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ULTRASONIC CAVITATION EFFECT ON THE FATTY ACID COMPOSITION OF LINSEED OIL-BASED EMULSIONS*

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Ultrasonic homogenization is a promising method of emulsion formation. Linseed oil containing polyunsaturated fatty acids in the optimal ratio was used as a control sample and the fat base of the emulsions. The effect of ultrasonic treatment can lead to a change in the percentage of fatty acids due to the acceleration and initiation of several chemical reactions. To assess the feasibility of using ultrasound in food production, the fatty acid composition of natural linseed oil and emulsions obtained from it, treated by ultrasound with different durations were studied. The study monitored the formation of radicals since increasing temperature and pressure during the collapse of cavitation bubbles initiates the formation of free hydrogen H- and hydroxyl OH-radicals within and next to them. These may initiate the oxidation of bioorganic compounds in the food product. The research results show that emulsions exposure to the ultrasound at a frequency of 20 kHz for 10, 20 and 30 minutes are capable to maintain the ratio of fatty acids while obtaining a homogeneous, finely dispersed and stable food emulsion. Deviations in the values characterizing the fatty acids content in the emulsions treated by ultrasound for 10, 20 and 30 minutes as compared with the control sample – linseed oil are insignificant (0.02±0.015% to 0.83±0.015). Using the method of electron paramagnetic resonance, the absence of free radicals was established both in the control sample – flax oil, and in emulsions obtained on its basis, homogenized using ultrasonic exposure.

Keywords: emulsions, linseed oil, ultrasonic, fatty acids, free radicals, Gas chromatography–mass spectrometry (GC-MS).

Introduction

Food emulsion production is one of the primary areas of the food industry. Emulsion food products are dispersed systems consisting of microscopic drops of liquid (dispersed phase) distributed in another liquid (dispersion medium). The main components of oils included in emulsion food products are triglycerides of higher fatty acids.

Currently, various methods of producing emulsions are explored, including ultrasonic homogenization which occupies a special place due to low energy consumption, high productivity and the possibility of producing fine and stable emulsions [1–3]. The emulsion droplets formation under the ultrasonic exposure occurs as a result of the collapse of cavitation bubbles filled with gas and the local release of significant energy with an immediate increase in pressure and temperature. At the same time, heterogeneous physical processes such as chemical reactions, formation of intense micro-flows and shock waves, ultrasonic glow appearance, etc. take place in producing emulsions. These processes contribute to emulsions' intensive mixing and production of homogeneous and finely dispersed emulsions simultaneously affecting the processed emulsions [4–6]. Increasing the ultrasonic exposure power to a certain limit contributes to the formation of smaller droplets of the dispersed phase. These processes can lead to a change in the percentage of fatty acids due to the acceleration and initiation of several chemical reactions. Moreover, the energy accumulation in very small volumes can contribute to the macromolecule's chemical bond breakdown [7–10].

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Both gas (GC) and liquid chromatography (HPLC) are widely used to analyze the fatty acid composition of oils. The determination of fatty acids by HPLC can be done without sample preparation, using the reversed-phase version (OP HPLC) and non-polar solvent. The only difficulty with this method is that non-selective detectors, particularly the Evaporative Light Scattering Detector (ELSD), have to be used for peak identification. This detector does not have a linear response, and in order to eliminate this drawback, additives of cholesterol, which forms molecular associations with triglycerides, and silver nitrate are added to the test sample to increase the analysis sensitivity [11]. An atmospheric pressure chemical ionization mass spectrometer (APCI-MS) is used as a detector. A sufficient number of works have been devoted to studies of fatty acid composition using HPLC [12–21].

Difficulties in determining the fatty acid composition by GC are due to the fact that fatty acids are poorly separated on capillary columns with a non-polar stationary phase (SP). Special polar capillary columns are used for this purpose, but in this case, the separation is hindered by significant deviations in the quantitative content of fatty acids in the studied objects, which in turn lead to peaks' masking. The most promising in terms of separation of unsaturated fatty acids are carbon-graphite columns [22]. The standard method of fatty acid composition analysis is the gas chromatography method. In addition, research on the development of the method of interpretation of chromatograms in the analysis of fatty acids by GC [23] is conducted. In addition, the use of high-power ultrasonic exposure can bring the thermal decomposition of water with the formation of H• and •OH radicals. The latter can cause the surfactant molecules decomposition on the surface of cavitation bubbles [24]. These radicals recombine to excite sonoluminescence as follows (1):

$$\begin{aligned} H_2O &\longrightarrow \dot{H} + \dot{O}H(Wi) \\ RH + \dot{O}H &\longrightarrow \dot{R} + H_2O \end{aligned} \qquad (1)$$

or diffuse into the volume and react with the solvent or dissolved substances, initiating radical chemical processes. The recombination of OH radicals produces hydrogen peroxide H_2O_2 as follows (2):

$$\dot{R} + O_2 \longrightarrow R\dot{O}_2 \quad \dot{H} + O_2 \longrightarrow H\dot{O}_2 \qquad (2)$$

It is well known that hydrogen peroxide is an active initiator of redox reactions with various substances in solutions. Free hydrogen H- and hydroxyl OH- radicals generated during sonolysis in the presence of oxygen initiate oxidation of bioorganic compounds in the product subjected to ultrasound. The oxidation of organic compounds in the liquid phase is well studied. This process is a complex radical-chain reaction with a degenerate branching. Under the action of ultrasound, the process is described by the following principal scheme. Chain nucleation, or forming radicals can be represented as follows (3):

$$\begin{aligned} H_2O &\longrightarrow \dot{H} + \dot{O}H(Wi) \\ RH + \dot{O}H &\longrightarrow \dot{R} + H_2O \end{aligned} \qquad (3)$$

where Wi is the rate of formation of H- and OH- radicals under the action of ultrasound, RH is the compound with the organic radical R and a mobile hydrogen atom H.

The continuation of the chain includes the following reactions, the totality of which represents the general scheme of oxidation of organic substances (4, 5):

$$\dot{R} + O_2 \longrightarrow R\dot{O}_2 \quad \dot{H} + O_2 \longrightarrow H\dot{O}_2 \qquad (4)$$

$$R\dot{O}_2 + RH \longrightarrow ROOH + \dot{R} \qquad H\dot{O}_2 + RH \longrightarrow H_2O_2 + \dot{R} \qquad (5)$$

where RO_2 is an organic peroxide radical;
ROOH – organic peroxide. Branching of the chain occurs as follows (6, 7):

$$ROOH \longrightarrow R\dot{O} + H\dot{O} \quad (6)$$

$$\left.\begin{array}{l} \dot{R} + \dot{R} \longrightarrow \\ \dot{R} + R\dot{O}_2 \longrightarrow \\ R\dot{O}_2 + R\dot{O}_2 \longrightarrow \end{array}\right\} \text{molecular products of oxidation} \quad (7)$$

Depending on the nature of RH bioorganic compounds and the oxidation conditions (Wi rate and concentrations of RH, O_2, ROOH), the mechanism and rate of the process can vary significantly. Cell membrane lipids are particularly easily oxidized. Therefore, the mechanism considered is widely known as lipid peroxidation. Accordingly, RH and ROOH should be understood as lipids and their peroxide [25]. Convincing proof of the formation of free radicals is their registration by electron paramagnetic resonance (EPR) methods [26].

The present study is aimed at quantifying the fatty acid composition of linseed oil as a component of food emulsions when exposed to the ultrasound with a frequency of 20 kHz of various exposure times and monitoring the free radical formation after the ultrasound treatment. Linseed oil was used as the main ingredient of the emulsions since it is rich in Omega-3 fatty acids and characterized by an optimal ratio of Omega-3 and Omega-6 fatty acids in its composition and affects the physiological functions of the human body [27–29].

Materials and Methods

Chemicals, reagents and equipment. Unrefined linseed oil (manufactured by Oleos LLC, Podolsk, Russia), emulsifier Tween 80 (Polysorbate 80, manufactured by Sibtechnopharm LLC, Novosibirsk, Russia), and distilled water were used to make the emulsions and to analyze their fatty acid composition.

Linseed oil was used as a reference sample (Sample 1) as well as food emulsions obtained based on linseed oil and water in a ratio of 2 : 1 containing Tween 80 surfactant in an amount of 1.5%. Samples 2, 3 and 4 were the emulsions subjected to high-intensity ultrasonic treatment at an operating frequency of 20 kHz and an amplitude of 40–70% using a 750 W ultrasonic homogenizer equipped with a probe with a diameter of 13 mm for 10, 20 and 30 minutes, respectively.

Ultrasound-assisted extraction and esterification of fatty acids. For the fatty acid composition analysis, the samples were prepared by alkaline hydrolysis of fat with releasing fatty acids and subsequent their esterification with methanol with subsequent methyl esters production of fatty acids according to the GOST 30418-96 [30] with some modification. For this purpose, 2 g of oil or the emulsion were added to a flat-bottom flask and mixed with 30 ml of hexane when heated (85–95 °C) with a refuse condenser. Then hexane layer with the dissolved fat was separated and completely transferred into a round-bottomed flask connected to the lower flask in a rotary evaporator. After that, the solvent was completely evaporated at a temperature of (70±2) °C, and the resulting fat fractions were used to prepare methyl esters of fatty acids. 0.10 g of the resulting fat fraction was taken from the resulting fat fraction and added to a test tube and dissolved in the 1.9 cm^3 of hexane. Then 0.1 cm^3 of a sodium methylate solution in methanol with a molar concentration of 2 mol/dm^3 was added to a test tube using a pipette. Then, the tube was closed with a stopper, and the resulting mixture after intensive stirring was centrifuged for 2 minutes and supernatant was taken for further analysis.

Gas chromatographic analysis. The fatty acid composition of the initial linseed oil (Sample 1) and food emulsions based on it (Samples 2–4) were measured on a gas chromatograph with a mass spectrometric detector and a quadrupole GCMS Shimadzu QP2010 SE Ultra analyzer. 1 ml of an oil or emulsion sample previously converted to the gas phase was injected into the chromatograph injector, and then, combined with the gas carrier helium, was passed through a polar column filled with polyethylene glycol (PEG phase). There was a gradual increase in the temperature of the column according to the following program. T=50 °C was kept for 1 minute, then increased up to 200 °C and 240 °C with a rate change of 25 °C/min and 3 °C/min, respectively. The constant temperature (plateau) was reached for 18 minutes.

Electron paramagnetic resonance (EPR). The EPR (electron paramagnetic resonance) experiment on the free radical presence in the samples studied was done by recording EPR signal with g=2.0023 on the EPR spectrometer PS-100X (Adani, Belarus) in the three-centimeter wavelength range at temperatures of 293 K (Fig. 1).

There were chosen optimal conditions of microwave power and magnetic field modulation amplitude to register the EPR spectra, namely, the microwave radiation frequency of 9.54 GHz, microwave radiation power decay of 6 dB, modulation amplitude of 1000 G. The EPR spectra of oil and emulsion samples were recorded in special 1mm capillaries placed in a quartz ampoule with a diameter of 4 mm.

Results and discussions

Gas chromatography analysis. Based on the gas chromatography analysis of linseed oil and identification of fatty acids by mass spectra, we found using an internal standard protocol that the reference sample (Sample 1) contains palmitic, stearic, oleic, linoleic and α-linolenic acids with a percentage content 6.2, 6.13, 23.06, 17.49 and 45.52%, respectively (A chromatogram is provided in the Electronic Supplement to the article).

The analysis showed that in linseed oil the content of saturated fatty acids such as palmitic and stearic acids were several times less compared to unsaturated (oleic, linoleic and α-linolenic) ones. It is well known that saturated fatty acids are consumed by the human body as an energy material, and polyunsaturated fatty acids, in particular, linoleic, and α-linolenic fatty acids, are constituents of cell membranes and other structural elements of tissues. They introduce some important functions for the body, including ensuring normal growth and metabolism, vascular elasticity, etc., and have high biological activity. The human body cannot synthesize polyunsaturated fatty acids that are therefore indispensable as compared to some amino acids and vitamins. Indeed, the complete absence of polyunsaturated fatty acids in the diet causes growth cessation, necrotizing skin damage, and changes in capillary permeability.

Effect of extraction time. Ultrasonic treatment of an emulsion for 10 minutes (Sample 2) provided the percentage content of fatty acids (using internal standard protocol) as follows: palmitic acid – 6.28%, stearic acid – 5.84%, oleic acid – 22.26%, linoleic acid – 17.18%, α-linolenic acid – 44.84%. Analysis results of fatty acid composition of emulsions, treated by ultrasound for 10, 20 and 30 minutes, are provided in the Electronic Supplement to the article.

The ultrasonic-assisted treatment of the emulsion for 20 minutes was characterized by the following content of fatty acids: palmitic – 6.18%, stearic – 5.71%, oleic – 22.23%, linoleic – 17.04%, α-linolenic – 44.74%.

The content of palmitic acid in sample 4 was 6.35%, the content of stearic acid was identical to its content in the reference sample and equaled 6.13%. Deviations in the content of oleic, linoleic and α-linolenic acids proved to be insignificant. The content of linoleic and α linolenic acid in Sample 4 were found to be 17.4% and 45.39% correspondingly.

Table shows the fatty acid composition results of lin-seed oil before exposure to the ultrasound (reference sample) and the fatty acids content in emulsions obtained by the ultrasonic cavitation (Samples 2–4).

Thus, from the table it is seen that deviations in the fatty acids content as well as fatty acid ratio of the emulsions treated with the ultrasonic sound for 10, 20 and 30 minutes compared to those for the reference sample are insignificant and are in the range of 0.02–0.83, which can be assigned to the measurement error, which is 0.015%.

Electron paramagnetic resonance (EPR). The EPR method revealed the absence of free radicals both in the reference sample of linseed oil and in the emulsions obtained on its basis, which were homogenized using ultrasonic exposure, as proved by the EPR free radical signal absence in the range of magnetic field values near the g-factor equal to 2.0023 (Fig. 2).

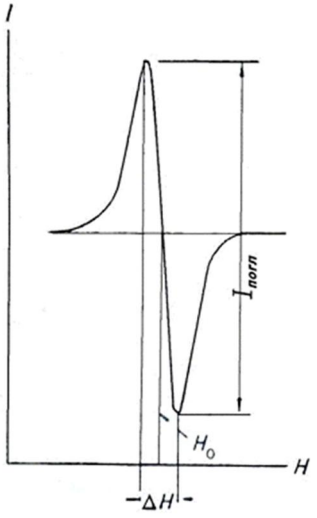

Fig. 1. Typical free radical EPR Spectrum, where I is the EPR signal intensity, H is the external magnetic field intensity [26]

Fatty acid composition of linseed oil before and after ultrasonic-assisted treatment

Fatty acid	Content, %			
	Reference sample	Sample 2	Sample 3	Sample 4
Palmitic	6.20	6.28	6.18	6.35
Stearic	6.13	5.84	5.71	6.13
Oleic	23.06	22.26	22.23	23.00
Linolic	17.49	17.18	17.04	17.40
α-linolenic	45.52	44.84	44.74	45.39
Acid ratio				

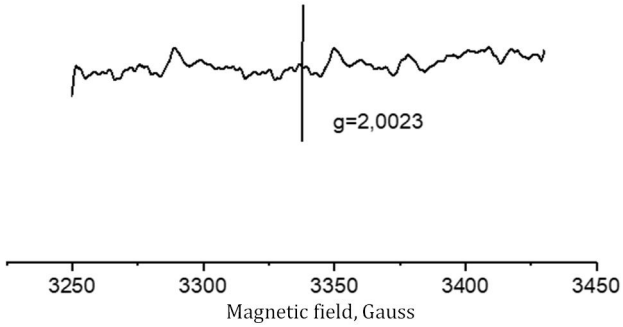

Fig. 2. EPR Spectrum of the Sample 4 (emulsion after ultrasonic-assisted treatment for 30 minutes)

Conclusions

The GC-MS method confirmed the fatty acid composition of linseed oil. Fatty acids percentage content as well as their ratio in the studied food emulsions were found. Analysis of the content of fatty acids in the studied samples showed that when obtaining emulsions with the use of ultrasonic homogenization there is a slight change in the percentage of each fatty acid in the emulsions compared to flax oil. The results showed that the duration of ultrasonic homogenization (10, 20 and 30 minutes) did not affect the qualitative and quantitative composition of the fat phase of emulsions. Consequently, when using it in food technology it is necessary to consider other technological parameters. The absence of free radicals in both control sample – in linseed oil, and in emulsions obtained on its basis, homogenized with the use of ultrasonic exposure has been established, indicating that the technological parameters used, including the duration of ultrasound exposure, allow to obtain emulsions with optimal properties for use as a base of emulsion food products.

References

1. Maa Y.F., Hsu C.C. *Pharmaceutical Development and Technology*, 1999, vol. 4(2), pp. 233–240. DOI: 10.1081/pdt-100101357.
2. Mason T.J. *Ultrasonics*, 1992, vol. 30(3), pp. 192–196. DOI: 10.1016/0041-624X(92)90072-T.
3. Canselier J., Jean J., Delmas H., Wilhelm A.M., Abismail B. *Journal of Dispersion Science and Technology*, 2002, vol. 23 (1–3), pp. 333–349. DOI: 10.1080/01932690208984209.
4. Abismail B., Canselier A., Wilhelm M., Delmas H., Gourdon C. *Ultrason Sonochem.*, 1999, vol. 6 (1–2), pp. 75–83. DOI: 10.1016/s1350-4177(98)00027-3.
5. Taylor G.I. *The scientific papers of GI Taylor*. Cambridge: Cambridge University Press, 1963, vol. 3, pp. 457–464.
6. Khmelev V.N., Golykh R.N., Shalunov A.V., Khmelev S.S., Karzakova K.A. *15th International Conference of Young Specialist on Micro/Nanotechnologies and Electron Devices*. Novosibirsk, 2014, pp. 203–207. DOI: 10.1109/EDM.2014.6882511.
7. Khmelev V.N., Golykh R.N., Khmelev S.S., Karzakova K.A. *Nauchno-tekhnicheskiy vestnik Povolzh'ya*, 2013, no. 2, pp. 249–251. (in Russ.).
8. Alegria A.E., Lion Y., Kondo T., Riesz P. *J. Phys. Chem.*, 1989, vol. 93(12), pp. 4908–4913. DOI: 10.1021/j100349a046.
9. Abismail B., Conselier J.P., Wilhelm A.M., Delma H., Gourdon C. *Ultrason Sonochem.*, 2000, vol. 7, pp. 187–192. DOI: 10.1016/s1350-4177(00)00040-7.
10. Krishna C.M., Kondo T., Riesz P. *International Journal of Radiation Applications and Instrumentation. Part C. Radiation Physics and Chemistry*, 1988, vol. 32(1), pp. 121–128. DOI: 10.1016/1359-0197(88)90025-2.
11. Mondello L., Tranchida P.Q., Stanek V., Jandera P., Dugo G., Dugo P. *Journal of Chromatography A*, 2005, vol. 1086 (1–2), pp. 91–98. DOI: 10.1016/j.chroma.2005.06.017.

12. Stein J., Kulemeier J., Lembcke B., Caspary W.F. *Journal of Chromatography A*, 1992, vol. 576(1), pp. 53–61. DOI: 10.1016/0378-4347(92)80174-o.
13. Nishiyama-Naruke A., Souza J.A., Carnel M., Curi R. *Analytical Letters*, 1998, vol. 31, pp. 2565–2576. DOI: 10.1080/00032719808005325
14. Van Eijk H.M., Bloemen J.G., Dejong C.H. *Journal of Chromatography B*, 2009, vol. 877(8–9), pp. 719–724. DOI: 10.1016/j.jchromb.2009.01.039.
15. Nguyen H.T.D., Ramli A., Kee L.M. *Journal of the Japan Institute of Energy*, 2017, vol. 96(12), pp. 532–537. DOI: 10.3775/jie.96.532.
16. Bravi E., Perretti G., Montanari L. *Journal of Chromatography A*, 2006, vol. 1134, pp. 210–214. DOI: 10.1016/j.chroma.2006.09.007
17. Guo H., Hu C., Qian J., Wu. D. *Journal of the American Oil Chemists' Society*, 2012, vol. 89, pp. 183–187. DOI: 10.1007/s11746-011-1898-5.
18. Rao M.S, Hidajat K., Ching C.B. *Journal of Chromatographic Science*, 1995, vol. 33, pp. 9–21. DOI: 10.1093/chromsci/33.1.9.
19. Ueno Y., Matsunaga H., Umemoto K., Nishijima K. *Chemical and Pharmaceutical Bulletin*, 1999, vol. 47(10), pp. 1375–1379. DOI: 10.1248/cpb.47.1375.
20. Wang F.H., Xiong X.J., Guo X.F., Wang H., Zhang H.S. *Journal of Chromatography A*, 2013, vol. 1291, pp. 84–91. DOI: 10.1016/j.chroma.2013.03.062.
21. Sun C., Zhao Y.Y., Curtis J.M. *Analytica Chimica Acta*, 2013, vol. 762, pp. 68–75. DOI: 10.1016/j.aca.2012.12.012.
22. Gaudin K., Chaminade P., Baillet A. *Journal of Separation Science*, 2004, vol. 27, pp. 41–46. DOI: 10.1002/jssc.200301622.
23. Rudakov O.B. *Khimija rastitel'nogo syr'ja*, 2001, no. 4, pp. 77–82. (in Russ.).
24. Koroleva M.Y., Yurtov E.V. *Russian Chemical Reviews*, 2012, vol. 81(1), pp. 21–43. DOI: 10.1070/RC2012v081n01ABEH004219.
25. Piskarev I.M., Ivanova I.P., Samodelkin A.G., Ivashchenko M.N. *Iniciirovanie i issledovanie svobodno-radikal'nyh processov v biologicheskih eksperimentah.* [Initiation and study of free radical processes in biological experiments]. Nizhny Novgorod, 2016, 106 p. (in Russ.).
26. *Elektronnyj Paramagnitnyj Rezonans Primenenie v Medicine. Elektronnyj Paramagnitnyj Rezonans. Faktory, Vliyayushchie na Celesoobraznost' Ispol'zovaniya Metoda EPR* [Electronic Paramagnetic Resonance Application in Medicine. Electronic Paramagnetic Resonance. Factors Influencing the Feasibility of Using the EPR Method]. URL: https://kadet-mvd.ru/pelevin-victor/elektronnyi-paramagnitnyi-rezonans-primenenie-v-medicine.html. (in Russ.).
27. Yu X., Tang Y., Liu P., Xiao L., Liu L., Shen R., Deng Q., Yao P. *Journal of Agricultural and Food Chemistry*, 2017, vol. 65(44), pp. 9635–9646. DOI: 10.1021/acs.jafc.7b03325.
28. Bykov M.I., Esaulenko E.E., Basov A.A. *Eksperimental'naya i klinicheskaya gastroenterologiya*, 2015, no. 6(118), pp. 53–56. (in Russ.).
29. Lisovaya E.V., Viktorova E.P., Borodkina A.V. *Tekhnologii pishchevoy i pererabatyvayushchey promyshlennosti APK – produkty zdorovogo pitaniya*, 2015, vol. 2(6), pp. 65–71. (in Russ.).
30. *GOST 30418-96. Masla Rastitel'nye. Metod Opredeleniya Zhirnokislotnogo Sostava.* [GOST 30418-96. Vegetable Oils. Method of Fatty Acid Composition Determination]. Moscow, 1998, 7 p. (in Russ.).

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