Experience in applying the technology of cavitation treatment of sunflower oil sludge for feeding ruminants

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Abstract. This paper presents research on evaluating the effectiveness of the technology of cavitation treatment of sunflower oil sludge intended for feeding ruminants. The evaluation on a general microbial content has been introduced, namely, quantitative and qualitative indicators of sunflower sludge before and after the cavitational processing have been described. During the experimental studies, the fact of a significant increase in digestibility of sunflower sludge after the cavitational processing by 21.1 % was revealed. At the same time, cavitation was not accompanied by any significant changes in the fatty acid composition of the feed, but it increased digestibility of a dry-matter in vitro due to an increase in bioavailability of the linoleic acid to 86.2 % versus 45.9 % in the native feed. The digestibility of other fatty acids either changed slightly, or decreased. The experiment shows the bactericidal and mycocidal effect caused by the cavitational processing. The effect caused by the cumulative jets of liquid when the cavitational microbubbles collapse caused the death of all microflora inherent to these types of products.

1. Introduction

One of the cutting-edge technologies directed at preparing feed to animals is the cavitational processing, which can be achieved by exposure to ultrasound or hydrodynamic effects. While processing in the testing environment under the influence of high-intensity fluctuations, various irreversible physical and chemical processes occur that contribute to increasing the nutritional value of the feedstock. The cavitation action concludes in shaping and collapsing of vapor-gas bubbles in a liquid environment caused by the ultrasonic action. The cavitational processing contributes to a change in feed composition due to a local pressure decrease in the liquid to a saturated vapor pressure, which is accompanied by the death of putrefactive and pathogenic microorganisms, as well as by destruction of mycotoxins, which are contained in the feed [1, 2].

Such a complex range of the cavitational processing is of interest to recycle such a waste product as sunflower sludge. For a variety of reasons, this feed has relatively low digestibility in the animal gastrointestinal tract that leads to the fact that the cavitational processing is able to increase the nutritional value and chemical composition of the raw materials under processing, thereby, its productive effect either increases [3] by sterilizing the feed received as the end product [4, 5].

In this regard, the cavitation processing effect with its influence on the fatty acid composition, fatty acids digestibility, and bacterial content on the sunflower sludge models is of certain scientific interest.
2. Materials and methods

The studies were conducted on young bulls of the Kazakh white-headed breed, aged 13 months. The ruminal fluid sampling was carried out among young bulls through the chronic fistula of the cicatrical tissue. The animal services and experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No.755 on 08/12/1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. During the experimental works, we undertook the efforts to minimize animals’ suffering and to reduce the number of samples used.

The sunflower sludge, as a waste of oil-fat industry, was used in the experimental works. The research scheme was intended to carry out a series of laboratory experiments, during which the cavitation effect on the chemical composition of a sunflower sludge was studied, and digestibility of the product was evaluated under *in vitro* conditions. The evaluation on a general microbial content has been introduced, namely, the quantitative and qualitative indicators of sunflower sludge before and after the cavitation processing have been presented.

The cavitation processing to sunflower sludge was carried out through ultrasonic processing at 28 kHz, at $t = 28 \, ^\circ\text{C}$. Installation was 220 V, the power was 5 W., the cavitation threshold was 19 kHz, the hydraulic module was 1:2.

The digestibility of dry matter was determined by the method *in vitro*, with “artificial cicatrical tissue KPL 01”. The studies were carried out by nylon bags method, with a 48-hour exposure. The original research methodology implied an evaluation of sunflower sludge splittability from a mixture with inert fillers (birch sawdust) at rate of 10 % by weight. Birch sawdust preparation included a processing with diethyl ether and drying ($t = 105 \, ^\circ\text{C}$) in a dry heat oven. The digestibility of dry matter of the feed *in vitro* was determined by the difference in weight of the feed sample together with a bag before and after two-stage incubation and by drying to constant weight at temperature of +60 °C according to the following formula:

$$K = \frac{(A - B)}{C} \times 100 \%$$

- $K$ – digestibility coefficient of dry matter of the feed (%);
- $A$ – the initial mass of the feed sample together with a bag (g);
- $In$ – the mass of the feed sample with a bag after digestion (g);
- $C$ – the initial mass of the feed sample without the mass of a bag (g).

The fats and oils composition was studied by gas chromatography according to GOST 31663-2012 Vegetable oils and fats on the gas chromatograph – Crystal Lux 400. The chromatograph is equipped with ZEBRON capillary column. The analysis of the sample was conducted for 50 min, at temperature from 60 to 200 °C. The temperature of the evaporator was 210 °C, the detector temperature – 240 °C, the gas flow rate (nitrogen) – 25 ml/min, hydrogen – 25 ml/min, air – 250 ml/min, dividing the stream – 1/40. Fatty acid separation was identified by comparison with the fatty acid mixture produced by Supelco TM Component FAME Mix.

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**Tools for statistical data.** Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with $p \leq 0.05$ considered as significant.

3. Results

During the experimental studies, the fact of a significant increase in digestibility of sunflower sludge after the cavitation processing by 21.1 % was revealed. In particular, if the digestibility of the native sunflower sludge *in vitro* was 47.9 %, then after processing it was 68.21, or 21.12 % more. In this case, the cavitation was not accompanied by any significant changes in the fatty acid composition of the sunflower sludge (Table 1).
Table 1. The cavitation processing effect on the fatty acid composition of sunflower sludge, %

| Fatty Acids | Sunflower sludge before processing | Sunflower sludge after processing |
|-------------|-----------------------------------|----------------------------------|
|             | M m | m       | M m | m       |
| C\text{16:0} | palmitic | 9.69 | 0.6 | 9.64 | 0.9 |
| C\text{16:1} | palmitoleic | 0.68 | 0.054 | 0.4 | 0.05 |
| C\text{18:0} | stearic | 3.62 | 0.09 | 3.64 | 0.11 |
| C\text{18:1} | oleic | 18.75 | 0.7 | 18.88 | 0.61 |
| C\text{18:2} | linoleic | 65.5 | 1.52 | 66.49 | 2.45 |
| C\text{20:0} | arachidic | 0.53 | 0.04 | 0.35 | 0.05 |
| C\text{22:1} | erucic | 1.23 | 0.22 | 0.6 | 0.07 |

The compared samples differed in palmitoleic levels by 0.28 %, linoleic by 0.99 %, arachidic by 0.18 % and erucic by 0.63 %. However, these discrepancies were not statistically significant. Meanwhile, against the background of the cavitation processing, the fatty acid composition of the sunflower sludge after exposure to the rumen environment \textit{in vitro} was subject to significant changes (Table 2).

Table 2. Digestibility and fatty acid composition of sunflower sludge after exposure and \textit{in vitro}, %

| Fatty acids | Sludge – native | Sludge – cavitated |
|-------------|----------------|------------------|
|             | Composition, % | Digestibility \textit{in vitro}, % | Composition, % | Digestibility \textit{in vitro}, % |
| C\text{16:0} | palmitic | 8.69±0.59 | 52.3 | 24.29±1.31 | 19.9 |
| C\text{18:0} | stearic | 3.66±0.25 | 48.0 | 7.83±0.68 | 31.6 |
| C\text{18:1} | oleic | 18.4±0.97 | 48.6 | 38.20±1.34 | 35.7 |
| C\text{18:2} | linoleic | 68.35±1.34 | 45.9 | 28.75±2.44\textsuperscript{b} | 86.2 |
| C\text{20:0} | arachidic | 0.3±0.04 | 66.9 | 0.38±0.04 | 65.5 |
| C\text{22:1} | erucic | 0.6±0.08 | 70.3 | 0.55±0.05 | 70.9 |

Significant difference in relation to control; \textsuperscript{b} p \leq 0.01.

The comparison of the fatty acid composition of the product with its digestibility made it possible to establish the apparent digestibility of individual fatty acids. As follows from the data obtained, the incubation of the processed sludge \textit{in vitro} was accompanied by the increase of linoleic acid digestibility up to 86.2 % against 45.9 % in the native food product. The digestibility of other fatty acids either changed slightly, or decreased.

The analysis of the sunflower sludge for the total content by microorganisms showed that as a result of microbiological analysis, bacterial, yeast and fungal colonies were found in the samples in 24 hours (Fig. 1, 2). Mold fungi were found in Petri dishes that are characterized by the appearance of fluffy cobweb-like or cotton-like plaque.

The total background bacterial content of sunflower sludge was 7.2×10\textsuperscript{3} CFU/g. It should be noted that a significant proportion accounted for yeast and mold fungi (more than 70 %). The number of yeast-like and mold fungi was 5.3×10\textsuperscript{3}, bacterial colonies p. Bacillus sp. did not exceed 1.2×10\textsuperscript{3} CFU/g. Bacteria of Escherichia coli group (BECG, coliforms), S. aureus and pathogenic bacteria, including salmonella, were not found in the studied samples of the sunflower sludge.

To study the effect of the cavitation process on the growth and development of microorganisms, we studied the sunflower sludge processed at the various storage periods. As a result of inoculation in Petri dishes at the first week of the experimental samples storage, any microorganisms were not detected. In future, from the end of the second week, some single colonies of mold fungi of the genera Aspergillus and Penicillium were observed in the samples, the appearance of which was probably associated with subsequent secondary contamination of the end product, as well as a few bacterial colonies of \textit{p. Bacillus sp}. The ability to form spores in bacilli allowed them to remain viable when processing the feed during cavitation. Nevertheless, the total number of microorganisms in the studied samples did not exceed 3.4×10\textsuperscript{2} CFU/g. BECG, S.aureus, and pathogenic bacteria, including salmonella, were not detected in the samples of the sunflower sludge after the cavitation processing.
4. Discussion

Low-frequency ultrasound and hydrodynamic cavitation are unique non-thermal methods that are widely used in the food industry [6, 7]. The growing interest in these types of food processing over the past two decades has been well described in a number of reviews and books [8, 9]. At the same time, there is very little data in the literature on the cavitation processing in feed preparation, the less scientific research devoted to the study of bioavailability of the feed products after this type of processing. Therefore, in research planning, we have undertaken the studies to evaluate the effect of the cavitation processing on the chemical composition and bioavailability of the sunflower sludge in animals bodies.

To consider the results obtained due to the experimental studies, we need to imagine clearly the mechanism on how the cavitation process impacts the organic matters in the aquatic environment. According to the research data, the cavitation process is triggered by converting electrical energy into physical vibration that generates sinusoidal pressure fluctuations inside the liquid. The first part of the wave is a wave of tension that generates a series of bubbles. The second part of the wave is a wave of compression and causes a strong collapse of the bubbles. The evolution towards the bubbles coming from the beginning of the process (formation) up to the further subsequent compression takes place in just a few microseconds [10, 11]. During the vibration process, the microbubbles, which are in the process as cores, increase in a size to a maximum of about 4–300 mm in diameter. In this case, microbubbles periodically and repeatedly expand and contract, causing the radial fluctuations during some acoustic cycles, which leads to microjets development at rate of 100 m/s and to shock wave development, the pressure of which reaches up to 103 MPa. This causes the matters to be cavitated in the liquid environment [13, 14].

Thus, sunflower sludge during the cavitational processing is subjected to complex effects at the level of individual molecules’ groups, which, as it follows from the data obtained by us, has not had a
pronounced effect on the fatty acid composition of the product (Table 1). However, judging by the feed digestibility, the cavitation was accompanied by the increase in bioavailability of sunflower sludge components with a cumulative increase in the digestibility of the in vitro dry matter by 21.2%. An important fact is not the equivalent increase in the digestibility of individual fatty acids (Table 2). As the data show, the digestibility of in vitro has increased only for linoleic acid up to 86.2% against 45.9% in the native feed. Taking into account some physics and chemical characteristics of the fatty acids, it can be assumed that the basic cavitation mechanism influencing the bioavailability of the sunflower sludge is in extraction increase. Increased extraction of lipids from plant raw materials after the cavitation processing has previously been described for a number of products. This allowed [16] to consider the cavitation among the most advanced methods of getting valuable food components by extraction.

The increase in bioavailability of sunflower sludge components after the cavitation can also be explained by the increase in the variability of the product, which is directly related to the digestibility of the feed. According to the data obtained, the cavitation processing applied to sunflower sludge was accompanied by the sterilization of the feed. With bacterial content of the native sludge 7.2 to 1659 3-1668 CFU/g, the sludge subjected to the cavitation processing did not contain any microbial bodies.

This phenomenon has previously been described in [19] as high pressure generation in the environment and destruction of microbial cell structures. This is accompanied by the lysis of microbial cells or by shaping of free radicals in chemical destruction reactions [20-23]. The use of this phenomenon in practice significantly increases the output of products in biotechnology.

5. Conclusion
The cavitation processing of sunflower sludge by low-frequency ultrasound does not lead to a change in the fatty acid composition, but is accompanied by a significant increase in the digestibility of in vitro of the dry matter due to the increase in bioavailability of the sludge linoleic acid. The cavitation processing allows to sterilize the sunflower sludge.

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References
[1] Yue C, Ben H, Wang J, Li T and Yu G 2019 Ultrasonic Preprocessingin Synthesis of Caprylic-Rich Structured Lipids by Lipase-Catalyzed Acidolysis of Corn Oil in Organic System and Its Physicochemical Properties Foods 8(11) 566 DOI: 10.3390/foods8110566
[2] Miroshnikov S A, Muslyumova D M, Bykov A V et al 2012 New approaches to the creation of feed products based on multicomponent vegetable-mineral mixtures subjected to cavitation processing Bull. of beef cattle breed. 3(77) 7–11
[3] Orsavova J, Misurcova L, Ambrozova J V et al 2015 Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids Int. J. Mol. Sci. 16(6) 12871–90 DOI: 10.3390/ijms160612871
[4] Lewinska A., Zebrowski J., Duda M et al 2015 Fatty Acid Profile and Biological Activities of Linseed and Rapeseed Oils Molec. 20(12) 22872–80 DOI: 10.3390/molecules201219887
[5] Deryabin D, Galadzhieva A, Kosyan D et al 2019 Plant-derived inhibitors of AHL-mediated quorum sensing in bacteria: Modes of action International Journal of Molecular Sciences 20(22) 5588
[6] Lohani U C, Muthukumarappan K and Meletharayil G H 2016 Application of hydrodynamic cavitation to improve antioxidant activity in sorghum flour and apple pomace Food Bioprod. Process 100 335–43 DOI: 10.1016/j.fbp.2016.08.005
[7] Lee A K, Lewis D M and Ashman P J 2012 Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements Biomass Bioenergy 46 89–101 DOI: 10.1016/j.biombioe.2012.06.034

[8] Chemat F and Khan M K 2011 Applications of ultrasound in food technology: Processing, preservation and extraction Ultrasound Sonochem. 18 813–35 DOI: 10.1016/j.ultrasonch.2010.11.023

[9] Gogate P R 2011 Hydrodynamic cavitation for food and water processing Food Bioprocess Technol. 4 996–1011 DOI: 10.1007/s11947-010-0418-1

[10] Cravotto G and Cintas P 2006 Power ultrasound in organic synthesis: Moving cavitational chemistry from academia to innovative and large-scale applications Chem. Soc. Rev. 35 180–96 DOI: 10.1039/B503848K

[11] Cravotto G, Mariatti F, Gunjevic V et al 2018 Pilot Scale Cavitational Reactors and Other Enabling Technologies to Design the Industrial Recovery of Polyphenols from Agro-Food By-Products, a Technical and Economical Overview Foods 7(9) 130 DOI: 10.3390/foods7090130

[12] Ashokkumar M. (2011). The characterization of acoustic cavitation bubbles – an overview Ultrasound Sonochem. 18 864–72 DOI: 10.1016/j.ultrasonch.2010.11.016

[13] Suslick K S and Skrabalak S E 2008 Sonocatalysis. In: Ertl G, Knözinger H, Schüth F, Weitkamp J ed Handbook of heterogeneous catalysis Weinheim: Wiley-VCH Verlag GmbH and Co. KGaA 2007–2017

[14] Nalajala V S and Moholkar V S 2011 Investigations in the physical mechanism of sonocrystallization Ultrasound Sonochem. 18 345–55 DOI: 10.1016/j.ultrasonch.2010.06.016

[15] Boffa L, Binello A, Mantegna S et al 2019 Cocoa bean shell waste valorisation; extraction from lab to pilot-scale cavitation reactors Food Res. Int. 115 200–8 DOI: 10.1016/j.foodres.2018.08.057

[16] Cravotto G and Cintas P 2006 Power ultrasound in organic synthesis: Moving cavitational chemistry from academia to innovative and large-scale applications Chem. Soc. Rev. 35 180–96 DOI: 10.1039/B503848K

[17] Sizova E A, Yausheva E V, Miroshnikov S A et al 2015 Element status in rats at intramuscular injection of iron nanoparticles Biosciences Biotechnology Research Asia 12 119-127

[18] Fisinin V I, Miroshnikov S A, Sizova E. A et al 2018 Metal particles as trace-element sources: current state and future prospects of the World’s Poultry Science Journal q1668 0679 74(3) 0689 523–40

[19] Feng H, Barbosa-Canovas G V and Weiss J 2011 Ultrasound technologies for food and bioprocessing (New York: Springer)

[20] Mason T J 2003 Sonochemistry and sonoprocessing: the link, the trends and (probably) the future Ultrasound Sonochem. 10 175–9 DOI: 10.1016/S1350-4177(03)00086-5

[21] Naveena B, Armshaw P and Pembroke J T 2015 Ultrasonic intensification as a tool for enhanced microbial biofuel yields Biotechnol. Biofuels 8 140 DOI: 10.1186/s13068-015-0321-0

[22] Bykov A V, Kvan O V, Duskaev G K et al 2019 Prospects of applying sunflower sludge after cavitational processing in poultry breeding IOP Conf. Ser. Earth and Environmental Sci. 341(1) 012060

[23] Duskaev G, Karimov I, Levakhin G et al 2019 Ecology of ruminal microorganisms under the influence of quercus cortex extract International Journal of GEOMATE 16(55) 59-66