DOI: https://doi.org/10.53040/cga12.56 UDC: 543.42.06:665.335.2+665.344.2

OIL CONTENT IN SEEDS BY NMR RELAXATION METHOD

Oleg HARCIUC[1], 0000-0003-3488-5321,
oleg.harciuc@sti.usm.md
Mehmet SÜTYEMEZ[2], 0000-0003-0417-6495
sutyemezmehmet@gmail.com
Marcela CHISTOL[1], 0000-0001-8594-151X,
marcela.chistol@sti.usm.md
Aliona MALII[1], 0000-0003-2674-000X,
aliona.malii@sti.usm.md
Aurelia STINGACI[1], 0000-0001-6621-9919,
aurelia.stingaci@sti.usm.md

[1]Institute of Genetics, Physiology and Plant Protection, Moldova State University, Chisinau, Republic of Moldova
[2]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Horticulture, Kahramanmaras, Republic of Turkey

Abstract. *The aim of our work was to evaluate the capability of NMR-relaxation method of the determination of oil content in walnut kernels and soybean seeds. NMR-relaxation spin-echoes for protons of the studied seeds were studied on a modernized NMR-relaxometer, using a sequence of 90°- and 180°- high-frequency pulses, with an interval 3.5 ms. As standards in NMR calibration for walnut kernels and for soybean seeds were used different oils. The oil content of commercial walnut kernels SRL "Monicol" is 69±1%, which corresponds to the typical range for the crop, 64-72%.*

After harvest 2021 the minimal oil content was in the seeds of soybean variety Pentata (14,6±0,2% for the seeds with dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg) and the maximal oil content was in seeds of soybean variety Ladutsa (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).

Keywords: NMR relaxation, oil, soybean, walnut.

INTRODUCTION

Seed oil content is important in the study of the seed biodiversity. Cultivated soybean seeds have an oil content of approximately 18–22%, whereas wild soybean seeds contain about 8–10% oil [1]. Fat is the main component of the nut kernel and generally accounts for 64%-72% [2]. As a biochemical utilization of a physical method, nuclear magnetic resonance and its improvements allow for numerous rapid determinations of seed oil content within a short period of time and greatly improve the throughput of analysis [3- 5]. Spin echo method is one of the elegant and

most useful features in pulsed nuclear magnetic resonance (NMR). Determination of oil content in seeds by NMR relaxation method is a clear, nondestructive, fast and accurate alternative to traditional liquid chemistry methods. The oil content determined by NMR relaxation method strictly corresponds to the amount of oil extracted from the walnut kernels by organic solvents [6]: oil was extracted with Petroleum Ether using a Soxhlet apparatus, dehusked seeds also were analyzed for oil content by NMR method. Comparison between NMR and Soxhlet method certified that for low oil content walnut seeds the data of NMR method were 62.58 ± 0.03% compared to 64.35 ± 0.28% for Soxhlet method; for medium oil content walnut seeds the data of NMR method were 67.99 ± 0.11% compared to 68.09 ± 0.66% for Soxhlet method; for high oil content walnut seeds the data of NMR method were 72.60 ± 0.01% compared to 73.17 ± 0.22% for Soxhlet method.

The aim of our work was to evaluate the capability of NMR-relaxation method of the determination of oil content in walnut kernels and soybean seeds.

MATERIALS AND METHODS

NMR-relaxation spin-echoes for protons of the studied seeds were studied on a modernized NMR-relaxometer [7], using a sequence of 90°- and 180°- high-frequency pulses [8], with an interval 3.5 *ms*. As standards in NMR calibration for walnut kernels and for soybean seeds were used different oils. For walnut kernels was used Pure Walnut Carrier Oil manufactured by RV Essential (Republic of India): Juglans Regia (100% Pure and Natural Cold Pressed). For soybean calibration was used soybean oil, obtained from soybean seeds variety Bucuria (the oil from the seeds was obtained from soybean meal after two extractions by toluene). Walnut kernels were obtained from walnut orchard of SRL "Monicol". Soybean seeds of different maturity groups (MG) were used: Ladutsa (MG II) and Pentata (MG III).

RESULTS AND DISCUSSIONS

Fig. 1 shows the samples and ampoule for determination of oil content in seeds by NMR relaxation method. The determination of oil content by NMR-relaxation requires significantly less material compared to biochemical extraction methods: while biochemical extraction uses 10 g of seeds [6], samples for measurement by NMR-relaxation, as a rule, do not exceed 0.2 g (and the sensitivity of the device, not less than 1 mg of oil, allows the use of small samples, weighing from 10 mg). For soybean the NMR measurements were performed on individual seeds. The oil content of seeds was determined by comparing echo-signals from seeds with echo-signal of calibration standards.



Figure 1. General view of the samples and ampoule for determination of oil content in seeds by NMR relaxation method
(208 mg soybean seed and 182 mg sample from native walnut kernel)

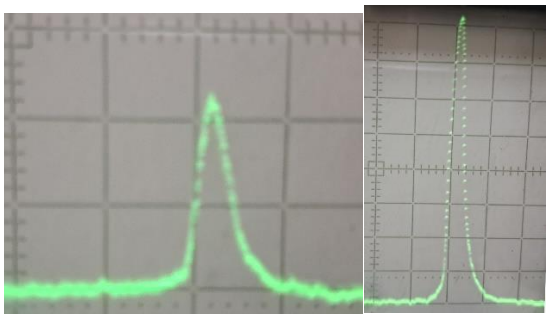


Figure 2. NMR spin echo signals recorded according to [8], at a time distance of 3.5 ms between 90°- and 180°-pulses for seed samples from different crops *(208 mg soybean seed and 182 mg sample from native walnut kernel)*

Figure 2 shows the differences of spin echo signals from seeds of different crops in intensity and shape: the echo signal from walnut kernel protons is significantly more intense and narrower. The echo signal from the protons of pure walnut oil used to construct the calibration curve is also characterized by a narrow shape, similar to the NMR spin echo signal from samples from native walnut kernels.

To avoid the influence of differences in the quality of the oils studied (for different crops or one crop of different origin), it is recommended to adjust the nuclear magnetic resonance relaxometer on a pure chemical standard (glycerol).

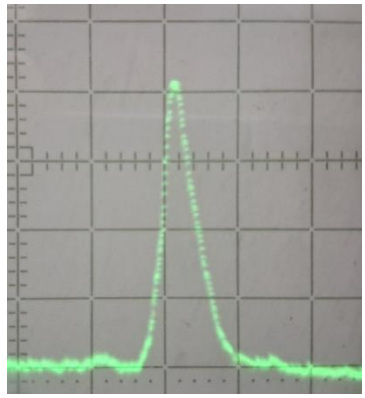


Figure 3. NMR spin-echo from standard of glycerol.

According to the NMR relaxation method, the oil content of commercial walnut kernels SRL "Monicol" is 69±1%, which corresponds to the typical range for the crop, 64-72%.

Oil content data for individual soybean seeds of Ladutsa and Pentata varieties (2021 harvest) are presented in Figure 4.

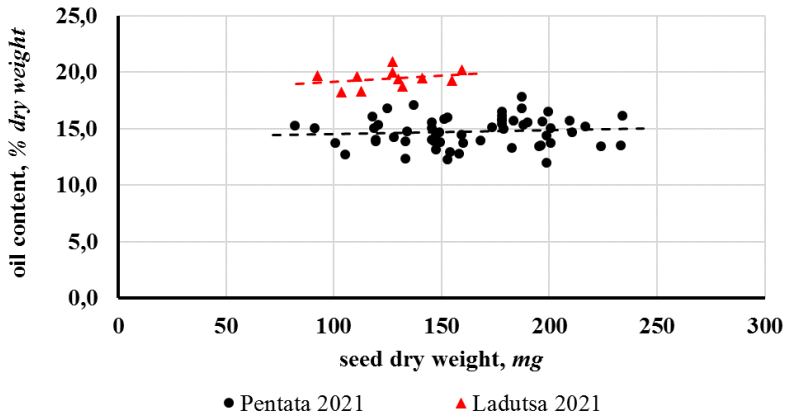


Figure 4. The oil content in soybean seeds of Pentata and Ladutsa varieties (harvest 2021)

Each dot corresponds to a separate individual seed

Table 1 shows the data on oil content determination by NMR relaxation method for different soybean varieties according the seed weight.

At harvest 2021 the minimal oil content in seeds was determined for soybean variety Pentata – 14,6-15,0% dry weight (14,6±0,2% for the seeds with dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg).

Table 1. Oil content in seeds of different weight for soybean varieties Pentata and

Ladutsa (harvest 2021)

Variety	Individual seed weight, *mg*		Oil content, *% dry weight*
	range	average	
Pentata	100-150	128±4	**14,6**±0,2
	150-200	177±2	**14,8**±0,2
	200-250	214±5	**14,9**±0,4
	250-300	269±16	**15,0**±0,3
Ladutsa	100-150	123±4	**19,3**±0,3
	150-200	157±2	**19,7**±0,5

The maximal oil content in seeds was determined for soybean variety Ladutsa – 19,3-19,7% dry weight (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).

CONCLUSIONS

The echo signal from walnut kernel protons is significantly more intense and narrower comparing to soybean seeds. The echo signal from the protons of pure walnut oil used to construct the calibration curve is also characterized by a narrow shape, similar to the NMR spin echo signal from samples from native walnut kernels. The oil content of commercial walnut kernels SRL "Monicol" is 69±1%, which corresponds to the typical range for the crop, 64-72%.

At harvest 2021 the minimal oil content in soybean seeds was determined for variety Pentata – 14,6-15,0% dry weight (14,6±0,2% for the seeds with dry weight 100-150 mg and 14,9±0,4% for the seeds with dry weight 200-250 mg). The maximal oil content in seeds was determined for soybean variety Ladutsa – 19,3-19,7% dry weight (19,3±0,3% for the seeds with dry weight 100-150 mg and 19,7±0,5% for the seeds with dry weight 150-200 mg).

To avoid the influence of differences in the quality of the oils studied (for different crops or one crop of different origin), it is recommended to adjust the nuclear magnetic resonance relaxometer on a pure chemical standard (glycerol).

Acknowledgments: Research was carried out within the research project 25.80013.5107.01TR "*Eco-farming for innovative protection of new walnut varieties: harnessing methodologies and technologies*", funded by TÜBITAK and NARD, he Sub-program 011103: "*Development of environmentally safe means to reduce the impact of agricultural crop pests in the context of climate change*" and the research project *"Manifestation of synergistic effects between nitrogen-fixing micro-organisms and biotic and abiotic factors aimed at increasing soybean protection and productivity"*, funded by doctoral school of biological, geonomic, chemical and technological sciences.

REFERENCES

1. PATIL, Gunvant, VUONG Tri D., KALE Sandip, VALLIYODAN Babu, DESHMUKH Rupesh, ZHU Chengsong, WU Xiaolei, BAI Yonge, YUNGBLUTH Dennis, LU Fang, KUMPATLA Siva, SHANNON J.Grover, VARSHNEY Radjeev K., NGUYEN Henry T. Dissecting genomic hotspots underlying seed protein, oil, and sucrose content in an interspecific mapping population of soybean using high-density linkage mapping. In: *Plant Biotechnology Journal*, 2018, vol.16, nr. 11, pp. 1939 – 1953. ISSN 1467-7652.
2. YI, Jianhua, SUN Yifei, ZHU Zhenbao, LIU Ning, & LU Jiali. Near-infraredreflectance spectroscopy for the prediction of chemical composition in walnut kernel. In: *International Journal of Food Properties*, 2017, vol. 20, nr. 7, pp. 1633–1642. ISSN 1094-2912.
3. KISTOL, Marcela, BUDAK Alexandru, KHARCHUK Oleg. Increasing of the information about oil content in seeds (NMR-relaxation study). In: International congress on oil and protein crops: Meeting of the EUCARPIA Oil and Protein Crops Section, dedicated to the 50th anniv. of Sci. Association of Geneticists and Breeders of the Rep. of Moldova, May 20-24, 2018: abstr. book. Chișinău, 2018, p. 37. ISBN 978-9975-3178-5-6.
4. MOGÎLDA, Anatolii, HARCIUC Oleg, BOTNARU Liuba. Analiza unor parametri cantitativi la genotipurile din cadrul colecției de Sesamum indicum L. In: Materialele Conferinţei ştiinţifico-practică cu participare internaţională "Instruire prin cercetare pentru o societate prosperă", Ed. a 8-a, 20-21 martie 2021: – Chişinău: UST, 2021, Vol. 1: Biologie (474 p.): pp. 95-101. ISBN 978-9975-76-327-1
5. CUȚITARU, Doina, HARCIUC Oleg, GANEA Anatolie. Determination of the oil content in the seeds of oil flax varieties preserved *ex situ* in the Republic of Moldova. In "*Agrobiodiversity for Improving the Nutrition, Health, Quality of People Life and Nature*", Book of Abstracts of the 6th International Scientific Conference. September 8, 2024, Nitra: AgroBio*Net*, p. 32. ISBN 978-80-552-2770-2.
6. POGGETTI, Luca, FERFUIA Claudio, CHIABÀ Cristina, TESTOLIN Raffaelle & Mario BALDINI. Kernel oil content and oil composition in walnut (*Juglans regia* L.) accessions from north-eastern Italy. In: *Journal of the Science of Food and Agriculture*, 2018, vol. 98, nr. 3, pp. 955-962. ISSN 1097-0010
7. PANOV, Pavel, KHARCHUK Oleg. A modernized NMR relaxometer for studying the state of water in plants and determining the oil content in seeds. - translation from Russian. In: Materialele Conferinţei ştiinţifice internaţionale (Ediţia a 5-a) „Genetica, fiziologia şi ameliorarea plantelor", 23-24 oct. 2014, Chișinău: S.n., 2014 (Tipogr. "Prin-Caro")., pp. 144-146. ISBN 978-9975-56-194-5.
8. HANH, Erwin L. Spin-echoes. In: *Physical Review*. 1950, vol. 80, nr. 4, pp. 580-594. ISSN 0031-899X.