MinnesotaCorn
RESEARCH & PROMOTION COUNCIL

PROGRESS REPORT

PROJECT TITLE: Recovery and Use of Value-Added Corn Functional Ingredients
PROJECT NUMBER:
REPORTING PERIOD: April 30, 2021
PRINCIPAL INVESTIGATOR: Padu Krishnan
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1.) PROJECT ACTIVITIES COMPLETED DURING THE REPORTING PERIOD. (*Describe project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

a. A state of the art analytical instrument was acquired for the specific purpose of tracking the fate of useful corn biofunctional agents in corn and corn fractions. While HPLC (High Performance Liquid Chromatograpy) is a work-horse instrument in most modern laboratories, when coupled with Photo Diode Array detection, the instrument will provide the analyst a more complete picture of the stability and distribution of compounds of interest originating in the corn kernel. Currently, we track the fate of these compounds by chemically measuring entire classes of compound (eg. Total Phenolics, Carotenoids, etc). While effective in giving us a general idea of potentially high yielding processes, chemical methods are a shotgun approach. HPLC-PDA will provide more detailed information (based on the use of pure standards) and we can estimate the doses in milligrams per serving size that we hope to retain in the corn fraction that will eventually be consumed by humans. This instrument will assist us in measuring and documenting the health promoting pigments that are valuable in food processing. The food industry is working to remove synthetic pigments and colorants that are generally viewed to be unhealthy by experts and consumers. Corn is naturally endowed with valuable pigments that have not yet been exploited for value addition. Pigments that migrate into wash streams in the production of food grade DDG also have economic value and need to be recovered. The system has been set up and training will begin shortly by the vendor.

Figure 1. High performance Liquid Chromatography with Photo Diode Array Detection for measurement of bioactive food functional compounds in corn and corn coproducts.

b. Graduate Student Patra Akaya graduated in December 2021. Her project dealt with particle size and food functionality of corn distillers grains. Her thesis was titled “Effects of Particle Size of Distillers Dried Grains and Increased Levels of Flour Enrichment - A Study of Nutritional Composition, Rheology, and Quality of Fiber and Protein Enriched Bread”. DDG used as a food ingredient in flour fortification needs to meet certain specifications. Particle size is one such variable that needs to be controlled. Increasing the level of fineness of grind (to match that of the flour) invariably increases surface area and has impact on the rheology or behavior of doughs. DDG fiber particles compete for the water in the food system that is critical to film formation, bubble stability and gas retention. DDG also dilutes the gluten strength that provides baked product their unique structure. Patra’s work investigated grinding using an ultracentrifugal mill and two mill screens (0.2mm and 0.5mm). She also used increasing levels of flour substitution (up to 20%).

Table 1. Texture evaluation of white pan bread made with bread flour and FDDG-flour blends containing 5-20% FDDG

Constituents	FDDG particle size	Substitution	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
			(g)			(g)	**(g)**	
Bread Flour	Control	0%	303.76d	18.93ab	1.053a	315.04d	5763.31bc	0.559b
FDDG	Fine	5%	246.27d	24.71a	1.029a	238.79d	5584.19bc	0.572b
FDDG	Fine	10%	510.40d	12.65b	0.846b	429.32d	4610.72bc	0.527bc
FDDG	Fine	20%	1255.86b	20.63ab	0.874b	1086.15b	19549.36a	0.624a
FDDG	Coarse	5%	457.81d	18.77ab	0.784bc	359.219d	5989.49bc	0.495c
FDDG	Coarse	10%	901.08c	15.11b	0.7015c	676.454c	9234.89b	0.564b
FDDG	Coarse	20%	2282.94a	1.11c	0.791bc	1789.007a	1909.17c	0.541bc

Legend:
BF: Bread Flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers Dried Grain
FDDG: Food Grade Distillers Dried Grain
Fine: FDDG ground using 0.2mm mesh
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

Bread made with increasing levels of DDG (fine or coarse) showed increased hardness in general. Bread springiness was also significantly reduced. Fine grind DDG did not have as significant an effect on springiness as coarse grind. Fineness or coarseness of FDDG yielded mixed results for other mouthfeel parameter such as cohesiveness, gumminess, chewiness and resilience. The 10% substitutions were acceptable products as judged by a sensory panel.

Table 2. Total Phenolic Content Retention in Food Grade Distillers Grains prepared in a 6 Liter Capacity Reaction flask

Nine Treatments	TPC of Raw DDG in mg	TPC retention in FDDG in mg	% TPC Recovery
ACE SEQ ETOH	412.24 (100g)	299.11 (97.5g)	72.56
ACE SEQ HEX	412.24 (100g)	330.68 (92g)	80.22
ACE NON SEQ ETOH	412.24 (100g)	187.90 (93g)	45.58
ACE NON SEQ HEX	412.24 (100g)	217.90 (95.25g)	52.86
ACE NON SEQ HEX/ETOH	412.24 (100g)	156.41 (91.5g)	37.94

c. Graduate student Brady Bury determined the effectiveness of a 6L glass ACE Extraction System to simulate FDDG processing conditions (Table 2). This system allowed for studying the mass balance of substances being removed from the residue (FDDG) and partitioned into the wash solvents. This system permitted safe study while the monitoring eluents. Sequential addition of solvents was effective in retaining Total Phenolic Content in the residue. 72.6 to 80.2% of TPC were recovered in the residue (FDDG fraction). Both Ethanol and Hexane were effective in the sequential extraction schemes. This establishes that useful phenolic moieties will be retained in the portion of the DDG intended for FDDG production.

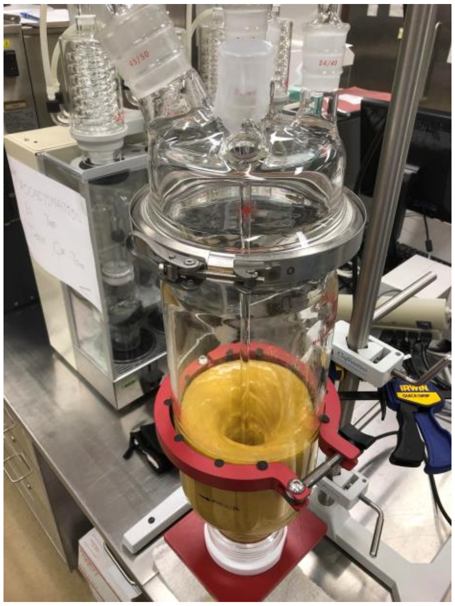

Figure 2. ACE Glassware (6L), used to show effects of scale, solvent type and extraction modes on selective recovery and retention of Total Phenolics and Total Carotenoids in high fiber-high protein fractions.

Three bench-scale extraction and refluxing apparatus were used employing fixed solvent-to-sample ratios, and three organic solvent systems (ETOH, Hexane, & HEX-ETOH). The use of 6L capacity ACE Glassware permitted handling of large solvent volumes for washing, decanting and efficient recovery of solvents. Soxhlet extractors (3 L and 0.5L) employed heating and water-circulated condensing systems in order to continually reflux small amounts of samples with hexane or ethanol. The focus of this report is the retention of TPC and TCC in the fiber-rich and protein-rich fraction. TPC and TCC recovery from oil residues will be reported elsewhere.

d. Scale-up and Commercialization efforts.
We are exploring scale-up of FDDG processing with Southern Illinois University and NCERC. The latter has pharmaceutical grade fermentation facilities for DDG production. NCERC is the National Corn-to-Ethanol Research Center. Protocols and equipment needs for 100 Kg to 200 Kg of FDDG are being explored to complement this system. This arrangement will enable larger batches of FDDG for standardization and commercializing efforts.

(20+) NCERC | Facebook

2.) IDENTIFY ANY SIGNIFICANT FINDINGS AND RESULTS OF THE PROJECT TO DATE.

TPC Content
ACE Sequential and ACE Non-Sequential Extraction Techniques retained 72.6% and 80.2%, respectively, of Total Phenolic Content in the Food Grade DDG Fraction compared to levels found in the starting material (Table 1). Both solvents, Ethanol and Hexane, were effective in retaining TPC in the fiber-protein fraction. This is a significant finding as TPC moieties in the high protein-high fiber fraction may contribute to health properties of food materials. One hundred grams of FDDG will deliver approximately 300 mg of TPC. A 10g portion used in conventional food product can deliver close to 30 mg of TPC in typical food portion sizes.

3.) CHALLENGES ENCOUNTERED. (*Describe any challenges that you encountered related to project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

No challenges were encountered.

4.) FINANCIAL INFORMATION (*Describe any budget challenges and provide specific reasons for deviations from the projected project spending.*)

No budget challenges were encountered.

5.) EDUCATION AND OUTREACH ACTIVITES. *(Describe any conferences, workshops, field days, etc attended, number of contacts at each event, and/or publications developed to disseminate project results.)*

Type	**Status**	**Year Published**	**Citation**
Journal article	Published	2021	Rathnakumar, K, Osorio-Arias, J.M., Krishnan, P.G., and Martinez-Monteagudo, S.I. 2021. Fractionation of spent coffee ground with tertiary amine extraction. Journal of Separation and Purification Technology. Volume 274, 1 November 2021, 119111 https://doi.org/10.1016/j.seppur.2021.11911 Fractionation of spent coffee ground with tertiary amine extraction | Elsevier Enhanced Reader While this article is not about corn, it is germaine to novel extraction protocols for recovering phenolic substances in agricultural materials.

M.S. Thesis	Published abstract in Proceedings	December, 2020	Patra Akaya. Effects of particle size of DDG on dough rheology and bread quality. December, 2021.

Appendix:

A national survey of ethanol plants was conducted to determine the current level of interest in value-addition from corn fractions from the ethanol plants. Current thinking about readiness of ethanol plants for alternate value-added production was surveyed using multiple choice and ranking tool. This survey was distributed electronically through the American Coalition for Ethanol (ACE). The response rate was 33%. A summary of findings from the survey is provided below. This information was also shared in a previous progress report.

Survey of Ethanol Plants
Food Applications for ethanol coproducts

Survey was sent to approximately 50 ethanol plants and there were 16 total respondents for a response rate of 32%.

The estimated ethanol production capacity of your plant (million gallons/year).
Key for interpretation of items: X percent of respondents chose the answer on the right.

50% produce 51-100 million gallons/year
25% produce 101-150 million gallons/year
13% produce 151+ million gallons/year
12% produce 0-50 million gallons/year

Your estimate of the percentage of distillers' grains sold in your plant as dry DDG (Distillers' Dried Grains) as opposed to modified wet distillers' grains.

69% greater that 71%
19% 51-70%
12% 0-20%

Which of the following is the most accurate response to "Does your plant employ additional steps to achieve advanced fiber or protein separations?"

75% No fermentation
13% Yes, pre-fermentation
12% Yes, post-fermentation

Since food applications require stainless steel contact surfaces for optimal sanitation and cleanability, what is your estimate of the proportion of stainless steel used throughout the plant during DDG processing?

31% Minimal less than 50%
31% Extensive greater than 80%
25% Moderate greater than 70%
13% Some 50-69%

In your estimation, what number of years will yield acceptable Return on Investment for discretionary capital investments?

37% 3 years
25% 2 years
13% 4 years
13% 5 years
12% 1 year

Food grade applications require clear separation of product streams from contaminants such as dust & debris, insects, cleaning reagents, antibiotics, environmental contaminates, etc. On a scale from 1-5 (with 5 being the highest), your interest in segregating DDG from non-food materials to provide product streams for food-grade applications.

38% 3
25% 1
19% 4
12% 2
6% 5

On a scale from 1-5 (with 5 being the highest), your level of interest and willingness to install and pay for elaborate cleaning and grading of incoming corn for future human food applications of DDG (based on a 5-year Return on Investment).

44% 3
38% 1
12% 2
6% 5
0% 4

Your estimate of which of these factors presents the greatest challenges for food-grade DDG?

44% GMOs
38% Mycotoxins
12% Antibiotics
6% Cleaning reagents

Your estimate of the proportion of your DDG production that you feel can be diverted for human food production assuming that you are leaving enough DDG for the livestock industry.

44% 30%
31% 20%
13% 15%
6% 10%
6% 5%

On a scale from 1-5 (with 5 being the highest), rank your level of interest in the recovery of bioactive compounds from product streams in the ethanol plant (e.g. phytonutrients, carotenoids, xanthophyll pigments, phenolics, antioxidants, specialty proteins, etc.) based on a 5-year Return on Investment.

31% 3
31% 1
13% 4
13% 5
12% 2

On a scale of 1 to 5 (with 5 being the highest), your level of interest in food-grade DDG products.

25% 1
19% 5
19% 4
19% 3
18% 2

On a scale of 1 to 5 (with 5 being the highest), your level of interest in acquiring production equipment for processing DDG for food applications (assuming a 5 year Return on Investment).

44% 1
25% 3
19% 4
6% 5
6% 2

Food application requires the use of ingredients that are Generally Recognized as Safe as approved by the Food and Drug Administration. On a scale of 1 to 5 (with 5 being the highest), your level of comfort with Generally Recognized As Safe (GRAS) approved reagents and chemicals and food-grade enzymes being used in your plant.

38% 5
31% 3
25% 4
6% 2
0% 1

On a scale of 1 to 5 (with 5 being the highest), your potential interest in the use of non-GMO corn as a starting material for specialized product processing in the ethanol plant (e.g. specialty proteins, specialty fibers, food antioxidants, natural pigments & colorants, health-promoting bioactive compounds, vitamins, etc.).

44% 1
31% 3
13% 4
12% 2
0% 5

Rank 1-6 (with 1 being the most beneficial to you), which of the following will benefit you the most in relation to human food applications for DDG and other value added corn constituents?

Bold/Italicized/Underlined are the highest percentages for each rank 1 through 6

Cost and economic returns for human food applications

<u>73% 1</u>
7% 6
7% 4
7% 5
6% 2
0% 3

Capital needs

<u>33% 2</u>
27% 3
20% 4
13% 5
7% 1
0% 6

Processing changes

<u>40% 3</u>
20% 5
20% 2
13% 4
7% 1
0% 6

Corn cleaning at the front end of the plant

<u>34% 4</u>
20% 6
20% 5
13% 3
13% 2
0% 1

Capture of CO_2 as an ingredient in food processing

<u>40% 5</u>
<u>40% 6</u>
7% 3
7% 2
6% 1
0% 4

Issues surrounding food applications for ethanol co-products

<u>33% 6</u>
27% 4
20% 2
13% 3
7% 1
0% 5