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**ORIGINAL RESEARCH PAPER**

# THE ANTIOXIDANT COMPOUNDS DETERMINATION OF VARIOUS BREWER'S SPENT GRAIN EXTRACTS

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**Abstract:** Brewer's spent grain is a brewing waste and contains a number of biologically active substances that not completely are extracted into malt wort during mashing, but are not use anywhere. The aim of the study was to obtain a comparative characterization of various surface active extractants, including cationic one, (benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride), in relation to phenolic compounds and amino acid content of brewer's spent grain, which can serve as the basis for the biologically active additives technology development. The extraction use made it possible to verify the soluble polyphenolic compounds (phenolic acids and rutin) and amino acids presence in the extracts. Studies have led to the conclusion that extractants can be presented in the following sequence in increasing extraction efficiency, i.e., in the strength of micelle formation, in increasing order: water, 0.01 % solution of benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride, 70 % solution of propane-1,2-diol, 70 % solution of ethanol. All extracts are contained 18 amino acids, including 9 essential, 8 functional ones, and 5 polyphenolic substances, including rutin with P-vitamin activity. The carrying out alcohol extraction avoided the loss of essential amino acids by 4.5 % and functional ones - by 3.8 % respectively.

**Keywords:** *amino acids, bioactive compound, brewer's spend grain, extraction, organic solutions, plant raw material, polyphenols*

## INTRODUCTION

Recently, much attention in the world has been paid to waste-free technologies. The food production by-products can be a rich source of bioactive compounds necessary for proper human nutrition.

In this context, much attention is paid to malt grains - brewing waste, which is processed in various ways due to the presence of fiber, protein, B, E vitamins, as well as trace elements [1, 2].

Researchers pay great attention to antioxidant substances of plant materials, including malt grains [3 – 8].

The main representatives of antioxidant substances are polyphenols in various forms: free and bound [9]. It should be noted that phenolic acids, represented by hydroxybenzoic (gallic, p-hydroxybenzoic, vanilla, syringic and protocatechuic) and hydroxycinnamic (homologues of coumaric, coffeic, ferulic and synapic) acids, are present in grain raw materials. Of the flavonoids in the grain, and therefore the brewer's spent grain, there are catechins, homologs of leukoanthocyanidin, prodelphinidin, proanthocyanidin, campferomil, etc. Moreover, the bound forms of phenolic compounds are localized in the outer layers of the malt shell and esterified with polysaccharides [10].

In order to extract plant-derived antioxidants, many physicochemical methods are used, including extraction with various solvents.

Extraction makes it possible to isolate biologically active substances into the liquid phase, including polyphenols, which have a wide spectrum of action.

Many authors have noted that biologically active substances extracted have an affinity for surface active extractants (SAE) [11]. SAE themselves have different polarity, which is important when micelles are formed around the extracted substance during the extraction procedure. In order of increasing polarity, these are: hexane, ethyl ether, ethyl acetate, butanol, and water – alcohol mixtures [12].

The use of SAE is based on the association taking place in a liquid medium and the formation of di-, tri- and tetramers of SAE molecules, resulting in the formation of micellar compounds with external polar groups. These groups contribute to the solubilization of extractable substances, including sparingly soluble polyphenols [13].

It was noted that with an increase in hydroxyl functional groups in SAE, their solvation activity increases, which affects the rate of micelle formation [14].

The aim of the study was to obtain a comparative characterization of various SAE, including cationic one (benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride) in relation to phenolic compounds and amino acid content of brewer's spent grain, which can serve as the basis for the biologically active additives technology development.

## MATERIALS AND METHODS

### *Samples*

The sample of brewer's spent grain (BSG) was obtained from a single lot after the filtration stage during the Pilsner beer production on pilot brewery (Bavaria, Germany).

BSG sample was carefully washed, ground, sealted in polyester bags, vacuum packed and stored at 4 °C during investigation.

***Chemical analysis methods***

Total phenolic content (TFC) was determined by the Folin-Ciocalteu reagent (FCR) colorimetric method [15]. Quantification was performed by a calibration curve using quercetin as an authentic phenolic standard (0-0.1 $mg \cdot L^{-1}$; $p<0.01$ and $R^2=0.99$) and results were given as mg of quercetin per 100µL of extract sample. 500 µL aliquot of BSG extracts was mixed with 7500 µL distilled water and 2000 µL FCR and 3000 µL 20 % sodium carbonate and 19500 µL of distilled water were added. The mixture was incubated in the dark during 30 minutes, at 25 °C. The absorbance was measured at 765 nm using a spectrophotometer (SF26, Russia). The measurement was performed against a control sample using water instead of extract.

***High performance liquid chromatography (HPLC) analysis***

The determination of the amino acids mass concentration was a high performance liquid chromatography method with an "Agilent Technologies 1200" ("Agilent", USA) diode array detector [16]. HPLS equipment was fitted column Luna 5u C18 (2) 250 x 4.6 mm 5 micron (Phenomenex, USA) with wavelength 338 nm.

Before measuring samples were derivatized by 1 $mg \cdot L^{-1}$ o-phthaldialdegyde in 0.1 $mol \cdot L^{-1}$ borate buffer solution (*p*H 9.5). After those samples and all standards solutions were injected at a volume of 20 µL in a reversed-phase column at 39 °C. The mobile phase was 0.1 $mol \cdot L^{-1}$ sodium acetate solution (*p*H 6.4) and acetonitrile solution. The eluent flow rate was 1000 $\mu L \cdot min^{-1}$.

The determination of the phenol acids mass concentration was a high performance liquid chromatography method with an "Agilent Technologies 1200" ("Agilent", USA) diode array detector. HPLS equipment was fitted column Hypersil 5u C18 250 x 4.6 mm 5 micron (Thermo, USA) with wavelength 270 and 310 nm. The samples and all standards solutions were injected at a volume of 20 µL in a reversed-phase column at 40 °C. The mobile phase was 0,025 $mol \cdot L^{-1}$ potassium dihydrogen phosphate solution (A) (*p*H 2.5) and acetonitrile solution (B) with the ratio (A : B - 87:13). The eluent flow rate was 1300 $\mu L \cdot min^{-1}$.

The determination of the quercetin and rutin mass concentration was a high performance liquid chromatography method with an "Agilent Technologies 1200" ("Agilent", USA) diode array detector. HPLS equipment was fitted column Luna 5u C18 (2) 250x4.6 mm 5 micron (Phenomenex, USA) with wavelength 290 nm. The mobile phase was 2 % acetic acid solution (A) and acetonitrile solution (B) with the ratio (A : B - 70 : 30). The eluent flow rate was 1500 $\mu L \cdot min^{-1}$.

The replication of experiments at all stages of the experiment - not less than 3. The results of experimental studies were processed by methods of mathematical statistics using Student's criterion.

**BSG extracts preparation**

Extraction was performed at the ratio 2:10 (w/v – solute/solvent) and mixtures were shaken for 1 hours on a shaker (US 1350, Russia), at 100 rpm and at 60 °C. Five different solvents with different active substances concentration in water were used:

benzyldimethyl-[3- (myristoylamino) propyl] ammonium chloride (M), 0.01 %; ethanol, (Et), 70 %; propane-1,2-diol (PG), 70 %, and water (control sample, K). After extraction all samples were centrifuged at 4000 rpm for 20 minutes, and supernatant were separated and stored at 4 °C.

## RESULTS AND DISCUSSION

The study results of the 4 samples extracts BSG by the biologically active substances content are presented in Table 1.

***Table 1.*** *The content of biologically active compounds in extracts from grains*

| Content indicators | The content of substances in extracts | | | |
|---|---|---|---|---|
| | K | 0.01 % M | 70 % Et | 70 % PG |
| TFC [mg·100μL] | 15.0 | 17.4 | 65.8 | 63.8 |
| amino acids [mg·L$^{-1}$] | | | | |
| aspartic acid | 4.88 | 5.07 | 5.72 | 5.76 |
| glutamic acid | 3.84 | 2.28 | 6.63 | 6.01 |
| asparagine | 2.98 | 3.11 | 5.22 | 5.75 |
| histidine | 1.38 | 1.43 | 2.74 | 2.42 |
| serine | 3.87 | 3.69 | 5.09 | 5.51 |
| glutamine | 4.64 | 4.92 | 8.30 | 8.77 |
| arginine | 0.90 | 0.60 | 1.45 | 1.42 |
| glycin | 3.33 | 3.29 | 7.45 | 7.57 |
| threonine* | 2.90 | 1.72 | 5.14 | 4.84 |
| alanin | 5.39 | 2.77 | 11.46 | 11.87 |
| tyrosine* | 2.31 | 2.36 | 3.36 | 3.44 |
| valine* | 3.17 | 2.37 | 6.42 | 6.05 |
| methionine* | 1.44 | 1.42 | 2.49 | 2.55 |
| tryptophan* | 2.80 | 2.54 | 5.83 | 5.43 |
| isoleucine* | 1.38 | 1.40 | 3.75 | 3.32 |
| phenylalanine* | 2.82 | 1.99 | 6.68 | 6.30 |
| leycine* | 2.62 | 1.86 | 6.78 | 6.20 |
| lysine* | 2.09 | 1.26 | 2.73 | 2.49 |
| *all essential amino acids | 21.53 | 16.92 | 43.18 | 40.62 |
| phenolic acids [mg·L$^{-1}$] | | | | |
| gallic | 0.714 | 0.627 | 0.507 | 0.582 |
| vanillic | н/о | 0.442 | 1.416 | 1.166 |
| syringic | н/о | 0.261 | 0.893 | 0.344 |
| synapic | 0.175 | 0.179 | 1.582 | 1.531 |
| flavanoides [mg·L$^{-1}$] | | | | |
| routin | not found | not found | 9.23 | 8.55 |
| quercetine | not found | not found | not found | not found |

The Table 1 data showed that SAE use promotes the extraction of biologically active substances or antioxidants to varying degrees.

According to the standard for polyphenolic compounds (Figure 1) and amino acids (Figure 2), it was possible to identify 5 types of polyphenols and 18 amino acids (Table 1 data).

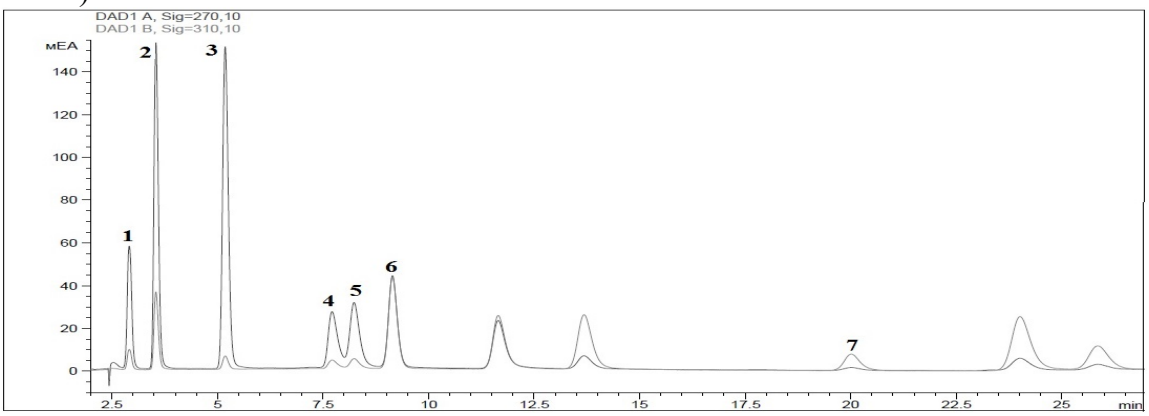


***Figure 1.*** *The composition of the HPLS standard phenolic acids: 1. Gallic acid; 2. 5-(Hydroxymethyl)furfural; 3. Furfural; 4. 4-Hydroxy-3-methoxybenzoic acid; 5. 3,5-Dimethoxy-4-hydroxybenzoic acid; 6. 5-Methyl-2-furaldehyde; 7. Sinapic acid*

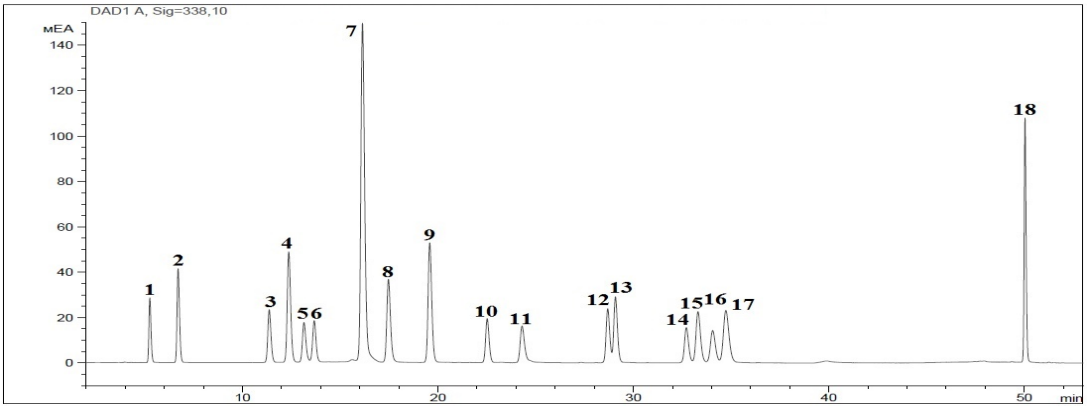


***Figure 2.*** *The composition of the HPLS standard amino acids: 1. L-Aspartic acid; 2. L-Glutamic acid; 3. L-Asparagine; 4. L-Histidine monohydrochloride monohydrate; 5. L-Serine; 6. L-Glutamine; 7. L-Arginine monohydrochloride; 8. Glycine; 9. L-Threonine; 10. L-Alanine; 11. L-Tyrosine; 12. L-Valine; 13. L-Methionine; 14. L-Tryptophan; 15. L-Isoleucine; 16. L-Phenylalanine; 17. L-Leucine; 18.L-Lysine monohydrochloride*

Generally, the total polyphenol content (TPC) measuring by Folin-Cocalteu method is directly proportional to the sum of the polyphenols determined in the study.

The obtained data led to the conclusion that aqueous alcohols solutions are most preferred for the extraction of both polyphenols and amino acids, which is consistent with published data [6 – 8].

The obtained data showed that the benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride solubilization activity is lower compared to ethyl and propane-1,2-diol extracts, which led to less extraction of biologically active substances in solutions. So, aqueous solution of benzyldimethyl-[3-(myristoylamino) propyl] ammonium

chloride made it possible to extract 35 % more total polyphenols compared to aqueous extraction and 70 % less compared to alcohol extraction.
Alcohol extraction also allowed 2 times more amino acids to be extracted compared to aqueous extraction and 2,5 times more compared to benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride extraction.
It should be noted that P-vitamin activity (i.e. the routin content) to the greatest extent has to 70 % water- ethanol solution. The used methods did not allow to isolate quercetin from BSG. In this regard, a thorough study of other approaches is required: repeated processing using various extractants, etc.
It is very important to note a number of amino acids presence in the extracts. For comparison, the data in Table 2 on the amino acids content in malt wort [17] and a water-ethanol extract from BSG are given.

***Table 2.*** *The amino acids content in malt wort and a water-etanol extract from BSG*

| Amino acids content | The content in samples [mg·L$^{-1}$] | |
|---|---|---|
| | malt wort | 70 % water-ethanol extract |
| aspartic acid | 160.0 | 5.72 |
| glutamic acid | 290.0 | 6.63 |
| asparagine | 65.0 | 5.22 |
| histidine | 62.5 | 2.74 |
| serine | 75.0 | 5.09 |
| glutamine | 30.0 | 8.30 |
| arginine | 100.0 | 1.45 |
| glycin | 45.0 | 7.45 |
| threonine* | 100.0 | 5.14 |
| alanin | 110.0 | 11.46 |
| tyrosine* | 110.0 | 3.36 |
| valine* | 140.0 | 6.42 |
| methionine* | 40,0 | 2.49 |
| tryptophan* | 50.0 | 5.83 |
| isoleucine* | 80.0 | 3.75 |
| phenylalanine* | 140.0 | 6.68 |
| leucine* | 180.0 | 6.78 |
| lysine* | 100.0 | 2.73 |
| *all essential amino acids | 940.0 | 43.18 |

The Table 2 data showed that essential amino acid contents was 4.5 % in the brewer's spent grain on the content in the wort. If the BSG is not disposed of, then the loss of amino acids may be 4.5% according to our studies.
It is known that all amino acids are divided into nutritionally essential and nonessential, i.e. synthesized by the human body. Based on recent research, some amino acids have been assigned functional status [18]. The arginine, cystine, leucine, methionine, tryptophan, tyrosine, glutamic acid, glycin, proline and taurine have been classified as functional amino acids. The role of functional amino acids are accelerate and regulate mane metabolic pathways to improve and maintain human health, survival, growth, development and reproduction.

The amount of BSG water ethanol extract functional amino acids was 30.63 $mg \cdot L^{-1}$, which is significant for the further use of extracts in order to obtain concentrates of biologically active substances.

## CONCLUSIONS

The preliminary results for 4 different extracts showed that 70 % water-ethanol and 70 % water-propane-1,2-diol solvents are the most effective for the polyphenolic substances and amino acids extraction. The solvation ability of the benzyldimethyl-[3-(myristoylamino) propyl] ammonium chloride solution was not sufficient for the extraction of the main biologically active compounds in comparison with alcohol solutions.
The extracts allowed to extract phenolic acids, flavonoids (rutin) and amino acids to different degrees from brewer's spent grain, i.e. those substances that were in a soluble state, unlike quercetin.
Studies have shown that extracts using brewer's spent grains contain up to 4.5 % of essential amino acids and up to 3.8 % of functional amino acids of the malt wort amino acids amount.
The obtained data on the functional amino acids and polyphenols content in the extracts, including those with P-vitamin activity, speak in favor of the prospect of further research in this area.

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