Composition of Corn Steep Water during Experimental Steeping

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To understand better the process of steeping and the potential uses of corn steep water, the composition of corn steep water was analyzed and compared at various times during steeping under four defined experimental conditions. Of particular interest were the analyses of corn steep water for bacterial flora, carbohydrates, amino acids, polypeptides, fatty acids, lactic acid and other organic compounds, hydrolytic enzymes, *myo*-inositol phosphates, heavy metals, and inorganic ions. The experimental model compares favorably with the industrial process, especially with respect to the high bacterial numbers in the steep water with the newest corn.

Keywords: *Corn steep water; compositional analysis; steeping*

INTRODUCTION

Most wet milling industries hydrate corn by counter-current steeping. Steeping consists of three critical features that set up good corn milling; they are, hydration of the kernels, sulfur dioxide-mediated changes in the corn, and a bacterial fermentation (Watson, 1964, 1967). The counter-current designation indicates that the corn proceeds to the mill against the flow of the water for hydration. The two incompatible phases of steeping in counter-current steeping, the fermentation and the sulfur dioxide phases, occur at opposite ends of a series of 10 to 30 steep tanks. The bacterial fermentation in steeping occurs at maximum rates at the end of the series where the new (driest) corn is added into the oldest water. During the 20 to 40 h of steeping, the corn is soaked in water with increasing sulfur dioxide concentrations that ultimately reach concentrations that inhibit the fermentation and kill the bacteria. The sulfur dioxide is added with the newest water to the oldest corn just before milling. A laboratory, batch steeping method has been constructed to compare the process with counter-current steeping and to limit the variables and the microbial contamination inherent with the use of multiple tanks in series.

MATERIALS AND METHODS

Materials. The sources of materials were as follows: the ion exchange resins, AG 50(H^+) and AG3(OH^-), from BioRad (Hercules, CA); the CarboPAc HPLC column from Dionex Corp. (Sunnyvale, CA); the sodium hydroxide solution (50%) for HPAEC-PAD from Fisher Scientific (Pittsburgh, PA); the OV-1 and DB-5 capillary gas chromatographic columns from J&W Scientific (Folsom, CA); the APIZYM kit from BIO MERIEUX SA (France); the amylase substrate, 4,6-ethylidene-(G7)-*p*-nitrophenyl(G1)-α-D-maltoheptaoside, from Boehringer Mannheim, (Indianapolis, IN); the sodium and lithium high-performance columns for total and free amino acid analysis, respectively, from Beckman (Palo Alto, CA); the C-18 reversed-phase HPLC column from Vydac (Hesperia, CA); a panel of saturated fatty acid methyl ester standards from Sigma (St. Louis, MO); and sugar standards from Pfanstiel (Waukegan, IL). Standard *myo*-inositol phosphates and lactic acid were obtained from Sigma Chemical Company (St. Louis, MO) or Calbiochem (San Diego, CA) and were shown to be chromatographically pure (>95%) by IP-RP-HPLC or HPAEC-CD. All other chemicals were of the highest purity available.

Bacteria. *Lactobacillus rhamnosus* ATCC 15820, along with *L. rhamnosus* ATCC 7469, and *L. fermentum* ATCC 8289 were obtained from the American Type Culture Collection (Rockville, MD) for comparisons with the industrial steep lactobacilli. Steep lactobacillus strains were isolated on germinated corn agar from industrial steep water. Following incubation of the plates at 45 °C for 48 h, the predominant colony morphology was 1 to 2 mm diameter, transparent, flat, and rough. Bacteria from these colonies were Gram-positive, rod-shaped, 6−10 μm long, and demonstrated chains up to two to three cells. The bacteria were nonmotile, catalase negative, fastidious, and did not form spores. These characteristics were consistent with a previous description of the steep lactobacillus (Kuznetsov, 1959). Single colonies were selected on the basis of date of isolation, industrial source, and subtle differences in colonial morphology to be catalogued as isolates and frozen in 50% glycerol at −70 °C for use in steeping experiments. In these studies an isolate identified as UI-2 was used.

Culture Media. Germinated corn medium (GCM) was made with corn that had been allowed to germinate in a moist humidor for three days at room temperature. The germinated corn was blended (Waring blender at high speed) with tap water (200 mL of packed corn with 400 mL of water) and made into an 800-mL slurry. The slurry was mixed with 20 g of yeast autolysate, 10 g of yeast extract, 15 g of proteose peptone (no. 3) and 20 g of agar. The volume was increased to 1 L, the pH was adjusted to 5.5, and the slurry was autoclaved to be poured as the bottom phase of a biphasic medium in plates. Top agar for the biphasic media consisted of 10 g of yeast extract, 10 g of tryptic soy broth, 20 g of dextrose, and 20 g of agar per liter (pH 5.5). Bacteria were also grown in MRS (Demain et al., 1960) broth and agar (Difco, Detroit, MI), in MRS medium with 40% industrial steep water (SW-MRS), and in MRS medium with both 40% steep water and 40% tomato juice (TJSW-MRS). These media were also prepared as top and bottom phases of biphasic media and were adjusted to pH 6.0 before autoclaving. Laboratory steep water (LSW) was constructed by adding 20% industrial steep water, 0.5% dextrose, and sterile tap water to yield a volume of 250 mL, and combined with 170 g of sodium bisulfite-disinfected corn.

Steeping Methods. Laboratory steeping, based on the method reported by Watson et al. (1951), was conducted in cylindrical glass tanks (55-mm diameter, 225-mm height, 1/60th reduction of an industrial tank) closed by no. 11 rubber stoppers. The glass steep tanks, stoppers, and circulating tubing were sterilized by autoclaving and were covered with aluminum foil until used. Dried corn (Gringer Seed Company, Iowa City, IA; 170 g) was cleaned of fines and placed in each

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sterile steep tank. Disinfection of the corn in the tank involved three consecutive washes with 300-mL volumes of 0.2% sodium bisulfite in tap water (pH 4.0), and incubating each wash for 1 min at room temperature. The bisulfite was washed away with two-300-mL volumes of sterile tap water so that the LSW medium could be constructed in the tank.

Sterile Steep Experiment. The tank was sealed with a stopper and placed in a 52 °C water bath, and the mixing pump started at 120 mL/min. Mixing the steep tanks consisted of drawing water from the bottom of the steep tank (Masterflex pump, Cole-Parmer, Chicago, IL) through a tube (3 mm i.d.) in the stopper for addition back to the top of the tank and was designed to be similar to the steep water recirculation in industrial tanks. After 20 and 40 h, corn steep water was recovered from this steep experiment, designated ES-1 and ES-2, respectively, and subjected to analyses.

SO_2 Experiment. After 20 h of steeping, a syringe pump containing 4.44% sodium bisulfite (7.1 g/100 mL of 65.55% sodium bisulfite) was started at 0.81 mL/h to construct a linear gradient of bisulfite over the subsequent 20 h. Bisulfite was measured by the pararosanaline assay (Miwak and Okuda, 1977; Westy and Gaeke, 1956). After 40 h of incubation, the tanks were drained and the corn was removed for milling (Peters et al., 1996). Corn steep water recovered at the 20- and 40-h time points from this steep experiment is designated ESA-1 and ESA-2, respectively.

Lactic Acid Experiment. Lactic acid [21.9% solution from T_0 to T_{20} (0.57 mL/h); 11.0% solution from T_{20} to T_{40} (0.57 mL/h)] was pumped into the steeping tank from the beginning of the experiment, increasing the concentration to 10 mg/mL at 20 h and 15 mg/mL at 40 h. Corn steep water recovered at the 20- and 40-h time points from this steep experiment is designated ESB-1 and ESB-2, respectively.

Bacteria-SO_2 Steep Experiment. Steep lactobacilli UI-2 were grown from freezer stocks for inoculating laboratory steep tanks. The initial biphasic MRS/TJ-SW-MRS medium (2 mL) following static incubation in screw-capped tubes at 45 °C for 24 h was inoculated into 100 mL of top MRS broth in a total of 200 mL of MRS/TJ-SW-MRS medium and incubated for 18 h at 45 °C. The bacteria were harvested by centrifugation at 7000 rpm for 15 min, suspended in 25 mL of sterile tap water, and added to the laboratory steep tank. After 20 h of incubation, a syringe pump containing 4.44% sodium bisulfite (7.1 g/100 mL of 65.55% sodium bisulfite) was started at 0.81 mL/h to construct a linear gradient of bisulfite over the subsequent 20 h. Bisulfite was measured by the pararosanaline assay. After 40 h of incubation, the tanks were drained and the corn was removed for milling (Peters et al., 1996). Corn steep water recovered at the 20- and 40-h time points from this steep experiment is designated ESC-1 and ESC-2, respectively.

Steep Water Analysis. Samples (5 mL) were taken from the recycle tubing at 20- and 40-h time points during laboratory steeping. The pH was determined immediately, and a 1-mL portion of the sample was mixed with 9 mL of 0.04 M tetrachloromercurate (TCM) to stabilize the bisulfite (Westy and Gaeke, 1956) for later analysis by the pararosanaline assay. A portion of the sample (1 mL) was reserved for dilution in sterile tap water for triplicate determinations of viable bacteria by plating on GCM. Plates were incubated at 45 °C for 2 days before counting colonies.

The samples were either clarified by centrifugation at 1000*g* for 15 min and/or filtered with a no. 1 Whatman filter or a 0.2-μm filter. The clear supernatant was stored at −20 °C until analyzed.

In preparation for carbohydrate or organic acid analyses, the steep water was subjected to ion-exchange treatment to separate the organic acid (anionic fraction) and the carbohydrate (neutral fraction). Clarified corn steep water was first passed over a cation exchange column (AG50, H^+, 1.5 × 3.0 cm). The column was washed with water (25−30 mL), and the washings, after adjustment to pH 8, were then passed over an anion-exchange column (AG3, OH^-, 1.5 × 3.0 cm). The column was washed with water (25−30 mL), and the neutral fraction, containing the carbohydrate, was recovered; its volume was measured to determine the dilution factor. The components of corn steep water that bound to the anion exchange resin (hereafter referred to as the anionic fraction) were eluted with 1.5 M pyridinium acetate (30 mL), and the eluate was lyophilized.

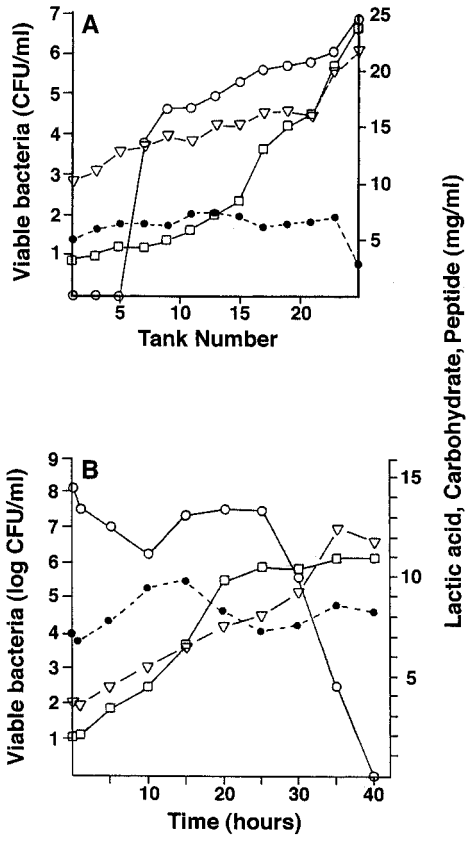

Figure 1. Comparison of the laboratory model with industrial steeping. Steep water taken from the tops of industrial steep tanks (A) and from a laboratory steep tank at increasing times (B) were analyzed for viable bacterial numbers (○) and lactic acid (□) by HPLC (Hull et al., 1996); peptides (▽) by the Lowry assay (Lowry et al., 1951); and carbohydrates (●) by the anthrone assay (Hanson and Phillips, 1981).

Analytical Procedures. Methods and instrumentation used in analyses for carbohydrates, hydrolytic enzymes, amino acids, polypeptides, nonprotein nitrogenous components, lactic and glycolic acid, *myo*-inositol phosphates, heavy metals, inorganic ions, fatty acids, and lipophilic components have been described in detail elsewhere (Hull et al., 1996; Hull and Montgomery, 1995).

RESULTS AND DISCUSSION

A laboratory model for steeping was constructed to study the fermentation and corn hydration phases of steeping. A comparison between industrial counter-current steeping and the laboratory batch method is displayed in Figure 1. In the counter-current process (Figure 1A), new corn progresses to the mill (to the lower tank numbers) while new water (mill water laced with bisulfite) progresses to the higher tank number. The maximum fermentation, noted by the highest bacterial numbers, occurs in the oldest water containing the lowest bisulfite concentrations in the industrial tanks. Industrial steeping does not start with pure water, rather with the combined washings from the preparation of starch, gluten, and other fractions of corn. Consequently, analysis of the early industrial steeps gives constituents that were in the feed water and the counter-current movement adds further to the difficulty in interpreting the steep water constituents. This difficulty does not occur in the laboratory batch method.

Table 1. Experimental Corn Steep Descriptions

stream	description	pH	CHO (g/L)[a]	protein (g/L)[b]
ES-1	sterile steep (21 h)	6.0	10.8	7.1
ES-2	sterile steep (40 h)	6.0	14.8	11.3
ESA-1	SO_2 steep (21 h)	5.9	14.2	8.1
ESA-2	SO_2 steep (40 h)	5.7	16.2	3.1
ESB-1	lactic acid steep (21 h)	3.5	12.6	2.7
ESB-2	lactic acid steep (40 h)	3.5	19.0	5.5
ESC-1	UI-2 + SO_2 steep (21 h)	3.6	7.4	8.0
ESC-2	UI-2 + SO_2 steep (40 h)	3.8	9.6	10.2

[a] The clarified streams were analyzed for total carbohydrate by the phenol−sulfuric acid assay (Dubois et al., 1956); expressed as glucose equivalents. [b] From the acid hydrolysis of each stream and includes both free amino acids, peptides, and proteins.

The batch method avoids any accumulation of compounds from previous steep cycles so that the compounds appearing in the water result from one set of experimental conditions (Table 1). The laboratory steep tank was inoculated with high numbers of bacteria to initiate a fermentation in the newest corn (Figure 1B). The major difference between the industrial and laboratory methods was the age of the corn; the oldest corn in the industrial process occurred in the low number tanks (Figure 1A) in contrast to the newest corn at early times of laboratory steeping (Figure 1B). Therefore, the disinfection of the corn by bisulfite occurred at opposite ends of the two processes. However, the water flow of industrial steeping resulted in both steeping systems showing similarities in the production of lactic acid from the bacteria as well as the accumulation of carbohydrates and peptides from the corn over the course of increasing tank numbers (Figure 1A) and increasing time (Figure 1B) for industrial and laboratory steeping, respectively.

The concentrations of the laboratory steep water components ranged from one-third to one-half of the industrial levels (Figure 1). The differences in these concentrations reflect the enormous scale of the industrial process, involving tanks containing thousands of bushels of corn with repetitive cycling of the steep water over multiple tanks of corn. The industrial water contains compounds left over from previous steep runs, and these mask the appearance of compounds from the fresh corn presently being steeped.

With the batch method, different steeping conditions were enacted to compare the contribution of the individual treatments with the complete steeping conditions employing both bacteria and bisulfite (Figure 1). Figure 2 reveals the higher levels of carbohydrate in steeps during sterile steeping, without additions of bacteria or chemicals (A), with the addition of lactic acid only (B), and with the addition of bisulfite only (C). The higher levels of carbohydrates in these steeps show the effects of the fermentation in the laboratory steep inoculated with bacteria (Figure 1B) and in the industrial steeping process (Figure 1A). Conversely without the bacterial fermentation process, the peptide concentrations (Figure 2) were one-half to one-third the amounts observed when steeping was conducted with bacteria (Figure 1). These general trends in appearance of bacterial product and corn hydration products were analyzed in greater detail.

Carbohydrates of Corn Steep Water. In all four experimental steeps, the total carbohydrate increases with steeping (Table 1). The carbohydrate seems to be higher in all steeps that do not contain the microorganisms, which is indicative of the utilization of sugar by the microorganisms as nutrient and conversion to lactic acid.

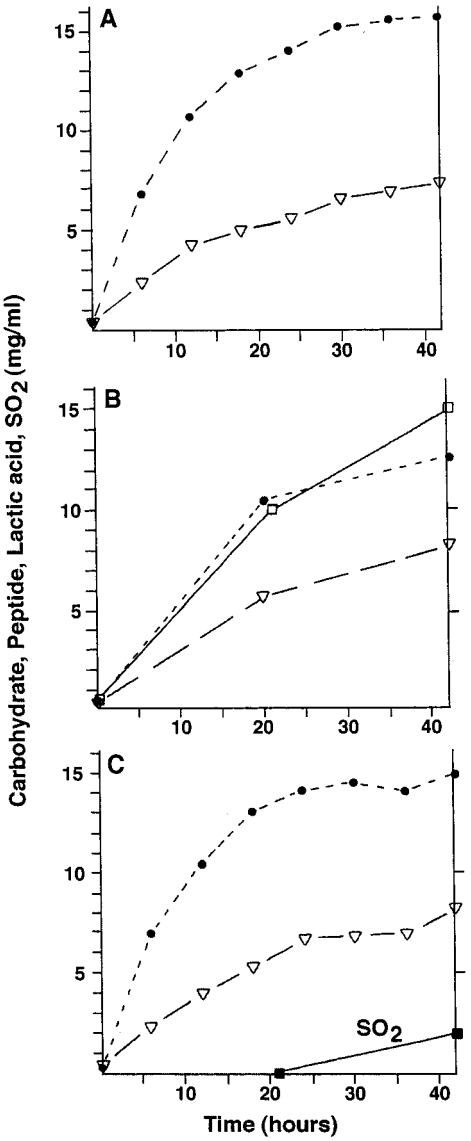

Figure 2. Steep water composition during laboratory steeping in the absence of bacteria. Laboratory steep tanks were started without the addition of bacteria or chemicals (A), with the addition of a gradient of lactic acid only (B), and the addition of a gradient of bisulfite only (C). Water was removed at times and assayed for lactic acid (□) by HPLC, for bisulfite (■) by the pararosaniline assay, for peptide (▽) by the Lowry assay, and for carbohydrate (●) by the anthrone assay.

The carbohydrates in each of the steeps were analyzed quantitatively by HPAEC-PAD (Table 2). The identification of the sugars was based on their chromatographic behaviors compared with carbohydrate standards by HPAEC-PAD and to their TMS derivatives by GLC. The oligosaccharides were also hydrolyzed with 2 N TFA, and the component sugars were identified (Table 3). Glucose and fructose are the predominant monosaccharides, with the exception of ESC (Table 1). In this experimental steep, the fructose is either not present (early) or greatly diminished (late). Smaller amounts of galactose and arabinose are present in every steep sample but, unlike in the industrial steep (Hull et al., 1996), there is no evidence for the presence of xylose in the experimental steeps. The oligosaccharides present in every experimental steep were trehalose, melibiose, and raffinose; sucrose and maltose were found in some (Table 2). There were little, if any, of the higher oligosaccharides in any of the steeps as determined by HPAEC-PAD (data not shown), an

Table 2. Simple Carbohydrates of Corn Steep Water[a]

	monosaccharides (g/L)				di- and trisaccharides (g/L)				
stream	Ara	Gal	Glc	Fru	trehalose	sucrose	melibiose	raffinose	maltose
ES-1	0.08	0.26	4.92	4.60	0.07	0.11	0.20	0.16	–
ES-2	0.13	0.46	7.24	5.39	0.10	0.13	0.19	0.28	–
ESA-1	0.07	0.11	3.59	2.85	0.03	–	0.25	0.02	–
ESA-2	0.06	0.29	4.63	2.84	0.05	–	0.19	0.02	–
ESB-1	0.04	0.12	2.63	2.90	0.04	–	0.12	0.19	–
ESB-2	0.10	0.30	3.91	4.47	0.07	0.07	0.12	0.24	0.02
ESC-1	0.07	0.17	2.03	—[b]	0.04	0.08	0.06	0.27	0.04
ESC-2	0.08	0.22	1.66	0.17	0.05	0.07	0.04	0.22	0.02

[a] Mono-, di-, and trisaccharides were identified and quantitated by HPAEC-PAD. [b] Not detected.

Table 3. Complex Carbohydrates of Experimental Corn Steep Water[a]

		concentration, g/L			% of oligomeric
stream	sugar	total[b]	free[c]	oligomeric[d]	as polysaccharide[e]
ES-2	Ara	0.64	0.13	0.51	100
	Gal	1.60	0.46	1.14	81
	Glc	19.16	7.24	11.92	97
ESA-2	Ara	0.56	0.06	0.50	100
	Gal	1.37	0.29	1.08	89
	Glc	15.75	4.63	11.12	98
ESC-2	Ara	0.22	0.08	0.14	100
	Gal	0.33	0.22	0.11	18
	Glc	2.81	1.66	1.15	83

[a] Complex carbohydrate of selected corn steeps was determined from the difference between the carbohydrate occurring as mono-, di-, and trisaccharide from the total acid hydrolyzate of the sample. [b] Determined by HPAEC-PAD after 2 N TFA hydrolysis. [c] Determined by HPAEC-PAD before 2 N TFA hydrolysis. [d] Total − free (i.e., oligo- and polysaccharides). [e] [Oligomeric − (sum of sugar expected from di- and trisaccharides)]/oligomeric × 100.

analytical procedure that separates the malto-dextrin series up to a 30-mer (Koizumi et al., 1989). However, acid hydrolysis of some of the steeps produced increases in arabinose, galactose, and glucose that exceeded the amount of these sugars expected from hydrolysis of the di- and trisaccharides present (Table 3). These results suggest that polysaccharides, composed of arabinose, galactose, and/or glucose, are present in the steep.

In the long process of steeping at an elevated temperature there are many reactions occurring during the extraction processes. The steeping, if done in the presence of lactic acid or lactic acid-producing microorganisms, is under acidic conditions (pH 3.5−4.3, Table 1). In addition, several enzymes have been demonstrated to be present. These enzymes are principally the glycohydrolases, α-amylase, β-D-galactosidase, β-D-hexosaminidase, and α-D-glucosidase, which, except for the latter, are consistently present throughout the steeping. It is noted in this complex system that, in general, as the steeping proceeds, the amounts of arabinose, glucose, and galactose increase. These increases are most likely due to acidic and/or enzymatic hydrolysis of cell wall hemicelluloses (Wilkie, 1979) extracted from the corn during steeping. Of note is the observation that in the steep in which the glucose concentration decreased as the steeping progressed and the fructose concentration was very low (ESC, Table 2), the di- and trisaccharides containing glucose and/or fructose also decreased. The decreased glucose and fructose concentration in this experimental steep (i.e., the only one with microorganisms) is due to their utilization by lactobacilli in fermentative processes.

Trehalose, a common component of several fermentation processes (Hull et al., 1995), has not been reported previously in corn, but is found in all experimental steeps.

Amino Acids, Peptides, and Nonprotein Nitrogenous Components of Corn Steep Water. Total amino acid analysis (Table 4) of corn steep water revealed a composition rich in glutamic acid/glutamine, leucine, proline, and aspartic acid/asparagine, with low levels of lysine, cysteine, and methionine, a composition characteristic of the various aqueous-soluble corn proteins (Wilson, 1987). A comparison between the sterile steep (ES) and the steep most similar to industrial steep (ESC) provides further evidence in support of the notion that the majority of the amino acid content in steep water originates from the corn rather than from fermentation products (Hull et al., 1996). Comparison within experimental steeps shows the amino acid content increases with steeping time, with the exception of ESA. This result could suggest that increased SO_2 levels at later steep times could be detrimental to extraction of protein and polypeptides in late steep.

From the free amino acid content (Table 4) of corn steep water it is clear that polypeptides are present. RP-HPLC analysis (Table 5; Figure 3) shows that the polypeptides in the four steeps were similar, with two major peptides (**2** and **5**) common to all experimental steeps. There are several minor peptides (**2b** and **3a**) that are unique to the ESB and ESC steeps, respectively. The total polypeptide content of corn steep water increased during steeping (Table 5) and, in turn, two major peptides showed an increase during steeping, except in ESC in which peptide **5** and the total peptide content decreased. This result is in contrast with the industrial steep polypeptide content which is ∼10 times greater (Hull et al., 1996). Enzymatic activities provided no evidence for proteases during steeping; however, the length of industrial steeping time (up to 30 h) coupled with the increased temperature (52 °C) and the presence of microorganisms could contribute to the enhancement of proteolytic activity during steeping.

Analysis of the corn steep samples for nonprotein nitrogenous components (Table 4) demonstrated the presence of significant amounts of phosphoserine and γ-aminobutyric acid in most of the corn steeps analyzed.

Lactic and Glycolic Acid of Corn Steep Water. Lactic acid is the most prominent organic constituent of corn steep water (Wright, 1987), an observation consistent with our analysis of the anionic fraction from four different industries (Hull et al., 1996) and with those analyses of the experimental steeps (Table 6). Glycolic acid was also present at 20−100-fold lower concentrations than the lactic acid. The L-lactic acid content was compared with the total lactic acid, from which the D-lactic acid isomer was estimated. The ES steeps contained mostly D-lactate, whereas the ESC steeps contained a mixture of D- and L-lactate (Table 6) in a ratio consistent with that seen in the industrial steeps (Hull et al., 1996). Of significance is the presence of predominantly D-lactate in the early times of the

Table 4. Total and Free Amino Acid Composition of Experimental Corn Steep Water[a]

	amino acid (g/L)																		
stream	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Cys	Ile	Leu	Tyr	Phe	His	Lys	Arg	P-ser[b]	γ-ABA[c]
ES-1	0.9	0.3	0.3	0.8	1.0	0.4	0.6	0.3	<0.1	0.2	0.3	0.5	0.2	0.2	0.2	0.4	0.5	0.03	0.60
	0.9	*0.1*	*0.1*	*0.3*	*0.9*	*0.1*	*0.4*	*0.1*	*0.1*	–[d]	*0.1*	*0.3*	*0.1*	*0.1*	*0.1*	*0.2*	*0.2*		
ES-2	1.3	0.5	0.5	1.3	1.3	0.7	0.9	0.5	0.2	0.3	0.5	0.7	0.4	0.3	0.3	0.7	0.9	0.05	1.10
	1.3	*0.2*	*0.2*	*0.5*	*1.3*	*0.2*	*0.6*	*0.3*	*0.1*	*<0.1*	*0.3*	*0.6*	*0.2*	*0.2*	*0.1*	*0.5*	*0.5*		
ESA-1	1.0	0.3	0.3	1.0	1.3	0.5	0.7	0.3	0.1	0.3	0.3	0.5	0.2	0.2	0.2	0.4	0.5	0.03	0.70
	0.9	*0.1*	*0.1*	*0.3*	*1.1*	*0.1*	*0.4*	*0.1*	*0.1*	–	*0.1*	*0.3*	*0.1*	*0.1*	–	*0.2*	*0.2*		
ESA-2	0.4	0.1	0.1	0.4	0.4	0.2	0.2	0.1	<0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.3	0.09	0.30
	0.3	*0.1*	*0.1*	*0.1*	*0.3*	*0.1*	*0.2*	*0.1*	*<0.1*	–	*0.1*	*0.2*	*0.1*	*0.1*	*0.1*	*0.1*	*0.2*		
ESB-1	0.3	0.1	0.1	0.4	0.3	0.2	0.2	0.1	<0.1	0.1	0.1	0.3	0.1	0.1	–	0.2	0.2	0.01	0.17
	0.3	–	–	*0.1*	*0.3*	–	*0.1*	*0.1*	*<0.1*	–	*0.1*	*0.2*	*0.1*	*0.1*	–	*0.1*	*0.1*		
ESB-2	0.5	0.2	0.3	0.7	0.5	0.3	0.4	0.3	0.1	0.1	0.2	0.6	0.2	0.2	–	0.4	0.5	0.01	0.27
	0.5	*0.1*	*0.1*	*0.2*	*0.4*	*0.1*	*0.2*	*0.1*	*0.1*	–	*0.1*	*0.5*	*0.1*	*0.2*	–	*0.3*	*0.3*		
ESC-1	0.7	0.3	0.4	1.2	0.8	0.4	0.6	0.4	0.1	0.2	0.3	0.9	0.3	0.3	0.1	0.4	0.6	0.05	0.31
	0.7	*0.1*	*0.2*	*0.4*	*0.6*	*0.1*	*0.4*	*0.3*	*0.1*	*<0.1*	*0.2*	*0.9*	*0.2*	*0.3*	*0.1*	*0.3*	*0.5*		
ESC-2	0.8	0.4	0.5	1.5	0.9	0.5	0.7	0.5	0.2	0.3	0.4	1.2	0.4	0.4	0.2	0.5	0.8	0.28	0.34
	0.8	*0.2*	*0.2*	*0.5*	*0.6*	*0.1*	*0.5*	*0.3*	*0.2*	–	*0.3*	*1.1*	*0.3*	*0.4*	*0.1*	*0.4*	*0.6*		

[a] Total amino acid composition of the early and late fractions from four different experimental steeps was determined, after acid hydrolysis, with a Beckman 6300 amino acid analyzer; free amino acid composition was determined after deproteinization by ultrafiltration (free amino acid contents are italicized). [b] Phosphoserine. [c] γ-Aminobutyric acid. [d] Not detected.

Table 5. Polypeptide Analysis of Experimental Corn Steep Water

	polypeptide (μmol/L)[a]							
stream	1	2	3	4	5	6	7	total
ES-1	0.28	0.55	0.05	0.16	0.37	0.11	0.10	1.62
ES-2	0.42	0.90	–[b]	0.24	0.76	0.10	0.10	2.52
ESA-1	–	0.27	–	0.09	0.25	0.08	0.05	0.74
ESA-2	–	0.53	–	0.14	0.29	0.07	0.09	1.12
ESB-1	–	0.42	0.05	0.04	0.26	–	0.13	0.90
ESB-2	–	0.72	0.10	0.06	0.38	–	0.12	1.38
ESC-1	–	1.67	0.17	0.09	0.88	–	–	2.81
ESC-2	–	1.74	0.24	0.09	0.47	–	–	2.54

[a] The peptides were separated by RP-HPLC (Figure 1) and were quantitated with respect to a tetrapeptide, Leu-Val-Trp-Ser, added as an internal standard; peaks are numbered on the basis of their order of elution. [b] Not present.

sterile steep, which must arise from the corn itself because no bacteria were detected in the sterile steep. Furthermore, comparison of the lactate levels between ES and ESC steeps revealed an increased amount in the ESC steep that could only be due to the production of lactate by the steep lactobacilli.

Lipophilic Components of Corn Steep Water. Lipophilic components of corn steep water extracted by dichloromethane comprised <0.1% of the total dry weight. Evidence for fatty acids was present only in ESB and ESC steeps, and the composition was similar to those in the industrial steeps (Hull et al., 1996).

***myo*-Inositol Phosphates of Corn Steep Water.** Analysis of experimental steep water for *myo*-inositol phosphates (Table 7) showed significant quantities of InsP3 and InsP4, which is consistent with observations of InsPs in industrial corn steepwater (Hull and Montgomery, 1995). Of particular note is the correlation of increased amounts of InsPs in the steeps with more acidic pH (ESB and ESC versus ES and ESA). The increased amount of InsPs in ESB-1, ESC-1, and ESC-2 suggests that the acidic pH of these steeps (Table 1) led to increased extraction of the InsPs from the corn. The decreased InsP content of ESB-2 was unexpected.

Heavy Metals and Inorganic Ions of Corn Steep Water. Iron is the most prevalent heavy metal present in corn steep water (Table 8) and is more prominent in the steeps containing lactobacilli. Though chromium and cadmium were not detectable, copper and nickel were present at ~5−100% of the concentration of iron. Lead was not a significant component of any of the experimental steeps. Physiologically relevant ions, such as potassium, sodium, inorganic phosphate, and chloride, were detected in corn steep water at significant levels. However, unlike in the industrial steeps, potassium was not the most prevalent (Table 8; Hull et al.,

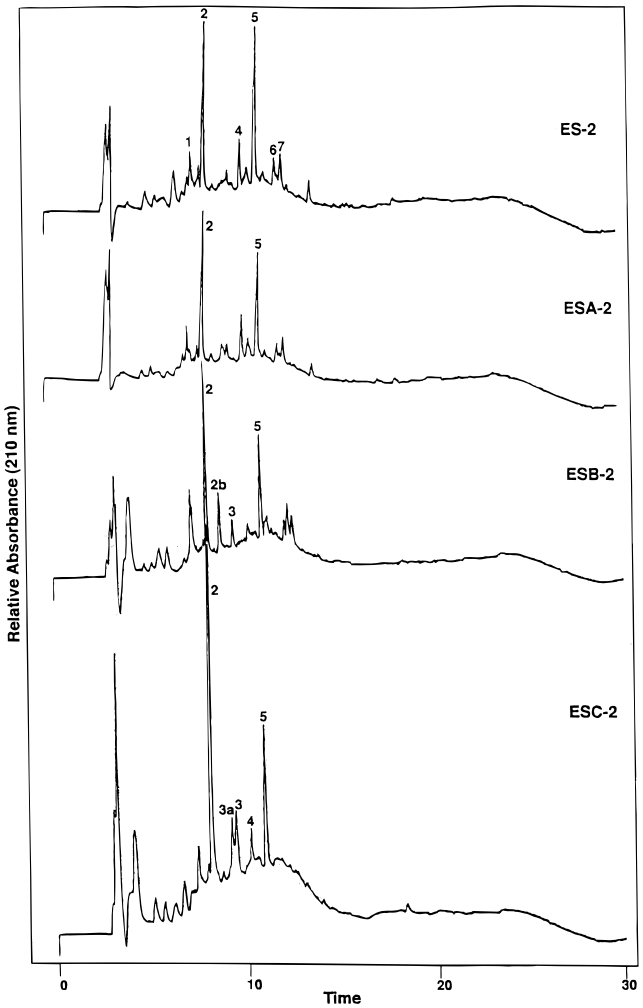

Figure 3. RP-HPLC analysis of the polypeptides of experimental corn steepwater. Late corn steepwater from each of four different experimental corn steeps was diluted as indicated and an aliquot (100 μL) was analyzed for polypeptides by RP-HPLC. Peak numbers were assigned on the basis of increasing elution time (see Table 5).

Table 6. Lactic and Glycolic Acids in Experimental Corn Steep Water[a]

		lactic acid, (g/L)		
stream	glycolic acid (g/L)[b]	total[b]	L-isomer[c]	D-isomer[d]
ES-1	0.1	2.5	0.1	2.4
ES-2	0.2	2.7	0.1	2.6
ESA-1	0.2	2.2	0.1	2.1
ESA-2	0.3	2.7	0.3	2.4
ESB-1[e]	0.1	10.7	6.0	4.7
ESB-2[e]	0.2	19.4	11.1	8.3
ESC-1	0.1	6.7	6.1	0.6
ESC-2	0.2	16.0	6.6	9.4

[a] Lactic and glycolic acids of early and late experimental corn steep were separated and identified by GLC-MS and quantified by GLC-FID. [b] Determined by GLC analysis. [c] Determined with L-lactate dehydrogenase assay (Hohorst, 1963). [d] Determined by difference of the determinations in *b* and *c*. [e] Lactic acid was added over the period of the steep, which makes the significance of the results questionable.

Table 7. *myo*-Inositol Phosphates of Experimental Corn Steep Water[a]

	myo-inositol phosphates (g/L)				
stream	IP3	IP4	IP5	IP6	total
ES-1	0.01	0.13	0.17	0.32	0.63
ES-2	0.03	0.23	0.22	0.49	0.97
ESA-1	—[b]	0.11	0.11	0.14	0.36
ESA-2	0.03	0.20	0.29	0.79	1.31
ESB-1	–	0.14	0.32	3.5	3.96
ESB-2	0.05	0.11	0.12	0.60	0.88
ESC-1	0.03	0.23	0.47	3.10	3.83
ESC-2	–	0.16	0.47	3.80	4.43

[a] The InsP content of early and late fractions of four different experimental corn steeps was determined by IP-RP-HPLC. [b] Not detected.

Table 8. Inorganic Components of Experimental Corn Steep Water[a]

	inorganic component							
stream	Fe[b]	Cu[b]	Ni[b]	Pb[c]	K^{+}[d]	P_i[d]	Na^{+}[d]	Cl^{-}[d]
ES-1	0.25	0.23	0.20	0.0	0.9	0.1	—[e]	–
ES-2	0.70	0.37	0.28	<6.0	1.2	0.2	–	–
ESA-1	0.08	0.32	0.18	0	0.2	1.4	0.1	0.8
ESA-2	0.72	0.36	0.31	<1.0	1.4	2.6	0.3	1.5
ESB-1	1.12	0.21	0.17	0	0.2	1.4	6.1	1.1
ESB-2	2.20	0.13	0.21	<1.0	0.2	2.3	11.2	1.7
ESC-1	2.97	0.41	0.24	0	0.3	2.2	6.1	1.7
ESC-2	6.36	0.40	0.29	<1.0	1.6	2.7	6.6	2.0

[a] The heavy metals present in the indicated experimental corn steep water fractions were ascertained by atomic absorption spectroscopy; the inorganic ions were determined by ion-selective electrochemistry (Cd and Cr were not found above background levels). [b] Expressed in mg/L. [c] Expressed in μg/L. [d] Expressed in g/L. [e] Not detected.

1996). The inorganic phosphate was in part a product of dephosphorylation of InsPs found in corn steep water (Hull and Montgomery, 1995), a hydrolytic process facilitated by the presence of phosphatases in corn steep water.

CONCLUSION

The primary objective of the steeping process is to enable an efficient milling of dried corn with good fractionation of the starch, protein, and oil components. Somewhat related is the quality of the corn steepwater, which is dependent on the reactions that proceed in the process. These reactions principally involve the roles of the SO_2, bacterial flora, and the resulting lactic acid, acting together in a complex system. The effects of each of these factors could not be analyzed in the industrial steeps. The experimental batch steeping method has permitted an evaluation of the effect of each factor on the composition of the resulting steepwater. Bisulfite-disinfected corn served to support the growth of the inoculated steep lactobacilli without introducing contaminating microorganisms. Steep tanks that were run without any additions (i.e., bacteria, bisulfite or lactic acid) did not reveal detectable bacteria following 48 h of steeping.

It is clear that much of the composition is determined principally by the sterile aqueous extraction of corn, the carbohydrate being similar in amount and composition, but the total amino acid content was significantly less in the batch process. Also extracted by the sterile steep were the same inorganic ions, except Na^{+} and Cl^{-}. It was interesting to note the presence of glycolic and lactic acids in the sterile steep, suggesting that corn has a significant amount of endogenous lactic and glycolic acid extractable by the steeping process. A notable difference in the sterile steeping was the amount of amino acid-polypeptide extracted. This amount is not significantly changed by the inclusion of SO_3^{2-} or lactic acid, and the amounts do not approach the industrial steeps until both SO_3^{2-} and bacteria are included, at which point the carbohydrates are reduced at the expense of the production of lactic acid.

It was of interest to evaluate the milling efficiency of the experimentally steeped corn. The results (Peters et al., 1996) showed that the yield of starch increased from 55% in the sterile steep to 71% in the steep with bacteria and SO_2 (Figure 1B), and lactic acid and SO_2 alone gave intermediate yields.

ABBREVIATIONS USED

BCA, bicinchoninic acid; GLC-FID, gas−liquid chromatography with flame ionization detection; GLC-MS, gas-liquid chromatography with mass spectral detection; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; InsP, *myo*-inositol phosphate; InsP3, *myo*-inositol trisphosphate; InsP4, *myo*-inositol tetrakisphosphate; InsP5, *myo*-inositol pentakisphosphate; InsP6, *myo*-inositol hexakisphosphate; IP-RP-HPLC, ion-pair reversed-phase high performance liquid chromatography; RP-HPLC, reversed-phase high-performance liquid chromatography; TMS, trimethylsilyl; GCM, germinated corn medium; MRS, Difco medium; SW-MRS, steep water added to MRS; TJSW-MRS, steep water and tomato juice added to MRS; LSW, laboratory steep water.

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