Metabolomics approach in digital assessment of fatty acids profile of cottonseed for biological activity improvement of cotton oil

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Abstract. Cotton is the main source of natural fiber worldwide, also seeds to process oil and meal. The fatty acids profile as the part of metabolomics profile of seeds studied in cotton accessions belong to Gossypium hirsutum L. (Upland), G. herbaceum L. (diploid), G. barbadense L. (Pima cotton) grown in South of RF. Gas-liquid chromatography and mass-spectrometry used. The digital peaks identification performed by mass spectrum libraries. The research aim was to characterize fatty acids profile of seeds, to select the best cotton cultivars accessions, valuable in seed biochemistry compound. Considered as a part of metabolomics profile linolenic acid tested in greatest amount among other fatty acids. The mean amount reached 117.2 mg 100 g−1 of seed among all studied varieties. The maximum amount reported in Abolin CFN variety, G. hirsutum (197.4). The content of di-unsaturated linoleic acid in Fors and Voitenok FRT reached 186.2 and 85.7 mg 100 g−1 respectively, and Tiamin 82.6 (G. barbadense). Oleic acid mean amount estimated as 70.9, which maximum contents detected in cultivars Cumbazic Maron (naturally colored fiber) and Fors. Improvement of biological activity of cottonseed oil, meals and cakes as well is in focus to implement the trend of healthy food and high-quality feed.

1. Introduction
A computerized flow of unified information about raw materials traits is able to reach the world commodity exchanges within the shortest possible time, actively influence on promotion of new products in market [1, 2, 3] owing to implementation of Internet technologies. The modern era has heralded a shift from the industrial society, in which natural resources are crucial input factors for the economy, towards a digitalized knowledge society. For sustainable economic development a digitalized data are significant, which insufficient production and underutilization can be a problem. The availability of quality digital data impact on sustainable industry development [4], scientific and economical knowledge management, distribution of innovative goods and services. Digital development that meets the needs of the present without compromising the ability of future generations to meet their own needs. Knowledge and digitalized artefacts could be considered as a vehicle to support sustainable technological development of society. Fundamental, applied and technological ideas related to digitalized data and solutions tend to quality accumulation become available and can be widely used [5, 6, 7].
Digital technologies have a fundamentally new opportunity for the study of biochemical features of oil crops seeds based on metabolomics approach – evaluation of plant cell life results on molecular genetics and biochemical levels. Analysis of metabolic profiles allows the identification of biochemical markers of biological processes. Gas-liquid and liquid chromatography (high pressure) using polar column and mass-spectrometry [8] are the methodological basis of study. The possibility of the analyses is significantly improved with usage of digital peaks identification by mass-spectrum (NIST Version 2.0). Molecular biological research, supplemented by digitalization, allow to study plant organisms at the genomic, proteomic and metabolic levels. It makes possible to estimate a process in complex process in plant organisms as diagnostics in agricultural biology to characterize quantity and quality [9, 10].

Cotton is the main worldwide source of textile fiber, but seeds as well, that used for seed oil process, meals and cakes. The world crop acreage increased up to 3 million hectares. Annual world cottonseed production reached 48.5 million tons. Cotton seed oil production increased on 3.67% this season, compared with 2017/2018 [11]. So, evaluation of fatty acids profile of cotton seeds may be considered as very important and significant aspect to improve the biological property of products. The perspectives of enlarged of cotton seeds application to produce high grade vegetable oil and functional food ingredients (FFI) assume the comprehensive fatty acids compound research, that is the main task of the present study.

2. Purpose of Investigations
The aims of research are to characterize fatty acids profile of seeds, to estimate the varieties’ seeds biochemical traits variability, the influence of varieties’ genetic specifications on fatty acids composition, to select the best accessions taking in to consideration the complex of seeds characters.

3. Material and Methods
The fatty acids profile as the part of metabolomics profile of seeds was studied in accessions of cotton varieties, that belong to three botanical species – Gossypium hirsutum L. (Upland cotton), G. herbaceum L. (diploid), G. barbadense L. (Pima cotton). Cultivars have been grown in Sothern Federal District, RF – in the South-Eastern part of delta of Volga in 2017-2018. Seeds were weighted, homogenized with ethanol in 1:10, the samples were insisted for 30 days at 5-6°C. The extract obtained was centrifuged at 14 thousand r m⁻¹ for 10 minutes. 100 μl of the extract was evaporated on CentriVap Concentrator «Labconco» (USA). 50 μl of bis (trimethylsilyl) trifluoroacetamide was added to the dry residue and held for 40 minutes at 100 °C on Digi-Block (USA). The analysis was performed on a capillary column HP-5MS 5% phenylmethylpolysiloxane (30.0 m, 250.00 μm, 0.25 μm) using gas-liquid chromatography with mass spectrometry (GLC MS) on an Agilent 6850 chromatograph with a quadrupole mass Agilent 5975B VL MSD Selective Detector by Agilent Technologies (USA). Analysis conditions: helium through a column of 1.5 ml min⁻¹. Heating program from +70°C to +220°C, at a heating rate of 4°C per minute. Detector temperature +250°C, injector temperature +300°C, sample volume 1 μl. The internal standard is a solution of tricosan in pyridine (1 μg μl⁻¹). The results were processed by the programs “AMDIS” and “UniChrom”. Peaks were digitally identified using the NIST Version 2.0 mass spectra library. The results of the analysis of the fatty acid composition as an integral part of the cotton seed metabolome were processed using the ANOVA analysis of the Statistica 10.0 (StatSoft Inc., USA).

4. Results and Discussion
Estimation of the fatty acids composition as a metabolomics profile of seeds shows, that the maximum amount among of all the rest polyunsaturated fatty acids in seeds was linolenic acid content. The total mean content measured as 117.2 mg 100 g⁻¹ of seed of all studied varieties (see table). The largest amount of this acid was detected in the Abolin CFN variety, that belongs to
Upland cotton, *G. hirsutum* (197.4). The variety Tutum (*G. herbaceum*, so named “huza”) also had a high content of linolenic acid in the seeds - up to 185.5.

**Table.** Amounts of polyunsaturated fatty acids (mg 100 g$^{-1}$) in cottonseeds of cotton varieties (*Gossypium* L.), Southern Russia, 2017-2018

| Fatty acids* | Mean (descending ranked) (n=18) | Variability of mean amounts between varieties listed |
|--------------|---------------------------------|----------------------------------------------------|
| Linolenic acid (CID: 5280934) | 117.2 197.4 137.0 123.4 185.5 7.1 2.2 38.6 10.3 | |
| Linoleic acid (CID: 5280450) | 107.6 66.5 85.7 126.8 95.0 186.2 67.1 82.8 10.1 | |
| Oleic acid (CID: 445639) | 70.9 48.4 53.9 73.4 67.5 123.6 42.1 63.4 7.2 | |
| Palmitic acid (CID: 985) | 60.8 41.8 51.0 65.9 54.9 99.9 41.3 51.8 6.4 | |
| Stearic acid (CID: 5281) | 20.4 21.7 22.6 21.4 19.8 19.2 16.6 16.7 2.4 | |
| Vaccenic acid (CID: 5281120) | 18.4 17.3 14.1 18.1 15.2 28.7 11.9 20.9 2.2 | |
| Docosanoic acid (CID: 8215) | 7.1 7.2 19.6 5.1 1.9 2.8 1.5 10.0 1.5 | |
| Lignoceric acid (CID: 11197) | 2.6 2.1 1.7 1.3 4.2 3.9 1.1 3.0 0.5 | |
| Behenic acid (CID: 8215) | 2.2 1.8 1.9 1.5 2.3 2.8 1.3 4.6 0.7 | |
| Pelargonic acid (CID: 8158) | 1.8 1.0 1.8 1.5 4.4 1.1 0.9 2.5 0.6 | |

*Chemical names according to PubChem, U.S. National Center for Biotechnology Information, ** G. herbaceum* L. (diploid), ***G. barbadense* L (Pima cotton). SD – standard deviation (significance level p=0.05)

The content of linoleic acid in cotton seeds reached the maximum values in the variety Fors 186.2 mg 100 g$^{-1}$, Voitenok FRT (85.7) and high grade fiber cotton (*G. barbadense*) Tyamin (82.8) as well.

The oleic acid average content estimated as 70.9 mg 100 g$^{-1}$ among of all seed accessions, but the maximum amount 73.4 was evaluated in the variety with naturally colored fiber Cumbazic Maron (*G. hirsutum*).

Palmitic acid is saturated fatty acid. It used to process of “cotton palmitin”, which is used to produce of high-grade margarine. The average amount in seeds of all evaluated cultivars is 70.8 mg 100 g$^{-1}$, but the maximum content (up to 99.9) studied in the Fors cultivar.

The rest of the identified fatty acids in the cotton seeds are stearic, vaccinic, docosanoic, lignoceric, behenic and pelargonic were detected in average amounts variated from 1.8 up to 20.4 mg 100 g$^{-1}$ of seeds of cultivar’s samples.

The research possibilities of liquid and gas chromatography in combination with atomic emission spectrometry and mass spectrometry now are used to solve a range of problems for the
qualitative and quantitative analysis of a complex of substances in food, to characterize phenols and flavonoids amounts and estimation of antioxidant activity [12, 13], functional products research [14], creation of high nutritional value, gluten free food [15]. The polyunsaturated linoleic and lenolenic fatty acids that found in the presented study of cotton seeds, according to the literature, have expressed biological activity. Proteomics identifies the gene products, whereas metabolomics determines whether the expressed substances are metabolically active and identify biochemical processes and the roles of various metabolites. Metabolome reflects the qualitative composition of a biological object (for example seeds). Metabolic profiling provides an instantaneous picture of what is occurring in the cell, for example, after seeds ripening, identifying key biochemical compounds that important for imparting quality. Monitoring of metabolite patterns can lead to quality improvement for nutrition value [16, 17]. The increase the biological activity of cottonseed oil, as well as cottonseed meal and cake, is relevant in implementing the trend of healthy nutrition and to create high-quality compound feeds for farm animals.

Gas-liquid chromatography and mass spectrometry using the digitalization capabilities of the obtained data significantly influenced to the development of studies of the metabolome and proteome of plant organs, complex structural and molecular biological macromolecular complexes [16, 17]. The presented studies can help to highlight cotton cultivars that combine the traits of valuable seed’s fatty acid composition in combination with the quality of naturally colored cotton fiber. The presented studies are important, since the production and sale of cotton processing products are the significant component of the global economy in the 21-st century.

Aspects of the biological activity of vegetable oil were discussed in the works of early authors [18]. The value and biological activity of vegetable oils was determined by the high content of polyunsaturated fatty acids, phosphatides, tocopherols and sterols. But it was underlined, that the total tocopherols amount in vegetable oil is not a criterion for high oil biological value. In modern evaluations, besides the amino-acid profile of proteins, unsaturated fatty acids are mentioned as significant in the formation of biological activity and nutritional value of vegetable oils [19, 20].

Cotton may be relevant and promising crop to improve nutritional value of seed and vegetable oil as the sources of functional food (FF). FF must be accepted as a number of food products with significant amounts of functional food ingredients (FFI) – that are physiologically active, valuable and healthy ingredients, that have the real properties to reduce the risk of developing nutrition-related diseases. FFI prevent or fill up the nutritional deficiencies. Besides the polysaccharides and polyhydric alcohols, there are: polyunsaturated fatty acids (PUFA), conjugated isomers of linoleic acid (CLA) and other substances in the FFI list. In order to obtain FFI, a number of vegetable oils have been recommended to process [21]. Conjugated fatty acids (CFA) – isomers of linoleic and linolenic acids (CLA, CLNA), as well as PUFA are able positively modulate inflammations and energy metabolism. Mentioned compounds contribute anticarcinogenic and antioxidant effects [22]. It proved [23] that like to CFA, unsaturated fatty acids – oleic and docosanoic, as well as linoleic and linolenic, have a positive effect on metabolism in cardio-vascular system («cardiometabolic health»). It assumed that CLA protects cell membranes from the negative effects of free radicals. The lowering cholesterol effect was investigated and found, that amount of high density lipoprotein (HDL) was not reduced. In present research the polyunsaturated fatty acids dominated in fatty acids profile of the evaluated cottonseeds accessions. The diversity of agro-climatic zones of the RF may allow the production of a wide range of textile materials and valuable food products as well. According to the table, there are totally 58% of polyunsaturated acids amounts in cotton seeds, as well as 80% of unsaturated ones in evaluated profile. Main of them are – linoleic, linolenic and oleic acids, that are valuable for producing a range of healthy foods, FFI and feed for animals.

It was considered that the main limit for cottonseed use was gossypol that is a phenolic compound, produced by glands of cotton plants – leaves, seeds, and flower buds [18, 24, 25] But it was underlined that just high concentrations of free gossypol may be responsible for acute
clinical signs of gossypol poisoning [24]. However, modern breeding and selection of recently produced modern cotton varieties significantly reduced in gossypol amounts in seeds [26] down to 0.15 %. It was described, that gossypol in slight concentrations, reveals significant effect as natural antioxidant for cotton oil stability [25]. It was earlier reported [18] that conventional oil extraction and refining by coal, may resulting significant reduce gossypol amount. Regretfully the mentioned refining process may reduce the amounts of natural antioxidants such as phosphatides (lecithin). However, there wasn’t reported evidence of PUFA significant oxidation, during crude oil refine process. By the way, modern cottonseed oil extraction by microwave radiation and elimination of any heat treatment of cottonseeds before extraction reveals the stability of fatty acids composition during crude oil treatment. The study didn’t show any significant differences between cooked and uncooked oil samples (P > 0.05) regarding physic-chemical characteristics [25]. The cooking process of the cottonseed oil also increased the total phenolic compounds and considerably decreased total gossypol content.

5. Conclusion
Digitized information about the quality of produced, stored and trading batches of oilseed raw materials is reproducible and informative because of the use of universal digital identification of mass spectrum. This information is unified and accessible from library PubChem (U.S. National Center for Biotechnology Information). As result of the research, the fatty acids profile of seeds of modern cotton varieties (Gossypium hirsutum, G. herbaceum L. and G. barbadense L. species) were assessed. Cultivars Abolin CFN, Voitenok FRT, Cumbasic Maron and Tiamin with high amounts of linolenic, linoleic and oleic acids in seeds were selected. Cotton varieties characterized in valuable content of unsaturated and polyunsaturated fatty acids may be used for food and feed production. Therefore, it seems practical to make use of produced as a by-product in Southern soil-climatic zones of Russia cotton seeds as the raw material for a food, oil and fat industries.

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