

Metal chelation of corn protein products/citric acid derivatives generated via reactive extrusion[☆]

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Abstract

Citric acid (CA) was thermochemically reacted with corn coproducts from the ethanol industry, i.e. corn gluten meal (CGM), produced from corn wet milling, and distillers' dried grains (DDG), produced from corn dry milling, to generate value-added, acid-insoluble reaction products with enhanced metal-binding properties. Heated CA produced an anhydride that reacted with the nucleophilic hydroxyl groups of starch and fiber as well as the hydroxyl, sulfhydryl, and amino groups of protein to produce ester or acyl derivatives. Short-term reactive extrusion of 1:1 w/w ratios of each corn product with CA by two passages at 20 rpm of each blend through a Brabender Plasti-Corder PL 2000, 1.9 cm single screw, 30:1 (L:D) fitted with a 1:1 screw and no die, through zone temperatures of 140, 200, and 196°C yielded reaction products that possessed similar degrees of carboxylation as a lengthy, 24 h oven-baking procedure at 120°C previously reported. Metal ion binding of the extruded derivatives increased with increasing pH from 3.0–5.0 where the solubility of the metal ions was unaffected by pH. At a pH of 4.5, where the charged ligand acts as an electron pair donor and at a metal ion concentration of 200 mg/l, the equilibrium binding efficiency for CGM/CA derivative under this set of conditions was: 0.54, 0.33, 0.85, 1.00, 0.59, 0.28, 0.30, 0.72, and 0.45 mmol/g for Cd²⁺, Co²⁺, Cu²⁺, Fe²⁺, Pb²⁺, Mn²⁺, Ni²⁺, Ag⁺, and Zn²⁺, respectively; whereas, for the DDG/CA derivative the metal binding was: 0.78, 0.66, 1.04, 1.08, 0.64, 0.51, 0.60, 1.02, and 0.73 mmol/g, respectively. The ability of citrate derivative to chelate metals varied with each derivative. Based on a comparison of metal binding data to heated CGM and DDG controls with no CA, the pendant carboxyl groups contributed by the CA moiety gave significantly higher metal ion binding. Comparison of metal binding ability for both the CGM/CA and DDG/CA derivatives with a sulfonic acid, styrene type analytical grade cation exchange resin, AG50W-X8, showed that CGM/CA possessed 13–44% of the capacity to bind metals, whereas DDG/CA possessed 23–47%. The corn-based

[☆] 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 USDA implies no approval of the product to the exclusion of others that may also be suitable.

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derivatives produced in this study were biodegradable and could be produced at a considerably lower cost compared with petroleum-based resins. Published by Elsevier Science B.V.

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1. Introduction

Petrochemically derived ion exchange resins as well as filtration polymers (including Zeolites), membrane separation, metal ion flocculents and base precipitation methods have been used to remove toxic heavy metals from industrial wastewaters that are generated by mining, metal finishing and electroplating operations. According to a recent industry study by The Freedonia Group (1997) the demand for ion exchange resins primarily for water treatment is predicted to advance 3.3% annually to 70 million kg, valued at US\$320 million by the year 2001. To allay societal concerns over the removal of toxic heavy metals from municipal disposal sites and ground water, investigation of biopolymers as heavy metal absorbing agents has intensified according to Hwang and Damodaran (1997). These researchers investigated the metal chelating properties and biodegradability of ethylenediaminetetraacetic acid dianhydride modification of soy protein to fulfill this need. To develop technologies for using agricultural materials as a renewable resource, Lehrfeld (1996) derivatized corn cobs, oat hulls and sugar beets by addition of phosphate, sulfate, succinyl, maleyl, and phthalyl ester groups to enhance the metal binding properties of these agricultural residues. Wing, and Sessa and Wing, investigated the copper binding properties of thermochemically modified citric acid (CA) derivatives of corn starch (Wing, 1996a), corn fiber (Wing, 1996b), and the coproducts of the ethanol industry, i.e. corn gluten meal (CGM) from corn wet milling and distillers' dried grains (DDG) from corn dry milling (Sessa and Wing, 1998a,b). In those studies, CA as an anhydride was reacted with the hydroxyl functional groups of corn starch or fiber as well as the protein nucleophilic groups to generate ester or amide derivatives. Because CA can generate a second anhydride that

can crosslink the starch, fiber or protein, these researchers evaluated conditions that limited this secondary reaction to optimize the carboxyl content and, therefore, enhance the copper binding capacities. The lengthy oven baking procedure of 24 h that they used would not be economically feasible for large-scale production of their derivatives.

The objectives of this research were: (a) to derivatize CGM and DDG with CA via a continuous extrusion process that would achieve the carboxyl content and metal binding properties equivalent to that obtained by the oven baking procedure; (b) to evaluate the metal binding properties of CGM/CA and DDG/CA derivatives with nine heavy metals where the metal chelating ability of each derivative could be compared to the binding capacity of a commercial petroleum-based ion exchange resin; and (c) to investigate the biodegradability of the CGM/CA and DDG/CA derivatives.

2. Materials and methods

2.1. Materials

DDG was supplied as Fibre-Plus by the Brown Forman Distillery (Louisville, KY). Fibre-Plus is the high fiber product from corn, rye, and malted barley that remains after yeast fermentation of the starch. The Fibre-Plus protein composition assessed by gel electrophoresis (Wolf and Lawton, 1997) demonstrated its pattern to be similar to DDG. The chemical composition was 27.5% protein, 5.6% moisture, 1.2% ash, 13.0% lipid, 52.0% dietary fiber (1.2% soluble and 50.8% insoluble) and 0.7% carbohydrates (by difference). CGM, obtained from Cargill Foods (Dayton, OH), possessed 69.3% protein, 9.4% moisture, 5.0% ash, 2.0% lipid, 11.0% starch, 0.7% crude

fiber and 2.6% other. Corn starch was Buffalo 3401 from CPC International (Englewood Cliffs, NJ). CA, anhydrous, granular, was provided by Archer Daniels Midland (Decatur, IL). AG50W-X8 resin, biotechnology grade, 100–200 mesh, 50% solids, was purchased from Bio-Rad Laboratories (Hercules, CA). AG50W-X8 is a strongly acidic cation exchange resin composed of sulfonic acid exchange groups attached to a styrene–divinylbenzene polymer lattice. All other chemicals used in this study were of reagent grade.

2.2. Thermal reaction/extruder

Blends of DDG with CA at 5:3 w/w or 1:1 w/w and CGM at 1:1 w/w were each prepared as described below where the starting material: DDG:CA:H₂O, 5:3:2 (w/w/v) possessed a moisture content of 21.0% (Table 1). For the 1:1 w/w blend used in Table 2, CA (250 g) was first dissolved in water (500 ml); dry DDG or CGM (250 g) was then slowly added to the CA solution while mixing at low speed with a Hobart mixer. To assure homogeneity an additional 15 min mixing was performed after the addition of dry corn product. The corn product/CA batters were dried in a forced air oven at 60°C for 24 h to remove surface moisture. The oven-baked (OB) procedure consisted of dry heating the corn product/CA batters after removal of surface moisture either 24 h at 110°C designated OBI or 24 h at 120°C labelled OB2. For the reactive extrusion (RE) procedure two replicates were performed on the

corn product/CA (1:1 w/w) blends with each moistened to 17%. These batches were passed twice through a Brabender Plasti-Corder PL 2000, single screw, 30:1 (L:D) fitted with a feed type screw configuration and no die. A screw speed of 20 rpm and zone temperatures: I, 140, II, 200, and III, 196°C were used to achieve a product that, when water-washed, possessed a carboxylation content similar to either the oven baking procedures OB1 or OB2. Water-washing the CA reaction products was accomplished by preparing an aqueous slurry of each product in 1.2 l distilled water and adjusting the dispersion to pH 2 with concentrated HCl. After mixing the slurry for 30 min, it was filtered on a Buchner funnel fitted with Whatman 541 filter paper. The insoluble, filtered residues were then extensively rinsed with 2 l distilled water. The filtrates were pooled and evaporated in a hot air oven to obtain a weight of unreacted CA; the washed, filtered residues were air dried. Reaction efficiency was calculated by the relationship: (wt. of insoluble reaction product)/(total wts. of insoluble reaction product + unreacted CA). Two replicates were run for each reaction condition.

2.3. Carboxyl determination

Air-dried samples, 1.000 g each, were slurried in distilled water (100 ml) and measured amounts of 0.100 N NaOH was added (Wing, 1996a). After stirring for 24 h while adjusting the pH between 9.5–10.5 with measured amounts of additional

Table 1
Effect of extrusion conditions on the carboxylation and Cu²⁺ binding of DDG reacted with CA^a

Treatment	Zone heat (°C)			RPM	Moisture (%)	COOH (mmol/g)	Cu ²⁺ (mmol/g)
	I	II	III				
1	130	170	166	4	3.6	2.05	0.85
2	130	170	166	6	3.6	1.93	0.74
3	130	170	166	10	5.2	1.79	0.61
4	140	200	196	10	2.4	2.82	0.96
5	140	200	196	15	3.0	1.88	0.76
6	155	232	228	15	2.1	2.90	0.85
7	155	232	228	30 ^a	2.3	1.13	0.43

^a Residence time, 2 min 18 s.

Table 2

Thermochemical reaction of CA with CGM and DDG—comparison of oven baking (OB) with reactive extrusion (RE) procedures

CA derivative/process	Reaction efficiency (%)	Kjeld N ₂ , db (%)	COOH (mmol/g)
CGM/OB1	75.5 ± 0.8	7.23 ± 0.03	4.18 ± 0.05
CGM/OB2	82.4 ± 1.2	7.10 ± 0.02	4.26 ± 0.01
CGM/RE	78.6 ± 1.8	7.30 ± 0.12	4.34 ± 0.01
DDG/OB1	94.0 ± 1.4	3.18 ± 0.07	3.48 ± 0.06
DDG/OB2	97.7 ± 1.5	2.74 ± 0.05	4.19 ± 0.04
DDG/RE	61.5 ± 3.8	3.72 ± 0.16	3.78 ± 0.06

base, the mixture was back titrated with 0.100 N HCl to an end point of pH 8.5. Conversion factors were determined using oxalic acid and citric acid as standards. DDG and CGM samples with no CA were used as controls. No corrections were made for deesterification since the pH was maintained between 9.5–10.5; at pH 11 and above some deesterification did occur.

2.4. Metal binding efficiencies

Samples (1.000 g) finely ground with a coffee mill to pass through a 40 mesh sieve, were each slurried in 100 ml distilled water containing metal ion concentrations calculated to be approximately 200 mg/l. The metal ions assessed were Cd²⁺, Co²⁺, Cu²⁺, Fe²⁺, Pb²⁺, Mn²⁺, Ni²⁺, Ag⁺, and Zn²⁺. The corn product/metal ion slurry was adjusted to a pH of 3.0, 3.5, 4.0, 4.5, or 5.0 with 1N NaOH and maintained at each pH for 24 h. The solids were filtered on Whatman 54 filter paper. To remove unbound metal ions, the solids were extensively rinsed with water and the volume was adjusted to 1 l. This solution was reserved for metal ion analysis. Rinsed solids, washed from the filter paper, were stripped of metal ions with 50% nitric acid and refiltered with a collection of the filtrate. Filtrates of unbound as well as bound metal ions, each diluted to 1 l, were then analyzed for metal ion content. As a control, AG50W-X8 cation exchange resin (1.000 g) was slurried with metal ions as described above except no pH adjustment was made since optimum metal binding occurred at its endogenous pH which was 2.1. Because this resin contained 50% moisture, its

metal binding was calculated and compared on a dry basis (db).

2.5. Analyses

Metal analysis was performed with a Perkin-Elmer Plasma 400 emission spectrometer calibrated with metal ion standards. Micro-Kjeldahl nitrogen, ash and moisture contents were determined by the American Association of Cereal Chemists' Approved Methods (American Association of Cereal Chemists', 1990). Statistical analyses were performed with the Statistical Analysis Systems software package (SAS/STAT, 1987).

2.6. Respirometry

CGM and DDG controls and their respective extruded citrate derivatives at 1.000 g were each placed into 250 ml glass bottles with 100 g prairie topsoil (Bluestem Products, Cedar Rapids, IA). Corn starch (Buffalo 3401) was evaluated for comparative purposes. The soil contained 0.9% N, had a pH of 7.9, and was adjusted to 60% moisture. The samples were maintained at 23°C with a water bath. CO₂ production and O₂ consumption were monitored over time with a Micro-oxymax respirometer (Columbus Instruments, Columbus, OH) by the procedure of Shogren et al. (1998). Net CO₂ production was calculated by subtracting the total CO₂ produced by a soil sample alone from the value for the sample tested. Values given are the average of duplicate sample chambers and are normalized to the total amount of carbon in each sample.

3. Results and discussion

3.1. Derivatization process

Both CGM and DDG are composite materials that will generate polyesters and amides of CA when thermally reacted with CA. The effect of extrusion conditions on the carboxylation and copper binding of DDG when reacted with CA is listed in Table 1. The feed composition for this extrusion scheme was 5:3:2 for DDG:CA:H₂O (w/w/v) where 300 g anhydrous CA was dissolved in 200 ml distilled water heated to 50°C; 500 g DDG was slowly added while mixing at slow speed with a Hobart mixer. In this experiment, the 3:2 ratio of CA to H₂O was the maximum amount of CA that could be completely dissolved in a minimum amount of warm water. When the dry DDG was blended into the dissolved CA, the resulting overall moisture level of the semidry product was about 21% which was ideal for extrusion. The zone heats of 140, 200, and 196°C at 10 rpm (treatment 4) gave the best carboxylation and Cu²⁺ binding (Table 1). Under these same zone heat conditions with the extruder speed increased to 15 rpm the degree of carboxylation and Cu²⁺ binding dropped significantly. The longer residence time for the reaction of DDG with CA accomplished at 10 rpm was essential for a more complete derivatization. However, when the zone temperatures were raised to 155, 232, and 228°C and the screw speed was 15 rpm (treatment 6) the carboxylation was similar to that achieved at 10 rpm with the lower zone temperatures, yet, the Cu²⁺ binding was much lower. Also in treatment 6, some product scorching was visually observed. These higher heating conditions could dehydrate CA to aconitic acid which in turn would give rise to mixtures of citric acid and aconitic acid esters and amides from reaction with DDG. The hydroxyl group of CA is known to participate in the chelation of metals. Therefore, the aconitic ester and amide would have the same degree of carboxylation but with less metal binding than the CA derivative.

Sessa and Wing (Sessa and Wing, 1998a,b) demonstrated that a 1:1 (w/w) blend of DDG with CA was required to optimize the carboxyl

content of the CA derivative formed by an oven-baking procedure. To achieve a similar degree of carboxylation via reactive extrusion, a 1:1 (w/w) blend was used. Intimate mixing of dry DDG with CA followed by wetting to 17% total moisture content with distilled water gave a moisturized blend that when subjected to the extrusion conditions described previously (i.e. 10 rpm through heat zones 140, 200, and 196°C) yielded DDG/CA derivatives with a carboxyl content of only 2.98 mmol/g, which degree of carboxylation was considerably lower than a blend of DDG that was intimately mixed with CA, which was completely dissolved in water. The complete wetting of corn product with CA dissolved in water was essential to effect a more complete derivatization. Therefore, 1:1 blends of CGM with CA dissolved in water, dried to remove surface moisture by heating in a hot air oven at 60°C overnight and moistened to 17% total moisture content were subjected to reactive extrusion twice through heat zones 140, 200, and 196°C at 20 rpm. Each sample was finely ground and remoistened to 17% before the second passage. Two passages at 20 rpm through the extruder gave equivalent values to one passage at 10 rpm. Feeding the semidry material at 20 rpm was much easier than at 10 rpm. These derivatized products proved to be as effective in the degree of carboxylation and metal-binding efficiency as the 24 h oven-baking procedure described by Sessa and Wing (1998a). The data in Table 2 shows the equivalent reaction efficiencies and carboxyl contents when CGM/CA derivatives either by oven-baking 24 h at 120°C or the mean of two replicates of the reactive extrusion are compared. However, when DDG was reacted with CA under the same set of conditions, the oven-baking procedure (i.e. 24 h at 120°C) gave a much higher percent reaction efficiency and carboxyl content compared with the reactive extrusion derivatization process, i.e. 97.7 versus 61.5% and 4.19 versus 3.78%, respectively. Despite the lower reaction efficiencies and carboxyl content, the DDG/CA derivatives subjected to reactive extrusion gave Cu²⁺ binding of 1.04 mmol/g (see the data in Table 3) versus 1.07 mmol/g reported by Sessa and Wing (1998a) who used the oven-baking derivatization procedure.

Apparently, the high heat and shear of this blend when subjected to reactive extrusion destroyed the protein and fiber conformations, which resulted in the exposure of more metal binding sites. For both the extruded CGM/CA and DDG/CA derivatives, the Kjeldahl nitrogen content was higher than either oven-baked derivatives, respectively. These higher nitrogen values for both CA derivatives indicate that some crosslinking of protein with CA may have occurred. If crosslinking of protein or carbohydrate moieties did occur, only one carboxyl and hydroxyl group would be available from the citric acid moiety to bind metals. The crosslinked CA derivative will not bind metals as effectively as the dicarboxyl groups where only one ester or amide linkage has been generated.

3.2. Product evaluation

CA is known to be an excellent chelating agent. A chelate is a complex resulting from the combination of a metal ion and a multidentate ligand such that the ligand forms two or more bonds with the metal, resulting in a ring structure that includes the metal ion. CA itself possesses the coordinating functions of three carboxylic and one hydroxyl group. All these groups are known to participate in the binding of metal ions. CA as an anhydride will react with the hydroxyl functional groups of corn starch or fiber (Wing,

1996a,b) as well as the nucleophilic NH_2 , SH, and OH groups of protein (Sessa and Wing, 1998a,b) to generate ester or amide derivatives. This derivatization of CA with biobased materials results in a water insoluble chelate with two carboxylic acid and one hydroxyl group. The electron-donating species in these CA complexes are commonly referred to as ligands. The principal electron donating atoms in these ligands are oxygen, nitrogen and sulfur where oxygen, nitrogen, and sulfur contributions reside with the protein moieties and oxygen resides with the carbohydrate and CA moieties. Charged ligands form more stable chelates than uncharged ligands, hence charged citrate ions will form more stable chelates than the carboxylic acid groups of CA.

The effect of pH on metal-binding of both CGM and DDG/CA derivatives formed by reactive extrusion was evident from the data in Fig. 1. To obtain a charged species from the citrate or protein moieties, a pH above 4.0 is needed where the pK of the carboxyl groups in citrate and/or protein is about 4.6. Most commonly, the pH of the system largely determines which metals are chelated preferentially. With the exception of Pb^{2+} binding to either CGM or DDG/CA derivatives, a pH of 5.0 gave the highest degree of binding. However, high concentrations of hydroxyl ion provided by NaOH for neutralizing the samples caused some turbidity with Fe^{2+} , which will form a hydroxide at pH 5.0. Therefore, the

Table 3
Carboxyl content/heavy metal binding (mmol/g) with CGM and DDG CA derivatives compared to cation exchange resin AG50W-X8

Biopolymer	CGM	CGM/CA	DDG	DDG/CA	AG50W-X8
COOH	1.11 ± 0.00	4.34 ± 0.01	1.11 ± 0.00	3.78 ± 0.07	–
Cd ²⁺	0.18 ± 0.01	0.54 ± 0.12	0.11 ± 0.01	0.78 ± 0.22	1.78 ± 0.15
Co ²⁺	0.07 ± 0.03	0.33 ± 0.07	0.06 ± 0.00	0.66 ± 0.01	2.65 ± 0.20
Cu ²⁺	0.27 ± 0.03	0.85 ± 0.15	0.25 ± 0.03	1.04 ± 0.15	2.30 ± 0.29
Fe ²⁺	0.55 ± 0.07	1.00 ± 0.06	0.50 ± 0.12	1.08 ± 0.08	2.29 ± 0.15
Pb ²⁺	0.20 ± 0.01	0.59 ± 0.04	0.14 ± 0.01	0.64 ± 0.04	1.40 ± 0.11
Mn ²⁺	0.07 ± 0.02	0.28 ± 0.07	0.07 ± 0.01	0.51 ± 0.05	2.19 ± 0.15
Ni ²⁺	0.03 ± 0.00	0.30 ± 0.00	0.08 ± 0.01	0.60 ± 0.09	2.27 ± 0.57
Ag ⁺	0.35 ± 0.03	0.72 ± 0.05	0.20 ± 0.01	1.02 ± 0.10	2.72 ± 0.24
Zn ²⁺	0.16 ± 0.01	0.45 ± 0.01	0.12 ± 0.01	0.73 ± 0.04	2.69 ± 0.41

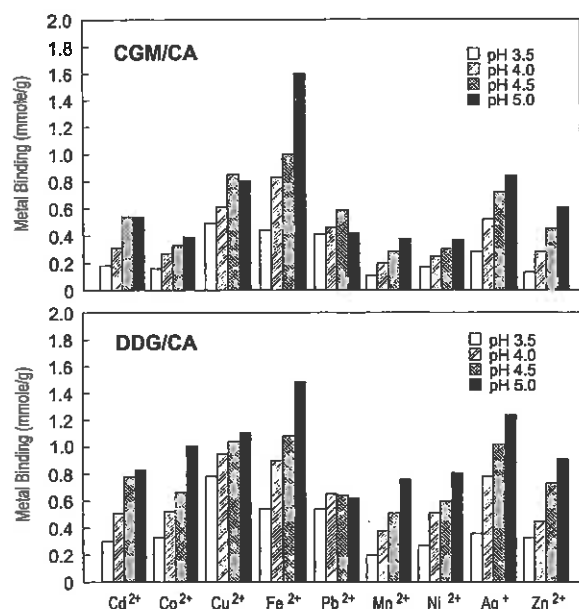


Fig. 1. Effect of pH on the metal binding (mmol/g) of CA derivatives of CGM and DDG.

higher values for Fe^{2+} binding at pH 5.0 for both the CGM and DDG/CA derivatives are not only due to metal chelation, but result from some hydroxide precipitation. Hence, to negate the effects of hydroxide precipitation a pH of 4.5, high enough to give a charged carboxyl species, was used in both our previous (Wing, 1996a,b; Sessa and Wing, 1998a,b) and current investigations. Of the metal ions evaluated, Ag^+ forms an hydroxide at a pH of approximately 8. To demonstrate the excellent metal binding of our CA derivatives at pH's higher than 5.0, we evaluated Ag^+ at a pH of 6.5 (data not tabulated). Here, the CGM/CA derivative chelated 0.86 mmol/g, whereas the DDG/CA derivative chelated 1.66 mmol/g. Our results indicate that the CGM and DDG/CA

derivatives have the ability to chelate metals in weakly acidic solutions of pH 4 and higher.

The *t*-test analysis showed a significant increase in the metal binding of the corn product based citrate derivatives compared with the corn product controls for all nine metal ions (see the data in Table 3). AG50W-X8, a strongly acidic sulfonic acid cationic exchange resin, was used at its endogenous pH of 2.1 as a comparative control. Below pH 3.0, this exchange resin possesses a charged sulfonic acid group available for binding metals. For direct comparison of metal binding, we used the ionically charged species for both resin and CA derivatives. All values for metal binding with the exchange resin were adjusted and recorded on a dry basis.

Hwang and Damodaran (1997) investigated the equilibrium binding efficiencies of divalent calcium, zinc, mercury, and lead ions by ethylenediaminetetraacetic acid dianhydride modified soy protein hydrogels. The binding efficiencies of Zn^{2+} and Pb^{2+} for DDG/CA derivatives in our current study, 0.73 and 0.64 mmol/g, respectively, compared well with their results of 0.65 and 0.70 mmol/g, respectively.

A *t*-test was carried out to distinguish the difference between the metal binding of nine metals to CGM and DDG/CA derivatives as a ratio with the binding of each of these metals to our ion exchange control (i.e. AG50W-X8). We established a ratio by dividing the means of the metal binding observations for each metal and corn product/CA derivative by the means of the metal binding to the AG50W-X8 control for each metal. The data, recorded in Table 4, showed that the CGM/CA derivative possessed 13–44% of the capacity to bind metals, whereas the DDG/CA derivative possessed 23–47%.

Table 4

Ratio of metal binding of CGM and DDG CA derivatives with cation exchange resin AG50W-X8

CA derivative	Cd^{2+}	Co^{2+}	Cu^{2+}	Fe^{2+}	Pb^{2+}	Mn^{2+}	Ni^{2+}	Ag^+	Zn^{2+}
CGM ^a	0.30	0.13	0.37	0.44	0.42	0.13	0.13	0.26	0.17
DDG ^b	0.44	0.25	0.45	0.47	0.46	0.23	0.27	0.37	0.27

^a Overall correlation coefficient $R^2 = 0.92$ at $P < 0.001$.

^b Overall correlation coefficient $R^2 = 0.86$ at $P < 0.001$.

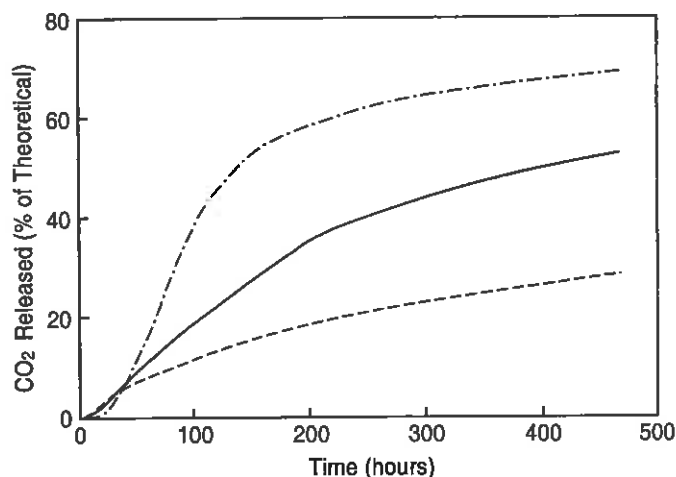


Fig. 2. Carbon dioxide released during biodegradation of CA derivatives of CGM and DDG. Corn starch (· · ·); CGM/CA (—); and DDG/CA (---).

3.3. Biodegradability

Carbon dioxide released versus time for corn starch as the control and citrate derivatives of CGM and DDG are shown in Fig. 2. About 69% of the starch was degraded in 500 h based on theoretical yield of CO_2 , whereas 52% of CGM/CA and 27% of DDG/CA were biodegraded. Because our evaluation involved theoretical CO_2 evolution and did not include assessment of the proteolytic breakdown of proteins to peptides, amino acids and/or ammonia, our findings only reflect the nonprotein components of the composite mixtures. Our results indicate that CGM/CA with less than 2% lipid biodegrades faster than DDG/CA. Because DDG/CA possessed 22.4% less protein than CGM/CA, its slower degradation may reflect either its 13% lipid content or its 51% insoluble fiber content.

In a second respirometry study, we determined whether the extraction of lipid from DDG and DDG/CA with chloroform would affect their rates of biodegradation. We also investigated how the CA derivatization process affects CGM and DDG biodegradation. As shown in Fig. 3 defatted DDG biodegraded to a significantly lesser extent in 650 h than the undefatted DDG, whereas both defatted and undefatted DDG/CA derivatives biodegraded similarly. Removal of the

13% lipid from DDG would yield higher proportions of protein and insoluble fiber. Apparently, insoluble fiber is more difficult to biodegrade. When the hydroxyl groups of insoluble fiber and the nucleophilic groups of protein from DDG are either esterified or acylated with CA, chloroform defatting made no difference in the biodegradation rate. CA acylated protein and CA esterified insoluble fiber biodegrade similarly and to a lesser extent in 650 h than the underivatized DDG.

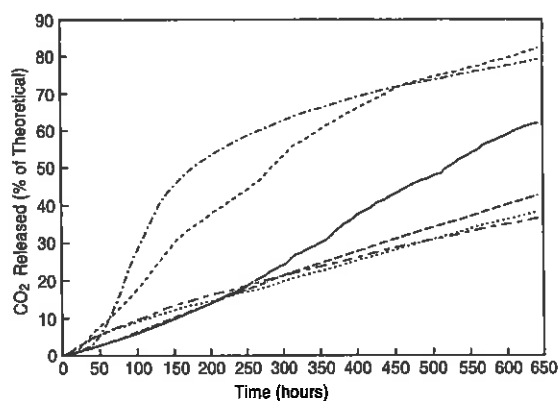


Fig. 3. Carbon dioxide released during biodegradation of corn gluten meal (CGM); distillers' dried grains (DDG), defatted DDG and respective citric acid (CA) derivatives. Corn starch (· · ·); CGM (---); DDG (—); defatted DDG (— —); DDG/CA (— · —); and defatted DDG/CA (· · ·).

Similarly, when we compared CGM biodegradation of 73% of the theoretical yield of CO₂ in 500 h with 52% for its CA derivative (data from Fig. 2), the 21% drop in the theoretical yield of CO₂ in 500 h can be attributed to the slower breakdown of ester or acyl bound CA with starch and protein.

4. Conclusion

CGM/CA and DDG/CA derivatives exhibited promising metal-chelating properties as well as biodegradability. Comparison of the metal binding ability for both CGM/CA and DDG/CA derivatives with a sulfonic acid, styrene type analytical grade cation exchange resin, AG50W-X8, showed that CGM/CA possessed 13–44% of the capacity to bind the nine heavy metals evaluated whereas DDG/CA possessed 23–47%. These corn-based derivatives can be produced on a continuous basis by reactive extrusion. Based on cost of CA at US\$1.32/kg, CGM at US\$0.77/kg, and DDG at US\$0.28/kg, the citrate derivatives at 1:1 when extrusion processed at an estimated cost of US\$1.15/kg would cost about US\$2.20/kg which equates to a considerable cost savings when compared with the petroleum based resins costing US\$17.60 + /kg. These derivatives show evidence for biodegradability assessed by respirometry. These derivatives have metal chelating ability above pH 4.0 and have potential usage in waste water treatment to scavenge heavy metals and also because of their biodegradability can be used to add essential micronutrients to soils.

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