Agronomy Research **16**(S2), 1457–1465, 2018
https://doi.org/10.15159/AR.18.096

The effect of sapropel extracts on microflora and physicochemical parameters of Dried Distillers' Grain

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Abstract. This article is devoted to the effect of ultradispersed humate sapropel extracts, obtained from air-dry samples of sapropel, from Seryodka deposit (Pskov region, Russia) by alkaline extraction under the action of ultrasonic radiation, on microbial and physicochemical parameters of Distillers' grain (DG) during storage. At the end of the distillation, wet DG was divided and treated with sapropel extract. The untreated sample served as a control. Both (treated and untreated) were then dried to 10% moisture content. Microbial and physicochemical parameters (moisture content, pH, titrable acidity (TA), acid value (AV) of fats) were assessed before storage of dried DG. A similar experiment was repeated after one week of storage but on microbial load. The microbial counts ranged from 2.3×10^4 CFU g^{-1} (untreated) to 1.77×10^4 CFU g^{-1} (treated) before storage while 2.5×10^4 CFU $g^{-1}$to 2.18×10^4 CFU g^{-1} accounted for after a week of storage. The pH had increased from 4.5 ± 0.1 to 6.1 ± 0.1 before and after treatment respectively. TA likewise decreased from 3.2 ± 0.4 to 2.03 ± 0.06%. The results showed that sapropel extracts had effects on microflora and physicochemical parameters of DDG.

Keywords: Grains, pH, Acid Value, microflora, humic acid, fungicidal properties.

INTRODUCTION

Distillers' grains (DG) are primary fermentation by-products obtained after the fermentation of cereal grains by yeast into alcohol (Clark et al., 1987). It is valuable secondary by-product after ethanol production but due to its high moisture content pose a challenge in storing it. Wet DG approximately contains 77–81% of moisture (Aliyu & Bala, 2011). High moisture, the residue of fermentable sugars and other nutrients make DG a niche for microorganisms resulting in rapid degradation (Russ et al., 2005).

To curtail this menace (microbial instability), multi-stage separation and drying of DG were adapted. Drying is an alternative for DG preservation with the additional merit of reduced DG volume, hence decreased transport and storage costs (Santos et al., 2003).

According to GOST 31809-2012, the shelf life of the dried DG is 6 months. However, improper storage of DG can lead to the development of foreign microflora, which results in excretion of mycotoxins. These mycotoxins can interfere with the immune system of livestock's predisposing them to diseases (Emtsev, 2006).

DG has been designated as high-protein-energy substitutes for feeding livestock. Series of research has been carried out concerning the feeding (nutritional) value of DG. With respect to energy, DG possesses equal to slightly greater feeding value in comparison to corn. Nevertheless, vital research has suggested that DG could be employed as a protein source, which is equivalent to soybean and other protein base feeds utilized in feeding cattle (Coupe et al., 2008). The sort of grain utilized, technology and drying procedure can significantly impact the end product of DG, thus varies in chemical composition (pH, proteins, reducing sugar, amino acids, total acidity, an acid value of fat, etc.) and physical parameters (moisture content, odour, colour, and lightness) (Cromwell et al., 1993).

According to Emeis & Weissert (2009), sapropel is the term used to describe 'organic-rich fine-grained sediments deposited in lake and oceans' water. Also, Avdeyeva et al. (2009) defined sapropel as dark-colour sediments that are rich in organic matter, formed under anaerobic conditions from a dead organic matter of anhydrobiotic microflora and microfauna.

It has been noted that three vital constituents of sapropel interact with each other: biologically active, organic and mineral (Kireycheva & Khokhlova, 1998). Our previous work has shown the fungicidal potentials of ultradispersed humic sapropel suspensions. Sapropel extracts have proven to have significant fungicidal properties depending on volume applied, even in small doses (Barakova et al., 2017).

The important role of sapropel on the stored Dried DG (DDG) cannot be ignored since it alters the chemical composition and physical parameters due to the humic acid contained in it. The biological effects of humic substances are based on the diversity of their reaction groups, which enable them to participate in a variety of biochemical transformations (Perminova, 2008; Savchenko, 2015). The presence of carboxyl groups allows participation in ion exchange, hydroxyl reactions, carbonyl in oxidation-reduction reactions, etc. (Perminova, 2008).

Sapropel extracts treatment of animal feed has reported improving livestock productivity (Kireycheva & Khokhlova, 2000). Sapropel is a unique organic feed, which is promising to use in various sectors of industry, agriculture, livestock farming, medicine and balneology (Plaksin & Krivonos, 2007).

The aim of the present study is to show the effect of sapropel extract on microflora, chemical composition and physical parameters of DDG obtained after production of ethanol from barley grains.

MATERIALS AND METHOD

The objects of the study were ultradispersed humic sapropel suspensions obtained in RAS Limnology Institute with alkaline extraction and ultrasound treatment of air-dry sapropel from Seryodka deposit (Pskov region, Russia). Sapropel extracts used was obtained from hot method extraction at 40 °C.

Analysis and optimization of sapropel extracts

A rotary evaporator EV 130 (LabTech) was used to increase the concentration of sapropel extracts. The sapropel obtained from hot method extraction had 3.5% of dry matter and the evaporation was carried out to increase the concentration to 20%.

Automatic Titrator 848 Titrino Plus (Metrohm) was used for determination of sapropel extracts pH. The device operates in two modes: titration with automatic endpoint determination and pH measurement. The latter was used in the experiments. Time of assay was no less than 5 minutes, and sometimes more in case of decimals other than the last digit being unstable.

Refractometer PTR 46 (Index Instruments) was used for measurement of the concentration of dry matter in sapropel extracts.

Determination of moisture and starch content of barley

Moisture analyser MOC-120H (Shimadzu) was used for assessment of all barley grains and flour moisture content according to ISO/TC 34 (ISO/TC 34, 2009).

The barley used in this experiment was harvested in Russia within the 2016 season.

The starch content of the barley was determined by using Polarimeter (PolAA FF55). The determination of the starch content of barley was conducted according to ISO/TC 93- Ewers polarimetric method (ISO/TC 93, 1997).

Production of ethanol

Barley grains (10 kg) were weighted and milled in a milling machine (SINBO SCM-2929). Mashing, fermentation and distillation were carried out in Dr Guber factory.

The milled barley was then mixed with 35 L of warm water (50 °C). An amount of alpha amylase (13.6 mL) and xylanase (13.7 mL) was then pipetted into the solution, for a rest period of 30 minutes. The temperature was then increased to 70 °C, for a rest period of 4 hours.

A sample of wort was taken at 30 min intervals and the concentration of dry matter (°Brix) was measured using a refractometer. The final wort was allowed to cool down to a temperature of 30 °C. *Saccharomyces cerevisiæ* (8.4 g) was reactivated, 10 min before pitching. Glucoamylase (5.6 mL) was then added to the pitched wort. All enzymes used are from Erbslöh (Germany).

Fermentation was then carried out at a temperature of 30 °C for 72 hrs. After the 72 hrs of fermentation, a distillation of the fermented wash was then performed.

Distiller's grain collection and treatment

After distillation, Wet distillers' grains was then collected and centrifuged at 4,600 rpm for 10 minutes and stored in the freezer before their analysis (Fig. 1).

An amount of 20 mL of sapropel extracts (20% of dry matter and pH 7) (Fig. 2) was sprinkled on 100 g of distillers' grain followed by uniform mixing. The mixture was allowed for a 30 minutes rest period (undisturbed). A control sample of the same mass (untreated) was also observed (Fig. 3). Samples (treated and untreated distillers' grains) were then dried in cabinet dryer (ES-4610) at a temperature of 100 °C.

Figure 1. Wet Distiller's grain in plastic packet before drying and analysis.

Figure 2. Sapropel extracts (20% of dry matter and pH 7) before sprinkling on wet distillers' grain.

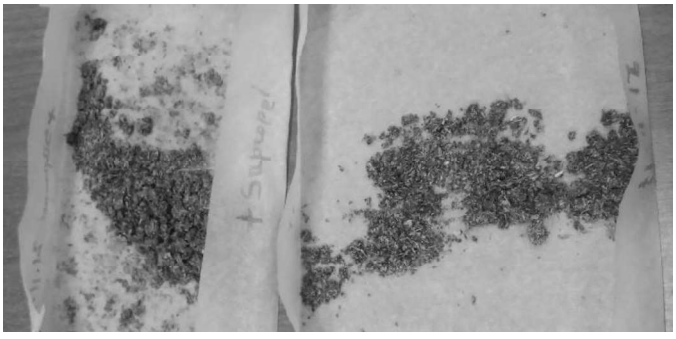

Figure 3. Dried DG 10% of moisture content, treated sample (left) and untreated (right).

Microbiological analysis of DDG

For microbiological analysis, 10 g of each sample (treated and untreated) of DDG, was taken and mixed with 100 mL sterilized distilled water in the different conical flask and the latter was shaken for 10 minutes. Quantification of the microbial load was then carried out according to the method described by Adadi and Obeng (2017). The

remaining DDG was stored in a thermostat TS-1/80 SPU at 37 °C for further microbial investigation after one week.

Physicochemical parameters determination

After microbiological analysis, physicochemical parameters (Moisture content, pH, Titratable acid (TA) and Acid value (AV) of fat) were then determined.

The moisture content of Dried Distiller's grains was measured according to ISO/TC 34 (ISO/TC 34, 2009).

The titratable or total acidity (TA) of DDG was determined according to GOST 13496.12-98 (GOST 13496.12, 1998).

Fat Acid Value (AV) was determined according to GOST 13496.18-85. In our research, the potentiometric titration method written in GOST 13496.18-85 was used to determine AV. The essence of this method consists in potentiometric titration of fatty acids extracted from the product by extraction with a mixture of chloroform and ethyl alcohol (GOST 13496.18, 1986).

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made at least in triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

The moisture and starch content of the raw barley grains determination

The moisture and starch content of the raw barley grains were determined in the present study and results are reported in Table 1.

The determination of the amount of moisture is one of the most fundamental and important analytical procedures that can be performed on a food product (Aurand et al., 1987). In brewing and whiskey ventures, starch content of the raw material is very crucial. This has a significant impact on the concentration of ethanol and composition of dried DG.

Table 1. Starch and moisture content of barley

Parameters	Concentration (%)
Moisture content	8.53 ± 0.07
Starch content	52.05 ± 0.05

Variation of the concentration of dry matter during mashing process

The concentration of dry matter was measured and results were recorded in Table 2.

Wort samples were taken at 30 min interval until the mashing was over (270 min) which allowed for the concentration of solids (°Brix) to be plotted against time (Fig. 4).

Table 2. Variation of the concentration of dry matter (°Brix) during mashing process

Time (min)	Concentration of dry matter (°Brix)
30	3.7 ± 0.3
60	12.3 ± 0.4
90	14.1 ± 0.5
120	16.2 ± 0.1
150	16.9 ± 0.1
180	17.6 ± 0.2
210	17.7 ± 0.1
240	17.9 ± 0.1
270	18.2 ± 0.1

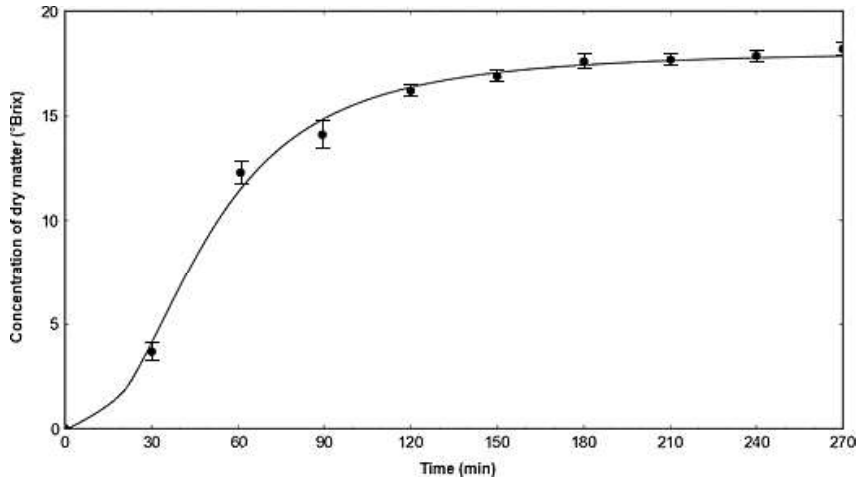

Figure 4. Variation of the concentration of dry matter (°Brix) during mashing process.

Amylase enzymes involved in the transformation of starch into fermentable sugars is temperature dependent, so as the mashing proceeds heat also increase aiding the activations of the enzymes. The increase in concentration of dry matter (°Brix) cannot remain persistent due to the deactivation of the enzymes at high temperature. Adadi et al. (2017) studied the concentration of dry matter (°Brix) kinetic during fermentation of beer supplemented with *Hippophae rhamnoides*.

The effect of sapropel on DDG's microflora

Microbiological analysis of DDG was carried out and after incubation, colonies were counted manually and the results were reported in Table 3.

Table 3. Microbiological results of un- and treated dried distillers' grain before and after one week of storage at 37 °C

Treatment	Before storage (CFU g^{-1})	After storage (CFU g^{-1})
Untreated DDG	2.3×10^4	2.5×10^4
Treated DDG	1.77×10^4	2.18×10^4

CFU - colony forming unit; g- gram of dried distillers 'grain.

In the present study, sapropel inhibited the growth of microflora on treated DDG. However, there was an inhibition of the untreated DDG, which could be a result of environmental effects (temperature, pressure, pH etc.). According to Adadi & Obeng (2017), pH is very important and has a major influence on microbial growth. Moreover, the antibacterial and antifungal properties of sapropel have been reported. Barakova et al. (2017) showed the fungicidal potency of sapropel extracts. However, these properties depend on the quantity utilized. Bactericidal properties of sapropel have also been elucidated by Buzlama & Chernov (2010), which are due to humates and humic substances (humic acids in humic acids group). These compounds could inhibit the proliferation of some groups of bacteria. Russell & Diez-Gonzalez (1998) explained how coupling mechanism is altered by acids transported across the cell membrane. This blocks the production of ATP thereby arresting the ability of anabolism.

The effect of sapropel extracts on physicochemical parameters of DDG

Physicochemical parameters of DDG were determined during this experiment and recorded in Table 4.

Chemical parameters of DDG were undoubtedly changed due to the application of sapropel extract. The chemical and physical characteristics of DDG were determined routinely as part of quality management. According to GOST 31809-2012, the moisture content of DG feed should be not less than 5 and not more than 10. DDG (treated and untreated) in the present study were in range: $5 < 9.96 < 10$ (Table 4).

Table 4. Physicochemical parameters of un- and treated dried distillers grain

Physicochemical parameters	Untreated DDG	Treated DDG
Moisture content (%)	9.96 ± 0.06	9.96 ± 0.06
pH	4.5 ± 0.1	6.1 ± 0.1
Titratable acidity (TA) (%)	3.2 ± 0.4	2.03 ± 0.06
Acid Value (AV) (mg g^{-1})	2.89 ± 0.04	1.26 ± 0.01

The pH had increased from 4.5 ± 0.1 to 6.1 ± 0.1 before and after treatment respectively. TA likewise decreased from 3.2 ± 0.4 to 2.03 ± 0.06%. Prior to the treatment, DDG was more acidic than after treatment. pH is important to assess the ability of a microorganism to grow in a specific environment. According to Adadi et al. (2017a) and Adadi (2017b), the lower a pH value, the higher TA.

The role in acidulation is influenced by inorganic acids like carbonic acid and phosphoric acid from carbon and phosphorus elements respectively whereas organic acids affect flavour, colour, microbial stability and keeping the quality of feed (Tyl & Sadler, 2017). Sapropel is not rich in inorganic acids but it could affect the TA of DDG due to its organic-rich sediments. A decrease (from 2.89 ± 0.04 to 1.26 ± 0.01 mg g^{-1}) in acid value (AV) was also observed. AV was expressed in milligrams of potassium hydroxide per gram of fat (Table 4).

CONCLUSION

In this study, sapropel extracts have shown its effect on microflora and physical parameters and chemical composition on DG. Sapropel extracts could be of great help to the food industries and other grain-related fields due to its unique organic acids. The potency of sapropel extracts depends on the amount used, its pH and concentration of dry matter hence these parameters should always be monitored for optimal results. Sapropel could aid to reduce the quantity of microorganisms in DG after treatment. On the other hand, sapropel can increase the growth rate of microorganisms during storage. Further research is needed in order to get better understanding of these relations.

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