The Canadian Society for Bioengineering
The Canadian society for engineering in agricultural, food, environmental, and biological systems.

CSBE | SCGAB

La Société Canadienne de Génie Agroalimentaire et de Bioingénierie
La société canadienne de génie agroalimentaire, de la bioingénierie et de l'environnement

Paper No. CSBE11-315

Protein Content and Antioxidant Activity of Distiller' Spent Grains (DSG) Dried under Different Conditions

Maria del Carmen Flores-Alvarez

Departamento de Ingeniería Química, Alimentos y Ambiental. Universidad de las Americas Puebla Ex-Hacienda Santa Catarina Mártir. Cholula, Puebla, Mexico 72810

Maria Elena Sosa-Morales

Departamento de Ingeniería Química, Alimentos y Ambiental. Universidad de las Americas Puebla Ex-Hacienda Santa Catarina Mártir. Cholula, Puebla, Mexico 72810. mariae.sosa@udlap.mx

Stefan Cenkowski

Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada R3T 5V6 stefan_cenkowski@umanitoba.ca

Jitendra Paliwal

Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada R3T 5V6

Written for presentation at the
CSBE/SCGAB 2011 Annual Conference
Inn at the Forks, Winnipeg, Manitoba
10-13 July 2011

ABSTRACT Distillers' spent grains (DSG) are the byproduct of bio-fuel and ethanol production. It has been reported than DSG contain high amounts of protein, fiber and other valuable components. Commonly, DSG are dried to extend their shelf-life and to reduce the cost of transportation, and probably, the drying method may affect the nutritional/functional value of the final product. The objective was to evaluate the protein content and the antioxidant activity of DSG dried under different conditions. Three drying procedures were followed: drying with near ambient air at 30°C, drying in a convection-oven at 150ºC and drying in superheated steam (SS) at 150ºC. Protein content ranged from 15.1 to 16.4% wb for all samples. With respect to phenolic content, ethanolic extracts (3.1-12.9 mg GAE/g) were more effective than aqueous extracts (0.87-2.9 mg GAE/g). The DSG samples dried with SS had the highest phenolic contents among the analyzed samples ($p<0.05$). Antioxidant activity ranged between 0.32-0.44 mg trolox/g for aqueous extracts and 0.54-0.57 mg trolox/g for ethanolic extracts. Superheated steam drying may be a suitable method to obtain dried DSG with good protein content and high phenolic content.

Keywords: Distiller' spent grains, superheated steam, drying, antioxidant activity.

INTRODUCTION Distillers' spent grains (DSG) are the byproduct of bio-fuel and ethanol production from corn, wheat, or other grains as the biomass for the fermentation process. Distillers' grains can be sold wet or dehydrated. Wet distillers' grains, distillers' dried grains, distillers' dried solubles and distillers' dried grains with solubles are all variations of the co-product from the ethanol production and they all vary in characteristics due to different types of treatments at the ethanol plant. Grains employed for ethanol production are a good source of phenolic acids (Adom and Liu, 2002), which are not metabolized during the fermentation by yeasts (Baranowski, 1980); thus, phenolic acids remains after fermentation, resulting in residues with certain content of beneficial compounds. Antioxidants are important because their frequent consumption is associated with a lower risk of cardiovascular disease and cancer (Temple, 2000). One of the methods used for determination of the antioxidant activity is to measure total phenolics. Others methods use ferric reducing antioxidant power (FRAP), 2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) assay (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Vignoli et al., 2011; Ye et al., 2011). Commonly, total phenolic content is measured using the Folin-Ciocalteau method. The reagent Folin-Ciocalteau is a mixture of acids which are reduced when they interact with phenolic compounds present in the sample (Gao et al., 2000). For measuring antioxidant activity, all the methods involve oxidative agents that accept electrons from reductants, which usually are treated as the antioxidants that are measured. The basis of the Trolox equivalent antioxidant capacity assay is the inhibition of the absorbance of the radical cation of ABTS by reductants (Cao and Prior, 2002).

There are reports that DSG have been added to bread formulations (Rasco et al., 1990; Liu et al., 2011) and used for animal feeding, such as weaned piglets (Pedersen et al., 2005) or dairy cattle (Penner et al., 2009). More novel applications will need to be considered, as the production of DSG is increasing around the world. Predictions indicate that there will be an over-supply of the product which could affect the future viability of the ethanol industry (Bonnardeaux, 2007). Commonly, DSG are dried to extend their shelf-life and to reduce the cost of transportation (Woods et al., 1994). Besides concerns about costs, a drying method employed may affect the nutritional and/or functional value of the final product (Tang et al., 2005; Inchuen et al., 2010). The objective of this study was to evaluate the protein content and the antioxidant activity of DSG dried under different conditions (near ambient air at 30°C, hot air at 150°C and superheated steam at 150°C).

MATERIALS AND METHODS The material used in this study was a mixture of 90% corn and 10% wheat stillage obtained from a local distillery (Mohawk Canada Limited, a division of Husky Oil Ltd., Minnedosa, MB). The whole stillage was centrifuged using a Sorvall General Purpose RC-3 centrifuge (Thermo Scientific Co., Asheville, NC). The centrifuge operated at a relative centrifugal force of 790xg, with 1000 mL sample containers rotation at a speed of 2200 rpm, on a radius of 0.14 m for 10 min. The centrifugation produced three fractions: liquid, semi-solid and the solid coarse fraction. Only the solid coarse fraction was used in the drying experiments and was named wet distillers grain (WDG). This fraction was placed in heavy duty plastic bags and stored in a freezer at -15°C before using it in further experiments. Before each set of drying experiments, the required amount of WDG was thawed at room temperature for two hours. The initial moisture content of WDG was 74.5% wb (AACC, 1999).

Drying methods. Three sets of different drying experiments were performed: (i) drying in near ambient air, (ii) drying in hot air, and (iii) drying with superheated steam. All drying experiments were conducted at the University of Manitoba, Canada and then dried samples were shipped to the Universidad de las Americas Puebla in Mexico for the analysis.

The near ambient air drying of WDG was conducted at 30°C, using a laboratory convection oven (Precision Thelco laboratory oven). The WDG samples were placed in aluminum dishes in a thin

layer (3 mm thick) and dried until final moisture was 9-10 % wb. Samples from different dishes in this temperature set-up were combined and used in further experiments.

The hot air drying of WDG was done in the same laboratory convection oven used in the near ambient air drying experiments. The temperature of the oven was set to 150°C. The wet material was spread in a thin layer in aluminum dishes and dried until the moisture of 9-10% wb was reached. Samples from different dishes were combined at the end of this experiment and stored in plastic bags for further experimentation.

The superheated steam (SS) drying was conducted at the Department of Biosystems Engineering, University of Manitoba, MB, Canada. The system which consists of a steam generator, superheater, steam-conveying pipes, drying chamber, condensation unit, data acquisition and control system has been already described by Pronyk et al. (2010). The SS temperature at the inlet to the drying chamber was at 150°C with the 1.5 m/s steam velocity passing through a sample. Drying experiments were conducted under or near to atmospheric pressure. Approximately 30 g of the wet material was placed in a thin layer on a thin wire mesh and dried in 150°C SS until final moisture was in the range between 9 to 10% wb. The experiment was repeated until required amount of dry material was obtained for further experimentation.

Determination of protein contents in DSG Protein content was calculated from the total nitrogen determination following the Kjedhal procedure (AOAC, 2000), by triplicate.

Preparation of extracts Two extracts were prepared for every sample of dried DSG: using 70% ethanol as solvent for ethanolic extracts or distilled water for aqueous extracts. A 0.1 g sample of homogenized dried DSG was dispersed in 25 mL of solvent and stirred for 2 h at room temperature. Next, the mixture was filtered through filter paper (Whatman #1) using a Büchner funnel. The obtained extract was placed in a threaded flask covered with aluminum foil, and kept in refrigeration until total analyses of phenolic compounds and antioxidant activity was performed.

Determination of phenolic compounds Total phenolic compounds content in both ethanolic and aqueous extracts was evaluated based on the Folin-Ciocalteau method (Gao et al., 2000). Two mL of distilled water, 100 µL of the extract and 200 µL of the Folin-Ciocalteau reagent were mixed in a 10 mL amber tube. A blank was also prepared. The mixture was left for 3 min at room temperature (~25 °C), and then 1 mL of Na_2CO_3 solution (20% w/v) was added. The tube was left for one hour in a dark place at room temperature, then the absorbance was measured at 765 nm using a spectrophotometer (SQ-2800 Single Beam Scanning UV-Visible, USA). The measurements were compared to a standard curve of gallic acid concentrations (previously developed), and expressed as milligrams of gallic acid equivalents per gram of DSG obtained “as is” after drying.

Antioxidant activity assay For measuring antioxidant activity *in vitro*, the ABTS method reported by Re et al. (1999) and modified by Kuskoski et al. (2004) was followed. ABTS (2,2-azinobis-3 ethyl benzothiazoline-6-sulphonic acid) was supplied from Sigma Chemical CO. (St. Louis, MO, USA. The $ABTS^+$ solution was prepared by reacting 7 mM of ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), with 2.45 mM of potassium persulfate. To achive this, 0.0194 g of the ABTS reagent was mixed with 0.0033 g of potassium persulfate and 5 mL of distilled water in an amber glass bottle. The bottle was also covered with aluminum foil and left undisturbed in a dark room at approximately 25°C temperature for 16 h - the time needed for the formation of radicals. The $ABTS^+$ solution was diluted by mixing it with absolute ethanol to an absorbance of 0.7+0.02 at 754 nm (approximately 100 µL of $ABTS^+$ solution for every 11 mL of ethanol). The absorbance at 754 nm was measured in 4 mL of solution containing 80 µL of the extract after 7 minutes of reaction. Results were expressed as grams of trolox per gram of dried DSG. The standard curve of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was performed previously.

Statistical analysis Results were statistically analyzed using Minitab Release 14 software, through an ANOVA test in order to identify if there were significant differences between data. Tukey´s pair wise comparisons were applied when differences were found at a significance level of p=0.05.

RESULTS AND DISCUSSION Protein content of DSG after drying ranged from 15.1 to 16.9% wb, and no effect of the drying method used was observed (p>0.05). Protein contents determined in this study are lower than values reported by Tang et al. (2005) for DSG dried at 145°C (27.3% db) or by Penner et al. (2009) for dried corn distillers' grain with soluble (29.7% wb). Differences are attributed to variability of grains.

Table 1. Protein contents of dried DSG under different methods.

Drying Method	Protein content (% wb)
Air	16.4 ± 2.1
Oven	15.1 ± 0.7
SS	16.9 ± 1.3

Ethanolic solution was more effective as extractant for both phenolic compounds and antioxidant activity, in comparison with distilled water (Figures 1 and 2). Total phenolic compounds content extracted with water was 0.88 for air drying, 1.29 for oven hot drying and 2.9 mg GAE/g for SS, while phenolic content extracted with ethanolic soluction was 4.62, 3.11 and 12.97 mg GAE/g of dried grain for air, hot oven and SS, respectively. DSG dried with SS had the highest phenolic contents among the analyzed samples (p<0.05). The phenolic compounds content determined in DSG dried with SS is considered good, as it is comparable to the value reported for fresh grape berries cv. Sonaka (11.2 mg GAE/g fresh weight), following a similar methodology (Kedage et al., 2007), but lower than those reported for mandarins by Ye et al. 2011 (values from 47.1 to 78.7 mg GAE/g).

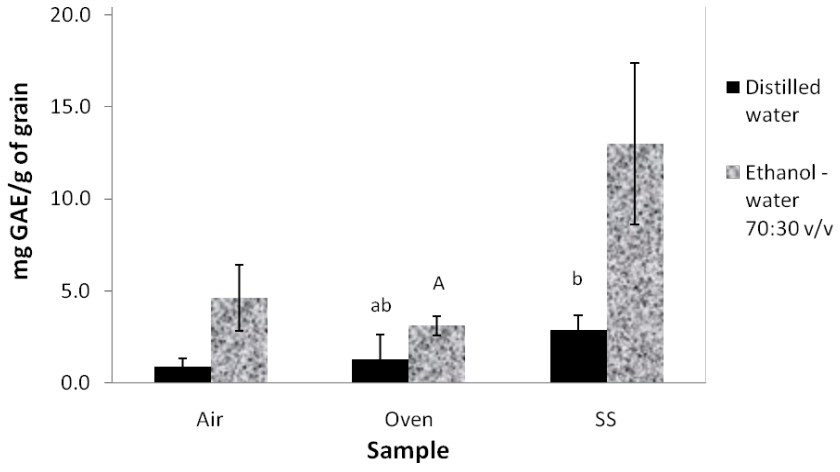

Figure 1. Total phenolic compounds content in dried DSG under different methods.

For antioxidant activity, aqueous extracts had 0.44, 0.39 and 0.32 mg trolox/g for air, hot oven and SS, respectively. Ethanolic extracts had 0.55, 0.57 and 0.54 mg trolox/g with respect to the same drying techniques used. Antioxidant activity of dried DSG is lower than values reported for grape by Kuskoski et al., 2005 (2.30 mg trolox/g) and values reported for light roasted Arabica coffee by Vignoli et al., 2011 (0.19 g trolox/g), even our method and the reported in these articles may vary. It can be seen that although the phenolic compounds content determined in DSG dried with SS is considered good, the antioxidant activity is relatively low in all samples using different drying techniques. It is difficult to explain ambiguous connections between antioxidant activity and the content of antioxidants based only on the quantitative analysis (Inchuen et al., 2010), as this correlation depends not only on the antioxidant quality and its concentration, but also on its interaction with other components which may produce synergistic or inhibitory effects (Kuskoski et al., 2005).

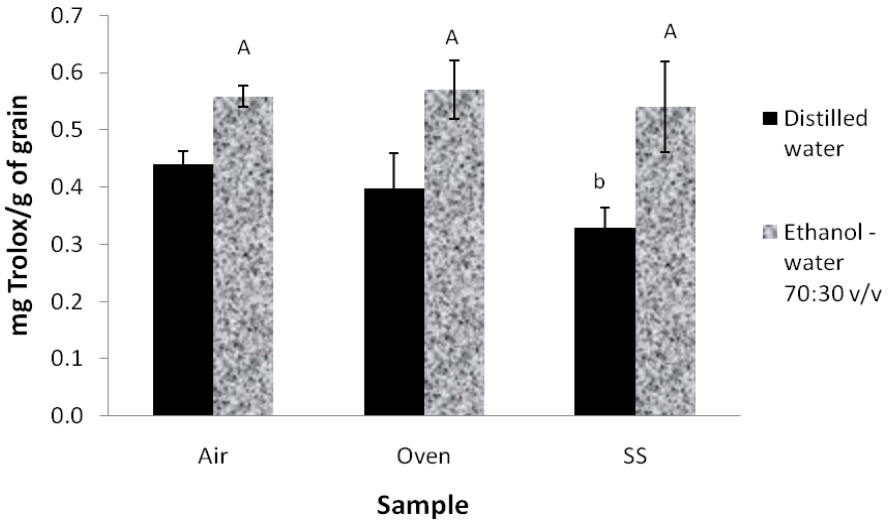

Figure 2. Antioxidant activity of dried DSG dried under different methods.

CONCLUSIONS Drying method did not have an effect on the protein content neither in the amount of the antioxidant activity for aqueous extracts of dried DSG, but it affected the antioxidant activity of ethanolic extracts and the phenolic compounds content in the product. Among the explored drying methods, the SS drying may be a suitable technique to obtain dried DSG with a good protein content and high phenolic content, which can be utilized in further applications.

Acknowledgements The authors acknowledge the financial support from the Natural Sciences and Engineering Research Council of Canada. Authors thank Praveen Johnson (graduate student) for his technical support and the preparation of dried sample of DSG.

REFERENCES

AACC. 1999. Standard #44-19.01. Air-Oven Method, Drying at 135°C. In: Approved Methods of the American Association of Cereal Chemists. AACC, St. Paul, MN.

Adom, K. K., and R. H. Liu. 2002. Antioxidant activity of grains. J. Agric. Food Chem. 50(21): 6182-6187.

AOAC. 2000. Official methods of analysis of AOAC International. Gaithersburg, MD, USA: AOAC International.

Baranowski, J. D. 1980. Inhibition of *Saccharomyces cerevisiae* by naturally-occurring hydroxycinnamates. J. Food Sci. 45: 592-594.

Bonnardeaux, J. 2007. Potential uses for distillers grains. Department of Agriculture and Food Western Australia 3 Baron-Hay Court, South Perth, WA 6151

Cao, G., and R. Prior. L. 2002. Measurement of total antioxidant capacity in nutritional and clinical studies. In "Handbook of antioxidants". Ed. E., Cadenas and L., Packer. p. 47. CRC Press.

Gao, X., M. Ohlander M, N. Jeppsson, L. Björk, and V. Trajkovski. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J. Agric. Food Chem. 48(5): 1485–1490.

Inchuen, S., W. Narkrugsa, and P. Pornchaloempong. 2010. Effect of drying methods on chemical composition, color and antioxidant properties of thai red curry powder. Kasetsart Journal. Natural Science. 44 (1): 142-151.

Kedage, V. V., J. C. Tilak, G. B. Dixit, T. P .A. Devasagayam, and M. Mhatre. 2007. A study of antioxidant properties of some varieties of grapes (*Vitis vinifera* L.). Crit. Rev. Food Sci. Nutr. 47: 176-185.

Kuskoski, M., A. Asuero, M. García-Parrilla, A. Troncoso, and R. Fett. 2004. Actividad antioxidante de pigmentos antociánicos. Ciencia y Tecnología Alimentaria 24(4): 691-693. (In Spanish)

Kuskoski, M., A. Asuero, A. Troncoso, J. Mancini-Filho, and R. Fett. 2005. Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. Ciência e Tecnologia de Alimentos. 25(4): 726-732. (In Spanish).

Liu, S.X., M. Singh, and G. Inglett. 2011. Effect of incorporation of distillers' dried grain with solubles (DDGS) on quality of cornbread. LWT – Food Sci. Technol. 44: 713-718.

Pedersen, C., S. Ross, H. Jonsson, and J. E. Lindberg. 2005. Performance, feeding behaviour and microbial diversity in weaned piglets fed liquid diets based on water or wet wheat-distillers grain. Archives Animal Nut. 59(3): 165-179.

Penner, G.B., P. Yu, and D.A. Christensen. 2009. Effect of replacing forage or concentrate with wet or dry distillers' grains on the productivity and chewing activity of dairy cattle. Animal Feed Sci. Technol. 153: 1-10.

Pronyk, C., S. Cenkowski, and W.E. Muir. 2010. Drying kinetics of instant Asian noodles processed in superheated steam. Drying Technology.28, 304-314.

Rasco, B., S.S. Gazzaz, and F.M. Dong. 1990. Iron, calcium, zinc and phytic acid content of yeast-raised breads containing distillers' grains and other fiber ingredients. J. Food Comp. Analysis 3: 88-95.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Medicine 26:1231-1237.

Tang, Z., S. Cenkowski and M. Izydorczyk. 2005. Thin-layer drying of spent grains in superheated steam. J. Food Eng. 67: 457-465.

Temple, N.J., 2000. Antioxidants and disease: more questions than answers. Nutrition Research 20, 449–459.

Vignoli, J.A., D.G. Bassoli, and M.T. Benassi. 2011. Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee:The influence of processing conditions and raw material. Food Chem. 124: 863-868.

Woods, B., H. Husain, and A. S. Mujumdar. 1994. Techno-economic assessment of potential superheated steam drying applications in Canada. Report 9138 U 888. Montreal, QC: Canadian Electrical Association.

Ye, X.-Q., J.-C. Chen, D.-H. Liu, P. Jiang, J. Shi, S. Xue, D. Wu, J.-G. Xu, and Y. Kakuda. 2011. Identification of bioactive composition and antioxidant activity in young mandarin fruits. Food Chem. 124: 1561-1566.