5}$ s^{-1}			Activation energy, Ea kJ/mol	Correlation coefficient, R
	90C	100C	110C		
1:2.5	2.43 ± 0.17	5.25 ± 0.36	8.83 ± 0.62	74.6 ± 7.1	0.996
2.5:1	0.60 ± 0.14	2.63 ± 0.18	3.67 ± 0.26	105.0 ± 3.6	0.945

Experiments with Different Temperatures

These experiments were aimed at establishing the dependence of the rate constants on the temperature for the interaction between fructose and glycine until nondialyzable melanoidines were obtained. The experiments were carried out at temperatures of 90, 100 and 110C for a duration of up to 50 h. The kinetic results and thermodynamic parameters obtained are presented in Table 2. It can be seen that the increase in temperature from 90 to 100C resulted in a 2.2 times increase of the reaction rate for molar ratio fructose:glycine = 1:2,5 and a 4.4 times increase for a ratio of 2.5:1. The acceleration of the reaction taking place within the interval from 100 to 110C was lower (1.7 and 1.4 times, respectively, for the two ratios). For the sake of comparison we shall point out that with the model system glucose-glycine (2.5:1 for an increase of temperature from 90 to 100C the reaction rate increased 2.4 times and from 100 to 110C it increased 2.3 times under the same reaction conditions (Obretenov *et al.* 1986). The activation energy of the process was calculated on the basis of the obtained kinetic data (Arabshahi and Lund 1985), in accordance with Arrhenius' equation:

(3)

where: k = rate constant, s^{-1}; Ea = activation energy, kJ/mol; R = gas constant; A = preexponential constant; T = temperature, K.

The data indicate that for an excess of glycine the reaction took place at a greater rate and a smaller E_a was necessary for the synthesis of melanoidines (74.6 kJ/mol) compared to the experiments with an excess of fructose (E_a = 105.0 kJ/mol). For the glucose-glycine model system (2.5:1) E_a was 99.7 kJ/mol at rate constants 2.33 10^{-5} s^{-1}, 5.75 $\times$ 10^{-5} s^{-1}, and 13.05 $\times$ 10^{-5} s^{-1} for the same temperatures (Obretenov *et al.* 1986).

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NEW METHOD FOR BATCH TESTING OF RED TART CHERRIES FOR THE PRESENCE OF PITS

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ABSTRACT

A method was developed to determine the number of pits in samples of red tart pitted (RTP) cherries. The method is based on pulping the sample in a modified kitchen blender and detecting pits either from the noise of pits rattling in the blender or by pouring the resulting pulp through a trap designed to retain the pits. The method is more rapid and convenient than the present method and allows the recovery of tested product as juice. Screening missed a total of 1½ pits when 1 pit was added to each of 233 samples consisting of fresh, fresh frozen, individually quick frozen and bulk frozen RTP cherries.

INTRODUCTION

Red tart pitted (RTP) (Montmorency cultivar) cherries form a major crop in U.S. agriculture, accounting for a total production of 86,000 metric tons (190 million lb) in 1991 (Binde *et al.* 1992). Substantially all (98%) of this crop is processed and sold frozen or canned. The Agricultural Marketing Service (AMS) of the U.S. Department of Agriculture (USDA) offers a voluntary inspection, grading, and USDA certification service, which covers frozen or canned RTP cherries. In 1991 almost 27,000 metric tons (60 million lb) were included in this grading program, requiring the testing of almost 25,000 samples. AMS carried out additional inspections of nonfrozen cherries, as did the industry itself of both frozen and nonfrozen cherries. Certification thus comprised a substantial effort.

The detection of pits or pit fragments remaining in pitted fruit, and in particular cherries, has been the subject of research and application for almost 30 years. For largely commercial reasons, such work has emphasized on-line, rather than batch, detection. Several methods have shown promise and are actively being pursued or even commercialized, although with limited success.

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Allen *et al.* (1966) patented a light (visible or IR) scattering method, which was investigated by Law (1973), who found a high, and undesirable, dependence on cherry orientation. This technique is the basis of a spectral reflection technique used in a commercial unit produced by Clayton Durand Manufacturing Co. (Durham, NC) and, it is claimed, is capable of detecting pits in cherries Timm *et al.* (1991). Light transmission was patented by Gillespie and Ricks (1987) and further improved by use of image analysis by Timm *et al.* (1991). Light transmission is the basis of a commercial device manufactured by Agrivision (Davis, CA). X-ray absorption is under investigation by SRS Technologies (1993). Rheological detection of pits was patented by Ross and Crawford (1979). Other methods, in particular weighing, microwave transmission, ultrasound transmission and pit-tube transmission were investigated by Timm *et al.* (1991). None of these methods are fully satisfactory, as shown by continuing work in on-line detection. In any event, detection capability to around 95–96% in the best of cases is not adequate to certify pit removal, as is required in batch tests and towards which the present work is directed.

Fresh cherries are delivered to the processing plant in pallet tanks of cold water after mechanical shake-down from the trees. Following visual sorting to remove extraneous material and obvious defects, cherries are immobilized and depitted by star shaped plungers which pass through them. The cherries are then processed in one of three forms. For bulk 5+1 frozen cherries, 30 lb are placed into plastic containers and covered with 1 part granular sugar to 5 parts fruit to protect the cherries from oxidation. The filled containers are then placed into a freezer and held in cold storage. Individually quick frozen (IQF) cherries are flash frozen to a free flowing product resembling marbles, then placed into containers and held in cold storage. Canned cherries are packed in #2 or #10 cans with or without sugar, followed by sterilization through heating.

Inspection may be done on-line in the plant or it may be done on the final packed product. The material to be inspected may thus vary in turgidity, water content, and even state (liquid or solid). From 3 to 29 samples are used, depending on the lot size (each sample contains 567 g or 20 ounces of cherries). For grade certification, the absence of pits is an important factor. For a lot to be of grade A, the overall pit count among all samples tested may not exceed 1 pit per 1,146 g (40 oz) with no single sample containing more than 2 pits. For grade B, the overall pit count must be less than 1 pit per 850 g (30 oz) and no sample may contain more than 3 pits. For grade C the pit count must be less than 1 pit per 567 g (20 oz) with no per sample restriction. Failing C the lot is termed substandard (USDA 1974). In actual fact, pit count is typically well below that; in 1991 80% by weight of cherries contained less than 1 pit per 3,400 g, while 60% contained less than 1 per 1,300 g for an overall pit count of 1 pit per 5,200 g (Binde *et al.* 1992). The industry would like to reach a consistent level of 1 per 28,300 g (1,000 oz), a level reached currently by over

35% of tested cherries (Binde *et al.* 1992). Testing for such low pit counts at the current levels of confidence will require even larger samples or a greater number of samples than are currently being tested.

The present method of testing for pits is laborious, time consuming, may be subject to error (missed pits) and consumes considerable product, which is usually discarded. A sample of 567 g (1,134 g or 40 oz is also used by the industry), containing at least 100 cherries, is spread single layer on a tray, thawed or partially thawed if needed, and each cherry is individually pressed by hand or instrument to ascertain the absence of pit material. The pitting plungers rarely produce pit fragments, so the test essentially counts whole pits remaining. What is needed is a method that is rapid, convenient, inexpensive in equipment and preferably nondestructive in that the tested sample can be returned to the process stream. This report presents a method substantially satisfying these requirements. In brief, the method consists of pulping the sample in a blender and determining the pit count either by washing the product through a slit strainer, which retains the pits, or by listening for the sound of the pits in the running blender. The pulped cherries can be returned to a cherry juice product line.

MATERIALS AND METHODS

Pitted cherries were obtained from a commercial source, through the offices of an ARS research station at E. Lansing, MI. Lots consisted of 45,000–68,000 g each of fresh cherries (ice packed, shipped overnight and tested within three days of receipt), bulk frozen cherries, IQF cherries (shipped frozen) and fresh canned cherries in #10 cans, packed with 17% syrup. A separate shipment of pits was obtained directly from ARS. To distinguish them from pits already present in the cherries as shipped, the separately received pits were dyed red.

For pulping cherries an Osterizer Cycle Blend blender (Sunbeam Oster Home Appliance Co., Laurel, MS)[2] was purchased locally. The blades of such a blender are sharpened for kitchen use. To avoid breaking the pits or jamming them against the container walls, all blade edges were ground until the edge was blunted to a width of 2.5 mm. The lower blades were ground to 19 mm in length (Fig. 1). No-load blade speed was measured by a Cole-Parmer photo-tachometer (model 08210, Niles, IL). At the lowest speed setting ("stir") of the blender the blade speed was measured to be 11,500 rpm, the next setting

[2]Reference to a company and/or product named in this article is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may perform in an equivalent manner.

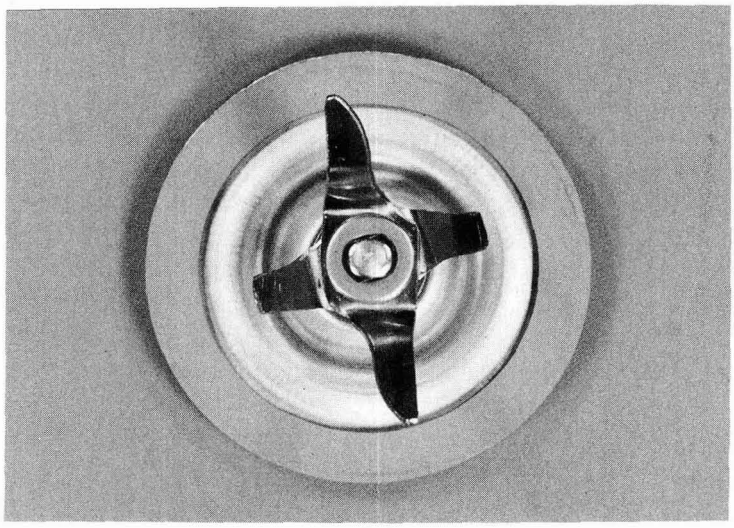

FIG 1. MODIFIED BLENDER BLADES
The edges have been dulled and the lower blades shortened to prevent pits being caught between the blade and the side of the pitcher.

("puree") was measured at 12,600 rpm. For lower speed a reduced voltage was supplied by a Powerstat variable transformer (Superior Electric Co., Bristol, CT). Operation at 9,000 rpm was achieved with 50 volts and a "liquify" setting. When the blender was filled with cherries the speed decreased substantially. However, blade speed under loaded conditions could not be measured as the blades were not visible. All speeds given below refer to unloaded conditions. Some samples were diluted with water before blending. Blending speed and time were chosen to optimize the pulping operation and minimize shattering of pits. A plastic food container (standard part, available locally) was used with the blender; a glass container was not satisfactory, as it caused pits to shatter.

For testing fresh cherries 567 g (20 oz) samples of drained cherries were placed in the blender; 473 ml (1 pint) of water and one pit was added to each sample. The first 26 samples were blended at 9,000 rpm for 1 min. Another 26 samples were pulped at 11,500 rpm. The noise of a pit rattling against the plastic container was listened for by the operator. At the end of 1 min the pulped sample was poured through a slitted trap (Fig. 2) constructed from aluminum. The pulp was rinsed through the trap with flowing water. The number of pits caught in the trap was visually counted.

Canned cherries were tested in a similar way. A total of 60 samples were tested, all at 11,500 rpm, but without added water. However, in this case the cherries as received contained a substantial number of pits. Accordingly, the pits

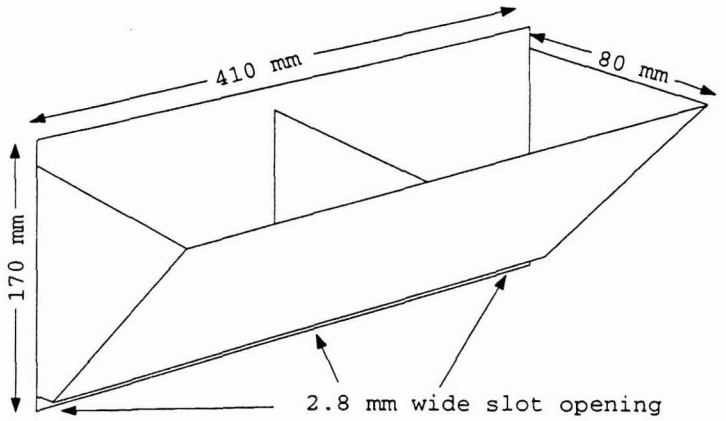

FIG 2. TRAP USED TO RETRIEVE PITS FROM PULPED CHERRIES
Material: 0.16 mm (1/16 in.) aluminum sheet stock.

received from ARS were used as additive only for the first 24 samples, after which recovered and dyed pits from the lot itself were added to the remaining samples.

A total of 49 samples of 5+1 bulk frozen cherries were tested. Five containers were thawed and drained, yielding approximately 5,600 g of cherries per container. One pint water and one dyed cherry pit were added to each 567 g sample, which was blended at 12,600 rpm for 20 s, followed by 40 s at 11,500 rpm. Pits received from ARS were added to the first 12 samples at 1 pit per sample; recovered, dyed pits were added to the remaining 37 samples.

A total of 72 567-g samples of IQF cherries were tested. After thawing, testing proceeded precisely as in the drained bulk frozen samples. Time of processing a set of 12 samples was measured. The sound of pits in the blending jar (or the absence of sound) was listened for, but no records were kept. The trap, shown in Fig. 2, was constructed from 0.16 mm (1/16 in.) thick aluminum sheet stock. Trap length amounted to 41 cm. The trap slot opening, which retained the pits, was 2.8 mm in width.

RESULTS AND DISCUSSION

Cherry pits can be visualized as ellipsoids. Micrometer widths on 50 of the pits as received from ARS yielded 9.4 ± 0.6, 7.9 ± 0.5 and 6.1 ± 0.4 mm for the three principal axes. The recovered pits yielded 8.4 ± 0.6, 7.3 ± 0.5 and 5.8 ± 0.4 mm, respectively. Received pits averaged 0.152 g per pit, recovered pits 0.146

g per pit. From an operational point of view, the difference is immaterial, as either type pit is caught by the trap.

The sample pit count and grade, based on the number of nondyed pits recovered, is shown in Table 1. Pit fragments were recorded as found, i.e., no account was taken of their size. It will be seen that grade covered the full range, from A to substandard. The lots had been selected to provide such a wide range. Since the trap opening was 2.8 mm in width, fragments smaller than that presumably could pass through and would not be recovered. Thus the fragment count may well be low.

TABLE 1.
NUMBER OF CHERRY PITS RECOVERED FROM COMMERCIAL TEST LOTS OF RTP MONTMORENCY CHERRIES

	Product			
Recovered pits/sample[a]	Fresh	Canned	Frozen	IQF[b]
0	49	6	32	39
1	3	18	11	25
2		11	6	4
3		17		4
4		7		
5		1		
Samples, no.	52	60	49	72
Total sample/pit, g	9828	274	1208	907
(ounces)	(346)	(10)	(43)	(32)
Samples with fragments	0	3	1	3
Grade, based on pit count	A	Sub Std.	A	B

[a] Added pits not included.

[b] Individually quick frozen

Recovery of added pits is shown in Table 2. In all but two cases all added pits were recovered. In one case a pit was lost (presumably shattered), in another a large pit fragment was recovered, for an overall recovery rate of $99.4 \pm 0.5\%$. Using this method 12 samples were processed in 20 min. This time does not include sample weighing, but does include filtering, wash out and pulp removal. The noise of a pit in the blender could be heard every time. This success will, of course, depend on the hearing acuity of the operator. The industry average for RTP cherries amounts to 1 pit per 9 567-g samples. No pit noise should therefore be heard in 8 of every 9 samples tested. If noise rather than screening were to be used, most of the sampled material could be returned as pulped, but nondiluted material.

TABLE 2.
DYED PITS RECOVERED, AFTER ADDITION OF 1 PER SAMPLE

	Product			
Pits	Fresh	Canned	Frozen	IQF[a]
Added	52	60	49	72
Recovered	52	59½[b]	49	71

[a] Individually quick frozen

[b] The half pit refers to a recovered fragment

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HEAT TREATMENT EFFECT ON TEXTURE CHANGES AND THERMAL DENATURATION OF PROTEINS IN BEEF MUSCLE

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ABSTRACT

Meat tenderness is one of the most important quality criteria when evaluating results of cooking conditions. Changes in tenderness and weight losses of heat-treated meat (semitendinosus muscle) for different time-temperature combinations were analyzed; the relationship between protein denaturation and textural changes was studied.

Heat treatments of meat samples (1.5 cm in diameter, 2 cm long) were performed in a thermostatic bath in the 60–90C range. Maximum heating times were 180 min. Meat hardness was determined by Warner-Bratzler measurements using an Instron testing machine. Protein denaturation was followed by Differential Scanning Calorimetry (DSC) analyzing peaks for myosin (I and II), sarcoplasmatic proteins and collagen (II) and actin (III).

Between 60 and 64C, hardness decreased with cooking time until reaching the lowest asymptotic values. This was related to protein denaturation of peak I and II. Between 66 and 68C, hardness decreased at first but increased later due to actin denaturation; at the temperatures 81 and 90C no modifications were observed and hardness remained at its higher values.

The kinetic model proposed fit the experimental results satisfactorily. Activation energies of tenderizing and toughening processes are similar to those of protein denaturation of peak II and III. Weight losses due to cooking were also modelled increasing through the entire temperature range.

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INTRODUCTION

The tenderness of meat is one of the main quality criteria to consider in the determination of the most desirable cooking conditions. During cooking, time/temperature combinations have strong influence on tenderness as well as other important quality factors such as juiciness, color and flavor (Martens *et al.* 1982).

Changes in meat tenderness that occur during cooking are generally associated with heat-induced alterations of the components (collagen and myofibrillar proteins) of the primary structure of the muscle tissue.

Collagen represents a low proportion of the muscle mass, generally about 2%; only a few muscles contain more than 10%. These muscles produce tougher meats suggesting a direct relationship between collagen content and texture. However, this relationship is not yet fully understood (Bailey 1984).

The myofibrils (60% of the total protein content) consist of several proteins, with myosin and actin being the major ones.

Paul (1963) and Laakkonen *et al.* (1970) suggested that heat solubilizes the connective tissue leading to meat tenderization, while meat toughening is due to the denaturation of myofibrillar proteins. Samples are more tender and present less cooking losses when heating temperatures are low (Bramblett *et al.* 1959; Marshall *et al.* 1960; Penfield and Meyer 1975).

Paul (1963) suggested that low temperatures associated with long cooking times in the 57–60C range, may promote greater degradation or softening of connective tissue without extensive hardening of muscle fibers.

Machlik and Draudt (1963) studied the effect of time and temperature on the shear patterns of small cylinders of semitendinosus muscle heated between 50 and 90C. They found a marked decrease in toughness between 58 and 60C and an increase between 65 and 75C. The decrease in shear force observed between 58 and 60C was attributed to the collagen shrinkage reaction.

Davey and Gilbert (1974) also working with small samples, determined the relationship between toughness and cooking temperature during a one hour treatment. They observed a toughness increase between 40 and 50C as well as between 65 and 75C. The first hardening phase was associated with the denaturation of the contractile system of muscle, while the second phase was attributed to the collagen shrinkage.

Bailey (1984) reported that as the temperature of a piece of meat is raised to between 40 and 50C, there was an increase in toughness as determined by the shear value, and this was interpreted as being due to denaturation of the myofibrillar proteins, primarily the actomyosin complex; denaturation resulted in a shrinkage of the muscle fiber. At higher temperatures, a slow increase in shear value was observed, and at 60–70C a second rapid increase was reported.

This shrinkage was considered to be dependent on the age of the animal, and is concomitant with the observed gelatinization of the collagen of the epi- and perimysium at about 65C. These conclusions differ from the accepted idea that collagen shrinkage leads to a softening of meat. Also, the second toughening phase takes place at temperatures slightly above those normally associated with collagen shrinkage reaction (Ledward 1979).

Bouton *et al.* (1974), working on meat slices cooked for one hour, observed a marked increase of WB shear force values at temperatures between 60 and 90C. Prolonged cooking at 90C (3 h) produces only a small decrease in shear force values even though adhesion values decreased significantly. Davey *et al.* (1976) found a marked decrease in toughness with long cooking times at 80C.

Studies performed with differential scanning calorimetry (DSC) related the heat-denaturation of meat proteins with the textural changes produced during meat cooking (Martens *et al.* 1982; Findlay *et al.* 1986).

On the other hand, studies performed with electron microscopy showed that heat caused both degradation of myofibrillar proteins and gelatinization of collagen (Cheng and Parrish 1976; Jones *et al.* 1977; Leander *et al.* 1980; Findlay *et al.* 1986).

Most of the water in meat is held within the myofibrils in the narrow channels between the filaments. Losses of water during cooking is accompanied by a shrinkage of the meat that occurs in two phases. According to Offer *et al.* (1984) at a temperature of about 45–60C, the shrinkage is primarily transverse to the fiber axis. At 60–90C, the shrinkage is parallel to the fiber axis, this would account for the decrease in sarcomere length and juiciness decreases with increasing temperatures (Laakkonen 1970; Bouton *et al.* 1975).

The objectives of the present work were to study and model the changes in tenderness and weight losses of meat submitted to thermal treatments with different temperature and time combinations and to establish the relationship between the denaturation process of the main protein systems and the textural changes produced in the cooked meat.

MATERIALS AND METHODS

For each experiment carried out at one bath temperature, cooking experiments were performed 24 h postmortem on 26 semitendinosus muscles from 26 steers (400 days age, carcass weight 220 kg). Epymisial connective tissue and adhering fat were removed from the muscle.

Thermal Treatment

Cylinders of meat were cut with a boring device (cores, 1.5 cm in diameter, 2 cm long) parallel to muscle fibers and packed in Cryovac CN Series 500 (Cook-In Materials) bags (Grace, Argentina). In each experiment, thermocouples were inserted in the thermal centers of two cores (control samples) to monitor the time-temperature relationship during the heating and cooling processes. The packed cylinders of meat were heated in a thermostatic bath at different temperatures (60, 64, 66, 67.5, 68, 81 and 90C). Processing times were calculated starting from the moment (zero time) the thermal center of the core reached a temperature that differed in 0.1C from the temperature of the fluid in the bath. Due to the small size of the samples this initial time was very short (lower than 2 min); maximum cooking times were of 180 min.

Three semitendinosus muscles from different animals were used for each experiment corresponding to one bath temperature. The cores of meat from each muscle were packed in groups of 11 samples. At each processing time, three packs (each one corresponding to a different animal) were removed from the thermostatic bath, cooled down by immersion in water at 0C until reaching 20C, before the determinations of hardness, weight losses and DSC scans. A minimum of 6 processing times were considered for each temperature treatment.

Texture Studies

Mechanical properties of the meat were tested using the Warner Bratzler shear on an Instron 1132 Universal Testing Machine (USA) using a cross head speed of 10 cm/min. Hardness was determined as the maximum force registered by 8 sheared cores and was expressed as the mean value.

Weight Losses

Samples were weighed before and after cooking to compare weight losses produced as a consequence of the different thermal treatments. Weight losses were expressed as a percentage of initial weight (W).

Because the present evaluation of weight loss is performed with small samples, the obtained data may not be fully representative of the water diffusion in larger pieces. However, the comparative analysis between time-temperature combination is still valid.

Differential Scanning Calorimetry (DSC)

DSC studies were performed in a Du Pont 910 Model (USA). For each temperature-time combination, 3 samples were analyzed. Meat samples of 12–25

mg were obtained from the center of the cooked cylinders. The samples were placed in aluminum pans and hermetically sealed. Meat samples were obtained with a boring device of the same size of the pan. A good contact between the sample and the bottom of the pan was assured. The heating rate of the scans was 10C/min within a range of 20 to 110C.

The reference pan contained 15 μl of distilled water. Peak areas of the thermograms were measured with an Image analyzer (Zeiss Morphomat 30, Germany).

RESULTS AND DISCUSSIONS

Texture Studies

Hardness values varied with heating time at different temperatures (Fig. 1). The lowest values of hardness were obtained at 60 and 64C showing larger reductions during the initial heating. This temperature range, characterized as the most effective for meat tenderization, was also mentioned by other authors (Machlik and Draudt 1963; Laakkonen *et al.* 1970; Penfield and Meyer 1975).

For 81 and 90C treatments, hardness showed relatively large values that remained approximately constant during the entire heat treatment. Machlik and Draudt (1963) observed that in the 80–90C range, the hardening of the samples was achieved quickly, but they also observed a toughness decrease at longer times.

It is interesting to observe that for an intermediate range of temperature (66 and 68C) hardness decreased very quickly, reaching a minimum value at a certain time for each temperature. From then on, hardness increased until a maximum value was reached; this value was similar for all temperatures.

These results show that two reactions with opposite effects take place, one producing tenderization and the other increasing meat hardness. This behavior is related to the denaturation of the different protein systems in muscle.

DSC Studies

Figure 2 shows thermograms of packaged meat samples heated for different times with water at 60, 66 and 68C. The three major endothermic transitions of beef muscle have been associated with specific changes in sensory texture (Martens *et al.* 1982). Peak I disappears for short treatment times at the three temperatures, while peak II disappears gradually at 60C and more rapidly at 66C. Peak II was not detected in samples treated at 68C. Peak III was not altered at 60C and disappeared when treated at 68C for 60 min. Stabursvik and Martens (1980) stated that at normal pH (5.4) the first peak of the thermogram

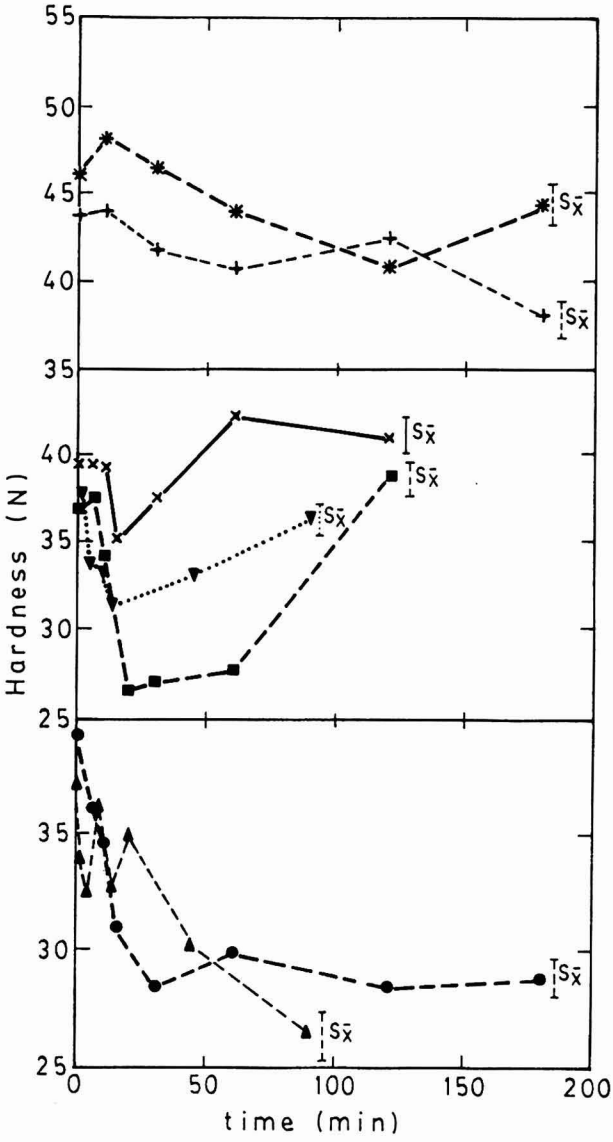

FIG. 1. HARDNESS (NEWTON) VS. COOKING TIME (MIN) RELATIONSHIPS IN SEMITENDINOSUS BEEF MUSCLE AT DIFFERENT PROCESSING TEMPERATURES

Bars indicate standard deviation of the mean.

(●)60C (▲) 64C (▼) 66C (■) 67.5C (×) 68C (+) 81C (*) 90C

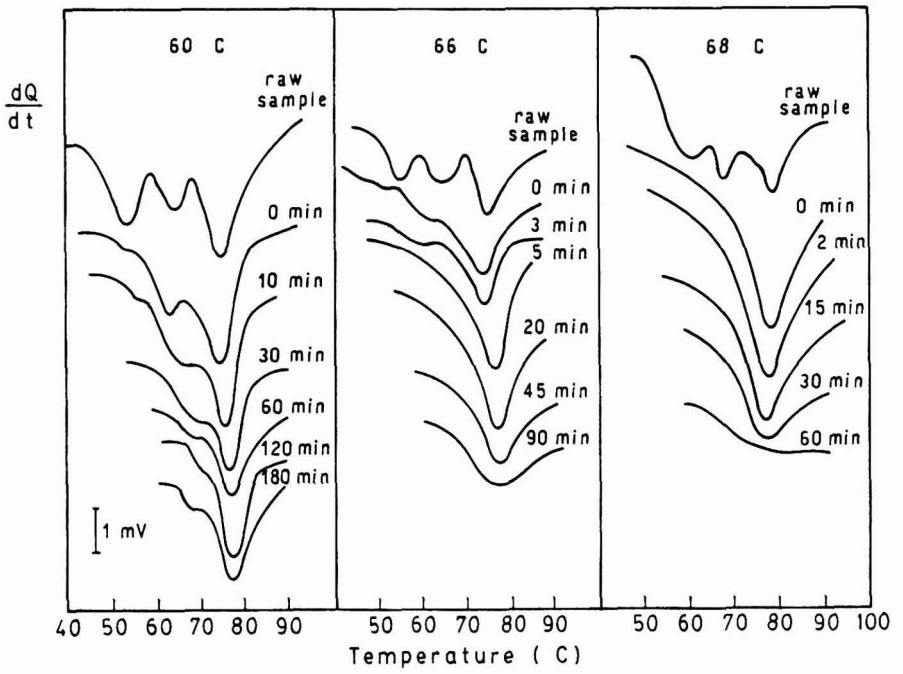

FIG. 2. THERMOGRAMS OF SEMITENDINOSUS BEEF MUSCLE PROTEINS AFTER DIFFERENT HEAT TREATMENTS

of muscle tissue corresponds to light mero-myosin, the second peak represents the denaturation of heavy mero-myosin, collagen and sarcoplasmic proteins, while the third peak mainly to actin. The temperature range of denaturation of the different protein components is characteristic of the species, the pH and the ionic strength of the muscle (Schmidt and Trout 1984).

According to Martens *et al.* (1982), due to the thermal lag in DSC samples scanned at 10C/min and to the fact that thermal denaturation of muscle protein in meat is irreversible, a protein in meat may be denatured at temperatures below its DSC peaks. Thus, it is difficult to read accurate denaturation temperature data directly from the thermogram of raw meat sample, but for a sample that has been heat treated prior to the DSC scan, it presents peaks whose areas are proportional to the amount of remaining native protein in the sample after the first heat treatment.

The percentages of native protein remaining after each heat treatment were obtained from the areas under the thermogram peaks (Tables 1, 2 and 3). At 81C the three peaks had disappeared for 0 min treatment.

TABLE 1.
THERMAL DENATURATION AT 60C OF THE MAIN PROTEIN SYSTEMS IN BEEF SEMITENDINOSUS MUSCLE AT DIFFERENT PROCESSING TIMES

	Percentage of native protein				
	time (min)				
	0	30	60	120	180
Peak					
I	7	0	0	0	0
II	81	63	36	29	18
III	100	100	100	100	100

Values correspond to the percentage of native protein remaining in each treatment and were obtained from the areas under the thermogram peaks.

TABLE 2.
THERMAL DENATURATION AT 66C OF THE MAIN PROTEIN SYSTEMS IN BEEF SEMITENDINOSUS MUSCLE AT DIFFERENT PROCESSING TIMES

	Percentage of native protein						
	time (min)						
	0	3	5	10	20	45	90
Peak							
I	3	0	0	0	0	0	0
II	13	6	0	0	0	0	0
III	100	100	100	64	58	47	30

Values correspond to the percentage of native protein remaining in each treatment and were obtained from the areas under the thermogram peaks.

TABLE 3.
THERMAL DENATURATION AT 68C OF THE MAIN PROTEIN SYSTEMS IN BEEF SEMITENDINOSUS MUSCLE AT DIFFERENT PROCESSING TIMES

	Percentage of native protein				
	time (min)				
	0	2	15	30	60
Peak					
I	0	0	0	0	0
II	0	0	0	0	0
III	100	100	61	46	0

Values correspond to the percentage of native protein remaining in each treatment and were obtained from the areas under the thermogram peaks.

The data show that the proteins corresponding to peak II underwent continuous denaturation at 60C. Only 18% of the native protein was detected at 60C for 180 min cooking, while at 66C for 5 min and at 68C for 0 min all the proteins were in their denatured form.

On the other hand, actin (peak III) began denaturation after 5 min at 66C and 2 min at 68C. Total denaturation was observed after 60 min at 68C. Martens *et al.* (1982) found that 5 min at 67C were necessary for actin denaturation to start.

They also found that after a treatment of about 70 min only 10% of the protein remained native. Similar studies done by Wagner and Añon (1985) found that 5 min at 67C were necessary to denature the proteins of peak II, while at 77C for 5 min practically no actin was found on its native form. Findlay *et al.* (1986) established that for 5 min at 57, 70 and 81C, proteins of peaks I, II and III, respectively, were denatured.

These results are supported by electron microscopy studies. Jones *et al.* (1977) carried out studies on meat samples cooked in a water bath at 50, 60 and 90C for 45 min. They found that at 50C the endomysial collagen did not demonstrate any change, while at 60C the endomysial sheath had lost its fibrous appearance and had probably started to gelatinize. However, at this temperature the essential structure of the myofibrils appeared to be intact. At 90C the sarcolemma had a granular appearance and the sarcomeres had shortened.

Leander *et al.* (1980), also working with electronic microscopy on samples cooked in an oven until reaching an internal temperature of 63, 68 and 73C, observed that at 63C, myofibrils were slightly affected by thermal treatment while the perimysial connective tissue evidenced thermal induced coagulation. At 68C the thermal contraction of the sarcomeres was observed without any alteration of the z-lines. Muscle fibers seemed intact but separate. Connective tissue sheaths that surrounded the individual fibers were coagulated and had a granular appearance. At 73C in some cases, the sarcomeres showed thermal contraction and breakage of the myofibrils at the z-line area.

Relationship Between Texture and Protein Denaturation

When DSC results and hardness values are compared, a relationship arises between the decrease in meat hardness and the denaturation of peak II proteins (collagen being one of them), and between hardness increase and denaturation of peak III proteins (actin).

The increased toughening associated with increased cooking temperature can be attributed to the contracted and likely hardened filamentous material present in the A-bands of the sarcomeres (myosin). Although increased disintegration of filaments occurred in the I-band (actin) as temperature was increased, these changes did not result in increased tenderness (Jones *et al.* 1977).

The activation energy of meat toughening process was calculated in order to verify the relationship between actin denaturation and the development of meat hardness. For that purpose hardness versus time curves at 66, 67.5 and 68C were used. Analysis was focused on the portion of the curve where only denaturation of peak III proteins exists. The zones analyzed were from 14 to 90 min at 66C; 20 to 120 min at 67.5C and 15 to 60 min at 68C. The following first order kinetic model was used to fit the experimental curves:

$$\frac{dD}{dt} = K_h D \tag{1}$$

with D = hardness at different time (N)
t = time (min)
K_h = kinetic constant

Integrating Eq. (1) resulted in:

$$\ln D = \ln D_o + K_h t \tag{2}$$

Table 4 shows K_h values obtained from Eq. (2) and the correlation coefficients (r) corresponding to each regression. An Arrhenius model was used to study the influence of temperature on K_h constant:

$$K_h = K_o \exp(-E/RT) \quad (3)$$

TABLE 4.
KINETIC CONSTANTS OF BEEF TOUGHENING
(SEMITENDINOSUS MUSCLE) DURING COOKING

T (C)	$K_h x 10^3$ (min^{-1})	r
66	1.989	0.998
67.5	3.835	0.886
68	4.105	1.000

r = linear correlation coefficient.

The activation energy calculated from the regression of K_h values was 362.4 Kjoule/mol (r = 0.988). This value agrees with the ones obtained for actin denaturation by Martens *et al.*(1982) and Wagner and Añon (1985), which were 349.9 Kjoule/mol and 378.7 Kjoule/mol, respectively.

Similarly, the activation energy (E) for the meat tenderization process was calculated. This process is related to the denaturation of peak II proteins, collagen being one of the proteins involved. The zone of hardness versus time curve at each tested temperature where only denaturation of peak II proteins exists, was analyzed. These zones were from 10 to 180 min at 60C, 2 to 90 min at 64C and 0 to 14 min at 66C. A first order kinetic analysis was used to obtain the kinetic constant K_t shown on Table 5 together with the correlation coefficients. The activation energy obtained was 604.6 Kjoule/mol (r = 0.997), similar to the value reported by Machlik and Draudt (1963) for meat tenderization during cooking and also with the activation energy for collagen shrinking reaction obtained in that work (589.38 Kjoule/mol). It should be mentioned that peak II reflects also the denaturation of a myosin portion that contributes to meat toughening (Paul 1963; Hamm 1966; Draudt 1972). However, the results do not show this hardness increase due to the higher effect of the connective tissue denaturation that is associated with meat tenderization.

TABLE 5.
KINETIC CONSTANTS OF BEEF TENDERIZATION
(SEMITENDINOSUS MUSCLE) DURING COOKING

T (C)	$K_t x 10^2$ (min^{-1})	r
60	0.031	0.948
64	0.287	0.925
66	1.300	0.934

r = linear correlation coefficient.

According to Davey and Niederer (1977), other factors besides denaturation of collagen and myofibrillar proteins may play a role when textural changes occur in heat treated meat. For example, proteolytic activity of cathepsins and other tenderizing enzymes may increase with temperatures between 55 and 65C. However, studies by Bouton and Harris (1981) showed that the role of proteases on textural changes at those temperatures is low compared to that of denaturation of collagen.

Weight Losses

Weight losses increased with time and higher temperature (Fig. 3). Results agree with those commonly found by all authors working on cooked meat (Laakkonen 1970; Bouton *et al.* 1975; Offer *et al.* 1984).

A first order kinetic model was used to fit the results of weight losses and the following equation was obtained:

$$W(t) = A - B \exp(-K_w t) \quad (4)$$

with W = weight loss at each time
A = weight loss at infinite time
B = difference between weight losses at infinite and initial time
K_w = kinetic constant

The k_w values and the correlation coefficients are shown in Table 6. The values of k_w increase with temperature following the Arrhenius model. The activation energy obtained was 54.93 Kjoule/mol (r = 0.912).

Martens *et al.* (1982) presented similar results working on the effect of temperature on weight losses. Actin and collagen denaturation caused structural

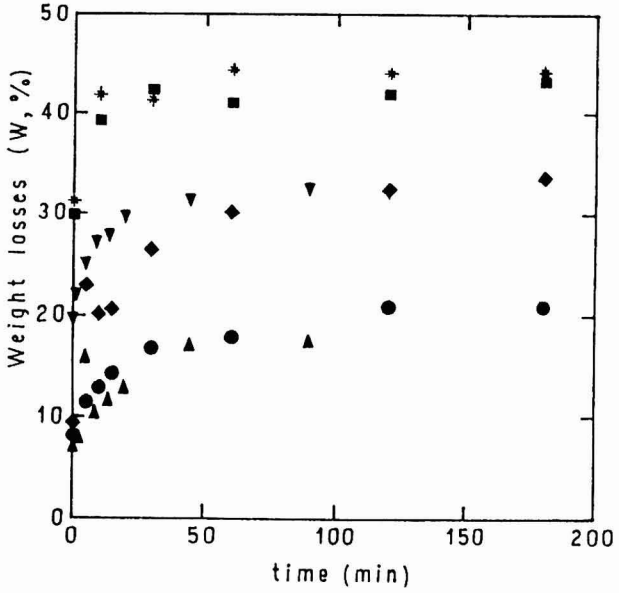

FIG. 3. WEIGHT LOSSES VS. COOKING TIME RELATIONSHIPS IN SEMITENDINOSUS BEEF MUSCLE AT DIFFERENT PROCESSING TEMPERATURES
(●) 60C (▲) 64C (▼) 66C (◆) 68C (■) 81C (*) 90C

TABLE 6.
KINETIC CONSTANTS OF WEIGHT LOSSES DURING COOKING IN SEMITENDINOSUS BEEF MUSCLE

T (C)	K_w (min^{-1})	r
60	0.037	0.990
64	0.043	0.970
66	0.091	1.000
68	0.044	0.990
81	0.141	1.000
90	0.190	1.000

r = linear correlation coefficient.

changes, which are responsible for water losses observed from meat tissue. The endomysium and perimysium act to expel the sarcoplasmic fluid from the fibers (Offer *et al.* 1984).

Findlay *et al.* (1986) found that the increase in cooking temperature produced shortening of the sarcomeres; this shrinkage correlated well with juiciness loss of the samples as evaluated by sensory analysis.

CONCLUSIONS

Both tenderness and weight losses of cooked meat (semitendinosus muscle) are affected by protein denaturation, which depends on the thermal history of the product.

Between 60 and 64C, hardness decreased with time until reaching the lowest asymptotic values. These temperatures produced denaturation of peaks I and II proteins, collagen being one of them. Between 66 and 68C, hardness decreased during the first period (denaturation of peak II proteins without modification of peak III ones) and increased later due to actin denaturation. Between 81 and 90C hardness was not modified and remained at the higher values, because actin was previously denaturated.

The kinetic model used allowed the determination of the activation energies of tenderizing and toughening processes. An excellent agreement was found between these activation energies and the denaturation of peak II and peak III proteins. Weight losses produced during cooking increased over the entire period and for the entire range of temperatures.

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EXTRACTION OF SALT AND ALKALI SOLUBLE PROTEINS FROM MECHANICALLY DEBONED TURKEY RESIDUE: EFFECT OF SOME VARIABLES USING RESPONSE SURFACE METHODOLOGY[1]

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ABSTRACT

Response surface methodology was used to study the extraction of proteins from mechanically deboned turkey residue (MDTR). The extraction conditions investigated were the solvent/MDTR (vol/wt) ratio, the duration of extraction, and the temperature and pH of the extraction medium. During the extraction of proteins from MDTR using an aqueous 1% sodium chloride solution, the amount of proteins extracted increased with increase in solvent/MDTR ratio, extraction temperature and pH. However, extractions carried out for longer than about 30 min did not increase the amount of proteins extracted into solution. Maximum extraction was achieved using a solvent/MDTR ratio of 5, and a duration of extraction of about 16 min at 37C and pH 10, leading to the extraction of 9.58 mg proteins per gram MDTR. This represented 5% of the total MDTR proteins.

INTRODUCTION

Turkey meat is usually used as a sole protein source both in the form of meat, and in such foods as luncheon meat and sausages where turkey protein extracts may be used with other protein sources. In the turkey deboning process, some usable meat is left on the bones. However, a modified deboning process has been used for some time to recover some of this meat from the bones by grinding or breaking the bones into small pieces and passing over a screen with

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some pressure in a deboner. Meat particles and fat pass through the screen while the bones are retained (Newman 1982). Nonetheless, even the modified deboning process leaves some valuable turkey meat on the bony residue. It is estimated that mechanically deboned poultry residue contains approximately between 13 and 20% protein of which about 18% can be extracted with aqueous solutions of salt and alkali (Lawrence *et al.* 1982; McCurdy *et al.* 1986).

Some research has been done on the recovery of protein from both poultry muscle and bony residue using sodium chloride and dilute alkali solutions (King and Earl 1988; McCurdy *et al.* 1986; Prusa and Bowers 1984; Saffle and Galbreath 1964). Proteins recovered using the methods proposed by these authors have been shown to be of good enough quality to be used as protein supplements in luncheon meats Jelen *et al.* 1982). It has been reported that the nutritional quality of both mechanically separated and alkali extracted chicken protein is high and equal to or better than casein (Ozimek *et al.* 1986; Young 1976).

Response surface methodology (RSM) consists of a series of statistical procedures used in the study of a process that may be controlled by numerous factors (Box *et al.* 1978; Cochran and Cox 1957). RSM defines the effect of each of the independent variables on the overall process as well as the effect of their cross interactions. The technique also determines the value of each input variable needed to maximize the response, and the nature of the response surface near the maximum response.

The principles of RSM have been incorporated into the RSREG (response surface regression) procedure of the Statistical Analysis System (SAS 1988) software. This facilitates the use of RSM on computers, as manual calculations are no longer necessary. Despite its usefulness, RSM has a major drawback in that conditions outside the experimental region cannot be predicted.

The purpose of this study was to use RSM to investigate the effects of four variables during the extraction of proteins from mechanically deboned turkey residue (MDTR) with an aqueous 1% sodium chloride solution. The conditions investigated were the solvent/MDTR (vol/wt) ratio (X_1), the duration of extraction (X_2), the temperature of the extraction medium (X_3) and the pH of the extraction medium (X_4). The solvent/MDTR ratio was limited to 5 (vol/wt) because there is a concern for the generation of large amounts of wastewater that may result from such a process. The temperature of the extraction medium was studied at levels below 37C to avoid heat coagulation of the proteins that could lower the yield of the extraction process. The pH of the extraction medium was studied at levels below 10 pH units because of the potential for the formation of potentially dangerous amino acid complexes like lysinoalanine at higher pH values.

MATERIALS AND METHODS

Mechanically deboned turkey residue from turkey frames was obtained from Farbest Foods Inc., Huntingburg, IN. The MDTR was frozen and stored at −20C until used. The bulk MDTR was thawed overnight in a cold room at 8C and working samples of 100–200 g were prepared from the bulk. Both the bulk MDTR and the working samples were returned to −20C storage. After usage, any unused portion was returned to the 8C storage but for no more than 2 days. All the chemicals used were of reagent grade purchased from commercial suppliers.

Protein Extraction Procedure

The procedure used during the extraction was adapted from those by Saffle and Galbreath (1964) and Kahn *et al.* (1974). The variables studied were solvent/MDTR ratio (vol/wt), holding time, and the temperature and pH of the extraction medium. The coded and uncoded levels of these variables are shown in Table 1.

TABLE 1.
VARIABLES WITH THEIR CODED AND PROCESS LEVELS
USED IN THE EXTRACTION OF PROTEINS FROM MDTR

Variables	Symbol	Coded level	Process level
Solvent/MDTR (vol/wt) ratio	X_1	-2	1
		-1	2
		0	3
		1	4
		2	5
Duration of extraction (min)	X_2	-2	5
		-1	15
		0	25
		1	35
		2	45
Extraction temperature (C)	X_3	-2	1
		-1	10
		0	19
		1	28
		2	37
pH	X_4	-2	6
		-1	7
		0	8
		1	9
		2	10

Twenty grams of the MDTR was washed twice, each time with approximately 200–300 ml of tap water (approximately 23C) for about 2 min. The washed MDTR was then placed in a Waring Blendor (Model 7012S, New Hartford, CT) with a predetermined amount of 1% sodium chloride solution (Table 1). The slurry was blended at low speed (speed 1) for 1 min and transferred into a beaker and held at a specified temperature. The pH of the slurry was adjusted to the predetermined level with 1 N hydrochloric acid or sodium hydroxide, and measured by a pH meter (Model 5984-50, Cole-Parmer Ind., Chicago, IL). The blended slurry was held in the beaker for a specified duration with occasional stirring after which it was centrifuged in a table-top centrifuge (Damon IEC model, Needham Hts., MA) at high speed (7) for 5 min. The fat layer at the top of the centrifuge tubes was skimmed off, the supernatant was collected and the bony residue was discarded.

Protein Analysis

The amount of protein in the supernatant was determined spectrophotometrically using the bicinchoninic acid method (Smith *et al.* 1985). Bovine serum albumin was used as the protein standard and the determinations were carried out using a spectrophotometer (Spectronic 20, Milton Roy Co., Rochester, NY).

Experimental Design

A central composite rotatable design (CCRD) of the second order, as shown in Table 2, was used. This design is based on four variables, giving a 2^4 factorial + star design (8 points) + 9 points in the center, with the radius of the design, $\alpha=2$ (Cochran and Cox 1957).

Statistical Analysis

The principle of RSM using the RSREG procedure of the SAS system was employed to analyze the data (SAS 1988).

RESULTS AND DISCUSSION

The results of the amount of proteins extracted from MDTR under various experimental conditions are shown in Table 2. These data were used to develop the polynomial model shown in Eq. (1).

TABLE 2.
CENTRAL COMPOSITE ROTATABLE SECOND ORDER DESIGN WITH FOUR VARIABLES AND THE MEAN[a] AMOUNT OF PROTEIN EXTRACTED FROM MECHANICALLY DEBONED TURKEY RESIDUE

RUN #	SOLVENT/ MDTR RATIO	TIME (min)	TEMP (C)	pH	YIELD[a] (mg/g MDTR)
1.	-2	0	0	0	2.83
2.	-1	-1	-1	-1	3.06
3.	-1	-1	-1	1	4.31
4.	-1	-1	1	-1	4.28
5.	-1	-1	1	1	4.75
6.	-1	1	-1	-1	3.64
7.	-1	1	-1	1	4.31
8.	-1	1	1	-1	4.52
9.	-1	1	1	1	5.00
10.	0	-2	0	0	4.00
11.	0	0	-2	0	5.51
12.	0	0	0	-2	4.42
13.	0	0	0	0	5.50
14.	0	0	0	0	5.08
15.	0	0	0	0	6.00
16.	0	0	0	0	4.83
17.	0	0	0	0	5.08
18.	0	0	0	0	5.25
19.	0	0	0	0	6.49
20.	0	0	0	0	5.00
21.	0	0	0	0	5.83
22.	0	0	0	2	5.34
23.	0	0	2	0	4.87
24.	0	2	0	0	3.91
25.	1	-1	-1	-1	4.27
26.	1	-1	-1	1	5.86
27.	1	-1	1	-1	5.88
28.	1	-1	1	1	8.21
29.	1	1	-1	-1	4.89
30.	1	1	-1	1	5.31
31.	1	1	1	-1	5.78
32.	1	1	1	1	7.43
33.	2	0	0	0	4.64

[a] Mean of duplicate runs

$$Y = 5.45 + 0.725X_1 + 0.007X_2 + 0.372X_3 + 0.446X_4 - 0.319X_1^2 - 0.270X_2^2 + 0.044X_3^2 - 0.033X_4^2 - 0.118X_1X_2 + 0.234X_1X_3 + 0.194X_1X_4 - 0.066X_2X_3 - 0.151X_2X_4 + 0.062X_3X_4 \quad (1)$$

The analysis of variance for the model [Eq. (1)] developed for this process, indicates that the model only moderately fitted the experimental data. The coefficient of determination (R^2) for the fit was only 73%. This may be due to the small sample size used (20 g). The model also had a nonsignificant lack of fit of 0.10. This nonsignificant lack of fit suggests that a good portion of the total error could be accounted for by the quadratic model. The model also failed to show any significant interaction effects ($P < 0.05$) between the variables and indicated that their importance could be ranked in the following order:

solvent/MDTR (vol/wt) ratio > pH of the extraction medium > temperature of the extraction medium > extraction time, as shown in Table 3. Only the solvent/MDTR (vol/wt) ratio (X_1) was found to be significant ($P < 0.05$).

TABLE 3.
ANALYSIS OF VARIANCE OF THE SECOND ORDER POLYNOMIAL MODEL DEVELOPED FOR PROTEIN EXTRACTION FROM MDTR

SOURCE	df		F-RATIO	P>F
Regression		R^2		
Linear	4	0.54	9.04	0.000^{s}
Quadratic	4	0.14	2.27	0.102
Crossproduct	6	0.06	0.64	0.698
Total	14	0.73	3.51	0.007^{s}
Residuals		MS		
Lack of fit	10	0.79	2.59	0.10^{ns}
Pure error	8	0.30	-	-
Total error	18	0.57	-	-
Factors				
X_1	5	17.40	6.07	0.002^{s}
X_2	5	2.87	1.00	0.445
X_3	5	4.38	1.53	0.230
X_4	5	5.85	2.04	0.121

^{s} Significant at $P<0.05$
^{ns} Not significant at $P<0.10$
R^2 = Coefficient of determination
MS = Mean square

A three dimensional response surface for the protein extraction process is shown in Fig. 1. The best way of finding the best conditions, within the experimental space under investigation, from this response surface, is to generate contour plots of the response surface. Contour plots are two dimensional plots, thus only two variables can be plotted at a time while the other two are held constant at defined levels.

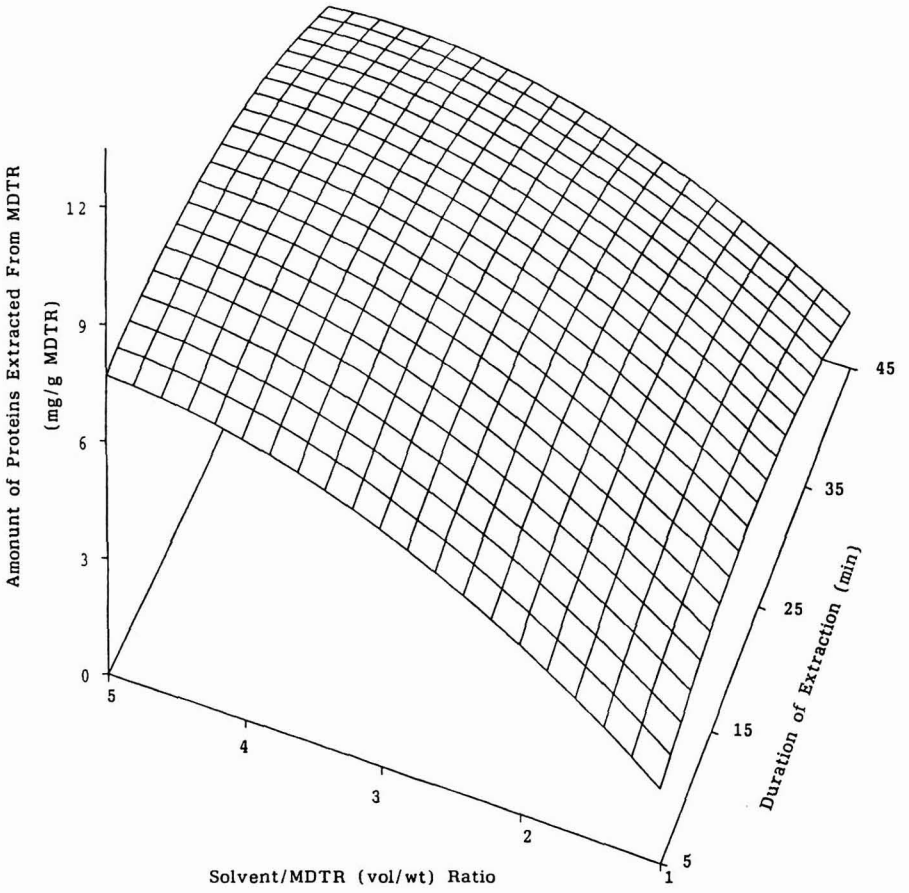

FIG. 1. THREE DIMENSIONAL RESPONSE SURFACE FOR THE EXTRACTION OF PROTEINS FROM MECHANICALLY DEBONED TURKEY RESIDUE AT 23.5C AND pH 10

A contour plot showing the effects of the solvent/MDTR (vol/wt) ratio (X_1) and the extraction time (X_2) on the amount of proteins extracted from MDTR (mg proteins per gram MDTR) is shown in Fig. 2. The temperature of the extraction medium (X_3) is set at 37C while the pH of the extraction medium (X_4) is set at 10 pH units. Maximum protein extraction occurred at solvent/MDTR ratios between 4 and 5 (vol/wt), and durations of extraction between 5 and 30 min. There was a small decrease in the amount of proteins extracted into solution after a duration of extraction of approximately 30 min. This may have been due to protein coagulation during prolonged extraction at this temperature (37C). The coagulation could have caused the formation of protein flocs that would have been precipitated and discarded with the MDTR waste. The increase in the amount of proteins extracted with increase in the solvent/MDTR ratio

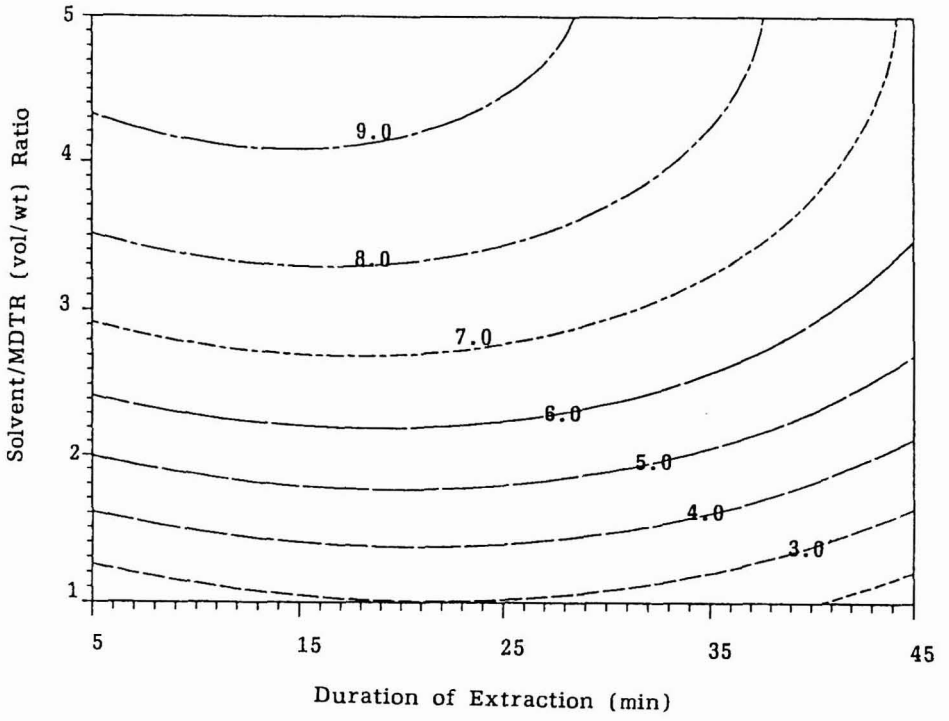

FIG. 2. CONTOUR PLOT SHOWING THE EFFECTS OF SOLVENT/MDTR (vol/wt) RATIO (X_1) AND THE DURATION OF EXTRACTION (X_2) ON THE AMOUNT OF PROTEINS EXTRACTED FROM MDTR (mg protein/g MDTR) AT 37C AND pH 10

shows the same trend as the trend reported by Diptee *et al.* (1989) during the extraction of proteins from brewer's spent grain. This increase in the amount of proteins extracted from MDTR into solution with increase in the solvent/MDTR (vol/wt) ratio can be attributed to a dilution effect. At high solvent/MDTR ratios, the protein solution is less saturated with proteins than at lower ratios. Thus more of the salt and alkali soluble proteins were solubilized at higher solvent/MDTR ratios than at lower ratios.

Figure 3 is a contour plot showing the effects of the solvent/MDTR (vol/wt) ratio and the temperature of the extraction medium (X_3) on the amount of proteins extracted from MDTR (mg/g MDTR) into solution. The duration of extraction is set at 16 min and the pH of the extraction medium is set at 10 pH units. Manipulation of the quadratic model with input from various points in Fig. 2 showed that the optimum value for the duration of extraction was approximately 16 min. At solvent/MDTR ratios between 1 and 2, only a small increase in the amount of proteins extracted from MDTR into solution occurred

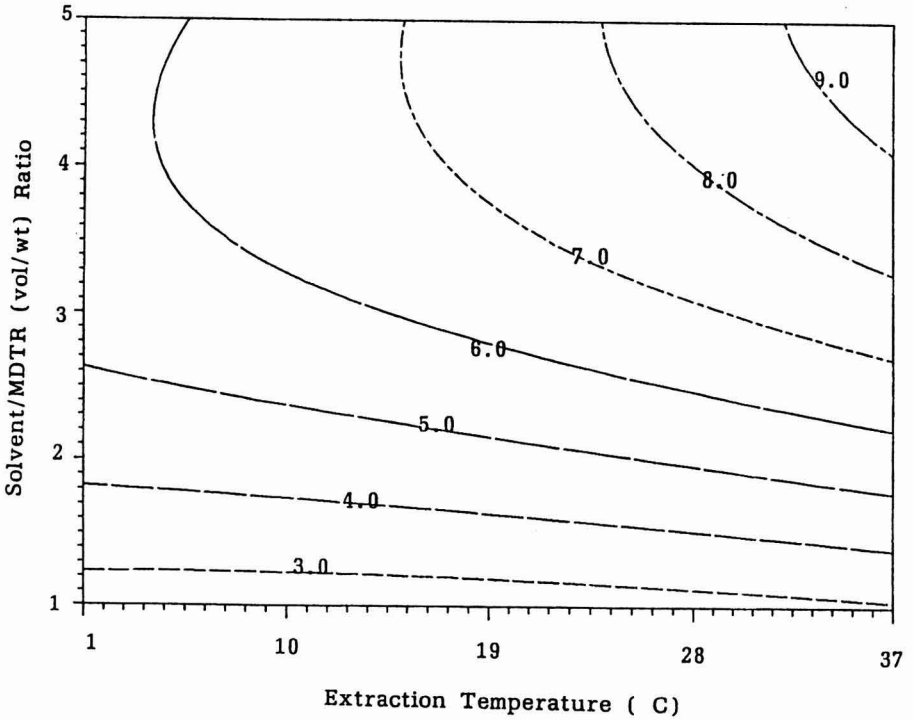

FIG. 3. CONTOUR PLOT SHOWING THE EFFECTS OF SOLVENT/MDTR (vol/wt) RATIO (X_1) AND EXTRACTION TEMPERATURE (X_3) ON THE EXTRACTION OF PROTEINS FROM MDTR DURING 16 MIN OF EXTRACTION AT pH 10

as the extraction temperature was increased from 1C to 37C, as can be seen in Fig. 3. Figure 3 also shows that at solvent/MDTR (vol/wt) ratios above 3.5, maximum extraction of proteins occurred with the amount of proteins extracted being much larger than the amount extracted at lower ratios, at all temperatures. At these same solvent/MDTR ratios (between 3.5 and 5), increase in the extraction temperature caused a larger increase in the amount of proteins extracted from MDTR than at lower solvent/MDTR ratios (lower than 2.5).

The amount of proteins extracted from MDTR into solution increased with increase in the pH of the extraction medium (X_4), as shown in Fig. 4. The duration of extraction is set at 16 min and the temperature of the extraction medium is set at 37C. This is because as the pH of the extraction medium was increased from pH 6 to pH 10, the proteins developed negative charges, and thus became more soluble in the dilute salt solution. The largest increase in the amount of proteins extracted from MDTR into solution, with increase in pH from pH 6 to pH10, occurred at solvent/MDTR (vol/wt) ratios greater than 3. The effect of the pH of the extraction medium was less apparent at lower solvent/MDTR (vol/wt) ratios.

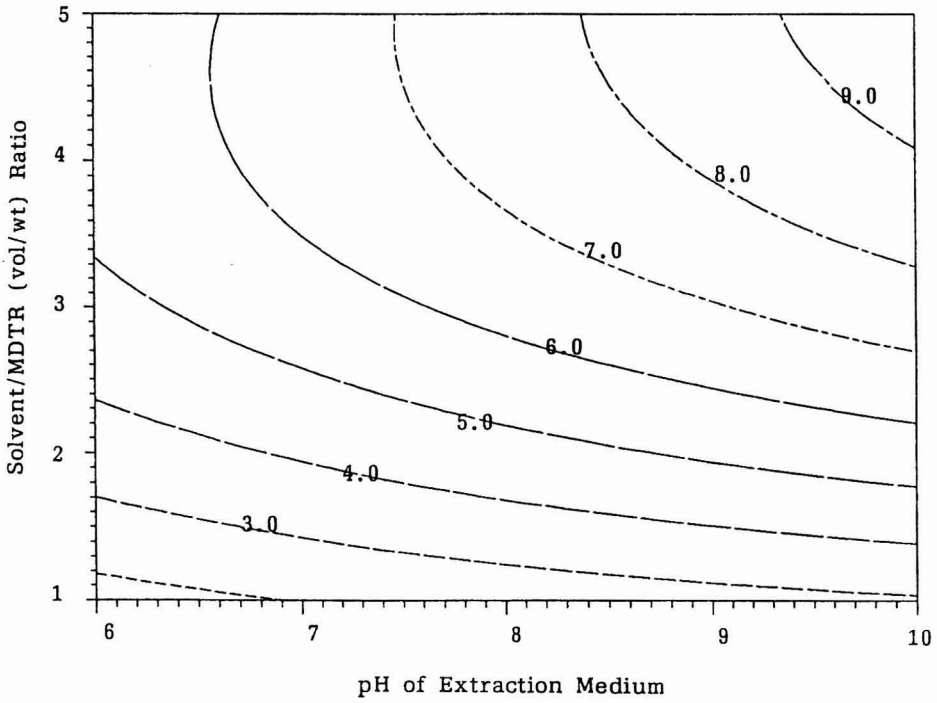

FIG. 4. CONTOUR PLOT SHOWING THE EFFECTS OF SOLVENT/MDTR (vol/wt) RATIO (X_1) AND pH (X_4) ON THE AMOUNT OF PROTEINS EXTRACTED FROM MDTR (mg protein/g MDTR) DURING 16 MIN OF EXTRACTION AT 37C

Verification studies were carried out to compare experimental results with those predicted by the quadratic model [Eq. (1)]. These results are shown in Table 4. The experimental and predicted results were very close to each other in magnitude, indicating that the model was adequate for the system.

The protein extraction process was predicted at laboratory temperature (about 23.5C in this case). A contour plot for the extraction process at a temperature of extraction of 23.5C and at pH 10 is shown in Fig. 5. The plot is similar to that in Fig. 2, except that the maximum amount of proteins that could be extracted has decreased from approximately 9.5 mg/g MDTR at 37C to approximately 7.5 mg/g MDTR at 23.5C.

The best extraction conditions determined for this extraction process were determined to be the following: a solvent/MDTR (vol/wt) ratio (X_1) of 5 with a duration of extraction (X_2) of approximately 16 min at a temperature (X_3) of 37C and pH (X_4) of 10, as shown in Table 4 (A). Under these conditions, 9.58 mg proteins were extracted per gram of MDTR. This represented approximately 5% of the total MDTR proteins and approximately 27.8% of the salt and alkali

TABLE 4.
CODED AND UNCODED VALUES OF THE VARIOUS VARIABLES
IN THE MAXIMIZING REGION OF THE RESPONSE SURFACE

	VARIABLE	LEVELS CODED	UNCODED
A)	Solvent/MDTR ratio (X_1)	2.0	5
	Holding time (X_2)	-0.9	16 min
	Temperature (X_3)	2.0	37 C
	pH (X_4)	2.0	10
	Predicted amount of proteins extracted = 9.64 mg/g MDTR		
	Actual amount of proteins extracted = 9.58[a] mg/g MDTR		
B)	Solvent/MDTR ratio (X_1)	1.0	4
	Holding time (X_2)	-0.9	16 min
	Temperature (X_3)	2.0	37 C
	pH (X_4)	2.0	10
	Predicted amount of extracted protein = 8.91 mg/g MDTR		
	Actual amount of extracted protein = 9.08[a] mg/g MDTR		

[a] Mean of four extractions

soluble proteins of MDTR. This yield is a little lower than the yield reported by McCurdy *et al.* 1986 (6–15%) in a laboratory scale experiment using chicken bone residue from processing chicken backs and necks in a Beehive deboner. Kijowski and Niewiarowicz (1985), however, made the point that the yield of proteins from the extraction process depends on factors such as the type of deboner from which the residue was collected, the raw material and the extraction method used during the extraction process.

The yield may also be low due to frozen storage of the MDTR at −20C during this investigation. Smith (1987) reported the loss of protein solubility in deboned meat with storage time at −20C. The magnitude of the loss in solubility in mechanically deboned meat was about 28% in 20 weeks of storage. The MDTR used in this investigation had been held in storage at −20C for at least six months prior to being used in this study.

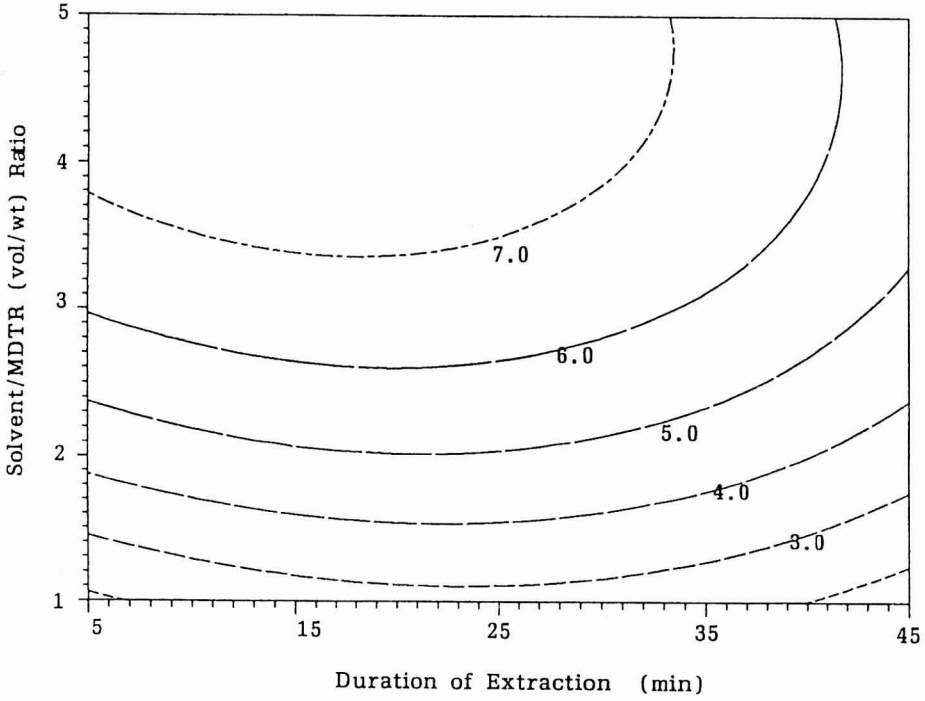

FIG. 5. CONTOUR PLOT SHOWING THE EFFECTS OF SOLVENT/MDTR (vol/wt) RATIO (X_1) AND DURATION OF EXTRACTION (X_2) ON THE EXTRACTION OF PROTEINS FROM MDTR AT 23.5C AND pH

CONCLUSION

In this process where proteins were being extracted from MDTR using an aqueous 1% sodium chloride solution, the solvent/MDTR ratio (X_1) was the only significant factor ($P < 0.05$). In order of significance, the other variables studied could be ranked as follows: pH (X_4) > extraction temperature (X_3) > duration of extraction (X_2). The extraction conditions chosen for the protein extraction process were a solvent/MDTR ratio of 5 (vol/wt) at 37C and pH 10 for 16 min of extraction. These conditions led to the extraction of 9.58 mg proteins per gram of MDTR, which represents approximately 5% of the total MDTR proteins and 27.8% of the salt and alkali soluble MDTR proteins.

There are numerous factors that could affect the yield during the extraction of proteins from deboner residue that have not been included in this study, such as the temperature of the water used in washing the MDTR, the type of deboner, and actual composition and source of the deboner residue. The yields from different methods used in the extraction of proteins from deboner residue cannot therefore be compared without taking all these factors into account.

This study was carried out to investigate the effects of variables that have been used in other protein recovery processes. The results from this study now allow the recognition of the most important variables that need to be utilized or more closely monitored than others in a larger scale (pilot plant scale) protein recovery process.

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EFFECT OF STABILIZERS AND PARTIALLY HYDROGENATED VEGETABLE OILS ON THE STABILITY AND QUALITY OF FILLED MILK[1]

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ABSTRACT

The objective of this study was to examine the effect of stabilizers and emulsifiers on the stability and sensory quality of vegetable oils in filled milk. Four formulations selected in a preliminary study from a total of 31 different combinations were pasteurized using a high-temperature short-time pasteurizer and evaluated for stability and sensory quality during storage at 2C. Results suggest that the four formulations were stable during storage and any of the four formulations could be used effectively for replacing the coconut oil-based formulation. However, the formulation with Sunflower HB 95/Stabilizer A possessed the best sensory characteristics after one week, whereas the formulation with Canola/Stabilizer B retained the best flavor and least off-flavor during three weeks of storage. In addition, the latter formulation was similar in flavor quality to coconut oil-based filled milk.

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INTRODUCTION

In the past two decades, imitation and filled milk products have shown some success in replacing their real milk counterparts in America as well as in Europe (Mann 1988). However, the quantity consumed is believed to be extremely small compared to the consumption of milk products. Filled milk is also consumed in some overseas U.S. military installations. Coconut oil, the main oil in many parts of the world, was used traditionally as a milk fat replacement in most filled milk formulations. The concern about elevation of serum cholesterol level by saturated fats and oils has led to attempts to replace coconut oil with less saturated vegetable oils.

Modler *et al.* (1970) investigated the use of several vegetable oils in filled milk formulations. Their study showed that, after one week of storage, the levels of oxidation and, consequently, oxidized flavor, in formulations containing safflower, corn, cottonseed, peanut, and olive oils were far too objectionable. However, the formulation made with lightly hydrogenated soybean oil was found to be acceptable, although it exhibited a mild oxidized flavor as well. Recently, a filled milk formulation, called a healthy alternative to whole milk, "Fit 'n' Lite" (Pritchitt Foods, UK), was introduced successfully in Europe. It was reportedly formulated with sunflower oil (Lane 1986). Using a similar concept, a frozen filled milk concentrate was developed by scientists in the U.S. Department of Agriculture (Kinzel 1990). Although not commercially available, the frozen milk concentrate when fully reconstituted would contain a minimum of 8.25% nonfat milk solids and up to 2% vegetable oil (soybean, corn, or peanut oil).

Emulsifiers are commonly used in filled milk products to keep oils dispersed in the milk system. Bundus (1970) reported that different types of emulsifying agents improved the flavor and mouthfeel (by affecting the body) of a filled milk product. However, little information is available about the effect of stabilizers and a combination of emulsifiers and stabilizers on flavor and textural characteristics of filled milk. The objectives of this study were to investigate the effect of partially hydrogenated vegetable oils and emulsifiers/stabilizers on the flavor and textural qualities of filled milk and to identify vegetable oils that are comparable to coconut oil in stability and acceptability. To achieve these objectives, eight partially hydrogenated vegetable oils were selected for their flavor stability. Using the oils and four emulsifiers/stabilizers, all possible combinations of filled milk formulations were made from which four most probable formulations were selected for this study.

MATERIALS AND METHODS

Materials

Based on preliminary studies of 31 different formulations generated by combining nine vegetable oils with different emulsifiers/stabilizers, four partially hydrogenated vegetable oils were selected for this study. These oils were coconut, canola, sunflower, and cottonseed. Those five oils that were not chosen were soybean, sunflower HB 105, natural sunflower, a modified vegetable fat, and a mixture of cottonseed and soybean. The coconut and canola oils were obtained from Anderson Clayton/Humko Products, Inc. (Memphis, TN). Sunflower and cottonseed oils were from SVO Enterprises (Eastlake, OH) and Beatrice/Hunt-Wesson, Inc. (Fullerton, CA), respectively. The selections were based on flavor characteristics of oils in filled milk formulations as well as storage stability tests conducted at the U.S. Army Natick RD&E Center (Natick, MA). All oils used had Active Oxygen Method (A.O.M.) values of not less than 100 h.

A mixture of mono- and diglyceride flakes (designated Emulsifier M) was obtained from Anderson Clayton/Humko Products, Inc. (Memphis, TN). Preblended mixtures of emulsifiers and stabilizers (Actoloid D22 Types A, B, and C) were obtained from Advanced Food Systems, Inc. (Somerset, NJ). The Actoloid D22 Type A (designated Stabilizer A) was composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate. The Actoloid D22 Type B (designated Stabilizer B) was composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate. The Actoloid D22 Type C (designated Stabilizer C) consisted of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate.

Low-heat, grade A, nonfat dry milk (NFDM) was obtained from Mid-America Dairymen, Inc. (Sabetha, KS).

Selection of Formulations

The filled milk formulation utilized was based on the minimum allowed standards for fat and milk-solids-not-fat (MSNF) used in the dairy industry for whole milk (CFR 1991). The basic formulation consisted of water (88.20%), NFDM (8.25%), vegetable oil (3.25%), and emulsifier/stabilizer (0.30%). Four formulations were selected for this study using four different oils and emulsifiers/stabilizers: cottonseed oil with Stabilizer C, sunflower HB 95 oil with Stabilizer A, canola oil with Emulsifier M, and canola oil with Stabilizer B. In addition, the formulation with coconut oil and Emulsifier M was used as a

reference. Selection of the four formulations was based on preliminary work involving a sensory evaluation of all possible combinations (31 formulations) of eight vegetable oils and four different emulsifiers/stabilizers. The four formulations selected had the best sensory characteristics of flavor, odor, mouthfeel, and overall quality according to the mean scores and comments made by a seven-membered sensory panel. The sensory work involved in this selection process was the same as described later in the sensory section.

Batching and Processing

Batching procedures depended largely upon the type of emulsifier/stabilizer used. In formulations where Emulsifier M was used, the NFDM was first dispersed in warm water using a blending pump to ensure proper dispersion. The temperature of the mix was then raised to 54C. In the meantime, the Emulsifier M flakes were melted and slowly incorporated into the vegetable oil at 49–54C. Next, the mixture of vegetable oil and emulsifier was blended in with the NFDM mixture at 49–54C and agitated for approximately 10 min. In formulations where Stabilizer A, B, or C was used, the stabilizers were first dry-blended with the NFDM and dispersed in warm water. The remaining steps in the process were the same as those for formulations using Emulsifier M.

All batching and processing of filled milk samples were done at the Kansas State University Dairy Processing Plant. Each batch (a total volume of 114 L) was pasteurized at 72C/15 s using a high-temperature, short-time (HTST) unit (De Laval Brand, Alfa-Laval Agri Inc., Everett, MA) and homogenized at 17,240 KPa (2,500 psig) with a single-stage homogenizer (APV Crepaco Inc., Rosemont, IL). The filled milk samples were packaged in plastic containers (1.89 L) using a plastic bottling machine (Federal Mfg. Co., Milwaukee, WI) and stored at 2C.

All four milk formulations were processed on the same day, and triplicate batches were made for each formulation by processing at weekly intervals. In addition, the formulation that was considered best out of the four was prepared in a single batch and compared with a coconut oil-based formulation.

Physicochemical and Microbiological Analysis

During storage, samples were analyzed for lipid oxidation and fat separation by the thiobarbituric acid (TBA) method (King 1962) and the Farrall Homogenization Index (Goss 1953; Farrall *et al.* 1941), respectively. The microbiological quality of the samples was monitored using the standard plate count (SPC) method and coliform test (Richardson 1985).

Sensory Evaluation of Filled Milk Samples

The sensory panel consisted of an average of six trained members, most of whom were members of the Kansas State University Dairy Products Judging Team. To be familiar with off-flavors and odors from vegetable oils, the panelists were trained with filled milk samples following the milk judging procedure (Bodyfelt *et al.* 1988). All samples were randomly coded and served in a random order. The samples were evaluated once a week using the formulation containing coconut oil and Emulsifier M as a reference.

All samples were tempered at 15C before evaluation. Samples were evaluated for off-flavors/odors (1 = none; 5 = extreme), mouthfeel (1 = very poor; 5 = excellent), overall milk flavor (1 = very poor; 9 = excellent), and overall quality (1 = very poor; 9 = excellent). The sensory characteristics and scales used were adapted from the American Dairy Science Association scorecard used for evaluating milk samples (Bodyfelt *et al.* 1988). The panelists were instructed to follow the dairy product judging procedures as described by Bodyfelt *et al.* (1988). They were encouraged to make additional comments for other sensory characteristics.

Consumer Test

The test population consisted of 100 individuals, 60% females and 40% males. The participants were made up mostly of students at Kansas State University who drank milk at least twice a week, and ranged between 18 and 44 years of age. The filled milk samples were randomly coded, and served in a random order. The 8 samples were served at about 2C in a small plastic cup. Each participant was asked to place a mark along a hedonic scale, labelled "like extremely" on one end and "dislike extremely" on the other end, indicating their degree of liking.

Statistical Analysis

The sensory data were computed for least-squares means using SAS General Linear Models (GLM) Procedures (SAS 1985).

RESULTS AND DISCUSSION

Results of the physicochemical analysis suggested that all batches of products tested were stable during storage at 2C. The Farrall Index of Homogenization ranged from 4.2 to 13.6. Considering that a Farrall Index of 12 is considered to be a satisfactory limit (Doan and Mykleby 1943), the

homogenization in this study appeared to be sufficient to disperse the vegetable oils into the milk proteins and to network them with the emulsifiers and stabilizers used. No visual fat separations were observed during storage. The TBA analysis indicated that lipid oxidation was negligible in all four formulations during storage. All formulations containing the partially hydrogenated oils showed an absorbance of less than 0.035 during three weeks of storage, and none of the formulations showed significantly higher TBA values ($P < 0.05$) than the formulation containing coconut oil. Furthermore, no members of the sensory panel reported oxidized off-flavor. Microbial counts were low during three weeks of storage. Both SPC and coliform counts were well below the legal limit for Grade "A" pasteurized milk and milk products (PMO 1989).

TABLE 1.
OFF-FLAVORS/ODORS OF FOUR FILLED MILK FORMULATIONS CONTAINING PARTIALLY HYDROGENATED VEGETABLE OILS AND STABILIZERS (LEAST-SQUARES MEAN ± STANDARD DEVIATION)[1,2]

	Formulation			
Time (week)	Cottonseed & Stabilizer C[3]	Sunflower & Stabilizer A[4]	Canola & Emulsifier M[5]	Canola & Stabilizer B[6]
1	2.45 ± 0.98	1.85 ± 0.59	2.44 ± 0.50	2.05 ± 0.70
2	2.44 ± 0.82	2.54 ± 0.67	2.14 ± 0.45	2.04 ± 0.83
3	2.22 ± 0.92	2.34 ± 0.66	2.66 ± 0.66	1.90 ± 0.63
Mean	2.37 ± 0.89	2.24 ± 0.65	2.41 ± 0.56	2.00 ± 0.72

[1]Mean of triplicates from 3 batches; Sensory scale (1 = none; 5 = extreme)

[2]Absence of superscripts within a row indicates no significant difference ($P > 0.05$).

[3]Composed of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate

[4]Composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate

[5]A mixture of mono- and diglycerides

[6]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

As shown in Table 1, all formulations had "slight" off-flavors as indicated by the mean scores when evaluated at 15C. However, none of the panelists indicated any major off-flavor or odor problems. Similar results were observed previously with these formulations during preliminary studies. According to the comments made by panelists, however, some differences were noticed in the off-flavor/odor characteristics among the four formulations. The formulation with Cottonseed/Stabilizer C was characterized as being "aromatic", "flowery", or "perfumery" in nature. The formulation containing Sunflower HB 95/Stabilizer A possessed few off-flavors/odors during the first week of storage. After two weeks, however, an "oily" off-flavor/odor became more pronounced. Meanwhile, formulations containing Canola/Emulsifier M or Stabilizer B did not have any identifiable off-flavors. During three weeks of storage, the formulation with Canola/Stabilizer B possessed the lowest mean scores for off-flavors/odors among the four formulations, although differences were not statistically significant.

TABLE 2.
OVERALL MILK FLAVOR OF FOUR MILK FORMULATIONS CONTAINING PARTIALLY HYDROGENATED VEGETABLE OILS AND STABILIZERS (LEAST-SQUARES MEANS ± STANDARD DEVIATION)[1,2]

	Formulation			
Time (week)	Cottonseed & Stabilizer C[3]	Sunflower & Stabilizer A[4]	Canola & Emulsifier M[5]	Canola & Stabilizer B[6]
1	$5.69^{cde} \pm 1.38$	$7.35^{a} \pm 1.70$	$5.90^{cde} \pm 1.72$	$6.52^{abc} \pm 1.54$
2	$5.64^{cde} \pm 1.88$	$5.64^{cde} \pm 1.17$	$6.94^{ab} \pm 1.22$	$7.04^{ab} \pm 1.24$
3	$6.00^{cde} \pm 1.47$	$6.10^{cde} \pm 1.06$	$5.15^{de} \pm 1.19$	$6.46^{abcd} \pm 1.26$
Mean	5.78 ± 1.57	6.36 ± 1.50	6.00 ± 1.52	6.67 ± 1.34

[1]Mean of triplicates from 3 batches; Sensory scale (1 = very poor; 9 = excellent)

[2]Mean scores within a row with different superscripts differ at $P < 0.05$.

[3]Composed of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate

[4]Composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate

[5]A mixture of mono- and diglycerides

[6]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

Table 2 shows sensory results for overall milk flavor of the four formulations during three weeks of storage. They indicated that the formulation containing Sunflower HB 95/Stabilizer A possessed a significantly better milk flavor than formulations with Cottonseed/Stabilizer C or Canola/Emulsifier M after the first week of storage and also showed a higher mean than Canola/Stabilizer B. After two weeks of storage, however, the Sunflower HB95/Stabilizer A formulation possessed less milk flavor intensity, partly from the loss of some of the full, creamy milk flavor characteristics and partly from the development of a persistent oily flavor. The formulation containing Canola/Stabilizer B, on the other hand, received consistently high milk flavor scores during three weeks of storage. The overall mean values suggest that the formulation with Canola/Stabilizer B had the most acceptable milk flavor among the four formulations, although the difference was not statistically significant.

All four formulations had acceptable mouthfeel (Table 3). There were no statistically significant differences among the formulations. The highest mean

TABLE 3.
MOUTHFEEL SCORES OF FOUR FILLED MILK FORMULATIONS CONTAINING PARTIALLY HYDROGENATED VEGETABLE OILS AND STABILIZERS (LEAST-SQUARES MEAN ± STANDARD DEVIATION)[1,2]

	Formulation			
Time (week)	Cottonseed & Stabilizer C[3]	Sunflower & Stabilizer A[4]	Canola & Emulsifier M[5]	Canola & Stabilizer B[6]
1	3.66 ± 0.74	4.19 ± 0.79	3.96 ± 0.77	4.01 ± 0.84
2	3.75 ± 1.20	3.75 ± 0.83	4.05 ± 0.67	4.35 ± 0.81
3	3.62 ± 0.87	3.94 ± 0.80	3.97 ± 0.70	4.01 ± 0.95
Mean	3.68 ± 0.95	3.96 ± 0.79	3.99 ± 0.70	4.12 ± 0.86

[1]Mean of triplicates from 3 batches; Sensory scale (1 = very poor; 5 = excellent)

[2]Absence of superscripts within a row indicates no significant difference ($P > 0.05$).

[3]Composed of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate

[4]Composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate

[5]A mixture of mono- and diglycerides

[6]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

scores were observed for the formulation containing Sunflower HB 95/Stabilizer A after the first week of storage; however, it did not retain these high scores. The Canola/Stabilizer B formulation showed consistently higher mean values during three weeks of storage. According to the panel comments, all four formulations had a slight "chalky" mouthfeel, but it was not regarded as a major defect.

As shown in Table 4, the overall quality of the four formulations followed the same pattern observed in the other three sensory characteristics for which these filled milk samples were evaluated. The mean values after one week of storage were highest for the formulation containing Sunflower HB 95/Stabilizer A but declined after two weeks of storage. The mean values suggest that the overall quality of the formulation with Sunflower HB 95/Stabilizer A improved minimally, but not significantly at three weeks of storage. The formulations containing Canola/Emulsifier M and Canola/Stabilizer B showed an improvement from one week to two weeks of storage. However, Canola/Stabilizer B

TABLE 4.
OVERALL QUALITY OF FOUR FILLED MILK FORMULATIONS CONTAINING PARTIALLY HYDROGENATED VEGETABLE OILS AND STABILIZERS (LEAST-SQUARES MEAN ± STANDARD DEVIATION)[1,2]

Time (week)	Formulation			
	Cottonseed & Stabilizer C[3]	Sunflower & Stabilizer A[4]	Canola & Emulsifier M[5]	Canola & Stabilizer B[6]
1	5.98 ± 1.83	7.24 ± 1.79	5.91 ± 1.79	6.59 ± 1.35
2	5.42 ± 2.38	5.22 ± 1.09	6.82 ± 1.03	6.92 ± 1.30
3	5.31 ± 2.05	5.61 ± 1.18	4.69 ± 0.92	6.23 ± 1.18
Mean	5.57 ± 2.08	6.02 ± 1.55	5.61 ± 1.56	6.57 ± 1.27

[1]Mean of triplicates from 3 batches; Sensory scale (1 = unacceptable; 9 = extremely acceptable)

[2]Absence of superscripts within a row indicates no significant difference ($P > 0.05$).

[3]Composed of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate

[4]Composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate

[5]A mixture of mono- and diglycerides

[6]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

retained its level of overall quality after three weeks of storage, whereas the formulation with Canola/Emulsifier M dropped considerably in its quality (Table 4). It may be worthwhile to note that the formulation containing cottonseed oil/Stabilizer C showed a considerable variation in overall quality as indicated by relatively large coefficients of variation (Table 4). This was largely due to the mild "aromatic, flowery or perfumery" off-flavor that was associated with the cottonseed oil. Some panelists liked the mild flavor note in the milk formulation, whereas others disliked it.

Results from the consumer acceptability test showed that the formulation containing coconut oil received the highest mean scores, although statistically it was not significantly different from the formulation containing Canola/Emulsifier M (Table 5). Results also showed that there was not a significant difference

TABLE 5.
MEAN SCORES FOR THE RESULTS OF FIVE MILK FORMULATIONS FROM CONSUMER ACCEPTABILITY TEST

Formulation	Mean scores[1,2]
Coconut & Emulsifier M[3]	5.34[a]
Canola & Emulsifier M[3]	4.93[ab]
Canola & Stabilizer B[4]	4.65[bc]
Sunflower & Stabilizer A[5]	4.19[cd]
Cottonseed & Stabilizer C[6]	4.05[d]

[1]Represent mean response of 100 individuals.

[2]Different superscripts differ at $P < 0.05$.

[3]A mixture of mono- and diglycerides

[4]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

[5]Composed of mono- and diglycerides, sodium caseinate, soy protein, carrageenan, and sodium citrate

[6]Composed of mono- and diglycerides, sodium caseinate, carrageenan, and sodium citrate

between the two formulations containing canola oil; however, the formulation containing Canola/Stabilizer B was significantly different from the coconut oil filled milk. According to mean scores, the formulation liked most by the participants was the coconut oil filled milk, followed by the formulation with Canola/Emulsifier M, Canola/Stabilizer B, Sunflower HB 95/Stabilizer A, and Cottonseed/Stabilizer C. It may be worthwhile to note that although the coconut oil filled milk scored the highest, it is still only slightly acceptable, based on the hedonic scale provided. The result of this test could have been affected by the preconceived notion about the filled milk that the individuals had, as they entered the testing area. This notion is supported by a consumer attitude study conducted by researchers (Hetrick 1969).

TABLE 6.
MEAN SENSORY SCORES (± STANDARD DEVIATION) FOR FILLED MILK SAMPLES CONTAINING PARTIALLY HYDROGENATED CANOLA AND COCONUT OILS[1,2]

	Sensory characteristics[3]			
Formulation	Off-flavors & odors	Overall milk flavor	Mouthfeel	Overall quality
Canola & Stabilizer B[4]	2.45 ± 0.71	6.90 ± 1.32	3.50 ± 0.80	6.90 ± 1.26
Coconut & Emulsifier M[5]	2.40 ± 0.72	6.60 ± 1.49	3.50 ± 0.84	6.20 ± 1.66

[1]Mean of 2 wk storage data

[2]Absence of superscripts within a column indicates no significant difference ($P > 0.05$)

[3]Scales: off-flavor (1 = none; 5 = extreme); milk flavor (1 = very poor; 9 = excellent); mouth-feel (1 = very poor; 5 = excellent); and overall quality (1 = very poor; 9 = excellent)

[4]Composed of mono- and diglycerides, soy protein, whey protein, carrageenan, sodium citrate, and disodium phosphate

[5]A mixture of mono- and diglycerides

Further examination of the sensory data collected over the three weeks of storage suggests that formulations with Canola/Stabilizer B and Sunflower HB 95/Stabilizer A were better than the other two formulations. After the first week of storage, the two formulations were characterized as having acceptable

mouthfeel and little off-flavors and/or odors. Also they were comparable to one another in overall milk flavor. However, the latter formulation did not retain its milk flavor during the rest of storage. The formulation with Canola/Stabilizer B showed much more consistency in off-flavor, overall milk flavor and quality. Therefore, an attempt was made to compare this formulation with a coconut oil-based formulation (control and liked most by the consumer panel) as shown in Table 6. During two weeks of storage, the formulations showed comparable results in off-flavors, milk flavor intensity, mouthfeel, and overall quality. When the two formulations were compared for flavor characteristics, the formulation with canola oil was characterized as creamy, whereas the coconut oil formulation was described as being slightly "flat" or "bland" in its flavor.

CONCLUSION

It appears that coconut oil in filled milk formulations can be replaced effectively with partially hydrogenated canola oil, or perhaps with sunflower oil or a combination of the oils, provided that appropriate stabilizers and emulsifiers are used. This study showed that any of the four formulations, which had been selected through the preliminary work, could be used effectively for replacing the coconut oil-based formulations, since there was no statistical significance. However, the formulation with cottonseed oil/Stabilizer C is least recommended because of a large variation in flavor perception due to a mild off-flavor associated with the vegetable oil.

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EFFECT OF POLYPHENOL OXIDASE AND ITS INHIBITORS ON ANTHOCYANIN CHANGES IN PLUM JUICE

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ABSTRACT

Total phenolics and chlorogenic acid in different plum cultivars ranged from 262 to 922 μ/g and 33 to 103, μg/g, respectively. Stanley plums had the highest polyphenol oxidase (PPO) activity and chlorogenic acid to total phenolics ratio as compared to all other cultivars. Degradation of anthocyanins in the juice made from Stanley plums was directly related to the level of PPO in the juice. Among PPO inhibitors, L-cysteine and sodium metabisulfite (at 0.5 and 1.0 mM) were effective in minimizing degradation of anthocyanins. Anthocyanins in the juice samples were heat stable and pasteurized juice showed acceptable microbial quality.

INTRODUCTION

Anthocyanins, a family of glycosidic pigments, responsible for most of the orange, red and blue color of flowers and fruits are found widely distributed in nature, mainly in flowers, fruits and vegetables (Mazza and Brouillard 1987). Naturally occurring enzymatic systems capable of decolorizing anthocyanins are present in molds, leaves, vegetables and fruits (Pifferi and Cultera 1974). Polyphenol oxidase (PPO), in addition to its role in the oxidation of colorless phenolic substrates (enzymatic browning), also catalyzes the degradation of anthocyanins to decolorize them (Schwimmer 1981). PPO catalyzed oxidation can influence food quality as demonstrated by Cash *et al.* (1976) on involvement of PPO in color loss of Concord grape juice.

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Proctor and Creasy (1969) reported that crude vegetable extracts require the presence of phenols for anthocyanin degradation. Oszmianski and Lee (1991) also reported that anthocyanins themselves are not substrates of PPO, but they are easily oxidized in the presence of chlorogenic acid. Sulfiting agents have been widely used in the food industry to prevent PPO action. However, the use of sulfiting agents as food additives is being reevaluated by the Food and Drug Administration (FDA), and, in some products, banned for use (Chen *et al.* 1991). It is, therefore, necessary to search for alternatives that exhibit effective inhibitory effect on PPO but do not pose any health concern to consumers.

Purple plums are an integral part of Michigan agriculture and are well positioned as a fresh market and/or processing crop that can be harvested between the cherry and apple crops. Currently, approximately one-half of the plums are used as table stock and the rest are processed and canned. New uses for plums, such as juice and paste production, which could further enlarge their market capabilities, are limited because of degradation of anthocyanin pigments, which give characteristic purple color to the plum juice. The objective of this study was to investigate PPO induced anthocyanin degradation in the plum juice and explore effective methods to minimize this loss.

MATERIALS AND METHODS

Ten different plum cultivars were harvested at maturity in September 1991 from orchards in Alma, Michigan, and immediately frozen. The plum samples were stored at −20C until further processing was required.

Plum Juice Extraction and Pasteurization

A 45-kg portion of Stanley plums was removed from −20C storage and allowed to thaw overnight at 5C. Debris (i.e., stems, leaves, shriveled fruit) were removed. The plums were heated to 65C and macerated in double jacketed stainless steel kettles. The macerated plums were cooled to 49C and a commercial grade pectinase was added (1 g pectinase/4.5 kg crushed fruit). After holding 6 h at room temperature the crushed fruits were pressed to obtain juice using a rack and cloth press. The yield of plum juice was about 60%. The soluble solids content (14°B) and pH (3.9) of the juice were determined using an Abbe-3L refractomater and a Corning 610A pH meter, respectively. Pasteurization of the juice was carried out at 88C for 1 min using a Spirotherm (Cherry-Burrel, Inc.). All the juice samples were held at −20C until further analysis.

Extraction and Assay of Plum PPO

Extraction and assay of PPO enzyme were carried out according to the method of Siddiq *et al.* (1992).

Total Phenolics and Chlorogenic Acid

Total phenolics and chlorogenic acid in plums were extracted by the method of Coseteng and Lee (1987). A 50 g sample of plums was homogenized at high speed in a Waring Blendor with 100 ml 80% ethanol for 2 min. The homogenate was boiled for 5 min under the hood to extract the phenolics. The extract was first filtered through nylon cloth and then through Whatman #4 filter paper under vacuum. The residue was mixed with an additional 100 ml 80% ethanol and boiled for another 10 min to reextract the phenolics. The extracts were combined and made to a final volume of 250 ml. This extract was used for the determination of total phenolics and chlorogenic acid according to the methods of Weurman and Swain (1955) and Mapson *et al.* (1963), respectively.

Total Anthocyanins in Plum Juice

The method of Cash *et al.* (1976) were used to determine changes in anthocyanins at room temperature (30C). Sample consisted of one part plum juice to two parts 0.025M citrate buffer, pH 4.5. Total anthocyanins were extracted by mixing 1 ml of diluted sample with 9 ml of 95% EtOH-1.5N HCl in an 85:15 ratio (Skalski and Sistrunk 1973). These samples were kept at 30C for 1 h to extract anthocyanins before reading the absorbance at 535 nm. Changes in anthocyanin pigments were followed hourly for the first 8 h and then at 16 and 24 h. All determinations were done in duplicate.

Effect of Heat Treatment and PPO on Plum Juice Anthocyanins

The diluted plum juice was heated to 65C (pasteurization temperature) and maintained at this temperature in a water bath. Anthocyanin degradation as a function of heating time was monitored at 10 min intervals for 70 min.

Crude PPO enzyme was added to the diluted juice samples at 1.0, 5.0 or 10.0% level and changes in anthocyanin pigments followed spectrophotometrically for 24 h as described above.

Effect of Pasteurization and PPO Inhibitors on Plum Juice Anthocyanins

Changes in total anthocyanins were monitored for 24 h at room temperature in pasteurized plum juice and in a model system consisting of 5% added PPO

enzyme to the plum juice in the presence of PPO inhibitors, ascorbic acid, L-cysteine and sodium metabisulfite at 0.5 and 1.0 mM each.

Microbial Analysis

Raw and pasteurized plum juice samples were held at 5C and analyzed in triplicate, for total plate (SPC), yeast and mold counts, at 0, 5 and 10 days and coliform count at 0 days using FDA (1990) procedures.

RESULTS AND DISCUSSION

Total Phenolics, Chlorogenic Acid and PPO Activity

Plum cultivars analyzed showed difference in total phenolics, chlorogenic acid and PPO activity (Table 1). Stanley plums followed by Pobeda and Abundance had the highest PPO activity. All other plum cultivars exhibited less than 20% PPO activity relative to Stanley. Total phenolics in different cultivars ranged from 262 to 922 C μg/g fruit tissue. 'Beauty' had the highest level of total phenolics and 'Stanley' the lowest. The concentration of chlorogenic acid ranged from 33 to 103 μg/g, with highest concentration found in 'Beauty' and the lowest in 'Wade'. Coseteng and Lee (1987) also reported that total phenolics and chlorogenic acid concentration in apples differed with respect to cultivar.

Stanley plums, which are of commercial importance in Michigan, had a higher chlorogenic acid to total phenolic ratio than any other cultivar. Involvement of chlorogenic acid in anthocyanin degradation has been demonstrated by Oszmianski and Lee (1991). Pifferi and Cultera (1974) reported that among the sweet cherry phenols, chlorogenic acid and pyrocatechol were active in anthocyanin degradation. Some researchers have shown that the degree of browning is to a large extent related to PPO activity only (Vamos-Vigyazo and Gajzago 1976). Others (Walker 1962; Ingle and Hyde 1968) have reported that discoloration is related to substrate content, like chlorogenic acid. However, Harel *et al.* (1966) demonstrated that both PPO activity and substrate concentration determine the degree of browning in apples.

Effect of Heat Treatment and PPO on Plum Juice Anthocyanins

Anthocyanin pigments of plum were relatively heat stable, and only 11 and 16% loss in anthocyanin was observed (Fig. 1) when heated at 65C for 30 and 70 min, respectively. Plum PPO was shown to be completely inactivated at 65C in 20 min (Siddiq *et al.* 1993). This indicates that plum juice can be pasteurized at this temperature with minimal loss of anthocyanins, although this long heating

TABLE 1.
TOTAL PHENOLICS AND CHLOROGENIC ACID CONTENT[1]
AND PPO ACTIVITY IN DIFFERENT PLUM CULTIVARS

Cultivar	Total Phenolics (µg/g)	Chlorogenic Acid (µg/g)	% PPO Activity[2]
Stanley	262	75	100.00
Pobeda	353	63	53.69
Abundance	437	38	30.24
Au Roadside	340	35	19.24
Wade	300	33	14.33
La Crescent	591	88	13.70
Beauty	922	103	12.80
Underwood	300	43	11.24
Pipestone	737	77	10.65
Shiro	296	46	6.22

[1] *Means of triplicate values*
[2] *Relative to Stanley plums*

time would not be especially compatible with most commercial juice processing procedures. Mok and Hettiarachchy (1991) reported no degradation of sunflower-hull anthocyanin when heated at 65C.

The loss of anthocyanin pigments in plum juice, over a 24-h period, was related to added PPO concentration. However, a significant difference in anthocyanin degradation ($p < 0.05$) was observed between 1.0% and 5.0 or 10.0% added PPO (Fig. 2). The loss of anthocyanins between 5.0 and 10.0% added PPO was not significant. Cash *et al.* (1976) reported that PPO was one of the most important factors involved in the loss of color of Concord grape juice.

Effect of Pasteurization and PPO Inhibitors on Plum Juice Anthocyanins

A significantly ($p < 0.05$) lower degradation of anthocyanin pigment was observed in the treated plum juice (pasteurized or treated with PPO inhibitors, L-cysteine and sodium metabisulfite) as compared to the raw juice (Fig. 3). Increasing the concentration of inhibitors from 0.5 to 1.0 mM did not show significant effect on minimizing anthocyanin degradation (data not shown). No significant difference in anthocyanin degradation was observed between

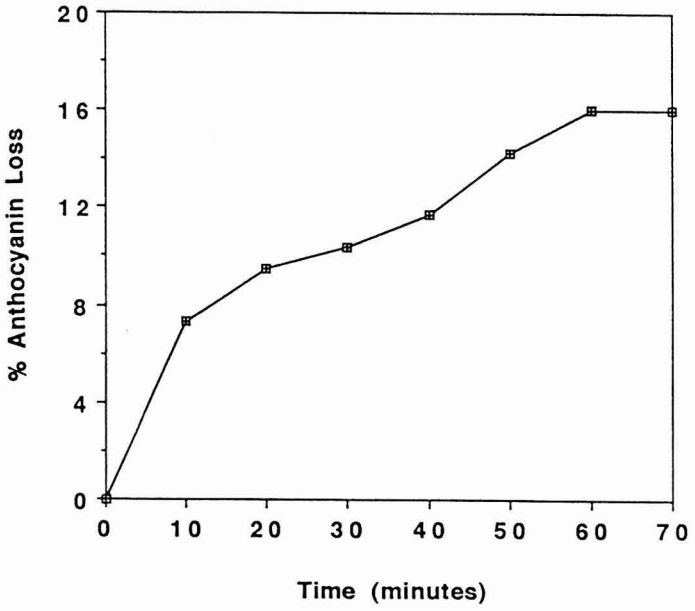

FIG. 1. THERMAL STABILITY OF PLUM JUICE ANTHOCYANINS AT 65C

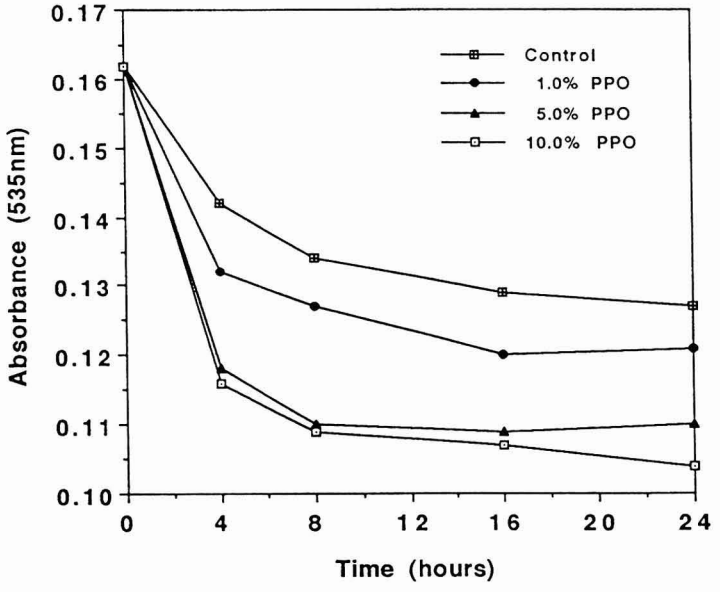

FIG. 2. EFFECT OF ADDED PPO ON ANTHOCYANIN LOSS IN PLUM JUICE AT 30C

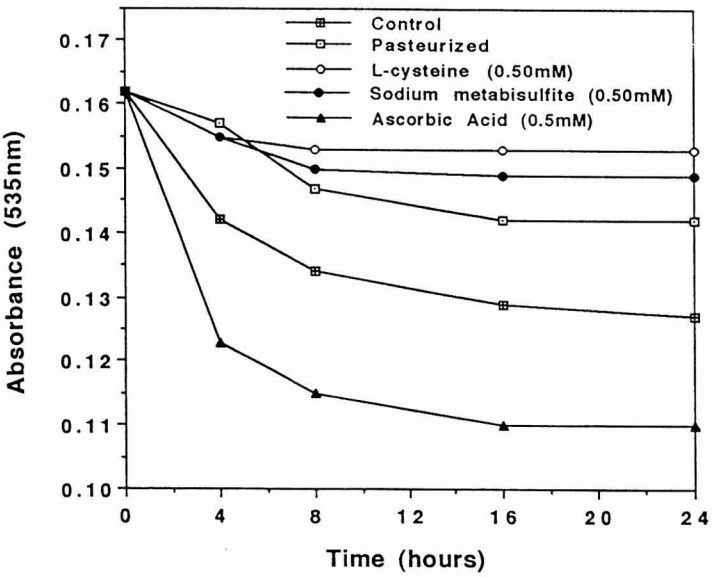

FIG. 3. EFFECT OF PASTEURIZATION AND PRO INHIBITORS ON ANTHOCYANIN DEGRADATION IN PLUM JUICE

pasteurized juice and that treated with L-cysteine (0.5 mM) or sodium metabisulfite(0.5 and 1.0 mM). L-cysteine alone or pasteurization in combination with lower concentrations of L-cysteine may be effectively used as an alternative to sodium metabisulfite for preserving color of plum juice.

L-Cysteine would have the advantage as a replacement for sulfites, since it is a naturally occurring amino acid and has GRAS status for use as a dough conditioner (Dudley and Hotchkiss 1989). These researchers reported that cysteine has two effects; first, it increases lag time for PPO activity and second, by combining with quinones it inhibits melanin formation. Murr and Morris (1974) suggested that cysteine, in addition to binding to quinones, also enhances protease activity, which, they believed, degrades phenolase, thus contributing to the prevention of discoloration.

Anthocyanin degradation in plum juice with added PPO and in the presence of ascorbic acid was most pronounced (Fig. 3). Ascorbic acid, also a native component of fruits, has been reported to have negative effect on anthocyanin stability. According to Davidek *et al.* (1990), the reaction mechanism is not understood but both ascorbic acid and anthocyanins are destroyed in the process. They postulated that hydrogen peroxide produced from ascorbic acid degradation most likely participates in the degradation of anthocyanins.

Microbial Analysis of Plum Juice

Table 2 shows the standard plate, yeast and mold and the coliform counts for raw and pasteurized juice samples held at 5C. In comparison to the pasteurized juice, raw juice samples had relatively higher microbial counts, which increased with holding. These results demonstrate that there is a need to heat the samples to achieve acceptable shelf-life and safe product because refrigeration alone is not adequate.

TABLE 2.
MICROBIAL ANALYSIS[1] OF PLUM JUICE HELD AT 5C

Analysis	Time (Days)	Raw	Pasteurized
1. SPC[2]	0	97	0.15
	5	143	0.21
	10	162	0.25
2. Yeast & Mold[3]	0	65	0.24
	5	148	0.40
	10	165	0.52
3. Coliform[4]	0	<1	<1

[1] *Means of triplicate values*
[2] *Standard Plate Count (CFU/ml x 10^4)*
[3] *Yeast and Mold Count (CFU/ml x 10^2)*
[4] *Coliform Count (CFU/ml x 10^2)*

CONCLUSIONS

In conclusion this study indicated that cultivar differences had a significant effect on total phenolics and chlorogenic acid concentration and PPO activity in plums. Stanley plums had higher chlorogenic acid to total phenolic ratio than any other cultivar. Pasteurization treatment could be effectively employed for plum juice preservation, as anthocyanin were observed to be relatively heat stable. Sodium metabisulfite and L-cysteine proved to be effective in controlling anthocyanin loss in plum juice. Presence of ascorbic acid in the system accelerated anthocyanin degradation. The HTST pasteurized sample showed acceptable microbial levels.

ACKNOWLEDGMENTS

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BOOK REVIEW

ANTIMICROBIALS IN FOODS. Edited by P. Michael Davidson and Alfred Larry Branen, Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016, 1993. $195.00.

Occasionally a book comes along that fills a niche previously unfilled, and that book becomes a classic, definitive reference in the field. The newly revised *Antimicrobials in Foods* is such a book. The first edition was published in 1983 and immediately became the standard reference in the field. If a researcher was new to the food antimicrobial field, a logical and necessary first step was to spend some time with Branen and Davidson's first edition.

Food microbiology has changed considerably since the publication of the first edition. Psychrotrophic pathogens like *Listeria monocytogenes* and *Yersina enterocolitica* were only just being recognized as major causes of foodborne disease. Refrigerated foods so common today, especially in Europe, were largely nonexistent. The Hazard Analysis and Critical Control Point (HACCP) technique for assuring food safety was only used in a few select segments of the food industry. Each of these factors have acted to increase the importance of food antimicrobials in assuring food safety.

When the first edition was published, the safety of some antimicrobials was being questioned. The editors pointed out in the 1983 edition preface that more natural and alternative antimicrobials should be found and tested. This did come to pass, and as they point out in the preface to the new edition, research on nearly all antimicrobials (especially the natural ones) has increased in the last 3 to 5 years. The progress in the area of Nisin and other bacteriocins alone has been staggering. It is difficult and maybe impossible for food microbiologists and preservation experts to keep up with the primary literature on each and every food antimicrobial. For these individuals *Antimicrobials in Foods* is an essential reference.

While the basic organization and structure of the first edition are still visible within the second, a number of changes are clearly apparent. The number of chapters has jumped from 13 to 17, the number of authors from 14 to 20, and the total number of pages from 465 to 647! Some of the chapter titles are unchanged (Introduction to Use of Antimicrobials; Sodium Benzoate and Benzoic Acid; Sanitizers; etc.) while others reflect the growth in the literature. The first edition chapter on "Nisin and Other Bacteriocins" has metamorphosed into two chapters, one covering Nisin and the other reviewing all bacteriocins. Other chapters (Natamycin, Indirect Antimicrobials and Methods for Evaluation) are completely new. In every case the chapter authors have been selected with care. The authors are all recognized experts (in some cases without peer) in their respective fields. Even where a chapter title and

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author(s) are unchanged from the first edition, it is clear that substantial revisions in the content have been made. A glance at the many references associated with each chapter reveals a conscientious effort by the authors to update the primary literature that supports the chapter material.

Finally, this reviewer was pleased to see that the ugly typeface used in so many Marcel Dekker food science references from the 1980's has been discarded in favor of one which is heavier and much more pleasing to the eye.

In summary, if you must understand food antimicrobial spectrum of activity, mechanisms of action or laws and regulations, this book is essential. Any food microbiologist or preservation expert should have ready access to a copy of this book.

DONALD W. SCHAFFNER

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

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

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

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MICHAELS, S.L. 1989. Crossflow microfilters ins and outs. Chem. Eng. *96*, 84–91.

LABUZA, T.P. 1982. *Shelf-Life Dating of Foods,* pp. 1–9, Food & Nutrition Press, Trumbull, CT.

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

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