

# Recovery of Lysine from Corn Steepwater

Dennis J. O'Brien<sup>1,2</sup> and James C. Craig, Jr.<sup>1</sup>

## ABSTRACT

Cereal Chem. 76(6):949-952

One approach to increasing the utilization of agricultural products is fractionation of low-value materials to yield high-value products. In this study, lysine recovery from corn steepwater, an internal processing stream generated in the wet-milling of corn, was investigated. A weakly acidic cation exchange resin was employed to selectively recover lysine from corn steepwater at pH 7.0. In column studies, the product from the ion exchange operation had a lysine content of 4–6% (db). The only other

amino acid in the product was arginine. The presence of sodium, potassium, and magnesium ions at significant concentrations in the steepwater limited the lysine content of the product because of competitive adsorption on the resin. The lysine-enriched product reported here is 4–10 times higher in lysine content than other corn milling coproducts and could potentially be useful as a lysine supplement in animal feeds.

Lysine is an essential amino acid for the growth of nonruminant animals and is becoming a widely used feed supplement. For example, lysine is the first limiting amino acid in swine diets based on soy meal and cereal-based grains (Bondi 1987). The primary uses for lysine are supplementation in the diets of poultry and swine. At present, worldwide consumption is 450 million pounds per year and is increasing rapidly (Lerner 1997). The current method of lysine production is fermentation, generally by mutant strains of the genus *Corynebacterium* (Demain and Solomon 1985).

Corn steepwater is an internal processing stream of the corn wet-milling process. It is produced by the steeping (soaking) of dry corn in water to which a small amount of sulfur dioxide has been added. Steeping releases corn solubles to the steepwater, which is subsequently concentrated by evaporation to ≈50% solids. The resulting liquid, corn steep liquor (CSL), is either used internally as part of the fermentation medium of the wet-milling plant or mixed with corn fiber and incorporated into gluten feed, a protein coproduct of the wet-milling process.

CSL is a rich source of nitrogenous compounds and other nutrients and growth factors (some of which derive from the lactic acid fermentation that occurs during steeping). Detailed compositional analyses of CSL have been published (Christianson et al 1965, Zabriskie et al 1980) that give a free amino acid content of 2.7% (dsb). The low value of corn steep liquor combined with the presence of significant amounts of free amino acids appear to offer opportunities for extracting higher valued products from this process stream.

Ion exchange is commonly used in the analytical separation and quantitation of amino acids due to the amphoteric nature of these compounds. It is also used as a unit operation in the recovery of single amino acids from fermentation broths (Samejima 1972). Of the common amino acids, lysine is one of only three that possess an overall positive charge at pH 7. Thus, it should be possible to employ ion-exchange technology to selectively adsorb lysine from the other amino acids present in corn steepwater. This study was undertaken to investigate the recovery of lysine from corn steepwater by cation exchange. The specific objective was to develop a product with a lysine content significantly greater than current corn milling by-products. The ultimate goal is an economically feasible process for production of a high-lysine feed supplement.

## MATERIALS AND METHODS

### Preparation of Corn Steepwater

CSL (≈50% solids) was obtained from Grain Processing Corp. (Muscatine, IA) and stored frozen until used. Corn steepwater contains 5–10% solids (Blanchard 1992). Thus, for this study, an 8% (w/w) solids solution was chosen as a representative corn steepwater. The steepwater was prepared as follows: corn steep liquor was diluted to 8%, centrifuged to remove suspended solids, and adjusted to pH 7 with NaOH. A precipitate formed which was removed by filtration. As the steepwater was prepared just before use, there was some variability in the feed composition of the steepwater for replicate experiments.

### Ion Exchange Resins

Amberlite IR-120 Plus and IRC-50 ion exchange resins are manufactured by Rohm and Haas Co. (Philadelphia, PA). IR-120 Plus is a gel-type, styrene-divinylbenzene-based, strongly acidic cation exchanger; IRC-50 is a macroporous-type, methacrylic acid-divinylbenzene-based, weakly acidic cation exchange resin. Before use, the resins were thoroughly washed in deionized water to remove impurities and leachable compounds. For operation on the Na cycle, the IR-120 Plus resin was conditioned with 10% NaCl. For IRC-50, the conditioning agent for the Na cycle was 1*N* sodium acetate adjusted to pH 7.0; 4% HCl was used for the H<sup>+</sup> cycle.

Ion-exchange experiments were performed in 11-mm diameter chromatography columns containing a measured amount of resin (2–3 g of dry resin). A peristaltic pump was used to feed all solutions to maintain a constant flow rate. After conditioning with the appropriate solution, the column was rinsed with deionized water (at least 10 bed volumes). The feed solution was then applied, followed by a deionized water rinse of one bed volume. The eluting solution (either 0.05*N*, 0.1*N*, or 1.0*N* HCl, except where noted) was applied and collected in fractions for later analysis. The pH of the column exit was monitored to estimate when elution was complete. After elution, a volume of 1*N* HCl was passed through the column to ensure complete exhaustion of the resin.

Experiments were conducted to determine both the total capacity (cation-exchange capacity) of the resins and the specific lysine capacity at several concentration levels. For total capacity measurement, the resin was conditioned with 4% HCl, and a known amount of resin was introduced into a flask containing a known volume of 0.1*N* NaOH in 5% NaCl. After equilibration, the solution was titrated with 0.1*N* HCl. Lysine capacities were determined in a column mode by recirculating standard lysine solutions through the column containing a known amount of resin for ≈18 hr until equilibrium.

### Analytical

In experiments in which lysine alone was used, lysine was measured spectrophotometrically by the ninhydrin method (Moore and Stein 1954). Equal volumes of sample and ninhydrin-hydrindantoin

<sup>1</sup> U.S. Department of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

<sup>2</sup> Corresponding author: Phone: 215-233-6601; Fax: 215-233-6795; E-mail: dobrien@arserrc.gov

reagent were boiled for 15 min in a water bath, cooled, and read at 570  $\mu\text{m}$ . Concentrations of the major metal ions in corn steepwater (Na, K, and Mg) were determined by atomic absorption spectroscopy with a model 1110B atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, CT). Amino acid analysis of steepwater samples was performed by the Pico-Tag system (Millipore Corp., Waters Chromatography Div., Milford, MA) in which phenylisothiocyanate is employed to derivatize primary and secondary amino acids which are then separated on a reverse-phase liquid chromatography column and detected at 254 nm. Only free amino acids were measured. Before analysis, the sample was adjusted to pH 11, and the samples were lyophilized.

## RESULTS

The IR-120 Plus and IRC-50 resins were first evaluated on the basis of their lysine capacity. As can be seen in Table I, IRC-50 exhibited a somewhat greater lysine capacity with sodium as the exchangeable ion for initial lysine concentrations in the range 35–42 mM. Because of the stronger affinity of the IRC-50 resin for hydrogen ions, the lysine capacity is much lower when the resin is in the hydrogen form (Table I). Based on these results, the IRC-50 resin in the sodium form was chosen for all further experiments.

To examine the dynamics of lysine adsorption and elution during column operation, experiments were performed initially with solutions containing only lysine at pH 7. Lysine solutions at 3.1–3.7 mM (approximately the concentration in corn steepwater) were passed through columns at 0.6 mL/min (0.1 bed volumes/min). As shown in Table II,  $\approx 90\%$  of the lysine is adsorbed under these conditions. Recovery of the adsorbed lysine was examined at two elution levels, 0.05N and 0.1N HCl. The elution of lysine from the resin was reasonably stoichiometric and efficient, resulting in recoveries of 83–100%. These results demonstrate that lysine can be efficiently adsorbed by the IRC-50 resin at the levels found in corn steepwater and effectively recovered therefrom.

Corn steepwater is a complex mixture of proteins, peptides, free amino acids, purines, and pyrimidines, lactic acid, metal ions, vitamins, and other growth factors. Of greatest relevance to the objectives of this study is the presence of metal ions at concentrations of 5–10 times that of the amino acids. The behavior of the three metals present at the highest concentrations in corn steepwater, potassium,

magnesium, and sodium, was followed during steepwater experiments, although it is realized that there are other positively charged species, both inorganic and organic, present which could affect the results of the experiments. The free amino acid composition of the corn steep liquor used in this study was determined analytically (Table III). Even though the alpha amino group of all amino acids is positively charged a pH 7, the only amino acids which carry an overall positive charge are lysine, arginine, and histidine. Amino acid analysis of the eluents from the corn steepwater experiments determined that lysine and arginine were the only amino acids adsorbed to any significant degree, as the histidine level in the steepwater was very low (Table III).

Corn steepwater experiments were conducted at pH 7 with three strengths of the HCl eluent, 0.05N, 0.1N, and 1.0N. The concentration of the eluent is important to the economics of an ion-exchange process as well as affecting further purification of the product, if required. A summary of the amino acid results is presented in Table IV. The degree of lysine recovery (as a fraction of that fed to the column) was in the range 0.07–0.15; for arginine it was somewhat lower, 0.03–0.13.

The amino acid recoveries are determined by the competitive equilibria among all the positively charged species present in the column feed, particularly the metal ions. Table V contains data on typical compositions of the column feed, column exit, and eluent streams for a corn steepwater experiment. On an equivalent basis, the metal ions exceed the amino acid concentrations by a factor of 38 in the corn steepwater feed. However, on a dry basis, the lysine content in the eluent stream was more than triple that in the feed, demonstrating an overall selectivity for lysine (as well as arginine) by the resin. The eluent compositions for all experiments are summarized in Table VI. Some variability in the data may have been introduced with the preparation of the corn steepwater. The steepwater was prepared from the corn steep liquor just before each column run. Given the heterogeneous nature of the steep liquor, the type of pretreatment, and the possibility of chemical, physical, and biological changes during storage of the steep liquor, some variability in the feed compositions can be expected. As expected, the concentrations of the recovered species in the eluent increase with the eluent strength. However, on a dry basis, the amino acid content remained relatively constant in the range 2–5%.

## DISCUSSION

The objective of this study was to recover an enriched lysine product from corn steepwater. Because of its similar chemistry and high isoelectric point, arginine was also adsorbed from the steep-

TABLE I  
Lysine Equilibria with Cation Exchange Resins

| Ion Exchange Resin | Type        | Ionic Form | Initial Lysine (mM) | Lysine Capacity <sup>a</sup> |
|--------------------|-------------|------------|---------------------|------------------------------|
| IR120-plus         | Strong acid | Na         | 14                  | 0.98                         |
|                    |             | Na         | 15                  | 1.03                         |
|                    |             | Na         | 35                  | 2.04                         |
|                    |             | Na         | 42                  | 2.18                         |
| IRC-50             | Weak acid   | Na         | 42                  | 2.98                         |
|                    |             | Na         | 112                 | 5.69                         |
|                    |             | H          | 41                  | 0.64                         |
|                    |             | H          | 41                  | 0.64                         |

<sup>a</sup> Lysine in mmol/g of dry resin.

TABLE II  
Lysine Adsorption and Recovery by Ion Exchange<sup>a</sup>

| HCl (N) | Lysine (mM) |             |        | Lysine Recovery |                     |
|---------|-------------|-------------|--------|-----------------|---------------------|
|         | Feed        | Column Exit | Eluent | %               | mmol/g of dry resin |
| 0.05    | 3.07        | 0.28        | 1.63   | 100             | 1.15                |
| 0.05    | 3.08        | 0.33        | 1.39   | 100             | 1.13                |
| 0.05    | 3.33        | 0.26        | 1.28   | 83.3            | 1.27                |
| 0.1     | 3.70        | 0.39        | 2.61   | 100             | 1.37                |
| 0.1     | 3.72        | 0.43        | 2.46   | 95.5            | 1.35                |

<sup>a</sup> Ion exchange resin: Amberlite IRC-50, Na cycle.

TABLE III  
Free Amino Acid Composition of Corn Steep Liquor

| Amino Acid    | % Weight |
|---------------|----------|
| Asparagine    | 0.571    |
| Aspartic acid | 0.038    |
| Threonine     | 0.227    |
| Serine        | 0.302    |
| Glutamic acid | 0.218    |
| Proline       | 0.704    |
| Glycine       | 0.138    |
| Alanine       | 0.840    |
| Cystine       | 0.013    |
| Valine        | 0.443    |
| Methionine    | 0.189    |
| Isoleucine    | 0.226    |
| Leucine       | 0.795    |
| Tryosine      | 0.181    |
| Phenylalanine | 0.335    |
| Histidine     | 0.012    |
| Lysine        | 0.440    |
| Tryptophan    | 0.000    |
| Arginine      | 0.842    |

water. No attempt was made to design experimental conditions to selectively recover lysine over arginine. Thus the resulting product contained roughly equal levels of lysine and arginine. Although for compounds such as amino acids, pH strongly affects their behavior in ion-exchange reactions, the pH of all experiments was fixed at 7.0 for two reasons. First, at this pH level, effectively, lysine and arginine would be the only amino acids adsorbed. Second, the isoelectric pH of lysine is 9.7, so as the pH increases >7.0, the net positive charge would decrease, thereby reducing its affinity for the resin.

The strength of the eluent stream is an important design parameter in that it affects the operating costs of an ion-exchange process. There is an obvious tradeoff between increasing chemical costs and higher product concentrations in the eluent stream, often leading to reduced purification requirements in downstream operations. Results of this study have shown that low elution levels (e.g., 0.05N HCl) can effectively be used to recover the adsorbed amino acids. Although higher elution strengths result in higher amino acid product concentrations, the lysine content of the product stream on a dry basis is not affected.

Experiments with corn steepwater have shown that only a small fraction (7–15%) of the lysine is removed under the conditions of this study. When evaluated from the perspective of the overall corn wet-milling plant, this low degree of removal is probably desirable, as it should not decrease the value of the CSL. CSL does have value, albeit low, based on its nutritive and protein contents for its uses as a fermentation additive and component of corn gluten feed (an animal feed), respectively. Thus, the recovery

of lysine with only a minimal decrease in the concentrations of two amino acids should not affect the economics of corn wet-milling operations.

The lysine content of the eluent stream, on a dry basis, was 2–5%. Even just considering the positively charged amino acids and the major metals present in the corn steepwater, lysine is selectively recovered from the steepwater feed. The low lysine contents are due the presence of significantly higher concentrations of sodium, magnesium, and potassium ions. The lysine content could likely be significantly increased by procedural or process changes: replacement of NaOH with ammonium hydroxide as the neutralizing agent for the CSL (the sodium in the steepwater is derived predominantly from this operation); use of NH<sub>4</sub>OH as the eluting agent, thereby eliminating chloride in the product (as has been done in the recovery of lysine from fermentation broths) (Shiio et al 1971); or anion exchange to remove chloride.

The presence of arginine in the recovered product in amounts approximately equal to lysine may have advantages in certain applications because arginine is one of five amino acids important in the formulation of diets for growing chickens (Bondi 1987).

This study was conducted to investigate strategies to increase the value of corn wet-milling coproducts through isolation of an enriched lysine product from corn steepwater. The proposed use of the product is as a feed supplement in the formulation of poultry and swine feeds. The lysine content of the product stream reported here (3–6%, db), is significantly greater than either corn (0.29%) (USDA 1989); the primary dry-milling coproduct, distillers' dried grains and solubles (0.78%); or the wet milling feed coproducts

**TABLE IV**  
Recovery of Amino Acids from Corn Steepwater by Ion Exchange<sup>a</sup>

| HCl (N) | Lysine (mmol) |           |                              | Arginine (mmol) |           |                              |
|---------|---------------|-----------|------------------------------|-----------------|-----------|------------------------------|
|         | Column Feed   | Recovered | Fraction Recovered from Feed | Column Feed     | Recovered | Fraction Recovered from Feed |
| 0.05    | 1.03          | 0.13      | 0.13                         | 1.75            | 0.13      | 0.07                         |
| 0.05    | 1.43          | 0.11      | 0.08                         | 2.13            | 0.1       | 0.05                         |
| 0.05    | 1.6           | 0.22      | 0.14                         | 2.82            | 0.12      | 0.04                         |
| 0.01    | 0.74          | 0.11      | 0.15                         | 1.37            | 0.13      | 0.09                         |
| 0.01    | 1.21          | 0.18      | 0.15                         | 2.21            | 0.19      | 0.08                         |
| 0.01    | 2.09          | 0.15      | 0.07                         | 3.66            | 0.12      | 0.03                         |
| 1       | 1.08          | 0.12      | 0.11                         | 1.86            | 0.23      | 0.13                         |
| 1       | 1.38          | 0.18      | 0.13                         | 2.57            | 0.17      | 0.07                         |

<sup>a</sup> Ion exchange resin: Amberlite IRC-50, feed: 8% (w/w) corn steep liquor, pH 7.0.

**TABLE V**  
Typical Stream Compositions for Corn Steepwater Experiments

| Analysis      | Column Feed | db <sup>a</sup> | Column Exit | db <sup>a</sup> | Eluent   | db <sup>a</sup> |
|---------------|-------------|-----------------|-------------|-----------------|----------|-----------------|
| pH            | 7.0         |                 | 7.7         |                 | 6.70     |                 |
| Solids, % w/w | 8.0         |                 | 8.0         |                 | 0.45     |                 |
| Lysine        | 0.45 g/L    | 0.71%           | 0.37 g/L    | 0.58%           | 0.10 g/L | 2.75%           |
| Arginine      | 0.91 g/L    | 1.37%           | 0.79 g/L    | 1.20%           | 0.12 g/L | 3.14%           |
| Magnesium     | 0.80 g/L    |                 | 0.22 g/L    |                 | 0.33 g/L |                 |
| Sodium        | 3.95 g/L    |                 | 4.80 g/L    |                 | 0.87 g/L |                 |
| Potassium     | 3.20 g/L    |                 | 2.60 g/L    |                 | 0.80 g/L |                 |

<sup>a</sup> Results on a dry basis as the hydrochloride.

**TABLE VI**  
Effect of Eluent Strength on the Adsorption and Recovery of Amino Acids and Metals from Corn Steepwater by Ion Exchange<sup>a</sup>

| HCl (N) | Lysine (g/L) | Arginine (g/L) | Magnesium (g/L) | Sodium (g/L) | Potassium (g/L) | Lysine (% db) <sup>b</sup> |
|---------|--------------|----------------|-----------------|--------------|-----------------|----------------------------|
| 0.05    | 0.13         | 0.15           | 0.24            | 0.95         | 0.80            | 3.6                        |
| 0.05    | 0.11         | 0.11           | 0.37            | 1.38         | 1.59            | 2.5                        |
| 0.05    | 0.11         | 0.14           | 0.82            | 2.02         | 2.00            | 2.0                        |
| 0.1     | 0.16         | 0.2            | 0.46            | 1.08         | 1.10            | 3.1                        |
| 0.1     | 0.17         | 0.22           | 1.17            | 1.72         | 2.28            | 2.3                        |
| 0.1     | 0.28         | 0.14           | 1.46            | 1.37         | 1.60            | 4.2                        |
| 1       | 0.56         | 1.34           | 2.03            | 4.42         | 4.68            | 1.4                        |
| 1       | 0.89         | 0.98           | 0.76            | 4.01         | 4.64            | 2.4                        |

<sup>a</sup> Ion exchange resin: Amberlite IRC-50, feed: 8% (w/w) corn steep liquor, pH 7.0.

<sup>b</sup> Results on a dry basis as the hydrochloride.

corn gluten feed (0.73%) and corn gluten meal (1.11%) (Weigel et al 1997). Given the current high selling price of lysine, \$2.28/lb (Lerner 1997), the value of an enriched lysine product should be greater than other corn milling coproducts that are priced primarily on their protein content. Ultimately, the costs of the ion-exchange process, including downstream concentration and drying, will determine whether manufacture of such a product is economically feasible.

#### ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Harold Farrell and Douglas Soroka to the amino acid analyses.

#### LITERATURE CITED

Blanchard, P. H. 1992. *Technology of Corn Wet Milling and Associated Processes*. Elsevier: Amsterdam.  
Bondi, A. A. 1987. *Animal Nutrition*. John Wiley and Sons: New York.  
Christianson, D. D., Cavins, J. F., and Wall, J. S. 1965. Identification and determination of nonprotein nitrogenous substances in corn steep

liquor. *J. Agric. Food Chem.* 13:277.  
Demain, A. L., and Solomon, N. A., eds. 1985. *Biology of Industrial Microorganisms*. Benjamin/Cummings: Menlo Park, CA.  
Lerner, M. 1997. *Chemical Marketing Reporter*. May 12. Schnell: New York.  
Moore, S., and Stein, W. H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* 211:907.  
Samejima, H. 1972. Methods for extraction and purification. In: *Microbial Production of Amino Acids*. K. Yamada, S. Kinoshita, T. Tsunoda, and K. Aida, eds. Kodansha: Tokyo.  
Shiio, I., Sano, K., Nakamori, S., Miyajima, R., and Katsuya, N. 1971. Method of Producing L-Lysine. US patent 3,616,218.  
USDA. 1989. U.S. Department of Agriculture Agricultural Handbook No. 8-20. *Composition of Foods: Cereal Grains and Pasta*. GPO: Washington, DC.  
Weigel, J. C., Loy, D., and Kilmer, L. 1997. Feed co-products of the dry corn milling process. National Corn Growers Assoc.: St. Louis, MO.  
Zabriskie, D. W., Armiger, W. B., Phillips, D. H., and Albano, P. A. 1980. *Traders' guide to fermentation media formulation*. Traders' Protein Co.: Memphis, TN.

[Received November 24, 1998. Accepted August 9, 1999.]