Inhibition of Spoilage Microorganisms in Refrigerated Raw Milk by Maillard Reaction Products

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Story in Brief

Different amounts (0, 0.21, 0.42 and 0.63 percent) of a "crude" mixture of Maillard reaction products (MRP) were added to refrigerated raw milk to evaluate their effect on growth of psychrotrophic microorganisms naturally present in raw milk. The samples containing 0.42 and 0.63 percent MRP had significantly lower numbers of microorganisms following four days of storage at 5C than did the samples containing 0 and 0.21 percent MPR ($P < .05$). The 0.63 percent MRP was significantly more inhibitory than the 0.42 percent MRP ($P < .05$).

Introduction

Despite considerable discussion in recent years about the possibility of hazards from the use of food additives, the world-wide crisis in the food supply demands that losses be reduced to a minimum. Consequently, the use of chemical preservatives alone, or as a supplement of other means of preservation is essential. Since the MRP are known to possess antioxidative properties and some evidence indicates they may play a role as inhibitors of microbial growth, this opens the feasibility of using these products as additives for improving the self-life of foods. The fact that the Maillard reaction is common in many processed foods, and people have been exposed for many years to the consumption of its products, encouraged us to assay these substances for antimicrobial activity in refrigerated raw milk.

Psychrotrophic microorganisms are present in almost all raw milk supplies. During growth in the milk, some psychrotrophs produce heat stable enzymes that can cause degradation of certain milk products after pasteurization. These heat resistant enzymes are of concern in the production of sterile milk products. They are much more resistant to heat than bacterial spores and can survive the heat treatment used to manufacture sterile milk. Thus, research looking for means of preventing or reducing the growth of psychrotrophs in raw milk takes on added significance.

The primary objective of this study was to find out if the addition of MRP to raw milk can prevent/or retard the growth of psychrotrophs during refrigerated storage.

Material and Methods

A crude mixture of Maillard reaction products, obtained by autoclaving (two hr at 121 C) an aqueous sugar-amino acid solution containing 0.2 M D-

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glucose (Fisher Scientific Co.) and 0.2 M D-L-Histidine, was used without any further fractionation for evaluation of its antimicrobial activity in raw milk. Based on the molar concentration, this solution contained seven percent solids.

Raw milk obtained from the Oklahoma State University Dairy Cattle Center was aseptically placed into a sterile 2000 ml flask in an ice-water bath for transport to the laboratory. Two sets of four sub-samples each were obtained from the original batch of milk. For each set, the sub-samples were prepared by aseptically delivering 90 ml portions of milk into each of four sterile 250 ml Erlenmeyer flasks containing respectively 0, 3, 6 and 9 ml of sterile MRP and sufficient sterile distilled water to make 10 ml volumes. Based on the molarity of ingredients in the MRP solution, the concentrations of MRP in the milk were 0, 0.21, 0.42 and 0.63 percent. After mixing, one set of samples was stored statically at 5 C and the other one was stored at 5 C in a water bath shaker adjusted to 120 RPM.

Samples for enumeration of microorganisms were taken prior to storage (day 0) and on the fourth day of storage (with or without agitation). The enumeration was done by plating the appropriate dilutions with Plate Count Agar (PCA; Difco Lab.) using the pour plate method (Speck, 1976) and incubation of 32 C for 48 hr. The results were statistically analyzed by means of an analysis of variance for a 2 x 4 factorial arrangement of treatment factors in a complete randomize block design. Further analysis for significantly different means was carried out using an accepted statistical procedure.

Results and Discussion

On day 0, no significant ($P>0.05$) difference was observed among the means for the samples containing MRP when compared to the mean count for the control samples (Table 1). This indicated that such products did not have an immediate deleterious effect on the microorganisms in the raw milk.

Table 1. Influence of the addition of MRP on growth of psychrotrophs in raw milk during storage at 5 C.

		Mean of Log_{10} Counts/ml[A]			
		Added MRP (%)			
Storage Condition	Day	0 (Control)	0.21	0.42	0.63
Agitated[C]	0	3.37 (a)[B]	3.38 (a)	3.34 (a)	3.34 (a)
	4	5.86 (a)	5.57 (a)	5.37 (b)	5.11 (c)
Static[C]	0	3.43 (a)	3.45 (a)	3.45 (a)	3.44 (a)
	4	6.34 (a)	6.10 (a)	5.63 (b)	5.04 (c)

[A]Each value represents the mean log_{10} count/ml from 8 trials.
[B]Numbers in same row followed by different letters are significantly different ($P<0.05$).
[C]Counts for the static samples were all significantly higher than for the agitated samples ($P<0.05$).

After four days of storage, the numbers of organisms in the milk were significantly ($P<0.05$) less numerous in agitated than in static samples, probably due to a decrease in the action of the inhibitory agent as a result of its interaction with the oxygen incorporated by continuous agitation. Based on the counts obtained on the fourth day samples, microbial growth decreased as the MRP concentration increased. The means of the $\log_{10}$ counts/ml from agitated milk were: 5.86 for the control, and 5.57, 5.37 and 5.11 for the samples containing respectively 0.21, 0.42 and 0.63 percent added MRP. For the corresponding static samples, the means of the $\log_{10}$ counts/ml were respectively 6.34, 6.10, 5.63 and 5.04. The fact that 0.63 percent MRP was significantly ($P<0.05$) more inhibitory than 0.42 percent MRP while 0.21 percent MRP was statistically similar ($P>0.05$) to the control indicated that there is a limiting concentration level below which the inhibitor(s) loses its effectiveness.

Failure of microorganisms to grow in culture media submitted to heat has been attributed to distruction and/or unavailability of amino acids or other nutrients due to the Maillard reaction rather than to the formation of inhibitory substances in this reaction (Rose and Peterson, 1949; Horn et al., 1968; Hagan et al., 1970). In the present work, neither destruction nor unavailability of nutrients appeared to be the cause of growth inhibition since the MRP used were performed materials incorporated into milk which should be nutritionally adequate to support microbial growth.

Although present studies suggest the feasibility of the use of MRP as preservatives to reduce the growth of psychrotrophs in refrigerated raw milk, further investigation is needed. Purification of the inhibitory agent(s) might provide a way to increase its effectiveness. Association, if any, of the inhibitor with brown-colored components needs to be investigated since browth discoloration would be a problem in a product such as milk.

Literature Cited

Hagan, S.N. et al. 1970. J. Agr. Food Chem. 18:273.

Horn, M.J. et al. 1968. J. Agr. Food Chem. 16:741.

Rose, D. and R. Peterson. 1949. Can. J. Res. B. 27:428.

Speck, M.L. 1976. *Compendium of Methods for the Microbiological Examination of Foods.* Am. Pub. Health Assoc.