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Effects of Ultradisperse Humic Sapropel Suspension on Microbial Growth and Fermentation Parameters of Barley Distillate

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Abstract: Barley and other cereal grains can be used in the production of ethanol. The quality and safety of the grains utilized have enormous effects on the overall yield and quality of the final product (ethanol). Therefore, the present paper seeks to elucidate the antimicrobial activities of ultradisperse humic sapropel suspensions (UDHSS) on barley, wort, fermentation, and the quality of the final product. A standard microbiological method was used to assess the biocidal activities. Physicochemical parameters and volatile compounds were determined. Treated samples exhibited least microbial growth (for grain: $1.145 \pm 0.120 \times 10^4$ cfu/g) when compared to the control ($3.425 \pm 0.33 \times 10^5$ cfu/g). Mash from the treated sample had less Free Amino Nitrogen (35.14 ± 0.02 mg/L) than the control experiment (41.42 ± 0.01). However, the levels of °Brix and Free Amino Nitrogen (FAN) were unaffected by the UDHSS treatments. After the chromatographic analysis, it was revealed that the barley distillate obtained from treated grains had high volatiles concentration when compared to the control experiment. The volume of the methanol quantified in the distillate was low, and hence safe, and might find applications in the food industries or in domestic consumption after rectification.

Keywords: antimicrobial effects; mashing; distillation; volatile compounds; gas chromatography; reactive oxygen species

1. Introduction

Ethanol or ethyl alcohol is a type of alcohol and its production is nothing new. In ancient times, Egyptians produced ethanol from vegetables while the Chinese discovered the technique of distillation, which increases the concentration of alcohol in fermented wash [1]. Ethanol can be produced from different grains such as corn, wheat, barley, sorghum, oat, and rice [2]. Distillation is used to produce rectified spirits. The latter is highly concentrated ethanol (drinking alcohol), which has been purified by means of rectification (repeated distillation). Rectified spirits, produced from grain, sugar beets, or potatoes, are used for multiple purposes, namely in the production of whiskey, brandy, gin, vodka, liqueurs, for medicinal purposes, and so on [3].

The safety of raw materials utilized in the production of ethanol technology has a significant effect on the quality and yield of ethanol as well as the by-products (distillers' grain). Maximizing the yield of ethanol is the main priority of every ethanol producer. However, yeasts are not the only living organisms that use the sugar or other nutrients in the wort. When contaminated grains are used to produce mash for ethanol production, bacteria and fungi compete with yeasts for the nutrients, thereby decreasing the yield and quality of the ethanol produced. The goal of every producer is to maximize profit. Encountering lower yields due to contamination by unwanted organisms will not guarantee this outcome, but rather lead to loss of profit, which could collapse an enterprise. According to Bischoff et al. [4], the class of lactic acid bacteria (LAB) that includes *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* causes the most problems during fermentation. During fermentation, yeast converts fermentable sugars (from starch degradation) to ethanol. Conversely, bacteria transform the same sugars to lactic or acetic acid. When bacteria are not controlled, yields can drop significantly, which is regarded as a huge economic loss for producers [5].

This has led to the wide application of antibiotics. Antibiotics such as penicillin, virginiamycin, erythromycin, tylosin, and tetracycline are effective against these LABs, killing them and leaving yeast unharmed. The most commonly used antibiotics in ethanol production are penicillin and virginiamycin [6]. Continuous use of these antibiotics can lead to the development of resistant strains, which could be difficult to manage. Therefore, using sapropel extracts as an alternative measure when dealing with the menace of contamination during ethanol production was proposed.

Sapropel is defined as the benthos, found in fresh water, formed under anaerobic conditions from a dead organic matter of hydrobiotic microflora and microfauna. It is principally composed of nutrients (i.e., sugars, minerals, lipids, etc.) and organic compounds known as humic substances (HS).

Sapropels and sapropel extracts have been previously reported to exhibit some antibacterial and antifungal properties, hence could be used as an alternative and novel antibiotic. The antimicrobial properties of sapropels can be attributed due to the presence of HS [7–9].

Therefore, the purpose of this paper is to study the antimicrobial potency of ultradisperse humic sapropel suspensions (UDHSS) and its effects on the chemical composition of barley grains, parameters of wort during and after mashing, fermentation, and on volatile compounds of ethyl alcohol.

2. Materials and Methods

The objects of the study were ultradisperse humic sapropel suspensions (UDHSS) obtained from the Russian Academy of Sciences (RAS) Limnology Institute, St Petersburg, Russia. The source of the sapropel is Seryodka Lake, Pskov, Russia.

The sapropel used was extracted via the hot method at 40 °C (104 °F) at pH 11.8 and 3.7 of the concentration of dry matter. Barley grains were purchased from the Narovny market, St Petersburg, Russia.

An amount of 20 mL of UDHSS 10% dry matter and pH solution of 6.7 was sprinkled on 100 g of barley grains followed by uniform mixing. The treated grains and the control samples were allowed to rest period (undisturbed) for 24 h. The treated sample was then air-dried in cabinet dryer ES-4610 (Reaktivsnab, Shymkent, Kazakhstan) at a temperature of 50 °C to 10–12% moisture content. Both treated and the control samples (10 g) were suspended in 100 mL sterile phosphate buffer solution (PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, 1 L distilled water, pH = 7.4) and mixed for 30 min on a shaker. Again, 1 mL of mash and the wash (treated and control) were aseptically pipetted into 100 mL sterile PBS. The mixtures were homogeneously mixed with the aid of a sterile glass rod. Serial dilution, inoculation, and quantification were carried out according to the method previously described in Reference [10]. Beef extract agar medium (Research Center for Pharmacotherapy, Saint Petersburg, Russia) was utilized in this study.

Moisture analyzer MOC-120H (Shimadzu, Tokyo, Japan) was used in assessing the moisture of the barley grains and flour based on the method previously described by ISO/TC 34 [11].

The starch content of the grains was determined using an optical activity AA-55 automatic Polarimeter (Optical Activity Limited, Cambridgeshire, UK) from the recommendations of the ISO/TC 93-Ewers polarimetric method [12].

The treated and untreated grains were milled separately using a coffee grinder (Sinbo SCM-2929, Istanbul, Turkey). Milled grain (500 g) was measured and transferred into hand-made mash tuns filled with 1.25 L of warm water (45 °C). The mixture was then placed in a water bath equipped with temperature regulators and a heating system (Figure 1). Enzyme preparation was then done by adding α-amylase (2.5 unit/1 g of starch) and Xylanase (1 unit/1 g of grains), in warm water (45 °C), to the mixture (Erbslöh, Geisenheim, Germany). The ratio of grains to water was 1:2.5. The temperature of the mash was then increased to 50 °C for 30 min followed by 70 °C with a rest time of 4 h.

Figure 1. Hand-made mash tun in a water bath during the mashing process.

During mashing, a portion of the mash was collected every 30 min and centrifuged with a centrifuge (ULAB, Beijing, China) at 5000 rpm for 10 min. The °Brix was measured using a refractometer PTR-46 (Shimadzu, Tokyo, Japan). The Free Amino Nitrogen (FAN) was determined by the Ninhydrin method [13].

Glucoamylase enzyme (7 unit/g of starch (Erbslöh, Geisenheim, Germany) was added to the mash after it was left to cool down. The yeast was reactivated 15 min before pitching (1 g per 10 mL of warm water (35 °C). The cool mash was pitched with *Saccharomyces cerevisiae* (1 g of yeasts per 1 L of mash (Lallemand, WI, USA), and kept in an incubator (Guangzhou Kenton Apparatus Company Limited, Guangzhou, China) and allowed to ferment at 30 °C for 72 h.

The degree of carbon dioxide (CO_2) was determined. Each handmade fermenter was equipped with a rubber hose (Figure 2), which was dipped in water to allow CO_2 to escape while preventing oxygen from entering the fermenter. The mass of each fermenter was measured before and during fermentation at 24 h intervals. The mass of the CO_2 eluting from the fermenters was then quantified using Equation (1).

$$X = \left(\frac{m - m_1}{m_2} \right) \times 100, \quad (1)$$

where X: Mass of carbon dioxide; m: Mass of fermenter and mash before fermentation; m_1: Mass fermenter and mash during fermentation; m_2: Mass of mash; and 100: Conversion of the mass of CO_2 in 100 g.

The titratable acidity (TA) of the wash was determined according to the method previously described in Reference [14] with some modifications. TA was determined by direct titration of the samples with phenolphthalein as the indicator until a slight pink coloring remains for 30 s.

After fermentation, the distillation was performed by measuring 100 mL of fermented wash into a EV311 rotary vacuum evaporator (Lab Tech, Milan, Italy). 60 mL of distilled water was used to rinse the measuring cylinder. The mixture (fermented wash and distilled water) was then transferred to a round-bottom flask, which was then connected to the distillation setup and evaporated at 75 °C

at 70 rpm. The distillation continued until 95 mL of distillate (alcohol and water) was obtained. Distilled water (5 mL) was then added to get 100 mL of distillate.

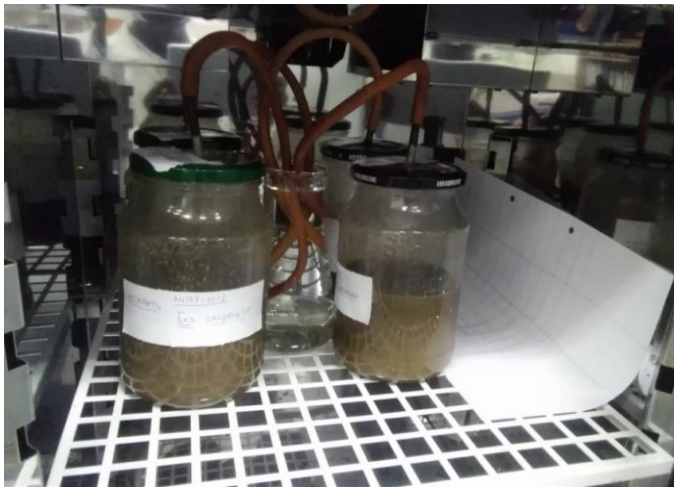

Figure 2. The hand-made fermenters with pipes in the incubator during fermentation.

Volatile compounds were determined according to Reference [15] and Reference [16]. The method is based on the chromatographic separation of micro impurities in a sample of alcohol-containing liquid and their subsequent detection by a flame ionization detector (FID). Gas chromatography "Crystal 5000.2", equipped with capillary column HP-FFAP (Santa Clara, CA, USA) 50 m × 0.32 mm × 0.52 µm, was used during the analysis. The temperature of the column prior to and at the end of the experiment was 76 °C and 200 °C, respectively. The temperature of the column thermostat was set to 5 °C/min up to 90 °C, and finally, it was set to 20 °C/min to 200 °C. The evaporation temperature was 180 °C. An injector with flow division: coefficient of the flow division was 1:26.7. The flame ionization detector (FID): Detector temperature was 210 °C. The air consumption was 200 mL/min, hydrogen consumption was 20 mL/min and blowing was 25 mL/min. The initial pressure of the carrier gas—compressed nitrogen (of particular purity) was 60 kPa. After 8.5 min, the pressure increases with a gradient of 30 kPa per minute up to 145 kPa. Without pre-treatment 0.3 µL of the ethanol was injected in the splitless mode (vent time, 60 s) and the compounds were identified by comparing the mass spectra obtained with Mass Spectral Library of the National Institute of Standards and Technology (NIST). The range of measured volume fractions of methanol was from 0.0001% to 0.0500% and the mass concentrations of other toxic micro-impurities from 0.5 to 10.0 mg/dm^3.

Data Analysis

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

3. Results and Discussion

3.1. Microbial Assessment

The incidence of microbial load for the treated sample ranged from 1.145 ± 0.120 × 10^4 cfu/g, 1.55 ± 0.212 × 10^3 cfu/mL, and 2.07 ± 0.127 × 10^2 cfu/mL for grains, mashed wort, and wash (sampled during fermentation), respectively. Whereas the control differs from 3.425 ± 0.33 × 10^5 cfu/g, 2.904 ± 0.141 × 10^4 cfu/mL, and 3.335 ± 0.205 × 10^3 cfu/mL for grains, mashed wort and wash, respectively (Table 1). With respect to the control (experiment) grains had the highest microbial load followed by the mashed wort and the least been the wash. On the other hand, microbial growth was recorded with the treated samples where grains showed the highest growth followed by the

mashed wort and the wash. From these results, it can be concluded that the UDHSS exhibited some antimicrobial properties, which had inhibited/reduced the proliferation of the microbes on the treated samples, as compared with the control.

Table 1. Microbial of load samples after treatment (Mean ± S.D × CFU/mL (g)).

Sampling Points	Treated	Control
Grains	$1.145 \pm 0.120 \times 10^4$ cfu/g	$3.425 \pm 0.33 \times 10^5$ cfu/g
Mashed wort	$1.55 \pm 0.212 \times 10^3$ cfu/mL	$2.904 \pm 0.141 \times 10^4$ cfu/mL
Wash	$2.07 \pm 0.127 \times 10^2$ cfu/mL	$3.335 \pm 0.205 \times 10^3$ cfu/mL

S.D = Standard deviation. Cfu/mL= colony forming unit per milliliter. Cfu/g = colony-forming units per gram.

UDHSS, and its isomers, were reported previously to exhibit antibacterial and antifungal properties [8,9,17], the biocidal actions were attributed to the fulvic acids (FA), humic acids (HA), mumie, and humin which are the principal constituents of HS in UDHSS [18–21]. Furthermore, HS has been documented to have inflicted damage on DNA with further growth arrest and apoptosis. This damage was ascribed to the reactive oxygen species (ROS) generated as the result of the HS [22]. Microbial contamination of grains is inevitable since the entire production process (during crop growth, harvesting, postharvest drying, and storage) is a possible source of contamination [23]. Contaminated grains could ruin (i.e., spoilage or off-flavour generation in beverage) an entire production line, thus causing financial loss to the brewer/distillers and dissatisfaction to the consumers since it is unpleasant to drink poorly flavored beverages. However, the presence of alcohol, the bitter compounds in hops, have low pH and exert antimicrobial effects on microbes in the products [24,25]. UDHSS has great potential in the fermentation since it was able to reduce the microbial load on the treated sample, and furthermore, its application will not create resistant strains and has no effects on the environment and to the consumer when compared with conventional antibiotics. The potency of the UDHSS could have been improved by evaporation to increase the concentration to 20%. For instance, 10 mL of UDHSS would have been more effective than the current 20 mL of the 10% applied.

3.2. Moisture and Starch Content of Barley Grains

The starch content of the treated and the control were 58.8 ± 0.2 and 58.3 ± 0.3, respectively (Table 2), with a 0.5% increase after treatment, which was not significantly different ($P > 0.05$). Starch is the major source of carbohydrates in cereals and therefore plays an important role in the production of alcohol. The hydrolysis of starch to glucose requires enzymes such as alpha and beta-amylase, glucoamylase, etc. The starch content of barley accounts for 55–70% of the total carbohydrates [26]. However, the starch content varies due to (1) genetics, (2) geographical location, (3) length of exposure to light (photoperiodism), and other such factors [27]. According to Patron et al. [28], the mutation of the *lys5* gene resulted in a drastic decrease in starch content. The alpha-amylase utilized is of a bacterial source and is thermostable hydrolyzing α-1, 4 bonds at random points on the starch molecule to rapidly reduce the viscosity of gelatinized starch solutions. It is a metal ion-containing enzyme requiring a calcium ion as its cofactor for maximum activity and stability.

Table 2. Moisture and starch content of barley grains before and after treatment.

Sample	Starch Content (%)	Moisture Content (%)
Treated	58.8 ± 0.2	10.57 ± 0.03
Control	58.3 ± 0.3	11. 62 ± 0.02

The second enzyme used, glucoamylase, hydrolyzes the maltose and dextrins from the non-reducing end of the molecule. Sammartino [29] has reported that glucoamylase hydrolyzes both α-1, 4 and α-1, 6 bonds to completely degrade the dextrins to glucose.

The moisture content of the treated grains (10.57 ± 0.03) was found to be lower when compared to the control ($P < 0.05$). The treated samples were dried after treatment and this could have caused a decrease in the moisture, as seen in Table 2. The low moisture content of malt or barley is good for brewers as it impedes the growth of microorganisms, thereby minimizing the risk of contamination. In contrast, high moisture content in grains supports the growth of fungi and other spoilage organisms, resulting in contamination of the grains. Contaminated grains are not utilized in ethanol production since it could affect the quality, contaminate the production lines, and decrease the overall yield and profit of a company. The moisture content of cereals indicates their safety, the quality, and shelf life [30]. As reported by Belitz and colleague [31], the moisture content of barley grains is in the range of 11–14%.

3.3. °Brix

The general overview of the fermentable sugars accumulation in the mash was measured, and the results are recorded in Figure 3. After 30 min of the mashing, the sugars were low in the control (5.967 ± 0.058) and the treated sample (6.133 ± 0.058) and this could be attributed to the fact that mashing began at low temperature, which had little influence on the enzymatic activities. However, an increase in temperature resulted in the drastic increase in enzymatic activities, consequently increasing the concentration of the fermentable sugars in the mash. This trend was consistent in the control and the treated sample. There is direct correlation between temperature and the rate of enzymatic activity coupled with the pH of the mash.

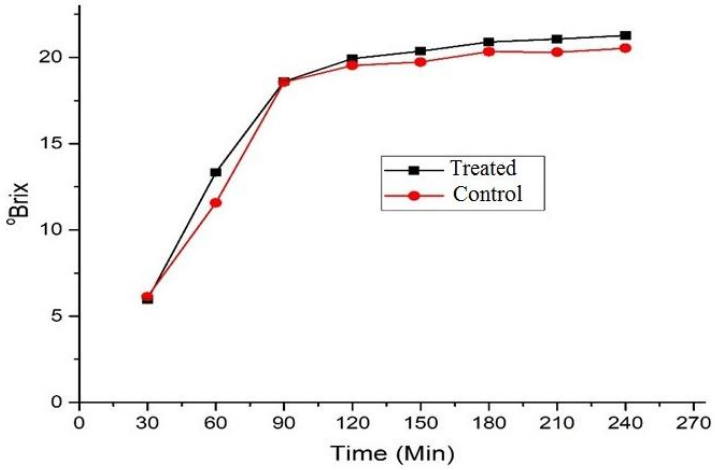

Figure 3. °Brix of the mash during the mashing of processes.

At the end of the mashing, the treated mash had slightly higher (21.267 ± 0.058) °Brix when compared to the control (20.533 ± 0.115). This could be attributed to the fact that the HS might slow the rate of the exogenous enzyme on the treated grains. According to Brigg et al. [32], 90–92% of the solids in the brewing wort are carbohydrates, which consist of sucrose, fructose, glucose, maltose, maltotriose, as well as dextrin. Moreover, 95% of those carbohydrates are products of the starch hydrolysis, which takes place in the mash tun.

The composition of carbohydrates in wort and its utilization by yeast has significant effects on fermentation efficiency and yeast metabolism, as well as the organoleptic profile of the final product [33]. β-amylase is not a thermostable enzyme and therefore prone to rapid denaturation at temperatures above 55 °C. Therefore, a thermostable α-amylase is employed to carry the reaction forward at a higher temperature to increase the yield of the ethanol [34].

3.4. Free Alpha Amino Nitrogen (FAN) of Mash

In the present study, the concentration of FAN in the treated sample was less (35.14 ± 0.02) than that of the control sample (41.42 ± 0.01) (Table 3). The amino acids constitute an important fraction of

the wort and the determination of FAN is of interest in experimental work and in routine control of products in order to establish its bioavailability. Yeasts consume amino acids as a source of nitrogen during fermentation. The determination of FAN is required to assess yeast performance [35].

Table 3. Free Amino Nitrogen of the mash.

Parameter	Control, mg/L	Treated, mg/L
Free Amino Nitrogen (FAN)	41.42 ± 0.01	35.14 ± 0.02

The yeast cells require nitrogenous compounds (e.g., individual amino acids, ammonium ions, and small peptides) for basic metabolism. Assimilable nitrogen or FAN, which can be defined as the sum of the individual wort amino acids, ammonium ions, and low molecular weight peptides [36]. The formation of volatiles is related to the concentration of these nitrogenous compounds, and their presence is not only vital for yeast performance, but also to obtain quality products. The low concentration of FAN in mash could be attributed to the humic substances (HA, FA, and humin) in the applied UDHSS. Proteases are vital mashing enzymes. During the mashing, proteases break down proteins into amino acids (via proteolysis). Proteolysis of the treated sample was inhibited due to the presence of HA. According to Ladd and Butler [37], HA inhibit proteolytic enzymes by binding to proteases via a cation-exchange mechanism, which allows the amino groups to link with the humic carboxyl groups. The inhibition of proteases affected proteolysis, thereby decreasing the amino acid concentration of the mash.

3.5. Variation of Carbon Dioxide during Fermentation

The mass (g) of CO_2released during fermentation was quantified and recorded in Table 4. The CO_2 released from the control fermenter (6.950 ± 0.031 g) was slightly higher when compared to the treated vessel (6.870 ± 0.020 g) after 24 h of fermentation. However, the dynamic changed after 48 h of fermentation where the treated vessel recorded the higher release (8.508 ± 0.022) than the control (8.474 ± 0.012 g). After 72 h, 8.846 ± 0.04 and 8.824 ± 0.013 g of CO_2 was released from the control and treated vessel, respectively.

Table 4. Carbon dioxide released during fermentation (g of CO_2 per 100 g of mash).

Time (hours)	Control (g)	Treated (g)
24	6.950 ± 0.031	6.870 ± 0.020
48	8.474 ± 0.012	8.508 ± 0.022
72	8.846 ± 0.04	8.824 ± 0.013

During fermentation, fermentable sugars are transformed to CO_2 and alcohol. The metabolic activity of yeast could be related to the amount of CO_2, alcohol, and energy released during fermentation. The increase in the evolving CO_2 in the present work showed that the yeast performance (i.e., metabolic activity) was high and could be molded to increase yield. However, HS (HA and humin), or other elements present in UDHSS [38,39], do not seem to have significant effects on the released CO_2.

3.6. Titratable Acidity

During fermentation, the TA of the samples were determined and the results were recorded in Table 5. The TA of both samples was low 0.443 ± 0.040 and 0.396 ± 0.015 on the first day, however, the control showed an increase, while the treated sample remained unchanged until the third day when it increased to 0.510 ± 0.010. There was a drastic decrease of TA (control) from 0.443 ± 0.040 to 0.406 ± 0.015 on the third day.

Table 5. Titratable acidity of treated and untreated fermented wash during fermentation.

Day	Control (°)	Treated (°)
1	0.396 ± 0.015	0.443 ± 0.040
2	0.443 ± 0.040	0.443 ± 0.040
3	0.406 ± 0.015	0.510 ± 0.010

TA correlates to the acid taste of a product. The lower the pH value, the higher the TA [40–43]. The increment of TA is very important during fermentation because it affects the pH of the mash. Yeast metabolites, i.e., lactic, malic, citrus, and acidic acids could be attributed to the changes in the TA [41]. Low pH promotes the growth of yeasts because they flourish best in pH as low as 2.0. The yeast, as a living organism, can regulate its own intracellular pH [44]. On the other hand, bacteria cannot tolerate acidic conditions. The increase in TA during fermentation inhibits the growth of bacterial curbing the menace of cross contamination in a production line. Lower beverage (i.e., beer) pH is one of the essential properties, which gives it microbial and physical stability [45].

3.7. Ethyl Alcohol Analysis: Volatile Compounds Determination

A total of 13 compounds were identified including two aldehydes, one ester, and 10 alcohols. Twelve volatiles were detected in the product produced from the treated samples, whereas 13 were detected in the control. Among the compounds, only 1-butanol was not identified in the treated samples, and on the other hand, 1-pentanol and hexanol were detected in the product (control). The concentration of volatile compounds in treated grains is higher than the control (Table 6). Acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, isobutyl alcohol, n-butanol, and isoamyl alcohol are the main volatiles detected in the in spirit drinks. The high concentration of volatiles in the treated sample could be attributed to the fact that UDHSS had elevated the amount of sugars in the grain hence increase the amount of the compounds detected. Higher alcohols (HA) are compounds that have more carbon atoms than ethanol and contribute to beverage flavor due to their solvent-like aroma resulting in a warm mouthfeel [46,47]. The efficient uptake and utilization of amino acid and sugar determined the concentration of HA [42]. The precursors for the formation of HA are formed or synthesized during proteolysis of proteins to amino acids in the mash. The type of mashing protocol adopted could significantly affect the quantity of HA [48].

Table 6. Volatile compounds found in distillate.

Number	Compounds	Control Sample, mg/dm^3	Treated Sample, mg/dm^3
1	Acetaldehyde	0.2581	0.4766
2	Ethyl acetate	0.2784	0.9619
3	Methanol *	0.0002	0.0004
4	2-propanol	0.6711	1.1315
5	Ethanol *	5.0884	7.6540
6	1-propanol	16.1658	30.0025
7	Isobutanol	14.8179	31.0407
8	1-butanol	0.2595	×
9	Isoamylol	38.495	83.834
10	1-pentanol	×	0.1992
11	Hexanol	×	0.4580
12	Benzaldehyde	14.3795	17.5549
13	2-phenylethanol	34.4960	33.9260

* Methanol and ethanol were determined in % *v*/*v* (volume per volume). (×) indicate absent of volatile compound.

HA was as follows: Isoamylol (0 and 38.495 mg/dm^3), isobutanol (31.0407 and 14.8179 mg/dm^3), 1-propanol (30.0025 and 16.1658 mg/dm^3), ethanol (7.6 and 5.0 mg/dm^3), 2-propanol (1.1315 and 0.6711 mg/dm^3), 1-pentanol (0 and 0.1992 mg/dm^3), methanol (0.0004 and 0.0002 mg/dm^3), and

hexanol, (0.4580 and 0 mg/dm^3) in the treated and the control, respectively. HA, also called fusel oil, has a negative impact on the quality products [48,49]. It can be observed that the ethanol content in the treated was more than 20% higher when compared to the control and the possible hypothesis is attributed to the number of nutrients in the UDHSS applied. The minerals, vitamins, etc., might have provided well-balanced nutritional requirements for the yeast resulting in rapid fermentation thus forming more ethanol than in the control. The nutritional composition of UDHSS was previously reported [8]. Similarly, Reference [50] identified 40 volatiles in spirit drinks.

The concentration of methanol (wood alcohol) detected was low in treated (0.0004% v/v) and the control (0.0002% v/v). Methanol is a toxic compound and is lethal to the consumer when a high volume (30–50 g) is ingested [51]. However, the amount detected in this study is low to cause any complication when consumed. Therefore, the distillate is safe for drinking after rectification and further analysis.

Esters are volatiles formed during a vigorous phase of fermentation by the enzymatic chemical condensation of acids and alcohols [32]. The quantity of ester (ethyl acetate) identified was 0.3 mg/L and 1 mg/L in the treated and control sample, respectively. Ethyl acetate is the most abundant ester in alcoholic beverages. The concentration of esters in spirits depends on the type of raw material, yeast strain employed, cleanliness of the environment, and the mash pH. The low concentrations of ethyl acetate mask bad flavor in beverages. However, at high concentrations, it gives a 'vinegar flavor' to spirits [52–54].

According to Ferreira et al. [55], aldehydes can be synthesized by the direct reaction of sugar (precursor) with amino acids or via transition metal ion-catalyzed oxidation of the amadori compound. 0.2 mg/L of acetaldehyde was detected in the treated sample, whereas 0.3 mg/L was found in the control experiment. Notably, benzaldehyde a predominant aldehyde in beer was 16 and 14 mg/L for the treated and the control experiment, respectively. Aldehydes (acetaldehyde and others) could negatively affect the quality of raw spirits. As reported by Reference [56], aldehydes cause an unpleasant taste and odor in spirits even when present at low concentrations. In previous studies [57,58], phenylacetaldehyde was detected in lager beer. Analysis of agricultural distillates (spirits) is of the utmost importance not only for the legal requirements that need be fulfilled to use them in the production of spirit-based beverages, but also because it is affecting the quality [59] and consequently consumer preference for a particular product.

4. Conclusions

In this study, the antimicrobial potency of UDHSS on barley grain, mash, and wash was assessed as well as parameters of wort and fermented wash during fermentation. The results revealed that UDHSS could replace conventional antibiotic currently employed in curbing the menace of microbial contamination of grains in the food and fermentation industries. However, a comparative study with traditional antibiotics is recommended in the future. The application of UDHSS is regarded as safe and poses no threat to the consumer and the environment, coupled with it effectively unleashing potent ROS, thus killing the microbes. The antimicrobial properties of UDHSS make it a promising agent in food industries. However, an overdose during application could also cause other spoilage organisms to grow because of the nutritional composition in the UDHSS [8] itself. Hence, an extensive study is required to establish the right volume to apply since the efficacy of the antimicrobial activities is also dependent on it. The study also showed that UDHSS affected some parameters of barley, fermentation, and the final product (ethanol). The results of gas chromatography prove that UDHSS had played a role in the increment of volatile compounds. Comparing the control and the treated sample revealed that the amount of methanol was too low to cause any complication when ingested. Moreover, the concentration in the former was high when compared to the latter. The lower concentration of volatile compounds in the distillate from the treated sample proves that it can find application in the food industry. After rectification, it could be used as drinking alcohol. However, rigorous and extensive study is required to decipher the effects of UDHSS on fermentation and the formation volatiles in the final product based on different types of barley.

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