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Effect of fertilization on the fat content and fatty acid profile of sorghum flour samples

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1. Summary

In our experiments, changes in the fat content and fatty acid composition in the flour of sorghum cultivated in growing areas treated with different nitrogen fertilizer doses were studied by the gas chromatographic analysis of the fatty acid methyl esters. The fat content exhibited a slight increase as a result of increasing fertilizer treatment, there were significant differences between the individual treatments. The fatty acid composition showed different results, depending on the treatments. We found that treatments had a positive effect on the fat content of the samples, however, increasing nitrogen dosages did not result in an outstanding increase in the case of any of the fatty acids. The ratio of fatty acids to each other did not change significantly as a function of the nitrogen dose. The average amount of unsaturated fatty acids was 83%, and the amount of saturated fatty acids was 17%. The fat content of sorghum is low (1.1-1.5%), but it can still be considered to be a good source of essential fatty acids. The average amount of omega-3 and omega-6 essential fatty acids in the samples analyzed by us was 50%, which also increases the nutrition physiology effect of sorghum.

2. Introduction

The fats found in sorghum play an important role both in terms of the nutrient content and the flavor, shelf life and usability of the foods made from it **[1]**. The fat content of sorghum flour is 3.17% **[2],** which is 3.32% on a dry matter basis, containing mostly unsaturated fatty acids **[3], [4]**. The fat content for the whole grain is 1-4 g/100 g **[5]**, while its values on a dry matter basis, according to different literature sources are 3.2 g/100 g **[6]**, 3.8 g/100 g **[7]**, 2.1 – 6.6 g/100 g **[8]** and 5.0% - 8.2% **[9]**. Fat contents of the main parts of the sorghum grain are summarized in ***Table 1***.

Lipids in the seed husk of sorghum mainly contain wax **[11]**. The waxy part contains chiefly aldehydes (46%), fatty alcohols (41%), fatty acids (7.5%), wax and sterol esters (1.4%), triacylglyceroloks (1%) and hydrocarbonates (0.7%) **[12]**. Apolar lipids (triglycerides) make up the largest amount. Of the polar lipids, phospholipids, glycolipids and unsaponifiable lipids (phytosterols, carotenoids, tocopherol) are found in sorghum **[13], [7]**. Free fatty acids are present in relatively small quantities **[5]**. Nearly 80% of the fatty acids found in sorghum are unsaturated fatty acids **[14]**, with the amount of polyunsaturated fatty acids

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(PUFA) being higher than that of monounsaturated fatty acids (MUFA) **[5], [9]**. ***Table 2*** shows the composition ranges of the fatty acids found in sorghum.

According to the publication of Wall and Blessin **[1]**, sorghum contains mostly linoleic acid (52%), followed by oleic acid (32%), palmitic acid (10%), stearic acid (4%) and linolenic acid (1%). Some sources say that the linoleic acid content of sorghum is 48.81%, its oleic acid content is 27.66% **[15]**. The fatty acid composition of some Nigerian cereals was studied by Adayeye and Ajewole **[16]**, and they found that the unsaturated fatty acid content of sorghum was higher than that of millet or maize (***Table 3***).

The growth and development of sorghum are influenced to a large extent by agrotechnical and ecological factors. Based on the research of Izsáki and Németh **[17]**, as well as Lásztity **[18]**, sorghum takes up 45% of the nitrogen before the end of the flowering period. Based on domestic long-term experiments it can be ascertained what the level of nutrient supply is which results in yield growth and higher produce quality, as well as when a deterioration of quality can be expected **[19]**. Our objective was to investigate the effect of various nitrogen treatments on the fat content of sorghum and on changes in its fatty acid composition.

3. Materials and methods

3.1. Materials

In the course of our research, the sorghum hybrid Zádor (*Sorghum bicolor* L. Moench) was examined. This sorghum hybrid received state recognition in 1998 **[20]**. The fertilizer used in the experiment was CAN (Pétisó) in doses of 0, 40, 80, 120, 160 and 200 kg ha^{-1}. The experiment was set up in four repetitions at the Karcag research Institute of the University of Debrecen, Institutes for Agricultural Research and Educational Farm in 2016. The soil of the experimental area was flat, balanced, and belonged to the meadow chernozem type in terms of its soil genetic classification. The humus content of the topsoil was 3.5% in the 0 to 25 cm layer, corresponding to a good humus content. The phosphorus and potassium content of the soil was also good. The sorghum samples obtained were ground using a Metefém FQC-109 laboratory mill **[21]**.

3.2. Method

Fat content determination of the samples was carried out on the basis of a petroleum ether extraction performed in a Soxhlet apparatus **[22]**.

The fatty acid compositions of the samples were determined in the laboratories of the Institute of Food Technology and Institute of Chemistry of the University of Debrecen. To determine the relative percentages by weight of fatty acid methyl esters, 3 gram portions of the flour samples were extracted with n-hexane. Following extraction, the retentate and the permeate were separated using filter paper. n-Hexane was removed by a rotary evaporator (Heidolph R210/215). The residual apolar fraction was dissolved in a small volume (1 ml) of n-hexane. For the preparation of fatty acid methyl esters, boron trifluoride (BF_3+CH_3OH) transesterification was used, the derivatizing reagent was applied in a three-fold excess for 20 minutes at 50 °C **[23]**. After derivatization, the sample was centrifuged for 5 minutes at 10,000 rpm. Until further instrumental analyses, the supernatant was stored at -20 °C.

The fatty acid composition of the samples was determined by a gas chromatographic (GC) method. The type of the gas chromatograph was HP5890 Series II, the autosampler was an Agilent 6890. The length of the SP2330 chromatographic column was 30 m, its internal diameter was 0.25 mm. Film thickness was 0.2 µm. The carrier gas was nitrogen, the flow rate was 1 ml/min. The initial temperature of the temperature program used was 75 °C for 2 minutes, the temperature gradient was 4 °C/min to 175 °C, and then 2 °C/min to 260 °C. Sample injection volume was 1 µl. The signal was obtained by a flame ionization detector (FID, 260 °C). All samples were analyzed in triplicate **[24], [25], [26]**. Statistical analysis was carried out by the SPSS 13.0 for Windows software, based on ANOVA, upon which Significant Difference ($SzD_{5\%}$) was built **[27], [28]**.

4. Analytical results

4.1. Fat content of the Zádor hybrid flour samples

The measured fat contents of the flour samples of the sorghum hybrid Zádor analyzed by us as a function of the amount of nitrogen applied to the growing area are summarized in ***Table 4***.

Sorghum samples treated with nitrogen fertilizer doses of 0, 50, 100 and 150 kg ha^{-1} were analyzed by Ayub et al. **[29]**, and their research showed that the fat content of the samples increased with the nitrogen amounts applied, the values being 1.59%, 1.77%, 1.82% and 1.95%, respectively. In our own experiment, the fat content of the ground sorghum flour samples varied between 1.1 and 1.5% as a function of the fertilizer doses applied. A significant difference could be observed between the values of the samples examined ($SzD_{5\%}$: 0.02). The fat content of untreated samples was 1.1%, while the fat content of the treated samples ranged between 1.2% and 1.5%.

4.2. Fatty acid composition of the flour samples analyzed in relative percentages by weight of the fatty acid methyl esters

The measured fatty acid methyl ester contents of the flour samples of the sorghum hybrid Zádor examined in our experiments as a function of the amount of nitrogen applied to the growing area are shown in ***Table 5***.

In the case of the samples examined by us, linoleic acid was present in the highest amount (48.04-48.73%), as was previously described by Wall and Blessin (52%) **[1]**, as well as Mehmood et al. (27.59%-50.73%) **[9]**. In our samples, the highest amounts after linoleic acid were found for oleic acid, with values between 31.59% and 32.50%. The amount of palmitic acid was between 14.40% and 14.61%, stearic acid was present in amounts between 1.84% and 2.36%, while linolenic acid between 1.56% and 1.81%. Behenic acid and lignoceric acid could be found in the samples in amounts below 1% (***Table 5, Figure 1***). There was no significant difference between the treatments in the case of palmitic acid ($SzD_{5\%}$: 0.38), palmitoleic acid ($SzD_{5\%}$: 0.34), stearic acid ($SzD_{5\%}$: 0.43), linoleic acid ($SzD_{5\%}$: 0.66), linoleic acid ($SzD_{5\%}$: 0.24) and lignoceric acid ($SzD_{5\%}$: 0.11). However, significant differences could be demonstrated between the sample treatments in the case of the measurement results of oleic acid ($SzD_{5\%}$: 0.30) and behenic acid ($SzD_{5\%}$: 0.06). Only in the case of oleic acid was it found that the oleic acid content of the treated samples were higher than that of the control samples. In the case of two fatty acids, palmitoleic acid and oleic acid, was it found that the values of the samples treated with the highest doses (200 kg/ha N) were the highest. In some cases (linoleic acid, behenic acid), the value of the control sample was the highest, while in another case (lignoceric acid), the value of the sample treated with the highest N dose was the lowest. Furthermore, it was observed that with increasing fertilizer treatment, the behenic acid content showed a decreasing tendency. The total values for essential fatty acids, such as linoleic acid (omega-6) and linolenic acid (omega-3), were between 49.93% and 50.44%, as a function of the treatments. Of this, the amount of omega-6 fatty acids was between 48.04% and 48.73%, while the amount of omega-3 fatty acids was 1.56% to 1.81% (***Table 5***).

The average linoleic acid content of fertilizer-treated samples was 48.28±0.19%, while the amount of linolenic acid was 1.70±0.09% (***Table 5, Figure 1***). In the untreated samples, 48.73% linoleic acid and 1.71% linolenic acid was found (***Table 5***).

The amount of unsaturated fatty acids in these samples ranged from 82.81% to 83.66%, with an average of 83%, while the amount of saturated fatty acids ranged from 16.34% to 17.19%, with an average of 17% (***Table 5, Figure 2***). In the untreated samples, the amount of unsaturated fatty acids was 82.8%, the amount of saturated fatty acids was 17.2% (***Table 5***).

The chromatogram of a sample treated with nitrogen fertilizer (160 kg/ha) is shown in ***Figure 3***.

5. Evaluation and discussion of the test results, conclusions

Based on the test results it can be stated that the fat content of the samples showed an increasing tendency as a result of treatments with increasing amounts, a significant, 1.1% to 1.5% difference could be observed between the treatments. According to the experiments of Ayub et al. **[29]**, the fat content of sorghum samples treated with nitrogen fertilizer doses (0; 50; 100; 150 kg ha^{-1}) alos increased (1.59%-1.95%). However, increasing nitrogen doses did not result in a remarkable increase in the case of any of the fatty acids, and the ratio of fatty acids to each other did not change significantly as a function of the nitrogen dose. It was observed in the case of oleic acid that treated samples exhibited higher values than the control samples. For two fatty acids, palmitoleic acid and oleic acid, it was found that samples treated with the highest dose (200 kg/ha N) showed the highest values. The average amount of unsaturated fatty acids was 83%, while the amount of saturated fatty acids was 17%. Hulse et al. **[13]** also report that the amount of unsaturated fatty acids in sorghum samples is 80%. In our samples, also linoleic acid and oleic acid were present in the highest amounts, followed by palmitic acid, stearic acid, linolenic acid, palmitoleic acid, behenic acid and lignoceric acid, as was also described by Wall and Blessin **[1]**. Total values for essential fatty acids, such as linoleic acid (omega-6) and linolenic acid (omega-3) were between 49.93% and 50.44%, depending on the treatments. Of this, the amount of omega-6 fatty acids was 48.04 – 48.73%, while the amount of omega-3 fatty acids was 1.56 – 1.81%. The fat content of sorghum is low, but it is a very good source of essential fatty acids, and this property increases its nutrition physiological effect.

6. References

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