FRI-LB-P-1-BFT(R)-04

BIOCHEMICAL ASPECTS OF WALNUT DAIRY FREE MILK

Ass. Prof. Cristina Popovici, PhD
Department of Food and Nutrition,
Technical University of Moldova
Tel.: +37368241547
E-mail: cristina.popovici@toap.utm.md

Ass. Prof. Alexei Baerle, PhD
Department of Chemistry,
Technical University of Moldova
Phone: +37379789301
E-mail: alexei.baerle@chim.utm.md

Prof. Pavel Tatarov, DcS
Department of Food Technology,
Technical University of Moldova
Tel.: +37379584862
E-mail: pavel.tatarov@tpa.utm.md

***Abstract**: Dairy-free milks are finding an audience in Europe, despite consumption still ranking well below the US. The market has increasingly benefited in recent years from the perceived health and taste benefits of non-dairy products. Nut milk is an useful beverage for patients with lactose intolerance, celiac disease, as well as vegans. In addition, nut milk is a source of aminoacids, vitamins and minerals complex. Current research is devoted to study different types of vegetable milk, walnut milk in particular as well as its biochemical and physico-chemical properties. In this paper as components for obtaining experimental samples of vegetable milk walnuts were used. The technology of walnut milk included following main steps: primary walnut preparation, extraction procedure and homogenization. Standard methods of analysis have been applied for evaluation of walnut milk chemical composition, basic quality properties as well as microstructure and rheological behavior. Study gives a detailed analysis of the fatty acid composition of the product by GC-chromatography; 20 fatty acids were found. The highest content is in the mono- and polyunsaturated fatty acids, namely the linoleic, linolenic and arachidonic acids, which are of great nutritive and biological value. Analysis of walnut milk microstructure showed that dimensions of oil drops in walnut milk are distributed in normal mode, the major part of oil volume is formed by drops with an average diameter of 2.70 microns. These results showed high potential and positive view on walnut milk production, in agreement with the current demand of healthy products.*

***Keywords:** Walnut, dairy free milk, health benefits, perspectives.*

INTRODUCTION

In the last few years, the population ratio demanding vegetable-based products is growing, either because of the increasing problems related with the intolerances to cow milk or because of changes in the food preferences (Bernat, N. *et al*, 2015). As a consequence of new consumer tendencies, food industries are currently producing new, high quality, nutritionally improved products with added value. Vegetable-based "milks" are included in these new products, which are available at any supermarket as an alternative to dairy products, with an increasing consumer acceptance. European sales of non-dairy milks are increasing by over 20% per year, Spain being the EU country in which the non-dairy drinks market grew the most (Bernat, N. *et al*, 2015, Organic Monitor, 2006). Similarly, total USA retail sales of soy, almond, rice and other plant milks reached $1.3 billion in 2011 (Packaged Facts, 2012).

There is a wide variety of vegetable-based milks, although most of the research activity has been focused on those obtained from soy. Research dealing with the use of non-soy vegetable milk is still scarce and most of it is related with the nutritional quality of such products. In this sense, walnuts can be used as an alternative to cow milk in lacto-intolerant people, allergic to animal proteins and vegans (Liu, S. *et al*, 2016, Popovici, C., 2017). Walnuts have an equilibrated mono-unsaturated fatty acid-polyunsaturated fatty acids ratio, which define the products which are healthy for people with heart disease (Baerle, A. *et al*, 2016). They are also considered helpful for maintaining cholesterol at healthy levels due to their high content of antioxidant compounds (Popovici, C., & Gubenea, O., 2016). Vegetable based milks are emulsified products where the nut fat is dispersed in an aqueous phase and where the rest of the components play different roles in the product stability. The different process steps, such as homogenisation and heat treatments usually produce changes in the arrangement of components, thus leading to modifications in the particle size, colour, viscosity and physical stability of the product. These physicochemical modifications have to be known to efficiently control the process and to implement the necessary improvements in the production lines.

The objective of the present study is to develop technology of walnut milk production and to analyse its chemical composition, basic quality properties as well as microstructure and rheological behavior in terms of storage.

MATERIALS AND METHODS

As components for obtaining experimental samples of vegetable milk walnuts of cultivars Calarash and Kogylnichanu from the State Enterprise "Forestry Iargara" were purchased. Walnuts used correspond to requirements for quality of the specifications and technical documentation.

Technology of samples preparation

The walnuts were cracked, shelled, soaking, peeled, and then grind into a powder. Walnut milk preparation was carried out essentially following the procedure presented in figure 1.

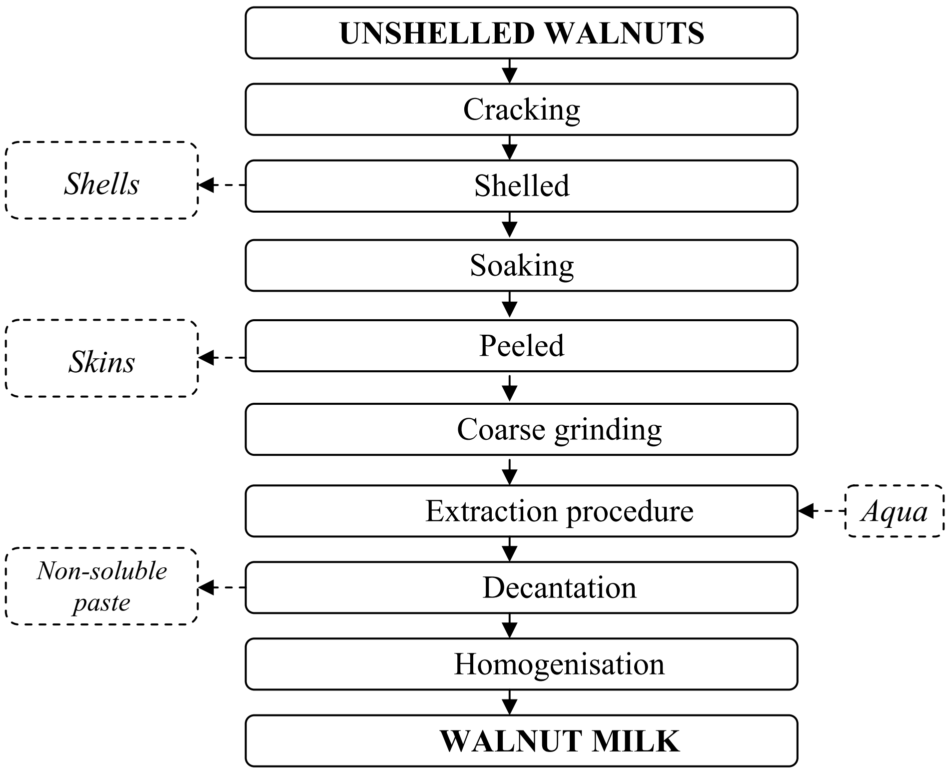


Fig. 1. Walnut milk processing flow diagram

Chemicals and reagents

Ethanol (99.9%), chloroform, glacial acetic acid, potassium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate ($Na_2S_2O_3 \times 5H_2O$) and starch were supplied by Eco-Chimie Ltd (Chisinău, Moldova). All used chemicals were of HPLC or analytical grade. Distilled water was used throughout.

Chemical composition

The quantification of ashes, fat content and proteins was carried out in the walnut milk according to the AOAC Official Methods AOAC 16.006, AOAC 945.16 and AOAC 958.48 respectively (Bernat, N., 2016). All the determinations were performed in duplicate.

Basic quality properties

Acidity of the walnut milk was determined by potassium hydroxide titration as described in AOCS Official Method Cd 3d-63 (AOCS, 1999). Peroxide value was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003). Water activity was determined using digital water activity analyzer with integrated temperature controller.

Microstucture and particle size distribution

The microstructure of walnut milk sample was determined using an optical digital microscope of advanced series, model "Motic DMB" (China). For this purpose a drop of the investigated sample of walnut milk was placed on subject glass, covered with its integumentary glass and then established in a microscope. Photos of walnut milk samples were obtained by digital camera connected to a microscope. Obtained photos of walnut milk emulsion were analized and particle size distributions were calculated.

Rheological properties

The rheological behavior of walnut milk sample was characterized by using a digital rotational rheometer (BROOKFIELD, model DV-III+, USA). Viscosity was measured by changing the rotation speed from 40 to 250s-1 at temperature 20ºC. All measurements were performed during 5 days.

Statistical analysis

Variance analysis of the results was carried out by least square method with application of Microsoft Office Excel program. Differences were considered statistically significant if probability was greater than 95% ($q < 5\%$). All assays were performed at room temperature, 20 ± 1ºC. Experimental results are represented according to standard rules.

RESULTS AND DISCUSSION

Characterisation of walnut milk: chemical composition and quality properties

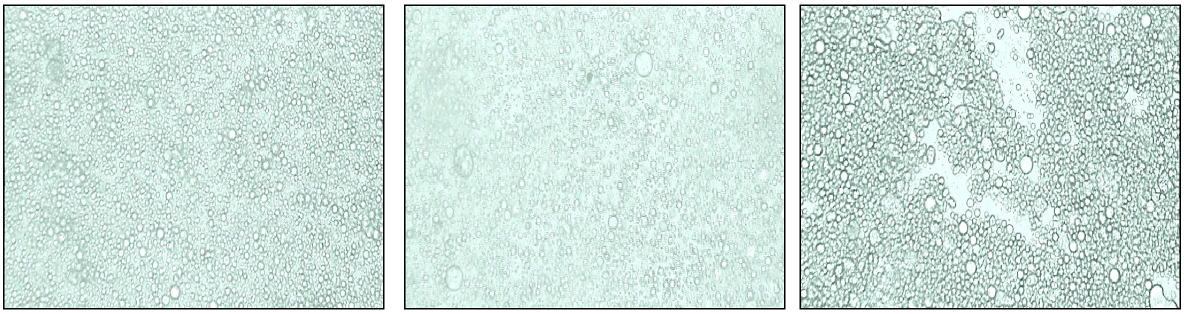
Results in walnut milk chemical composition has been summarised in Table 1. As was observed during the extraction process, the major losses of walnut components occur in the protein fraction, which remained in the waste by-product during the extraction process. In comparison with cow milk (3.2 and 3.4 g/100 ml of fat and protein, respectively), walnut milk has a higher fat content (18 g/100 ml) and a lower protein content (1.3g/100 ml). Nevertheless, walnut milk contain minerals and monounsaturated and polyunsaturated fatty acids respectively present in the lipid fraction.

Table 1. Chemical composition (g / 100ml) and quality properties of walnut milk

No.	Chemical composition/quality properties	Walnut milk
1.	Protein content, [g]	1.30 ± 0.01
2.	Lipid content, [g]	18.00 ± 0.05
3.	Ashes, [g]	0.41±0.01
4.	Acid Value, [KOH/g]	0.21 ± 0.02
5.	Peroxide Value, [mol/kg]	1.10 ± 0.03
6.	Water activity (a_w)	0.975 ± 0.005

Walnut milk microstructure

Visual comparison of images (figure 2) obtained by digital camera incorporated in optical microscope, denotes that stratification of walnut milk begins at the second day, and on the third day become significant. This is evident by the appearance of large oil drops, largest than 10 microns in diameter on the corresponding images. The real dimensions of each image are 250 × 170 microns.



1-st day *2-nd day* *3-rd day*

Fig. 2. Evolution of walnut milk microstructure during storage

Polidispersity of emulsion was analysed by zoom of representative areas of the images and subsequent mathematical processing. Oil-drops of certain sizes were manual counting in the zoomed images. Then, volume of the oil-drops with certain average diameter, the corresponding volume of fraction and the percentage of the fraction in the oil phase were calculated, using formulas (Baerle, A. *et al*, 2014):

$$V_{drop,i} = \frac{\pi < d_i >^3}{6}; \qquad V_i = N_{drops,i} \cdot V_{drop,i}; \qquad \varphi_i = \frac{V_i}{\sum V} \cdot 100\%,$$

in which: $V_{drop,i}$ – volume of single oil-drop with average diameter $< d_i >$; V_i - volume of fraction with number i, Ndrops,i – number of drops in the fraction i, φi – volume fraction of the drops with average diameter $< d_i >$; $\sum V$ - total volume of all oil-drops in the analyzed area.

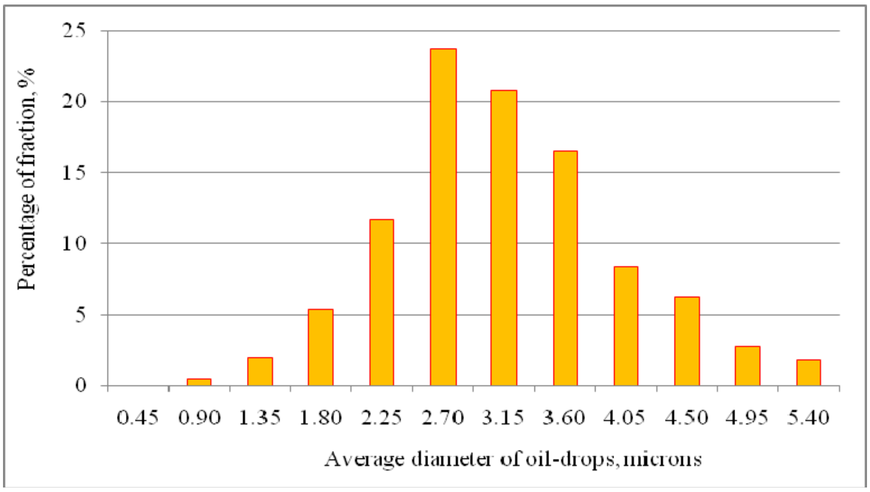


Fig. 3. Particle size distribution curve for walnut milk in terms of percentage of particles volume

Dimensions of oil drops in walnut milk are distributed in normal mode in the first day (Fig. 3). Sizes of particles are in the range of 0.45…5.40 microns. The major part of oil volume has an average diameter of 2.70 microns.

Rheological behavior of walnut milk

Rheological parameters play a key role in the definition of textural and sensory perception of a new product. Figure 4 shows changes of these parameters of walnut milk during 5 days of storage.

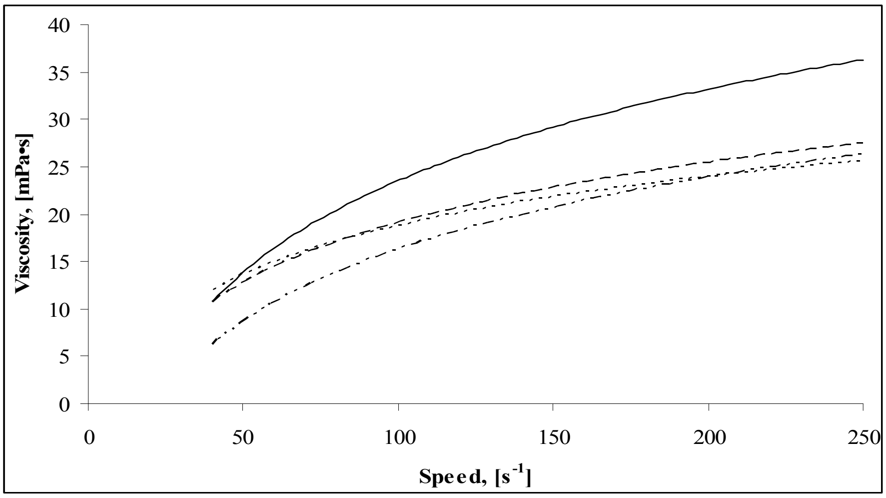


Fig. 4. Up-flow curves of walnut milk samples at different storage times

Results showed that the inner structure of the walnut milk were not significantly modified during the first days of storage at 4…6 ºC. Viscosity of the walnut milk sample increased after the third day of storage.

CONCLUSIONS

The development of walnut milk fully meets the current trends towards an increased consumer demand for healthier products. In the development of this product, some important technological deficiencies have been found, mainly related to the product´s physical stability during its shelf life. To this end, the optimisation of processing techniques must be encouraged and more studies focusing on the microstructure and arrangement of the different components of the walnut milk after processing are needed in order to clarify and understand how to improve the appearance and texture of the final product. These studies would also allow to decrease the amount of additives added (hydrocolloids and/or emulsifiers) and thus, to reduce the economic costs.

Acknowledgements

This work was done in the framework of Project 15.817.02.30A "*Development of methods and techniques for modernization of nuts (Juglans Regia L.) processing technology using their biologically active constituents in the functional foods*", cofounded by the Academy of Science and by the Technical University of Moldova.

REFERENCES

AOCS, (1999). *Official Methods and Recommended Practicles of the American Oil Chemists' Society*. Method Cd 3d-63. Champaign: AOCS Press.

AOCS, (2003). *Official Methods and Recommended Practicles of the American Oil Chemists' Society*. Method Cd 8-53. Champaign: AOCS Press.

Baerle, A., Dimova, O., Zadorojnai, L., Tatarov, P., Zenkovich, A. (2014). Electrophoresis of Oil-Containing Edible Microcapsules with Protein-Polyuronic Shells. *Ukrainean Food Journal*, Vol. 3, Is. 2, 211-217.

Baerle, A., Popovici, C., Radu, O., Tatarov, P. (2016). Effect of synthetic antioxidants on the oxidative stability of cold pressed walnut oil. *Journal of Food and Packaging Science*, Technique and Technologies, Year V, No. 9, 19-24.

Bernat, N., Chafer, M., Rodriguez-Gacia, J., et al. (2015). Effect of high pressure homogenisation and heat treatment on physical properties and stability of almond and hazelnut milks. *LWT – Food Science and Technology*, Vol. 62, Is. 1, Part 2, 488-496.

Bernat, N, Chafer, M., et al. (2014). Vegetable milks and their fermented derivative products. *International Journal of Food Studies*, Vol. 3, 93–124.

Horwitz, W. (2000). *Official Methods of Analysis of AOAC International* (17th ed). Gaithersburg, USA: Association of Official Analytical Chemists.

Liu, S., Liu, F., Xue, Y, Gao, Y. (2016). Evaluation on oxidative stability of walnut beverage emulsions. *Food Chemistry*, Vol. 203, 409–416.

Organic Monitor. (2006). *The European market for soya milk and non-dairy drinks*. Dublin: Research and Markets.

Packaged Facts. (2012). *Dairy alternative beverages in the U.S.: Soy milk, almond milk, rice milks and other dairy milk alternatives*.

Popovici, C., Gubenia, O. (2016). *Fatty acids composition and radical scavenging activity of walnut oil (Juglans regia L.) from central Moldova*. Paper presented at the 8th Central European Congress on Food 2016 - Food Science for Well-being, 297.

Popovici, C., Baerle, A., Tatarov, P. (2017) *Innovative solutions for dairy free milk processing*. Paper presented at the 4th North and East European Congress on Food (NEEFood) “Global and Local Challenges in Food Science and Technology”, Kaunas University of Technology, Lithuania, 11-13 September 2017, 99.

Popovici, C., Oprea, O.B., Boaghi, E. (2017) *Biochemical and physico-chemical evaluation of walnut milk*. Paper presented at the 8th International Symposium “Euroaliment – Mutatis mutandis in food”. University Dunarea de Jos of Galati, Romania, 7-8 September 2017, 95.