

that the interaction of tannin with proteins is affected by the types of tannin and protein, pH, and concentration. Due to the nonspecific nature of this interaction, it is possible that the tannins referred to in this work can inhibit other enzymes in the gastrointestinal tract besides trypsin. This property and its heat resistance indicate that tannins may play a more important role than the heat-labile proteinaceous trypsin inhibitor in heat-processed beans. This can be demonstrated more definitively by animal feeding experiments.

ACKNOWLEDGMENT

We greatly appreciate the generous gift of winged bean from Louis Lazaroff of the Asia Foundation. The assistance of Pilar Reyes, Ester Wong, Anna Cheng, and Sandra Burkhardt in some of the experiments and in evaluating the manuscript is greatly appreciated.

LITERATURE CITED

- Batugal, P. A., International Projects, Philippine Council on Agriculture and Resource Research, College, Laguna, The Philippines, personal communication, 1979.
- Elias, L. G., De Fernandez, D. G., Bressani, R., *J. Food Sci.* **44**, 524 (1979).
- Goldstein, J. L., Swain, T., *Phytochemistry* **2**, 371 (1963).
- Goldstein, J. L., Swain, T., *Phytochemistry* **4**, 185 (1965).
- Jirgensons, B., *Makromol. Chem.* **91**, 74 (1966).
- Kakade, M. K., Hoffa, D. E., Liener, I. E., *J. Nutr.* **103**, 1772 (1973).
- National Academy of Sciences (Ad Hoc Panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development), "The Winged Bean: A High-Protein Crop for the Tropics", National Academy of Sciences (USA), U.S. Government Printing Office, Washington, DC, 1975.
- Price, M. L., Butler, L. G., Rogler, J. C., Featherston, W. R., *J. Agric. Food Chem.* **27**, 441 (1979).
- Rackis, J. J., McGhee, J. E., Booth, A. N., *Cereal Chem.* **52**, 85 (1975).
- Rockland, L. B., Hayes, R. J., Metzler, E., Binder, L. J., U.S. Patent No. 3318708, May 9, 1967.
- Sherman, L., Luh, B. S., Hinreiner, E., *Food Technol.* **7**, 480 (1953).
- Sohonie, K., Bhandarkar, A. P., *J. Sci. Ind. Res.* **13B**, 500 (1954).
- Sosulski, F., *J. Am. Oil Chem. Soc.* **56**, 711 (1979).
- Tamir, M., Alumot, E., *J. Sci. Food Agric.* **20**, 199 (1969).
- Thompson, A. E., The Rockefeller Foundation, Yogyakarta, Indonesia, personal communication, 1979.
- Van Buren, J. P., Robinson, W. B., *J. Agric. Food Chem.* **17**, 772 (1969).
- Worthington Biochemical Corporation, "Worthington Enzymes", Freehold, NJ, 1977, p 221.

Received for review October 18, 1979. Accepted January 10, 1980. This work was partially supported by Biomedical Research Support Grant (NIH).

Interaction of Iron(II) with Lactose

Karen Bachran and Richard A. Bernhard*

A change in specific rotation of a lactose-FeCl₂ mixture indicated the formation of a soluble lactose-FeCl₂ complex. Maximum complex formation occurred within 1 h. Addition of NaOH to solutions containing lactose and FeCl₂ precipitated insoluble Fe(OH)₂ and lactose. Maximum lactose precipitation occurred at molar ratios NaOH/FeCl₂ = 2.0 and FeCl₂/lactose = 6.0 with 60% yield. Reaction reached completion in less than 30 min. Concentration of iron remaining in solution was some 15 times greater than expected, indicating the formation of a soluble lactose-Fe(OH)₂ complex. The lactose-iron precipitate is a gel consisting of approximately 70% water. The remainder is composed of an insoluble lactose-Fe(OH)₂ adduct and insoluble Fe(OH)₂. The amount of complex and lactose precipitated is a function of the relative insolubility of the metal hydroxide and the solubility of the adduct.

Complexing of metal ions with lactose has important biological and environmental implications. In the manufacture of cheese, enormous amounts of whey solutions and effluents are produced as byproducts. Lactose comprises about 5% of these whey solutions. Lactose is an excellent substrate for microorganisms. This presents a disposal problem since direct addition of the whey to sewage systems would raise the biological oxygen demand of the system appreciably. Removal of the lactose eliminates much of this problem. Methods for the removal of lactose include reverse osmosis and ultrafiltration (Sammon, 1974; Fenton-May et al., 1972) and precipitation of lactose using alkaline-earth metals (Olano et al., 1977a,b; McCommins et al., 1980; Kwon, 1979). Most researchers believe these precipitation reactions are a consequence of complex formation between lactose and the metal ion (Cerbulis,

1973; Herrington, 1934a,b; Kwon, 1979; McCommins et al., 1980; Olano, 1977a,b).

Most studies with carbohydrates have utilized alkali or alkaline-earth metals for complexation, but a few have been directed to iron-carbohydrate interactions. Charley et al. (1962) used iron(III)-fructose chelates to study penetration and transport of iron across biological membranes. Davis and Deller (1966) also studied the binding of iron(III) to carbohydrates. Cerbulis (1973) used FeCl₃ and NaOH alone, and in combination with CaO, to recover proteins and lactose from acid whey. Imamura and Kawamoto (1974) and Imamura et al. (1975) prepared and purified an iron(III)-lactose complex with a molar ratio of 1:1 and molecular weight of 5-10 000.

Very little is known about the reaction of lactose with divalent nonalkaline earth metals. There is still much to be learned about the precipitation process itself and the chemistry of lactose complexation. This study, utilizing iron(II), is an attempt to fill some of these informational gaps. The specific objectives of this study were to inves-

*Department of Food Science and Technology, University of California, Davis, California 95616.

tigate the complexation of lactose and iron(II) in aqueous solution, to study the effects of the molar ratios of reactants, time, temperature, and cations on the recovery of lactose, and to optimize the parameters for lactose precipitation.

EXPERIMENTAL SECTION

The preparation of lactose solutions, procedures for measurement of all optical rotations, pH, and titrations have been described previously (McCommins et al., 1980). Measurement of the specific rotation of the iron-lactose complex was carried out using the procedure described by Swartz et al. (1978).

Precipitation of Lactose from Aqueous Solutions Varying the Molar Ratio of NaOH/FeCl₂. A series of centrifuge bottles, each containing 50 mL of 0.555 M lactose solution (20% w/v), was cooled in ice water (5 ± 1 °C), and 16.55 ± 0.01 g (0.8325 mol) of FeCl₂·4H₂O was added to each bottle with constant stirring. After allowing 15 min for the complete solution of FeCl₂·4H₂O, 50 mL of suitable concentrations of NaOH was added to give solutions with molar ratios of NaOH/FeCl₂ from 0.5 to 4.0. All reactions were stirred for 60 min after addition of NaOH solution. The solution mixtures were then centrifuged at 5 ± 1 °C at 7970g (7000 rpm) for 20 min.

In each case after centrifugation, the supernatant solution was separated from the precipitate, and the pH of the solution was measured. If the pH was greater than 7.0, the solution was titrated with 3 N HCl. The optical rotation of all supernatant solutions was then determined.

Precipitation of Lactose from Aqueous Solutions Varying the Molar Ratio of FeCl₂/Lactose. Suitable amounts of FeCl₂·4H₂O and required amounts of NaOH in 50 mL of water were added to 50 mL of a standard solution of 0.555 M lactose to give solutions with molar ratios of Fe(II)/lactose from 1.0 to 6.0.

The precipitation reaction was carried out, and optical rotation measurements were made as above. Molar ratios Fe(II)/lactose greater than 6.0 were not used because the solution became too viscous to stir.

Time Studies Using NaOH and FeCl₂. The precipitation reaction, as described previously, was performed using 33.10 g of FeCl₂·4H₂O and 50 mL of 6.66 M NaOH (molar ratio = 2.0 NaOH/FeCl₂) or 50 mL of 5.66 M NaOH (molar ratio = 1.7 NaOH/FeCl₂). All reactions contained 50 mL of 0.555 M lactose (molar ratio = 6.0 FeCl₂/lactose). Reaction times used were 30, 60, 90, and 120 min. Optical rotation measurements were made immediately following centrifugation.

Temperature Studies. Precipitation of lactose was achieved using 33.10 g of FeCl₂·4H₂O and 50 mL of 6.66 M NaOH (molar ratio = 2.0 NaOH/FeCl₂) mixed with 50 mL of 0.555 M lactose as described above. Reaction time was 30 min. Temperatures used were 5 and 25 °C. Optical rotation measurements were made immediately following centrifugation.

Precipitation of Lactose from Aqueous Solutions Using FeCl₂ and Potassium Hydroxide. Fifty milliliters of 0.555 M lactose was mixed with 33.10 g of FeCl₂·4H₂O (molar ratio = 6.0 FeCl₂/lactose) as described previously. In place of NaOH, 50 mL of KOH was added, using either 6.66 M KOH (molar ratio = 2.0 KOH/FeCl₂) or 5.66 M KOH (molar ratio = 1.7 KOH/FeCl₂). Time studies were conducted using reaction durations of 30, 60, 90, and 120 min. Optical rotation measurements were made immediately following centrifugation.

Iron Analysis. The precipitation reaction was carried out as above. After centrifugation, the supernatant solution was diluted to 1 L and acidified using deionized water

Table I. Effect of Varying Molar Ratio NaOH/FeCl₂ on the Precipitation of Lactose^a

molar ratio NaOH/ FeCl ₂	% lactose precip ^b	pH of supernatant soln	color of supernatant soln
0.5	18.4 ± 2.6	5.3	medium green
1.0	35.4 ± 1.6	5.5	pale green
1.5	41.7 ± 1.3	6.0	yellow-green
2.0	49.3 ± 3.3	10.9	pale gold
3.0	39.1 ± 2.4	11.8	pale gold
4.0	36.1 ± 3.4	11.9	pale gold

^a Molar ratio FeCl₂/lactose = 3.0. ^b Mean of six replicates.

and 6 N HCl. Analysis for Fe(II) and total iron was then performed using the standard phenanthroline method (Taras, 1971).

Specific Rotation of Lactose-FeCl₂ Complex. The optical rotation of various lactose-FeCl₂ mixtures was determined and a mean value for the specific rotation of the complex calculated, +60.6° mL/dm·g (Swartz et al., 1978). Because this value is substantially greater than the specific rotation of lactose alone (+55.3° mL/dm·g), this value was used for calculations involving the specific rotation of lactose in solutions which contained significant amounts of FeCl₂ which had not reacted with NaOH to form Fe(OH)₂, i.e., when the molar ratio of NaOH/FeCl₂ ≤ 1.5.

RESULTS AND DISCUSSION

Effect of Varying the Molar Ratio of NaOH/FeCl₂. Results of these experiments are presented in Table I. As is evident from the data, the molar ratio of NaOH/FeCl₂ has a substantial effect on the reaction, with the molar ratio of 2.0 being a dividing point in the results. At ratios of less than 2.0, the solution is acidic with a green color caused by the acidic ferrous salt which has not been completely converted to Fe(OH)₂. At 2.0 the conversion reaction



is essentially complete as evidenced by the sudden jump in pH and by the change in the color of the solution.

The percentage of lactose precipitated likewise increases up to a molar ratio of 2.0. At ratios greater than 2.0, the percentage of lactose recovered in the precipitate decreases with increasing molar ratios, indicating that the optimum molar ratio of NaOH/FeCl₂ is 2.0.

Analysis of variance (AOV) of these data show that there is a significant difference between lactose recoveries at the 0.001 level of confidence ($F = 109.75$) and no significant difference between replications ($F = 1.74$). This indicates that the ratio of 2.0 should be used throughout the remainder of the experiments to obtain maximum lactose recovery.

Effect of Varying the Molar Ratio of FeCl₂/Lactose. Results of these experiments are presented in Table II. These results indicate a continuing increase in the percentage of lactose precipitated with increasing molar ratios of FeCl₂/lactose. The pHs show a slight increase through the series; however, at all ratios, the solution is highly alkaline. AOV for the amount of lactose precipitated indicates that the percentages are significantly different at $p = 0.001$ ($F = 140.18$) and that the replicates are not significantly different ($F = 0.79$).

A plot of percent lactose recovered vs. molar ratio FeCl₂/lactose is curvilinear and appears to exhibit a leveling-off effect in the area of molar ratios of 7-8. Higher ratios of FeCl₂/lactose were not examined to determine

Table II. Effect of Varying Molar Ratio of FeCl₂/Lactose on the Precipitation of Lactose^a

molar ratio FeCl ₂ /lactose	% lactose precip ^b	pH of supernatant soln
1.0	34.5 ± 3.2	10.6
2.0	42.0 ± 0.9	10.8
3.0	50.2 ± 1.6	11.0
4.0	52.4 ± 1.2	11.1
5.0	55.5 ± 1.0	11.3
6.0	59.8 ± 2.0	11.4

^a Molar ratio NaOH/FeCl₂ = 2.0. ^b Mean of five replicates.

Table III. Effect of Time on Precipitation of Lactose Using NaOH and KOH^a

base	molar ratio NaOH/ FeCl ₂	% lactose precipitated ^b			
		0.5 h	1.0 h	1.5 h	2.0 h
NaOH	2.0	59.6	59.6	57.6	52.7
	1.7	41.4	44.1	45.6	45.5
KOH	2.0	57.9	59.2	58.1	54.5
	1.7	42.2	43.9	46.4	44.9

^a Molar ratio FeCl₂/lactose = 6.0. ^b Mean of five replicates.

definitely if leveling-off does occur, for the increasing viscosity of the reaction mixture prevented adequate stirring at molar ratios greater than 6.0. The ratios studied do indicate that the highest experimentally possible molar ratio of FeCl₂/lactose (6.0) should be used to achieve maximum lactose precipitation.

Effect of Time on Precipitation of Lactose Using NaOH and KOH. Historically, studies pertaining to lactose precipitation utilizing metal salts combined with strong base used NaOH for economic reasons. Following this tradition, the current study was initiated using NaOH. From the previous two sections the optimal conditions for lactose precipitation were determined to be the following: molar ratio NaOH/FeCl₂ = 2.0; molar ratio FeCl₂/lactose = 6.0. These conditions, although useful, give little information about the reaction mechanisms. This reaction information can be obtained, in part, from time studies. The results from these studies are given in Table III. Data for use of NaOH at a molar ratio to FeCl₂ of 2.0 indicate that the reaction has reached completion in less than 30 min with a constant amount of lactose remaining in the precipitate up to 1 h. At 1.5 h some lactose seems to have resolubilized and at 2 h the resolubilization effect is even more striking. The amount of lactose precipitated using a molar ratio of NaOH/FeCl₂ = 1.7 shows a different pattern than that observed at a ratio of 2.0. In this case the reaction seems to come to completion after approximately 1.5 h.

Molar ratios = 2.0 and 1.7 OH⁻/FeCl₂ were chosen so that precipitation of lactose could be studied with time under both optimal and nonoptimal conditions. The specific use of molar ratio = 1.7 OH⁻/FeCl₂ allowed examination of lactose recovery at near optimal conditions yet without achieving stoichiometric balance.

Most probably this difference is a consequence of the complete conversion of FeCl₂ to Fe(OH)₂ in the first instance and an incomplete conversion in the second. However, the reaction is within 11% of completion after 30 min at the 1.7 ratio. The fact that the reaction does approach completion in such a short time, and the difference in the results at the two ratios, imply that the precipitation of lactose is due, in large part, to the for-

Table IV. Temperature Studies for Precipitation of Lactose Using FeCl₂ and NaOH^a

temp, °C	reaction time, min	% lactose precip ^b
5 ± 1	30	59.2 ± 0.3
25 ± 0.2	30	58.2 ± 0.1

^a Molar ratio NaOH/FeCl₂ = 2.0; molar ratio FeCl₂/lactose = 6.0. ^b Mean of three replicates.

mation of the less soluble hydroxide from the highly soluble chloride salt. Treatment of the data by AOV confirms the significance of the effect of time at both ratios and the significant difference in lactose precipitation between the two molar ratios of NaOH/FeCl₂.

In conjunction with this, the use of NaOH to obtain conversion of FeCl₂ to Fe(OH)₂ causes two other free species to be found in solution after conversion, Na⁺ and Cl⁻. These species have been shown by Swartz et al. (1978) to complex with lactose in aqueous solution.

In addition, Charley et al. (1962) have reported the formation of a carbohydrate complex involving iron and sodium complexed with fructose. These findings suggested that sodium in some form may be involved in the precipitation reaction. It is possible that a lactose complex may be formed with both iron and sodium. The findings of Swartz et al. (1978) indicate that NaCl may complex with lactose in solution in a manner competitive with any complex formation between lactose and the newly formed Fe(OH)₂.

In order to confirm or reject either or both of these possible effects, a strong base with a different cation must be used in place of NaOH. Potassium hydroxide seems to be ideal since it is readily available and the potassium ion has been found not to complex with lactose in discernible amounts (Swartz et al., 1978). Any difference in the amount of lactose precipitated using KOH, as opposed to NaOH, would be indicative of a sodium effect. Results of experiments utilizing KOH are presented in Table III. From these results, it can be concluded that the sodium ion is not involved in the reaction, as either an inhibitor or as an enhancer. This further confirms that the amount of lactose precipitated is due simply to a hydroxyl effect; that is, precipitation is due mainly to the formation of the metal hydroxide from metal salt plus strong base. Comparative AOV data for NaOH and KOH confirm that KOH and NaOH do not give significantly different results under identical experimental conditions. These AOV treatments also show that the only interaction involved in the precipitation reaction is the interaction between ratios and time.

Effect of Temperature on Precipitation of Lactose Using FeCl₂ and NaOH. From the time study it can be observed that, under experimental conditions that result in optimal lactose precipitation (molar ratio NaOH/FeCl₂ = 2, molar ratio FeCl₂/lactose = 6), reaction times of 30 min are sufficient for attainment of maximum lactose recovery.

Temperature studies were therefore conducted using a reaction time of 30 min to minimize degradation of lactose under the alkaline conditions used (Corbett and Kenner, 1953). Results in Table IV indicate that the percentage of lactose precipitated was essentially the same at 5 and 25 °C.

Effect of Time on the Concentration of Iron in Solution. From experiments previously discussed, it can be determined that precipitation of lactose is strongly dependent on the formation of iron hydroxide; but this sheds little light on the reaction mechanism. It is possible

Table V. Effects of Time on the Concentration of Iron Remaining in Solution^a

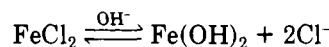
	time, h			
	0.5	1.0	1.5	2.0
% total iron in soln ^b	0.014	0.014	0.014	0.020
% total iron in soln as iron(II) ^b	0.009	0.006	0.005	0.004

^a Molar ratio NaOH/FeCl₂ = 2.0; molar ratio FeCl₂/lactose = 6.0. ^b Mean of three replicates.

that either adsorption, coprecipitation, complex formation, or any combination of the three is involved in the precipitation of lactose. In addition, no explanation is thus far possible for the resolubilization of precipitated lactose after 1 h. Iron analysis, studied as a function of time, provides some information in these areas.

Results of iron analysis for total iron and for iron(II) are given in Table V. As can be seen, the absolute amounts of iron remaining in solution after the addition of NaOH are extremely small—less than 1% of the iron in all cases. This seemingly negligible amount may be deceptive, however, when considered in relation to the reaction and the solubility of Fe(OH)₂ and Fe₂O₃.

Under the conditions of the experiment, the reaction



has gone to completion and almost all Fe(OH)₂ has precipitated. The portion remaining in solution is undergoing oxidation in basic solution, as evidenced by the results in

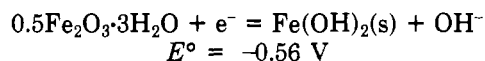


Table V.

Comparison of the amount of total iron in solution with the solubility of Fe(OH)₂ (1.5 × 10⁻⁴ g/100 mL of H₂O) as reported by Linke (1958) indicates that, even if all the iron remains in the form iron(II), at least 14 times more iron remains in solution than can be accounted for by the solubility. Considering the continuous oxidation of Fe(II) to Fe(III) and the solubility of Fe₂O₃ (3 × 10⁻¹⁰ g/100 mL of H₂O), the amount of iron remaining in solution (0.00236 mol or 0.212 g) becomes significant. This is especially true for the appreciable increase in soluble iron reported after 2 h.

The increase at 2 h can be explained as a consequence of the amphoteric nature of Fe(OH)₂. Although it readily redissolves in acids, it also redissolves in NaOH (Cotton and Wilkinson, 1972). This resolution of iron, in turn,

helps to explain another observed effect—the increase in the amount of lactose in solution with time. As the iron redissolves concomitant with this, lactose appears to resolubilize too.

The results of iron analysis, therefore, confirm the existence of a complex species lactose·Fe(OH)₂ with a solubility at least 15 times greater than that of Fe(OH)₂.

Comprehensive Reaction Interpretation Based on Experimental Results. The above results can now be brought together to obtain a proposed unified reaction mechanism. It is evident from the data obtained that lactose precipitation is dependent upon both the molar ratio of OH⁻/FeCl₂ and the molar ratio of FeCl₂/lactose. The nonlinear nature of the relationship between molar ratio of FeCl₂/lactose and the amount of lactose precipitated indicate that the precipitation reaction is not principally caused by adsorption. Under the experimental conditions employed, as the molar ratio of FeCl₂/lactose increases, the surface area for possible adsorption also increases by the same factor due to the stoichiometric formation of the insoluble Fe(OH)₂. If precipitation is due mainly to adsorption, the relationship between the molar ratio and the percent lactose precipitated should be linear because of this constant increase in surface area.

On the basis of the results of KOH and NaOH studies and under the experimental conditions employed, it was demonstrated that only iron was involved in lactose precipitation and that any complexation was attributable to the formation of a lactose·Fe(OH)₂ species.

The results in Table VI indicate the large amount of water incorporated into the precipitate relative to the total weight of the precipitate. The color of the solid leads one to the conclusion that the iron has remained in the ferrous state. It does not seem likely in this case that this large amount of water could be attributed solely to the presence of water of hydration for the precipitated Fe(OH)₂ or for the lactose. Alternatively, this would imply formation of a complex that is colloidal in nature as suggested by Waterman and van Aken (1927) for the sucrose-CaO interaction.

Although the large amount of water incorporated into the solid portion substantially decreases the volume of water available for retaining the lactose in solution, the calculations in Table VI (based on the solubility of lactose and the amount of water remaining in the supernatant solution) show that the incorporation of water into the solid cannot account for the amount of lactose precipitated. Temperature studies at 5 °C and at room temperature further serve to confirm this. The solubility of lactose at 5 °C is substantially different from the solubility at 25 °C

Table VI. Examples of Reaction Data under Varying Conditions

	molar ratio NaOH/FeCl ₂		
	0.5	2	2
	molar ratio FeCl ₂ /lactose		
	3	3	6
total weight of all reactants and solvent, g	121.54	126.07	147.83
weight of precipitate, g	26.48	53.75	70.52
% of reaction mixture precipitated	22	42	47%
% H ₂ O in precipitate ^a	64	77	71%
moles of lactose precipitated	0.0063 (8%) ^b	0.0139 (9%) ^b	0.0166 (8%) ^b
moles of Fe(OH) ₂ precipitated	0.0832 (28%) ^b	0.0832 (14%) ^b	0.1665 (21%) ^b
moles of H ₂ O in supernatant solution	4.32	3.21	2.77
color of precipitate	dark green	dark green	dark green
theoretical moles of lactose precipitated due to removal of water from solution without supersaturation of the solution	0.00	0.005	0.006

^a Calculated by difference. ^b Weight percent of precipitate.

(Nickerson, 1974). If the solubility is a major factor, this difference should cause appreciable differences in the amount of lactose precipitated at 5 and 25 °C. This was not the case as shown in Table IV.

The texture of the precipitate, slimy and gelatinous, and the large amount of water in it imply that the precipitate is actually a colloid—with the nature of a heavy suspension at nonoptimal conditions and approaching gel-like properties at optimal conditions for lactose precipitation. The relatively increased solubility of $\text{Fe}(\text{OH})_2$ in solution does indicate that a soluble adduct lactose- $\text{Fe}(\text{OH})_2$ exists with a solubility greater than that of $\text{Fe}(\text{OH})_2$ and less than that of lactose. This argues that the precipitate consists primarily of a lactose- $\text{Fe}(\text{OH})_2$ adduct, free $\text{Fe}(\text{OH})_2$, and water.

The increased recovery of lactose in the precipitate using $\text{Fe}(\text{OH})_2$ when compared with the results using $\text{Ca}(\text{OH})_2$ (McCommins et al., 1980) and $\text{Mg}(\text{OH})_2$ (Kwon, 1979) indicate that the amount of complex precipitated may be a function of the relative insolubility of the metal hydroxide. The percentage of lactose recovered decreases in the order $\text{Fe}(\text{OH})_2 > \text{Mg}(\text{OH})_2 > \text{Ca}(\text{OH})_2$, which can be correlated with increasing solubilities through this series [$\text{Fe}(\text{OH})_2 < \text{Mg}(\text{OH})_2 < \text{Ca}(\text{OH})_2$]. From this one can argue that the best metal hydroxide for lactose precipitation would be the one least soluble in water.

The lack of precipitation reported by Charley et al. (1962) with fructose and iron(II) even at highly alkaline pHs after addition of NaOH may be due to the difference in binding reported by Davis and Deller (1966). The 100-fold stronger iron binding ability of fructose may in some manner enable the complex to remain soluble after the addition of NaOH as compared with the relatively small chelating ability of lactose in which case the insolubility of $\text{Fe}(\text{OH})_2$ predominates—forcing the lactose out of solution with the $\text{Fe}(\text{OH})_2$.

The adduct model of Moulik and Khan (1975) is compatible with the findings of this study. Using their model, the incomplete conversion to $\text{Fe}(\text{OH})_2$ at molar ratios of $\text{NaOH}/\text{FeCl}_2 < 2$, could be rationalized for there is a variety of iron(II) species in solution. This could account for the additional time necessary for the reaction to reach completion at these ratios.

The total amount of lactose precipitated is a function of the solubility of the metal hydroxide and the solubility

of the adduct formed. This is in agreement with the work of McCommins et al. (1980). They found that the increased surface area provided by the addition of CaCO_3 for possible adsorption in the reaction mixture did not increase the amount of lactose recovered when using $\text{Ca}(\text{OH})_2$.

LITERATURE CITED

- Cerbulis, J., *J. Agric. Food Chem.* **21**, 255 (1973).
 Charley, P. J., Sarkar, B., Stitt, C. F., Saltman, P., *Biochim. Biophys. Acta* **69**, 313 (1962).
 Corbett, W. M., Kenner, J., *J. Chem. Soc.*, 2245 (1953).
 Cotton, F. A., Wilkinson, G., "Advanced Inorganic Chemistry; A Comprehensive Text", Wiley, New York, 1972.
 Davis, P. S., Deller, D. J., *Nature (London)* **212**, 404 (1966).
 Fenton-May, R. I., Hill, C. C., Jr., Amundson, C. H., Auclair, D. D., *A.I.C.H.E. Symp. Ser.* **68**(120), 31 (1972).
 Herrington, B. L., *J. Dairy Sci.* **17**, 701 (1934a).
 Herrington, B. L., *J. Dairy Sci.* **17**, 805 (1934b).
 Imamura, T., Kawamoto, H., *J. Fac. Fish. Anim. Husb., Hiroshima Univ.* **13**, 181 (1974).
 Imamura, T., Hatanaka, C., Kawamoto, H., *J. Fac. Fish. Anim. Husb., Hiroshima Univ.* **14**, 241 (1975).
 Kwon, S., M. S. Thesis, University of California, Davis, 1979.
 Linke, W. F., "Solubilities Inorganic and Metal-Organic Compounds", Vol. 1, 4th ed, D. Van Nostrand, Princeton, NJ, 1958.
 McCommins, D. B., Bernhard, R. A., Nickerson, T. A., *J. Food Sci.*, **45**, 362 (1980).
 Moulik, S. P., Khan, D. P., *Carbohydr. Res.* **41**, 93 (1975).
 Nickerson, T. A., "Fundamentals of Dairy Chemistry", Webb, B., Johnson, A., Alford, J., Eds., Avi Publishing Co., Westport, CT, 1974, p 273.
 Olano, A., Nickerson, T. A., Bernhard, R. A., *J. Food Sci.* **42**, 1481 (1977a).
 Olano, A., Nickerson, T. A., Bernhard, R. A., *J. Food Sci.* **42**, 1484 (1977b).
 Sammon, D. C., *Pure Appl. Chem.* **37**, 423 (1974).
 Swartz, M. L., Bernhard, R. A., Nickerson, T. A., *J. Food Sci.* **43**, 93 (1978).
 Taras, M. J., Ed., "Standard Methods for the Examination of Water and Waste Water", 13th ed, American Public Health Association, Washington, DC, 1971.
 Waterman, H. I., van Aken, J. S. A. J. M., *J. Soc. Chem. Ind.*, 411T (1927).

Received for review August 17, 1979. Accepted December 26, 1979. This research was supported by a grant from the Dairy Council of California.

Quantitative Determination of Individual Betacyanin Pigments by High-Performance Liquid Chromatography

Steven J. Schwartz and Joachim H. von Elbe*

A preparatory high-performance liquid chromatography (LC) method is employed to obtain crystalline betacyanin pigments. The betanin and betanidin hydrochloride molar absorptivities (ϵ) were determined to be 65 000 and 54 000, respectively. Spectrophotometric evidence and an enzymatic method were used to unequivocally identify peaks obtained from LC chromatograms. Pure solutions of pigment were used to calibrate an analytical LC apparatus to quantitate pigment mixtures. By employing visible detection at 535 nm, colorants are selectively screened for the red pigments. This method can accurately quantitate individual pigments rapidly without interference from other components.

Concern over the questionable safety of synthetic red food dyes as additives has prompted research in the use

Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin 53706.

of natural pigments as food colorants. Water soluble extracts or powders of the red beet (*Beta vulgaris*) have been shown to be useful alternatives (Pasch et al., 1975; von Elbe, 1977).

Methods for the production of highly pigmented con-