Drawert, F., Postel, W., Weyh, H., *Brauwelt*, 113(21), 407 (1973).
Drawert, F., Radola, B., Mueller, W, Goerg, A., *Eur. Brew. Conv. Proc. Congr.* 14, 479 (1971).
Drawert, F., Radola, B., Mueller, W., Goerg, A., Bednar, J., *Eur. Brew. Conv., Proc. Congr.* 14, 463 (1973).
Fertman, G. J., Gorinstein, S. B., *Fermentn. Spirit. Prom.* 34, 15 (1968); *Chem. Abstr.* 69, 75576a (1969).
Gertner, A., Grdinic, V., *Acta Pharm. Jugosl.* 15(4), 209 (1965).
Gordon, A., Keil, B., Sebesta, K., Knessl, O., Sorn, F., *Coll. Czech. Chem. Commun.* 15, 23 (1950).
Gorinstein, S., *J. Food Sci.* 38(7), 1264 (1973a)
Gorinstein, S. *Food Eng.* 45(8), 74 (1973b).
Gorinstein, S., *Brew. Dig.*, March, 46 (1974a).
Gorinstein, S., *J. Food. Sci.* 39, (8), 953 (1974b).
Gorinstein, S., *Brew, Dig.* June, 50 (1974c).
Gorinstein S., *J. Assoc. Off. Anal. Chem.* 58(4), 793 (1975).
Gorinstein S., *J. Assoc. Off. Anal. Chem.* 59(6), 1380 (1976).
Groves, W. E., Davis, F. C., Jr., Sells, B. H. *Anal. Biochem.* 22, 195 (1968).
Hampel, W., *Eur. Brew. Conv. Proc. Congr.* 14, 25 (1973).
Heatherbell, D. A. *Confructa*, 21 36 (1976).
Hebert, J. P., Strobbel, B., *Bios (Nancy)* 3, 335 (1972).
Hejgard, J., *Boeg*-Hansen. T. C., *J. Inst. Brew, London*, 80(5), 436 (1974).
Hudson, J. R., *Eur. Brew. Conv. Proc. Congr.* 14, 157 (1973).
Klimova, V., *Osnovn. Mikrometod. Anal. Org. Soedin.*, 71 (1967).
Kurganova, G. V., Saenko, N. F., Ivanova, N. N. *Vinodel. Vinograd. SSSR* 2, 15 (1974).
Laszlo, E., Joth, M., *Soripar* 19(3), 87 (1972).
Lie, S., *J. Inst. Brew., London*, 79(1) 37 (1973).
Maendl, B., Wullinger, F., Wanger, D., Piendl, A. *Brauwissenschaft*, 27, 285 (1974).
Marinelli, L., *Proc. Am. Soc. Brew. Chem.*, 33(3), 88 (1975).
Moll, M. Vinh, T., Flayeus, R., *Bios. (Paris)* 4(3), 145 (1973).
Narziss, L., Roettger, W., *Brauwissenschaft* 26(5), 148 (1973a).
Narziss, L., Roettger, W., *Brauwissenschaft* 26(8), 237 (1973b).
Phillips, R. E., *Inst. Brew. (Aust. Sect.), Proc. Conv.* 12, 85, (1972).
Reiner, L., Piendl, A., *Brauwissenschaft* 21(1), 1(1974).
Savage, D. J., Thompson, C.C., *Eur. Brew. Conv., Proc. Congr.* 14, 33 (1973).
Sielicka, B., *Rocz. Technol. Chem. Zywn.* 23(1), 93 (1973).
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* 30, 1190, (1958).
Sommer, G., *Monatsschr. Brau.* 27, 242 (1974).
Srikanta, S., Rao, M. S. *J. Agric Food Chem.* 22, 667 (1974).
Steiner, K., *Brau-Rundsch.* 83(10), 193 (1972).
Steiner, K., *Brau-Rundsch.* 84(12), 241 (1973).
Ten Hoopen, H. J. G., *J. Inst. Brew., London* 79(1), 29(1973).

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Effect of Ethanol on Optical Rotation, Velocity of Mutarotation, and Equilibrium Constant of Lactose

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The effects of ethanol on polarimetric readings of lactose were studied. The specific rotation of both α- and β-lactose was less in ethanol solutions than in water. Also, as the percentage of ethanol increased, the specific rotation of lactose decreased. The relation was linear between the percentage of ethanol and the final specific rotation of lactose. The equilibrium constant of lactose was less in ethanol than in water, decreasing proportionately with the percentage of ethanol, $K = 1.68651 - 0.00415(\%\ \text{EtOH})$. Therefore, there was a direct relation between percentage of ethanol and percentage of α-lactose at equilibrium. The mutarotation of lactose was 2.3 times as fast in water as in 50% (v/v) ethanol. Both k_1 and k_2 were less in ethanol solution than in water, but k_1 decreased more (to 41%) than k_2 (to 46.5%) in 50% (v/v) ethanol compared to their values in water. This is responsible for the lower equilibrium constant of lactose in ethanol solutions than that in water.

Mutarotation, a phenomenon characteristic of lactose, is influenced by many factors (Nickerson, 1974). It is accelerated by increased temperature, becoming almost instantaneous at about 75 °C, and also by bases and acids (with the former more effective). Mutarotation rate is minimal at about pH 5.0, whereas at pH 9 equilibrium is established within a few minutes (Troy and Sharp, 1930). The action of salts is variable. Many salts can accelerate the mutarotation of lactose; for example, Haase and Nickerson (1966) have shown that whey–salt solutions increased the rate of interchange. Other substances can retard mutarotation. For example, mutarotation of lactose slows in the presence of sucrose (Patel and Nickerson, 1970), alcohols, and acetone (Herrington, 1934a; Richards et al., 1927). Various workers are not in complete agreement on the value of the mutarotation constant.

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The ratio of β to α at equilibrium, which defines the equilibrium constant ($K = \beta/\alpha = k_1/k_2$), is influenced by various factors. Temperature has a slight effect; with rising temperature the equilibrium constant decreases (Gillis, 1920; Nickerson, 1956; Parisi, 1930). Also influencing this value is the nature of the solvent. Bleyer and Schmidt (1923) stated that the equilibrium was displaced toward the formation of α-lactose in very strong acid solutions, and toward the formation of β-lactose in strong bases. Values for K, k_1 (the velocity constant of the α to β change), and k_2 (the velocity constant of the β to α change) have been calculated at different temperatures and pH by various workers (Hudson, 1904; Parisi, 1930).

The specific rotation of lactose varies with temperature and solvent. The specific rotation of lactose is increased in glycerol solutions and decreased in solutions of ethyl or methyl alcohol or acetone (Herrington, 1934b; Hudson and Yanovsky, 1917).

Previously (Majd and Nickerson, 1976) it was shown that alcohols decrease lactose solubility and that the

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composition of the recovered lactose changes with crystallization time. This suggested that the rate and extent of mutarotation was affected.

This experiment was done to obtain data on the effect of ethanol on polarimetric readings and mutarotation coefficient ($k_1 + k_2$) of lactose. Also, we wished to measure the effect of ethanol on equilibrium constant (K), the velocity constant of the α to β change (k_1), and the velocity constant of the β to α change (k_2) of lactose. Such data may be useful in developing new methods for lactose preparation and recovery.

EXPERIMENTAL PROCEDURE

Materials. α-Lactose hydrate powder, β-lactose powder, and absolute ethanol were used. Optical rotations were determined with a Perkin-Elmer Model 141 automatic polarimeter, using a jacketed polarimeter cell 1 decimeter in length maintained at 25 °C and a sodium lamp at 589 nm as light source.

Optical Rotation of Lactose in Ethanol. This experiment determined the final optical rotations of lactose in pure water and in 10, 40, 50, 60, 70, and 80% v/v of ethanol. Pure lactose solutions of 1–8% (w/v hydrate basis) were prepared at 1% intervals. In an attempt to have similar numbers of observations at each ethanol level, the concentrations of lactose and the increments of concentration were made smaller as the ethanol content was increased. However, only a few observations could be made at higher concentrations of ethanol due to limited solubility of lactose under these conditions. The ranges of lactose concentration were as follows: 1–8% in water; 1–7% in 10% (v/v) ethanol; 1–5% in 40% (v/v) ethanol; 1–4% in 50% (v/v) ethanol; 0.5–3% in 60% (v/v) ethanol; 0.4–1.6% in 70% (v/v) ethanol; and 0.3–0.6% in 80% (v/v) ethanol.

Lactose hydrate powder was dissolved in distilled water (by heating when necessary and subsequent cooling to room temperature). Then, distilled water or distilled water and ethanol was added to reach the concentration desired.

After the solutions reached equilibrium in room temperature, a period of 24 h for lactose in water solution and of 48 h for lactose in ethanol solutions, observations were made on the final specific rotations of lactose in pure water and in different percentages of ethanol. Two relationships were studied: (1) concentration of lactose vs. observed rotation; (2) ethanol percentage vs. final specific rotation.

Equilibrium Constant (K), Mutarotation Coefficient ($k_1 + k_2$), and Individual Coefficients (k_1 and k_2) of Lactose in Ethanol. The equilibrium constants of lactose in pure water and 10–80% (v/v) of ethanol were determined at 25 °C. Both initial and final specific rotations of α- and β-lactose were needed for calculation of the equilibrium constant and mutarotation coefficients. When the distilled water was first added to dissolve the lactose powder, a stop watch was started. As soon as the solutions were prepared and the polarimeter tube filled, a reading was taken. Additional readings were taken at 2-min intervals for a total of 11 readings. These readings, plotted as a function of time, showed a linear relation. Extrapolation to zero time gave the initial rotation of the sample. Also, further readings were taken for an average period of 5 h at larger intervals for use in calculating the mutarotation coefficient. This was done only with the samples of water and 50% (v/v) ethanol. The final rotations of all the samples (18 samples) were obtained after the solutions reached equilibrium in the water bath (25 °C), a period of 24 h for lactose in water solution and of 48 h for lactose in ethanol solutions. From these data, the rate of transformation of α- to β-lactose (k_1) and the rate of transformation of β- to α-lactose (k_2), using both α- and β-lactose as starting material, were statistically calculated in water and 50% (v/v) ethanol. The mutarotation coefficient ($k_1 + k_2$) also was statistically calculated for these conditions.

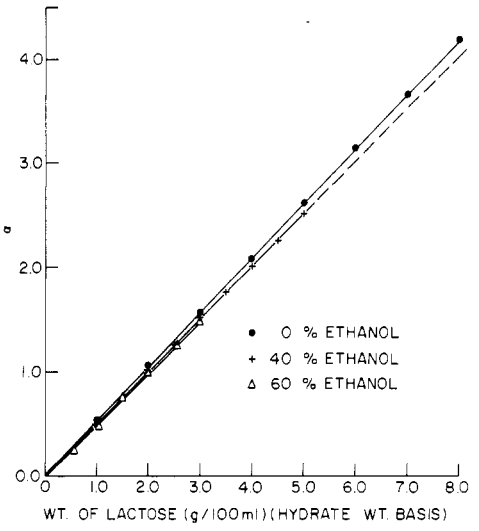

Figure 1. Relation of angular rotation and lactose concentration in water and in ethanol solutions (40 and 60%).

Analytical Methods. The equilibrium constants were calculated on an anhydrous basis from optical rotations with the equation:

$$K = \frac{k_1}{k_2} = \frac{r_\alpha - r_\infty}{r_\infty - r_\beta} \quad (1)$$

where r_α = initial specific rotation of α-lactose, r_β = initial specific rotation of β-lactose, r_∞ = the final or equilibrium rotation. Hudson's (1904) equation

$$k_1 + k_2 = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty} \quad (2)$$

in which r_0 = the initial rotation, r_t = the rotation at time t, r_∞ = the final or equilibrium rotation, was used to calculate the mutarotation coefficient. Solving eq 1 and 2 simultaneously yielded values for k_1 and k_2.

RESULTS AND DISCUSSION

Effect of Ethanol on the Optical Rotation of Lactose. Reports in the literature (Herrington 1934b; Hudson and Yanovsky, 1917) indicated that the specific rotation of lactose was different in ethanol from that in water, though few data were available. It was decided to develop sufficient data to show the relationship between optical rotation and alcohol content since composition of the recovered lactose is influenced by the alcohol (Majd and Nickerson, 1976).

Specific optical rotation shows a direct relationship between concentration in water and angular rotation. The present work shows a similar relation between concentration of lactose in different percentages of ethanol (up to 70%) and angular rotation, α, of these solutions (Figure 1). All were straight lines, with slightly different slopes, which decreased as percentage of alcohol increased.

The specific rotation of lactose in different percentages of ethanol was calculated (Table I), being less in ethanol solutions than in water. As the percentage of ethanol increased the specific rotation of lactose decreased linearly.

Equilibrium Constant (K), Mutarotation Coefficient ($k_1 + k_2$), and Individual Mutarotation Constants (k_1 and k_2) of Lactose in Ethanol. As mentioned, the nature of the solvent affects the equilibrium

Table I. Effect of Concentration of Ethanol on Final Specific Rotation of α-Lactose

Percent ethanol (v/v)	Final specific rotation[a] Experimental data	Final specific rotation[a] Calculated data[b]
0	52.5	52.5
10	52.1	52.1
20		51.8
30		51.3
40	50.8	50.8
50	50.4	50.5
60	50.0	50.1
70	49.4	49.4
80	48.9	49.0

[a] Hydrate weight basis. [b] Recalculated from final specific rotation (anhydrous weight basis).

constant of lactose. Values have been reported for the equilibrium constant of lactose in acid and base solutions, though there are no published data for this constant in alcohol solutions. This work determined the equilibrium constant (K) of lactose in 0–80% (v/v) ethanol. It was less than in water. As the percentage of ethanol increased, the initial and final specific rotation of both α- and β-lactose as well as the equilibrium constant of lactose decreased (Table II). For example, the equilibrium constant decreased from 1.69 in pure water to 1.35 at 80% (v/v) ethanol. This smaller value means that the proportion of lactose in the α form increased in ethanol solution at equilibrium. The proportion of α-lactose at equilibrium was 37.2% in water but increased to 40.2% in 50% (v/v) ethanol and to 42.6% in 80% (v/v) ethanol. Figure 2 shows the linear relation between ethanol concentration and equilibrium constant of lactose. The equation for the line is $K = 1.68651 - 0.00415$ (% EtOH), where K is the equilibrium constant, and EtOH is the ethanol concentration (v/v).

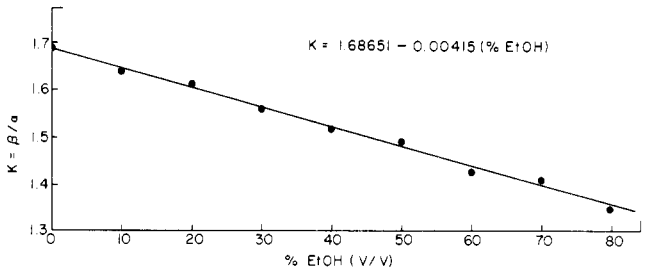

Figure 2. Effect of ethanol concentration on the equilibrium constant (K) of lactose.

As given in the literature, $K = k_1/k_2$, where K is the equilibrium constant of lactose, k_1 is the velocity constant for the change of α to β, and k_2 is the velocity constant for the change of β to α. From this equation there are four possibilities to explain a smaller K in ethanol than in water: (1) k_2 is larger in ethanol than in water, and k_1 remains constant. (2) Both k_1 and k_2 are larger in ethanol than in water, but k_2 shows a larger increase. (3) Both k_1 and k_2 are smaller in ethanol than in water, but k_1 decreases more. (4) k_1 is smaller in ethanol than in water, and k_2 remains constant.

Since it is known that mutarotation is retarded by alcohol (Herrington, 1934a; Richards et al., 1927), the only possibilities that can be valid are 3 and 4. The experimental data (Table II) also show that either 3 or 4 must occur because the percentage of α-lactose at equilibrium is higher in ethanol than in pure water. This means that ethanol either retards k_1 only, or that it affects both k_1 and k_2 but retards k_1 more.

To clarify this point, it was necessary to determine the mutarotation coefficient and the k_1 and k_2 of both α- and β-lactose in ethanol. The mutarotation coefficients of lactose were determined in water and 50% (v/v) ethanol. The values for α- and β-lactose were different in both water and in 50% (v/v) ethanol (Table III). The rate constants

Table II. Effect of Ethanol Concentration on Specific Rotation and Equilibrium Ratio of β- to α-Lactose at 25 °C (Anhydrous Weight Basis)

Percent ethanol (v/v)	Type of lactose	Specific rotation Initial	Specific rotation Final	β/α	% α-lactose	% β-lactose
0	α	88.8	55.3	1.69	37.2	62.8
	β	35.5	55.3			
10	α	87.2	54.8	1.64	37.9	62.1
	β	35.0	54.8			
20	α	86.4	54.5	1.61	38.3	61.7
	β	34.7	54.5			
30	α	85.5	54.0	1.56	39.1	60.9
	β	33.8	54.0			
40	α	84.7	53.5	1.52	39.7	60.3
	β	33.0	53.5			
50	α	84.7	53.2	1.49	40.2	59.8
	β	32.1	53.2			
60	α	84.2	52.7	1.43	41.2	58.8
	β	30.7	52.7			
70	α	83.6	52.0	1.41	41.5	58.5
	β	29.6	52.0			
80	α	83.2	51.6	1.35	42.6	57.4
	β	28.2	51.6			

Table III. Velocity of Mutarotation of Lactose in Water and 50% v/v Ethanol at 25 °C

Lactose form	Solvent	$K = \frac{k_1}{k_2}$	$k_1 + k_2$[a]	k_1[b]	k_2[b]
α	Water	1.69	0.00779	0.00489	0.00290
β	Water		0.00753	0.00473	0.00280
α	50% v/v ethanol	1.49	0.00338	0.00202	0.00136
β	50% v/v ethanol		0.00321	0.00192	0.00129

[a] The logarithmic base 10 and the time in minutes. [b] Average of eight replications.

($k_1 + k_2$) of α and β were 2.3 times faster in pure water than in 50% (v/v) ethanol solution. Table III also shows that both k_1 and k_2 were less in 50% (v/v) ethanol than in water though k_1 was reduced in ethanol to only 41% of its value in water, k_2 was reduced to 46.5% of its value in water. Therefore it can be concluded that the smaller equilibrium constant for mutarotation of lactose in ethanol is due to a greater decrease in the change of α to β (k_1) than the reverse reaction (k_2). Consequently, less α is changed to β (by a smaller k_1) while more β is changed to α (by a relatively larger k_2), with the resulting increase in α overall, this yielding a smaller equilibrium constant ($K = k_1/k_2$).

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LITERATURE CITED

Bleyer, B., Schmidt, H., *Biochem. Z.* **141**, 278 (1923).
Gillis, J., *Recl. Trav. Chim. Pays-Bas* **39**, 88 (1920).
Haase, G., Nickerson, T. A., *J. Dairy Sci.* **49**, 127 (1966).
Herrington, B. L., *J. Dairy Sci.* **17**, 659 (1934a).
Herrington, B. L., *J. Dairy Sci.* **17**, 701 (1934b).
Hudson, C. S., *J. Am. Chem. Soc.* **26**, 1065 (1904).
Hudson, C. S., Yanovsky, E., *J. Am. Chem. Soc.* **39**, 1013 (1917).
Majd, F., Nickerson, T. A., *J. Dairy Sci.* **59**, 1025 (1976).
Nickerson, T. A., *J. Dairy Sci.* **39**, 1342 (1956).
Nickerson, T. A., Lactose. "Fundamentals of Dairy Chemistry", Webb, B. H., Johnson, A. H., Alford, J. A., Ed., Avi Publishing Co., Westport, Conn., 1974.
Parisi, P., *G. Chim. Ind. Appl.* **12**, 225 (1930).
Patel, K. N., Nickerson, T. A., *J. Dairy Sci.* **53**, 1654 (1970).
Richards, E. M., Faulkner, I. J., Lowry, T. M., *J. Chem. Soc.*, 1733 (1927).
Troy, H. C., Sharp, P. F., *J. Dairy Sci.* **13**, 140 (1930).

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Fate of [^{14}C]Carbon Monoxide in Cooked or Stored Ground Beef Samples

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Lean ground beef samples were exposed to a 1% [^{14}C]carbon monoxide atmosphere for 3 days, resulting in about 30% saturation with CO of the total myoglobin content. Following such exposure, the samples were either stored or cooked for varying periods of time. Aqueous and fat extracts were made, and the amounts of radioactivity in these fractions, and in residual precipitate fractions, were determined. Activity in the aqueous fraction could be attributed entirely to carboxymyoglobin, and that in the lipid fraction was at the limit of detection and not significant. Less than 0.09 ppm ^{14}C as CO remained in precipitate fractions following cooking or bacterial spoilage, possibly in the form of denatured globin carbon monoxide hemochrome. In the various experimental samples, CO was lost during storage, with a half-life of about 3 days. The maximum loss from cooked patties was about 85%. The use of low levels of CO may prevent the discoloring noted when red meats are stored in elevated levels of CO_2.

It has long been known that carbon dioxide atmospheres inhibit microbial growth in meat (Haines, 1933). When beef is preserved by carbon dioxide, however, there may occur undesirable surface discoloration due to oxidation of oxymyoglobin (MbO_2) to metmyoglobin (MetMb). While reports vary to some degree, it appears that at levels of CO_2 lower than 20%, discoloration is not a major problem (Clark and Lentz, 1969, 1972, 1973). Others report that samples held in air had better color than those stored in 20 to 25% CO_2 (Huffman et al., 1975). A procedure in which a chemical system for continuous carbon dioxide generation was incorporated inside packages presented no problem with discoloration (Benedict et al., 1975). Elevated levels of oxygen in atmospheres containing high levels of CO_2 help prevent pigment changes (Clark and Lentz, 1973; Taylor and MacDougall, 1973).

However, at higher levels of CO_2 (30 to 60%) there is pronounced discoloration. Some workers have reported development of a greyish tinge which was not attributed to MetMb (Ledward, 1970; Ledward et al., 1971). More recently, the fairly rapid development of severe surface browning as a result of MetMb formation has been reported for beef samples held in elevated levels of CO_2 (Silliker et al., 1977). This undesirable oxidation could effectively be prevented by the incorporation of 1% carbon monoxide, due to the formation of carboxymyoglobin (MbCO), which is more stable in such atmospheres than MbO_2 (Wolfe et al., 1976). The beneficial effect of CO on color of refrigerated beef in different systems has been reported by others (El-Badawi et al., 1964; Besser and Kramer, 1972; Clark et al., 1976).

The present study, using radioactive CO, was designed to determine the extent and stability of the association of CO with beef during storage and cooking.

EXPERIMENTAL SECTION

Meat. Ground beef was chosen as a limiting case of high surface area for maximum CO incorporation, as well as bacterial loading, to enhance possible reactions. The leanest grade of ground beef was purchased at a local retail chain store. After thorough mixing of the meat, 50.0 ± 0.1 g patties (nominally 0.97 cm thick) were formed using a die and piston of 8.0 cm diameter. Avoiding all mutual

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