Quality of Life
(Banja Luka)

VOLUME **11** ISSUE **3-4** August 2020 ISSN 1986-602X
The Republic of Srpska, Bosnia and Herzegovina EISSN 1986-6038

UDC 614

A Multidisciplinary Journal of Food Science,
Environmental Science and Public Health

APEIRON
ΑΠΕΙΡΟΝ

VOLUME 11
ISSUE **3-4**

Quality of Life
(Banja Luka)

Izdavač/Published by Panevropski univerzitet ''Apeiron'' Banja Luka/ Pan-European University ''Apeiron'' Banja Luka
Urednik izdavača/Editor of University Publications Aleksandra Vidović, Bosnia and Herzegovina

Editorial Office **Quality of Life**
Pan-European University Apeiron
Pere Krece 13, 78 000 Banja Luka, B&H
Tel: +387 51 247 910
Mob: +387 65 141 619
Fax: +387 51 247 921
e-mail: qol@apeiron-edu.eu
sekretar@qol-au.com
redakcija@qol-au.com
URL: http://www.qol-au.com

Secretary Aleksandra Vidović
Language Editor (English, Serbian)
Technical Editor/Layout Sretko Bojić
Web design Alen Tatarević
Printed by Markos design & print studio, Banja Luka
Printed in 300 copies

Aim and Scope

Quality of Life publishes original research papers and reviews and aims to provide a forum for the rapid dissemination of significant novel research in the various disciplines encompassing the Science and technology of food, Public health engineering, Sanitary inspection and control, Environmental and public health. Topics covered by the journal include:

- Dietetics; Nutrition principles applied to foods
- Food Technology; Production and preservation of foodstuffs; Food preservation technique
- Industrial microbiology; Science and technique of applied microbiology; Applied mycology
- Public Health, environment and hygiene
- Hygiene of air, water, soil; Pollution and its control
- Water; Sanitation; Water treatment
- Sewage; Treatment, disposal, utilization of sewage
- Urban hygiene; Wastes; Refuse; Rubbish; Garbage; Collection and disposal of town wastes
- Measures against industrial and other nuisances
- Occupational health hazards; Occupational health and hygiene
- Ecology; Environmental engineering, sustainability and health
- Related topics

UDC 614 Quality of Life is registered with the Ministry of Science and Technology of the Republic of Srpska by serial registration code 07.030-053-160-4/10, date 03.03.2010.
Quality of Life (ISSN 1986-602X) is an international journal published two times a year.

Indexed in:

ebscohost.com
citefactor.org/contact
scholar.google.com
doisrpska.nub.rs
crossref.org
road.issn.org
cosmosimpactfactor.com
erihplus.nsd.no
esjindex.org

UDC 614

ISSN 1986-602X (Print)
ISSN 1986-6038 (Online)

Quality of Life

Vol. 11(2020) No. 3-4 (73-136)

Contents

Annual subscription is 30 EUR
Full-text available free of charge at http://www.qol-au.com

Dear readers and authors,

As Editor-in-Chief of the journal ***Quality of Life***, I look forward to the challenge of creating a journal that will enhance the quality of research in the various disciplines encompassing the Science and technology of food, Public health engineering, Sanitary inspection and control, Environmental and public health in our country, region as well as at the international level. The goal that we have set is high but not unachievable.

The journal ***Quality of Life*** was registered in the Register of Public Media in 2010 by the Decision of the RS Ministry of Education and Culture. Over the past years, this journal has published a large number of original scientifi c research papers, communications and review papers. ***Quality of Life*** is published twice a year by Pan-European University "Apeiron" Banja Luka.

All the papers published so far have undergone a thorough review by the editorial board and the reviewers, made up of experts from both RS/B&H, the surrounding and other countries, from proven and recognized university and research institutions. As a result of a professional approach to selecting and reviewing papers, and raising the quality of the journal, ***Quality of Life*** was classifi ed in the fi rst category of journals in 2019 by the Ministry of Education and Culture.We are proud to say that ***Quality of Life*** has been well received by the scientifi c and the general pub-lic in a relatively short period of time, which gives the editorial board a strong motivation for further work.

The editorial team would like to thank our many reviewers who helped to maintain the journal standard; our many authors who submitted their best work to the journal; and, most important, our readers for your continuing support.

I shall assure all our readers that our consistent efforts will be aimed toward increasing the visibility, impact, editorial cycle time, citations and the overall quality of our journals. We very much look forward to strengthening the reputation of our publications, and we want to attract more higher-quality submissions. I hope our readers and patrons share a similar vision, and we look forward to a productive, challenging and successful 2020 ahead. In the spirit of continuous improvement, any constructive input on streamlining our processes is very welcome.

Please help us grow by citing articles that you read in ***Quality of Life***. We look forward to receiving your contributions in the near future.

Editors

DOI: 10.7251/QOL2003077R **UDC:** 502.55(282.2:497.6Republika Srpska)

Original scientific paper

DIVERSITY AND ECOLOGY OF THE FRESHWATER CRAYFISH IN THE NORTHWEST OF THE REPUBLIC OF SRPSKA

Rajko Roljić
University of Banja Luka, Genetic Resources Institute University City, Banja Luka, Republic of Srpska
rajkoroljic@gmail.com

Abstract: The existence of three autochthonous crayfish species is confirmed in the northwest of the Republic of Srpska: Astacus astacus, Pontastacus leptodactylus and Austropotamobius torrentium. There is a lack of information on the European crayfish population status in the Republic of Srpska comparing to other European countries. Our aim is to generate the latest information on crayfish distribution and population status, and make it the basis for managing and preserving natural population. The present study was implemented in the period from April 2018 until September 2019. Mostly distributed crayfish species in the Republic of Srpska is A. torrentium registered in 12 locations at altitude ranging from 201 to 846 m in the Vrbas river basin, but it is also present in the tributary streams of the Sana river, in ecologically-like habitats, in particular in relatively clear waters with low quantity of organic substances (I and II water category). A. astacus is mainly present in the Crna river basin, forming both river and lake population (a great number thereof is present in the Balkana lake) – waters with oxygen concentration over 8 g.O2.m-3 and with BOD5 values below 0.5 g.O2.m-3. P. leptodactylus was found only in two locations: the Matura river (in Srbac) and the Vrbas river (upstream from Razboj), in the I-III category waters. The identified possible threats for autochthonous crayfish in fresh water ecosystems of the Republic of Srpska require urgent water management and preservation actions.

Key words: freshwater crayfish, Astacidae, Republic of Srpska.

INTRODUCTION

Basis for any astacological study of certain area fauna is gaining knowledge on population of living organisms in that area, specifically crayfish, and collecting relevant information on the presence of certain species, their number and possible endanger thereof.

More information is available on distribution and zoogeography of European crayfish species than of other species of aquatic invertebrate; the situation is not the same in all parts of Balkan Peninsula. Crayfish information is quite well available in Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, Kosovo and Metochia (Simić *et al*., 2008; Trožić-Borovac, 2011; Rajković, 2012; Živić *et al*., 2014). Partial information is available for other places in the Balkan Peninsula, and practically no published scientific information is available for the Republic of Srpska.

Local species are differently distributed in the European countries. The existence of four autochthonous species from the Astacidae family was reported: *Astacus astacus*, *Pontastacus leptodactylus*, *Austropotamobius torrentium* and *Austropotamobius pallipes* which are typical for the Balkan Peninsula (Obradović, 1984; Maguire and Gottstein-Matočec., 2004; Karaman, 1976; Bedjanic, 2004; Simić *et al*., 2008; Rajković, 2007; Trožić-Borovac, 2011; Živić, 2014). Globally, the *A. astacus*, *A. pallipes* and *A. torrentium* species are so pruned and threatened, and thus included in the international list of threatened species (IUCN Red List), and their habitats included in the Habitat Directive. The *A. astacus* species is classified as species at risk (VU) on the International Red List, *A. pallipes* as threatened species (EN) and the *A. torrentium* is classified as insufficiently known species (DD). Given the different levels of this species' endanger and their habitats, a special conservation approach is required in many parts of their distribution.

This study paper is intended for confirming the presence of the Astacidae family species on the northwest of the Republic of Srpska, and making contribution to knowledge on their distribution and status of their population.

MATERIALS AND METHODS

Observation of physical and chemical water features in researched locations and collection of crayfish samples was performed once a week in the period from April 9th, 2018 until August 23st, 2019.

In line with crayfish sampling, the Institute for Public Health of the Republic of Srpska conducted studies of physical and chemical water parameters in selected areas for the purpose of this study. A portion of selected results was taken over from biological and ecological studies carried out by Crnogorac *et al*. (2013) and Lolić *et al*. (2017) to compare new results with results obtained from previous studies.

Crayfish samples were collected manually, LiNi trap with baits and nets. Traps were placed by the watercourse edge, under stones and roots of the littoral vegetation and left overnight. Fresh crayfish were observed, both in the field and laboratory. Afterwards, all collected organisms were classified up to the species level by applying illustrated guidance for identification of European crayfish from the Astacidae family (Maguire, 2010). Morphometric features were measured by a vernier scale. Weighing was carried out by weighing scale produced by Kern PFB, Version 2.2 with maximum weight of 1200 g and accuracy of 0.01 g.

RESULTS

In the northwest of the Republic of Srpska the existence of three European crayfish species from the Astacidae family was reported: *A. astacus*, *P. leptodactylus* and *A. torrentium* (Figure No. 1). Observations were performed on the Crna, Rudnička, Sokočnica, Vijaka, Stanikova, Mlinska, Korana rivers and on the Marjanovića, Matića, Dževerov, Zelenikovac, Ponor, Dobraš, Šargovački, Ledenac streams. Downstream of the Matura and Vrbas rivers was observed, as well as the Malo lake of the Balkana lake and Veliko lake of the Balkana lake.

Table 1. Finding locations of crayfish on northwest of the Republic of Srpska

Locality	Number of individuals	Species	Geographical coordinates	Elevation (m)	Date
Balkana	58	A. astacus	44.41547°N 17.04797°E	787	03.08.2018.
Crna river	26	A. astacus	44.41792°N 17.04739°E	754	03.08.2018.
Rudnička river	3	A. astacus	44.82929°N 17.09777°E	262	10.09.2018.
Sokočnica	1	A. astacus	44.27234°N 17.05662°E	467	31.03.2019.
Vijaka	1	A. astacus	44.80849°N 17.61730°E	165	22.08.2019.
Matura	24	P. leptodactylus	45.12330°N 17.43471°E	90	30.05.2019.
Vrbas	35	P. leptodactylus	45.06337°N 17.45603°E	92	31.05.2019.
Stanikova river	1	A. torrentium	44.73713°N 17.46004°E	328	09.04.2018.
Mlinska river	31	A. torrentium	44.75088°N 17.46322°E	267	22.08.2018.
Rudnička river	24	A. torrentium	44.82929°N 17.09777°E	262	10.09.2018.
Marjanovića stream	67	A. torrentium	44.71530°N 17.29464°E	310	21.10.2018.
Korana	46	A. torrentium	44.316525°N 16.93559°E	637	11.11.2018.
Matića stream	11	A. torrentium	44.88761°N 17.22211°E	233	12.11.2018.
Dževerov stream	1	A. torrentium	44.41230°N 16.98980°E	767	30.03.2019.
Zelenikovac	31	A. torrentium	44.394331°N 16.97244°E	846	30.03.2019.
Ponor	1	A. torrentium	44.28353°N 16.58584°E	680	30.03.2019.
Dobraš	7	A. torrentium	44.87569°N 17.24475°E	253	31.03.2019.
Šargovački stream	1	A. torrentium	44.82323°N 17.16140°E	201	29.06.2019.
Ledenac	1	A. torrentium	44.61569°N 16.99916°E	841	23.08.2019.

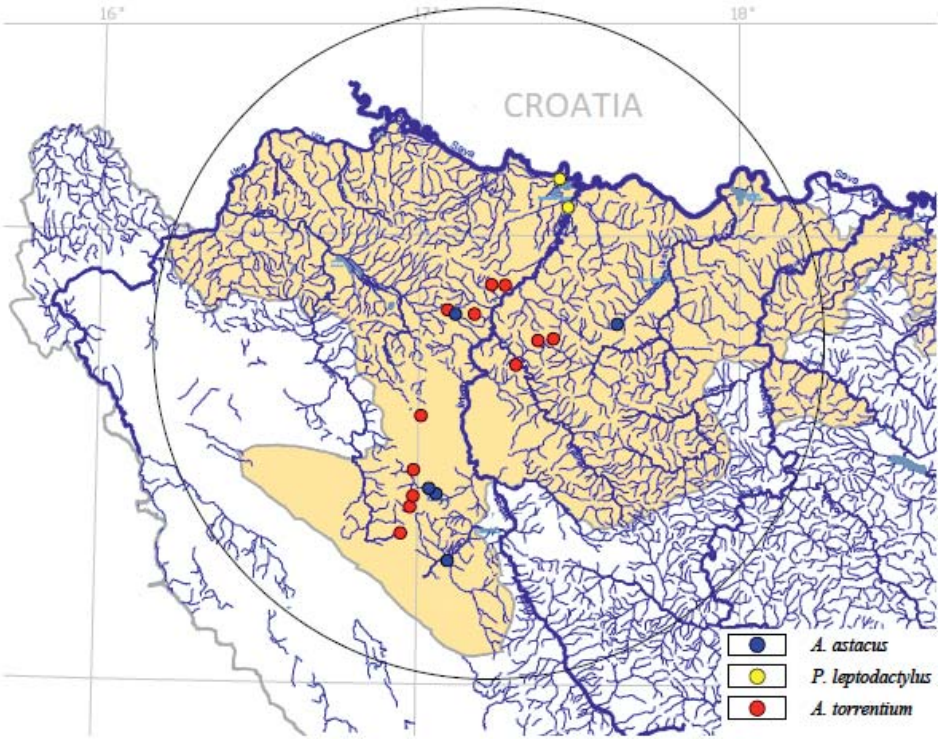


Figure 1. Distribution of A. astacus, P. leptodactylus and A. torrentium in the northwest of the Republic of Srpska

The European crayfish *A. astacus* is distributed in the north and the west of the Republic of Srpska in the downstream of the Crna, Rudnička, Sokočnica and Vijaka rivers and the Balkana lake. The observed aquatic habitats are located in highland area with altitude ranging from 165 to 787 m (Table 1). The water temperature in the part of the Rudnička river upstream is relatively low (12.8°C). While observing, the water temperature in the Crna river is higher (16.6°C). In all observed habitats the water was subalcaline (pH value ranges from 7.2 to 8.22). In all observed habitats, oxygen saturation was over 80%. Increased concentration of BOD_5 was registered on the Crna river with 3.54 $g \cdot O_2 \cdot m^{-3}$ and BOD_5 value on the upstream of the Rudnička river and Balkana lake was below 0.5 $g \cdot O_2 \cdot m^{-3}$. Analyzed parameters for the most observed aquatic habitats meet values anticipated under the Regulation referring to the first category of surface waters, implying to satisfactory water quality.

The Danube crayfish *P. leptodactylus* is distributed in the northern part of the Republic of Srpska, in the Matura river downstream and in the Vrbas river upstream in Srbac. Given its distribution, this species was observed on an altitude ranging from 90 to 92 m (Table 1). Waters in these watercourses with the Danube crayfish present, has relatively high values of diluted oxygen (> 8 $g \cdot O_2 \cdot m^{-3}$) and BOD_5 value which is rather low >1.92 $g \cdot O_2 \cdot m^{-3}$. Temperature measured in the sampling period fluctuated from 8 to 18°C. In the subsequent studies, females (*P. leptodactylus*) with eggs were registered in the Vrbas location, referring to the successful existence of relatively stable population distributed on the surface of 13 km of the Vrbas river watercourse (from Razboj) all the way to the Sava river mouth (up to Srbac).

The existence of stone crayfish *A. torrentium* was reported on numerous locations in the Stanikova, Mlinska, Rudnička and Korana rivers and the Marjanovića, Matića, Dževerov, Zelenikovac, Ponor, Dobraš, Šargovački, Ledenac streams. Observed streams run through the highlands at altitude from 201 to 846 m (Table 1). Most locations have relatively small number of populations reported, and the biggest number was reported in the Marjanovića stream (in Čelinac) with 67 analyzed specimens. Presence of female with eggs was reported in the population found in the Marjanovića stream. Water temperature in all observed

locations fluctuated from 11.5 to 15.5°C. Measurement results of diluted oxygen showed no big difference in concentration between observed locations and fluctuated from 5.17 to 10.12g·m^{-3}. In all observed locations the water saturation with oxygen was always over 80%. Given the pH value, all observed locations are situated in subalkaline area. The values of pH were ranging from 7.15 to 8.28. Increased BOD_5 values were registered in the Ponor area with 2.99 g·O_2·m^{-3}, and in other locations was below 0.5, in particular 0.6 g·O_2·m^{-3}.

Morphometric analysis was carried out on the European crayfish samples from the Balkana lake, the Danube crayfish from the Vrbas river and stone crayfish from the Marjanovića stream. According to the results, the longest body of the European crayfish was 114.87 mm, and the heaviest one was 55.9 g. The length of the Danube crayfish varied from 73.22 to 135.36 mm, with weight from 13.9 to 64.3 g. Smaller size, characteristic for stone crayfish, was measured and reached maximum of 117.9 mm with weight of 46 g (Table 2).

Table 2. Values of morphometric features of the Astacidae representative specimens from the northwest of the Republic of Srpska

A. astacus (Balkana)								
	Males (N=38)				**Females (N=20)**			
	Mean	**Min**	**Max**	**St.dev.**	**Mean**	**Min**	**Max**	**St.dev.**
TBL	107.74	100.8	114.4	5.13	88.03	71.23	105.6	13.55
W	42.68	31.9	55.9	8.69	21.86	9.7	45.6	13.81
P. leptodactylus (Vrbas)								
	Males (N=22)				**Females (N=13)**			
	Mean	**Min**	**Max**	**St.dev.**	**Mean**	**Min**	**Max**	**St.dev.**
TBL	103.91	79.3	135.36	15.92	89.61	73.22	112.73	14.73
W	30.82	20.9	64.3	11.98	20.8	13.9	39	8.31
A. torrentium (Marjanovića stream)								
	Males (N=33)				**Females (N=34)**			
	Mean	**Min**	**Max**	**St.dev.**	**Mean**	**Min**	**Max**	**St.dev.**
TBL	104.01	82.64	117.9	8.86	93.47	69.48	114.37	8.12
W	28	12	46	6.95	16.67	6.6	28.6	4.85

Analyzing sex structure, we may notice the male majority, being presumably consequence of the period when female were laying eggs and were less active (Streissl and Hodl, 2002; Maguire *et al*., 2002; Rajković, 2012).

DISCUSSION

The new records presented in this study paper include three species. Number of registered species was fairly uniform in comparison to information on registered species in Slovenia (three species - Bertok *et al*., 2003, 2004), Croatia (four species - Maguire and Gottstein Matočec, 2004), Srbiji (three species – Simić *et al*., 2008) and Montenegro (three species - Rajković, 2012).

The existence of the *A. astacus* species was reported in Europe at altitude ranging from 100 to 1600 m (Subchev and Stanimirova, 1998), and from 165 to 787 m in the northwest of the Republic of Srpska, although this species was found in Bosnia and Herzegovina and lowlands at altitude of 98 m, and high moun-

tains up to altitude of 1128 m (Trožić-Borovac, 2011). Machino and Füreder (1998) states that upper height limit of the European crayfish distribution in Austria is at altitude of 1516 m (Prebersee). Rajković (2012) believes that this species population significantly decreased in Europe mostly due to plague and habitat degradation. According to studies, this species appeared along with *A. pallipes* in Europe, in France (Souty-Grosset *et al*., 2006). During 2002 the presence of this species along with white-clawed crayfish *A. pallipes* were reported in the Boracko lake, as well as in the location of Mostarsko blato (Šanda and Petrusek, 2008). Results of general ecological conditions where the European crayfish lives in Europe, somewhat match the conditions found in the Republic of Srpska. This species inhabits waters with lower temperature (around 14.8 °C) and high oxygen percentage (over 80%). Further, individual ecology studies of the European crayfish were carried out in the region (Maguire, 2010; Rajković, 2012; Trožić-Borovac, 2012). It is stated that ideal habitats for *A. astacus* are clear streams with low water temperature (optimal from 12 to 14 °C), good oxygen saturation, loamy, rocky or gravel layer and abundance of water vegetation (Obradović, 1988).

Today's studies in Europe show that the Danube crayfish inhabits lentic and slow-flowing rivers. Habitats are located at altitudes ranging from 0 to 858 m (Trichkova *et al*., 2013), and from 90 to 92 m in the northwest of the Republic of Srpska. In Bosnia and Herzegovina this species is recently officially registered 2005 (Trožić-Borovac, 2011); today we know that it inhabits the Sava river (the Brčko area) and Miljacka (upstream from Sarajevo). Uncontrolled, scientifically unjustified introduction of the Danube crayfish to numerous European rivers, lakes, ponds, swamps, fishponds, has significantly spread its areal (Zaikov *et al*., 2009). The *P. leptodactylus* species behaves as invasive species not only in areas where introduced but also in areas where it is native in particular where spread naturally (Maguire and Dakić, 2011; Maguire 2010; Holdich, 2002). Although widespread, a little information is available thereof (Maguire and Dakić, 2011; Trožić-Borovac, 2011).

The existence of the *A. torrentium* species is registered at altitude ranging from 201 m (the Šargovački stream) to 846 m (Zelenikovac). It is mostly distributed in places ranging from 300 to 900 m, rarely below 200 m and over 1200 m (Trichkova *et al*., 2013). Subchev and Stanimirova (1998) state that it is distributed at altitude ranging from 100 to 200 m, rarely over 1600 m. Bohl (1987) believes that these crayfish were displaced from their natural habitats in lowlands to higher altitudes due to anthropogenic influence to their habitats as confirmed in the Republic of Srpska, because majority of these ecosystems is out of settlements. *A. torrentium* was found along with the European crayfish *A. astacus* in location of the Rudnička river in the village of Motike. According to studies in Bulgaria, this species occurs together with the *A. astacus* and *P. leptodactylus* species. Such cohabitation of the *A. torrentium* species with other crayfish is not usual for the species, and is rarely observed (Todorov *et al*., 2014). Analysis results of general ecological conditions where stone crayfish lives in Europe partially match conditions in the Republic of Srpska. Trožić-Borovac *et al*. (2007) state that this species is related to highland zone with beech trees. Namely, specimens prefer habitats with high quality degree (I/II), lower temperature (around 13°C) and high oxygen percentage (over 80%). In addition, similar studies were carried out in the region regarding exclusive relationship between water quality and status of the stone crayfish population (Maguire, 2010; Rajković, 2012; Trožić- Borovac *et al*., 2007). It is emphasized that the ideal habitats for habitation of the *A. torrentium* species are clear flowing waters with low water temperature, rich oxygen saturation, rocky or gravel layer and abundance of aquatic vegetation.

CONCLUSION

This paper presents information on distribution and individual ecology of crayfish in the northwest of the Republic of Srpska. Former studies confirmed an inhabitation of the northwest of the Republic of

Srpska with three autochthonous European species: *A. astacus*, *P. leptodactylus* and *A. torrentium*. Total of 19 locations belonging to Black Sea watershed was observed. The *A. astacus* and *A. torrentium* species inhabit mountain ecosystems, in relatively clear waters with low quantity of organic substances (I and II water category). The altitude where the distribution spot of the *A. astacus* and *A. torrentium* species is present, ranges from 165 to 846 m. Parts of lowlands of the Vrbas river (altitude of 92 m) and the Matura river (altitude of 90 m) were inhabited by specimens of the Danube crayfish *P. leptodactylus*. Former results of studies shows significant astacological values in the northwest of the Republic of Srpska, and thus observation should be continued to a greater extent in the forthcoming years.

ACKNOWLEDGEMENT

The paper presents the result achieved by realization of Program for the conservation and sustainable use of genetic resources of Republic of Srpska, financed by the Ministry of Scientific and Technological development, Higher Education and Information Society performs of the Republic of Srpska and implemented by the Institute of Genetic Resources of the University of Banja Luka. Expert opinion on the research of biodiversity was issued by the Republic Institute for the Protection of Cultural, Historical and Natural Heritage (No. 30/625-723/18, 09.10.2018th year; number 07/1.30/625-020/19, dated 23.1. 2019).

REFERENCES

Bedjanič, M. (2004). Novi podatki o razširjenosti raka navadnega koščaka *Austropotamobius torrentium* (Schrank, 1803) v Sloveniji (Crustacea: Decapoda). *Natura Sloveniae*, 6(1): 25-33.

Bertok, M., Budihna, N., Povž, M. (2003). Strokovne osnove za vzpostavljanje omrežja Natura 2000: ribe (Pisces), piškurji (Cyclostomata), raki deseteronožci (Decapoda): Končno poročilo. Naročnik: Ministrstvo za okolje, prostor in energijo, ARSO. Zavod za ribištvo Slovenije, Ljubljana, 370.

Bertok, M., Budihna, N., Povž, M., Seliškar, T. (2004). Ribe, piškurji, sladkovodni raki deseteronožci. *Proteus*, 66(9-10): 462-468.

Bohl, E. (1987). Comparative studies on crayfish brooks in Bavaria (*Astacus astacus* L., *Austropotamobius torrentium* Schr.). *Freshwater Crayfish*, 7, 287-294.

Crnogorac, Č., Trbić, G., Rajčević, V., Dekić, R., Pešević, D., Lolić, S., Milošević, A., Čelebić, M. (2013). Riječna mreža opštine Mrkonjić Grad (fizičkogeografska i ekološka istraživanja). Geografsko društvo Republike Srpske, Banja Luka.

Holdich, D. (2002). General biology. U: Holdich D. M. (ur.): Biology of freshwaetr crayfish. *Blackwell Science*, Oxford, 1-27.

Karaman, M. (1976). Fauna na Makedonija. Desetonogi rakovi-Decapoda (Malacostraca –Crustacea). Prirodonaucen muzej –Skopje.

Lolić, S., Dekić, R., Manojlović, M. (2017). Procjena kvaliteta vode vodotoka na području opštine Mrkonjić Grad, SKUP, 8 (1): 45-51.

Machino Y and Füreder L (1998). Der Steinkrebs *Austropotamobius torrentium* (Schrank, 1803) im Haldensee (Tirol, Österreich) und weitere Nachweise von Flußkrebsen in hochgelegenen Gewässern. Ber. Naturwiss.-Med. Verein Innsbruck, 85: 223–229.

Maguire, I. (2010). Slatkovodni rakovi, Priručnik za inventarizaciju i praćenje stanja. Državni zavod za zaštitu prirode, Zagreb, 44.

Maguire, I., Dakić, L. (2011). Comparative analyses of *Astacus leptodactylus* morphological characteristics from Croatia and Armenia. *Biologia*, 66(3): 491-498.

Maguire, I., Erben, R., Klobučar, G.I.V., Lajtner, J. (2002). A year cycle of *Austropotamobius torrentium* (Schrank) in streams on Medvednica mountain (Croatia). Bulletin Francais de Pêche et Pisciculture, 367, 943-957.

Maguire, I., Gottstein-Matočec, S. (2004). The distribution pattern of freshwater crayfish in Croatia. *Crustaceana*, 77 (1), 25-49.

Obradović, J. (1988). Slatkovodni rakovi. Ribar. Jugoslavija, 43, 55-59.

Obradović, J. (1984). Problem terminologije slatkovodnih rakova. Bilten Društva Ekologa Bosne i Hercegovine. Serija B-Naučni skupovi I savjetovanja. Broj 3- III Kongres ekologa Jugoslavije, knjiga II: 165-169.

Rajković, M. (2012). Distribucija, filogenija, ekologija i konzervacija rakova iz familije Astacidae na području Crne Gore. Doktorska disertacija. Prirodno- matematički fakultet, Univerzitet u Kragujevcu.

Rajković, M. (2007). Održivo korišćenje populacije riječnog raka *Astacus astacus* (Linnaeus 1758) u vodenim ekosistemima gornjeg toka rijeke Zete. Magistarski rad. Univerzitet u Kragujevcu.

Šanda, R., Petrusek, A. (2009). Distribution and diversity of crayfishes in the Adriatic drainage of Bosnia and Herzegovina. In: Kozák P. and Kouba A. (eds.), Abstract Book, Future of Native Crayfish in Europe, Regional European Crayfish Workshop, 7th–10th September 2009, Písek, 50.

Simić, V., Petrović, A., Rajković, M., Paunović, M. (2008). Crayfish of Serbia and Montenegro – The population status and the level of endangerment. *Crustaceana*, 81, 1153−1176.

Službeni glasnik Republike Srpske, br. 42. (2001). Uredba o klasifikaciji voda i kategorizaciji vodotoka.

Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P., (ur.) (2006). Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France, 187.

Streissl, F., Hödl, W. (2002). Growth, morphometrics, size at maturity, sexual dimorphism and condition index of *Austropotamobius torrentium* Schrank. *Hydrobiologia*, 477, 201-208.

Subchev, M. and Stanimirova, L. (1998). Distribution of freshwater crayfishes (Crustacea: Astacidae) and the epibionts of the genus Branchiobdella (Annelida: Branchiobdellae), Hystricosoma chappuisi Michaelsen, 1926 (Annelida: Oligochaeta) and Nitocrella divaricata (Crustacea: Copepoda) in Bulgaria. *Historia Naturalis bulgarica*, 9, 5–18.

Todorov, M., Antonova, V., Hubenov, Z., Ihtimanska, M., Kenderov, L., Trichkova, T., Varadinova, E., Deltshev, C. (2014). Distribution and current status of stone crayfish populations *Austropotamobius torrentium* (Decapoda: Astacidae) in Natura 2000 protected areas in Bulgaria. *Acta Zool Bulg*., 66, 181–202.

Trichkova T., I. Botev, Z. Hubenov, L. Kenderov, M. Todorov, D. Kozuharov, C. Deltshev and L. Füreder (2013). Freshwater crayfish (Decapoda: Astacidae) distribution and conservation in Bulgaria. *Freshwater Crayfish*, 19(2): 243-248.

Trožić-Borovac, S. (2011). Freshwater crayfish in Bosnia and Herzegovina: the first report on their distribution. *Knowledge and Management of Aquatic Ecosystems*, 401, 26.

Trožić-Borovac, S. (2012). Opće karakteristike plemenitog raka *Astacus astacus* Linneanus, 1758 (Decapoda, Astacidae) i njegova distribucija u vodama BiH. Časopis agencije za vodno područje rijeke Save Sarajevo, 80, 24-29.

Trožić-Borovac, S., Deljanin, L., Dautbašić, M. (2007). Ekološko-biosistematske karakteristike potočnog raka *Austropotamobius torrentium* (Shrank, 1803.) iz Nahorevskog potoka, Radovi Šumarskog fakulteta, 1, Univerzitet u Sarajevu, 39−55.

Zaikov, A., Hubenova, T. and Vasileva, P. (2009). Use of narrow-clawed crayfish (*Astacus leptodactylus*) in recreational and commercial fisheries and aquaculture in Bulgaria. EIFAC Occasional Paper No. 44, Rome, FAO.

Živić N., Atanacković A., Milošević S. and M. Milosavljević (2014). The Distribution of Astacidae (Decapoda) Fauna in Ko1sovo and Metohija, Serbia. *Water Research and Management*, 4(4): 47-52.

Recived: February 14, 2020
Accepted: March 6, 2020

DOI: 10.7251/QOL2003084C **UDC:** 616.921.5:598.2]-036.21

Original scientific paper

Study of the Influence of Solvent and Column Temperature on the Separation Effectiveness of LMW Glutenins by RP-HPLC

Vesna Gojković Cvjetković[1], Radoslav Grujić[2], Željka Marjanović-Balaban[3], Danijela Rajić[1]

[1]*University of East Sarajevo, Faculty of Technology Zvornik, Bosnia and Herzegovina, vesna.gojkovic@yahoo.com*
[2]*State High School of Medical Science, Prijedor, Bosnia and Herzegovina*
[3]*University of Banja Luka, Faculty of Forestry Banja Luka, Bosnia and Herzegovina*

Abstract: Gluten contains two fractions, which are represented in equal amounts. These are soluble gliadins and insoluble glutenins. Glutenin fraction is responsible for the dough and gluten viscoelastic properties. The dominant type of protein in glutenins are LMW glutenins.

The aim of this paper was to examine the influence of solvent type and column temperature on the separation effectiveness of LMW glutenins. Extraction was performed with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added. Separation of LMW glutenins was performed on HPLC Agilent Techologies 1260 Infinity apparatus.

After the extraction with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added, the highest number of proteins was observed after extraction with 50% (v/v) 1-propanol and at a column temperature of 40 °C (Xsr=20, respectively RC=69.56%), and the lowest number at a column temperature of 45 °C (Xsr=14.17, respectively RC=66.42%). The obtained results were read at a wavelength of 210 nm.

After the extraction of LMW glutenins with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and detected at a wavelength of 280 nm, the highest number of proteins was observed after extraction with 50% (v/v) isopropanol and at a column temperature of 50 °C (Xsr=24.17, RC=56.47%) and the lowest number of proteins after extraction with 50% (v/v) ethanol and at a column temperature of 50 °C (Xsr=12.17, RC=56.45%).

Key words: RP-HPLC, C3 column, LMW glutenins.

INTRODUCTION

Glutenins are one of the gluten proteins fraction. These are polymers formed by binding polypeptides to disulfide bonds. They are sparingly soluble proteins and the action of strong reducing agents (β-mercaptoethanol and dithioerythritol) breaks the intermolecular disulfide bonds. As a result, glutenin subunits are released and they are soluble in aqueous alcohol (Urade et al., 2018; Bonilla et a. 2020).

The molecular weight of glutenins varies from 500,000 to 10 and more million daltons (Wieser et al., 2006). Glutenins are divided into two subunits. These are glutenins with high molecular weight (HMW-glutenins) and glutenins with low molecular weight (LMW-glutenins) which give elasticity and viscosity to the dough (Wang et al. 2020; Gao et al., 2017).

The dominant type of glutenin subunits are low molecular weight glutenins (LMW-GS). They make up approximately 60% of total glutenin proteins. Their share in the total gluten protein content is approximately 20% (Wieser and Kieffer, 2001). Glutenins with low molecular weight (LMW-GS) are similar to α+β and γ gliadins in terms of amino acid composition and molecular weight.

They contain the N-terminal region, which consists of repeating units of glutamine and proline. In addition to the N-terminal, they also contain a C-terminal region. This region is homologous to α+β and γ

gliadins. Glutenins with low molecular weight (LMW-GS) contain 8 cysteine residues (Grosch and Wieser, 1999; Wieser, 2003; Huang et al., 2018). Of these, 6 residues are in a position homologous to α+β and γ gliadins. Two additional cysteine residues are unique to glutenins with low molecular weight (LMW-GS) (Wieser, 2007).

Although LMW glutenin subunits are similar to gliadins in primary and secondary structure, they still differ in one very important characteristic. In addition to intramolecular, LMWs also form intermolecular disulfide bonds. These bonds are incorporated into glutenin polymers (Delcour et al., 2012).

The contribution of LMW-GS to dough quality showed complex factors. Studies indicated that both the molecular structures and expression levels of glutenins had important effects on dough quality (Beom et al., 2018). Different types of LMW-GS also affect the dough quality.

Considering the significance of LMW glutenins on dough quality and the fastest and most efficient identification, the aim of this study was to examine the influence of solvent type and column temperature on the efficiency of protein separation by high pressure liquid chromatography with reversed phase.

MATERIAL AND METHOD

Material

Glutenin proteins were extracted from wheat flour type 500 purchased from the market of Bosnia and Herzegovina. 50% (v/v) ethanol, 1-propanol and isopropanol were used for the extraction.

Glutenin extraction

The extraction of glutenin proteins was performed according to a modified method of Wiser et al. (1998) and Gojkovic et al. (2019). First, albumin and globulins (soluble in aqueous and salt solution), then gliadins (soluble in alcohol) were extracted from wheat flour. After these protein fractions were removed, glutenins were extracted. Glutenins were extracted under a nitrogen atmosphere at 60 °C with 50% (v/v) aqueous ethanol (REAHEM, Srbobran), isopropanol (Lach-Ner, Czech Republic) and 1-propanol (Lach-Ner, Czech Republic) in which Tris-HCl (0.05 mol/l, Sigma-Aldrich, United States, pH=7.5), urea (2 mol/l, Sigma Aldrich, Germany) and dithioerythritol (1%, ACROS Organics, Switzerland) were added, at a room temperature, 20 °C.

The extraction was performed twice with 1.0 ml of appropriate solvent each (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol). Each time after the extraction solvent was added, the sample was homogenized on a vortex (Advanced Vortex Mixer ZX3, Velp scientifica) for 2 minutes. Then stirring was continued on a magnetic stirrer (Velp scientifica) for 10 minutes. After homogenization was completed, centrifugation of the samples in a centrifuge (Hettich zentrifugen, rotina 380 R) was carried out for 20 minutes at 7000 rpm. The supernatants were made up to 2.0 ml with an appropriate extraction solvent. Samples were filtered through a 0.45 μm membrane filter (RC syringe filters, Filtratech, France) before the analysis began.

Figure 1. shows a chromatogram of glutenins extracted with 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) and separated at a column temperature of 40 ºC and at a wavelength of 210 nm.

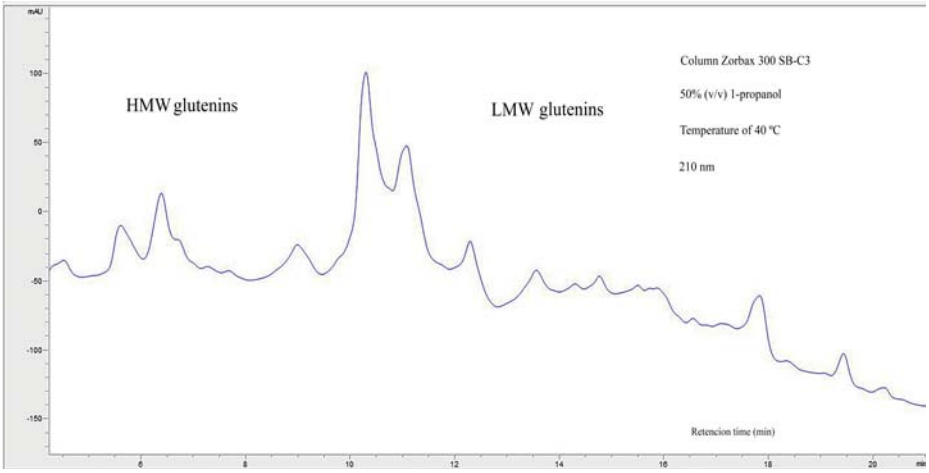


Figure 1. Chromatogram of glutenin proteins

RP-HPLC chromatography

RP-HPLC chromatography was performed on an HPLC Agilent Technologies 1260 Infinity apparatus. The separation of glutenin proteins was performed on a Zorbax 300 SB-C3 column (Agilent Technologies), size 4.6 x 150 mm, with a particle size of 5 µm. The column temperature was 40, 45 and 50 °C. Two mobile phases were used. These are deionized water and 0.1% trifluoroacetic acid (TFA, Acros, France) in acetonitrile (ACN; Biosolve, Chimie, France). The flow rate was 1 ml/min. The injection volume was 70 µl and the detection wavelength was 210 and 280 nm, respectively.

Statistical data processing

Statistical data processing was performed in IBM SPSS, Statistics 26. Descriptive statistical analysis calculated the average value, standard deviation and 95% confidence interval of the average value. Variance analysis of different groups was used to evaluate the effect of column temperature on the number of detected proteins and the relative concentration of LMW glutenins.

RESULTS AND DISCUSSION

Table 1. shows descriptive indicators of the number of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C), aborbance measured at 210 nm. The number of detected LMW glutenin proteins and their relative concentration is calculated relative to total proteins and total relative concentration.

Table 1. Descriptive indicators for LMW glutenin proteins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 210 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	18.00	0.89	0.36	17.06	18.94	17	19
	45	6	16.33	1.03	0.42	15.25	17.42	15	18
	50	6	16.50	1.05	0.43	15.40	17.60	15	18
50% (v/v) 1-propanol	40	6	20.00	0.63	0.26	19.34	20.66	19	21
	45	6	14.17	1.17	0.48	12.94	15.39	13	16
	50	6	15.50	0.55	0.22	14.93	16.07	15	16
50% (v/v) isopropanol	40	6	18.17	0.75	0.31	17.38	18.96	17	19
	45	6	18.83	1.33	0.54	17.44	20.23	18	21
	50	6	18.67	0.52	0.21	18.12	19.21	18	19
ANOVA (LMW) 50% (v/v) ethanol, F(2.15)=5.11, Sig.=0.020, eta square=10.11/24.94=0.40									
ANOVA (LMW) 50% (v/v) 1-propanol, F(2.15)=81.37, Sig.=0.000, eta square=112.11/122.44=0.92									
ANOVA (LMW) 50% (v/v) isopropanol, F(2.15)=0.83, Sig.=0.454>0.05									

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=18). The lowest number of proteins was obtained at a column temperature of 45 °C (Xav=16.33). As the column temperature increases, the number of proteins decreases, and then slightly increases (Table 1).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=20). The lowest number of proteins was obtained at a column temperature of 45 °C (Xav=14.17). As the column temperature increases, the number of proteins decreases, and then slightly increases (Table 1).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=18.83). The lowest number of proteins was obtained at a column temperature of 40 °C (Xav=18.17). As the column temperature increases, the number of proteins increases, and then slightly decreases (Table 1).

Based on the obtained results (Table 1), it can be seen that the highest number of LMW glutenin proteins was obtained by extraction with 50% (v/v) with 1-propanol and chromatographic separation at a column temperature of 40 °C (Xav=20). One-factor analysis of variance examined the effect of column temperature at a constant solvent concentration (50% v/v 1-propanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, glutenin protein separation time and absorbance measurement) on the number of proteins within the LMW glutenin fractions. It was found that there is a statistically significant difference in the number of proteins within the LMW glutenin fractions.

Table 2. shows descriptive indicators of the relative concentration of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and

50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C).

Table 2. Descriptive indicators of the relative concentration of LMW glutenins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column dimensions 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 210 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	84.99	1.47	0.60	83.46	86.54	83.48	87.34
	45	6	80.59	2.34	0.96	78.13	83.05	78.00	84.80
	50	6	71.48	1.94	0.79	69.45	73.52	69.75	75.23
50% (v/v) 1-propanol	40	6	69.56	0.74	0.30	68.78	70.34	68.61	70.70
	45	6	66.42	2.49	1.01	63.81	69.03	64.01	70.55
	50	6	63.25	2.28	0.93	60.86	65.65	60.21	66.89
50% (v/v) isopropanol	40	6	79.93	1.72	0.70	78.13	81.74	78.29	83.12
	45	6	88.66	1.93	0.79	86.64	90.69	85.39	91.05
	50	6	75.00	3.18	1.30	71.66	78.34	70.65	80.34
ANOVA (LMW) 50% (v/v) ethanol, F(2.15)=74.89, Sig.=0.000, eta square=569.83/626.90=0.91									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=15.00, Sig.=0.000, eta square=119.45/179.16=0.67									
ANOVA (LMW) 50% (v/v) isopropanol, F(2.15)=51.33, Sig.=0.000, eta square=574.47/658.41=0.87									

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=84.99%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=71.48%). As the column temperature increases, the relative concentration decreases (Table 2).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH= 7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=69.56%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=63.25%). As the column temperature increases, the relative concentration decreases (Table 2).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=88.66%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=75.00%). As the column temperature increases, the relative concentration increases and then decreases.

Based on the obtained results (Table 2), it can be seen that the highest relative concentration of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 45 °C (RC=88.66%). One-factor analysis of variance examined the effect of column temperature at a constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, glutenin protein separation time and ab-

sorbance measurement) on relative concentration of the LMW glutenin fractions. It was found that there is a statistically significant difference in the relative concentrations of LMW glutenin fractions.

Table 3. shows descriptive indicators of the number of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C), aborbance measured at 280 nm.

Table 3. Descriptive indicators for LMW glutenin proteins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 280 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	15.00	1.26	0.52	13,.67	16.33	14	17
	45	6	16.33	1.03	0.42	15.25	17.42	15	18
	50	6	12.17	0.75	0.31	11.38	12.96	11	13
50% (v/v) 1-propanol	40	6	17.33	0.82	0.33	16.48	18.19	16	18
	45	6	14.00	0.63	0.26	13.34	14.66	13	15
	50	6	15.50	1.52	0.62	13.91	17.09	13	17
50% (v/v) isopropanol	40	6	19.83	0.41	0.17	19.40	20.26	19	20
	45	6	16.67	1.37	0.56	15.23	18.10	14	18
	50	6	24.17	0.75	0.31	23.38	24.96	23	25
ANOVA (LMW), 50% (v/v) ethanol, F=(2.15)=25.21, Sig.=0.000, eta square=54.33/70.50=0.77									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=14.90, Sig.=0.000, eta square=33.44/50.28=0.66									
ANOVA (LMW), 50% (v/v) isopropanol, F(2.15)=98.14, Sig.=0.000, eta square=170.11/183.11=0.93									

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=16.33). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=12.17). As the temperature of the column increases, the number of proteins increases and then decreases (Table 3).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=17.33). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 45 °C (Xav=14). As the temperature of the column increases, the number of proteins decreases and then increases (Table 3).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 50 °C (Xav=24.17). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 45 °C (Xav=16.67). As the temperature of the column increases, the number of proteins decreases and then increases (Table 3).

Based on the obtained results (Table 3), it can be seen that the highest number of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 50 °C (Xav=20.17), at wavelength of 280 nm. One-factor analysis of variance examined the effect of column temperature at constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%, separation time glutenin protein and absorbance measurement) to the number of proteins within the LMW glutenin fraction. It was found that there is a statistically significant difference in the number of proteins.

Table 4. shows descriptive indicators of the relative concentration of LMW glutenins, after extraction with different solvents (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, at a different column temperatures (40, 45 and 50 °C).

Table 4. Descriptive indicators of the relative concentration of LMW glutenins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and a column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 280 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	64.29	1.21	0.49	63.02	65.56	62.88	66.35
	45	6	60.89	2.59	1.06	58.17	63.60	56.86	64.18
	50	6	56.45	2.56	1.05	53.76	59.14	53.48	61.14
50% (v/v) 1-propanol	40	6	37.75	3.32	1.36	34.26	41.24	34.23	43.62
	45	6	62.09	2.88	1.17	59.07	65.11	56.86	64.32
	50	6	52.56	2.82	1.15	49.61	55.52	48.48	55.14
50% (v/v) isopropanol	40	6	59.81	2.20	0.90	57.50	62.12	56.47	62.96
	45	6	69.86	4.24	1.73	65.41	74.30	63.76	75.30
	50	6	56.47	3.66	1.50	52.63	60.32	52.43	63.12
ANOVA (LMW), 50% (v/v) ethanol, F=(2.15)=18.86, Sig.=0.000, eta square=185.31/258.99=0.71									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=99.21, Sig.=0.000, eta square=1804.99/1941.45=0.93									
ANOVA (LMW), 50% (v/v) isopropanol, F(2.15)=24.12, Sig.=0.000, eta square=582.32/763.38=0.76									

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=64.29%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=56.45%). As the column temperature increases, the relative concentration of LMW glutenin decreases (Table 4).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/ l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=62.09%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 40 °C (Xav=37.75%). As the column temperature increases, the relative concentration of LMW glutenin increases and then decreases (Table 4).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=69.86%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=56.47%). As the column temperature increases, the relative concentration of LMW glutenin increases and then decreases (Table 4).

Based on the obtained results (Table 4), it can be seen that the highest relative concentrations of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 45 °C (RC=69.86%), at wavelength of 280 nm. One-factor analysis of variance examined the effect of column temperature at constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%, separation time glutenin protein and absorbance measurement) to the relatice concentrations of LMW glutenin fractions. It was found that there is a statistically significant difference in the number of proteins.

Based on the obtained results (Table 1 and Table 3), it can be seen that a higher number of proteins was obtained by separation at a wavelength of 280 nm (Xav=24.17), than at a wavelength of 210 nm (Xav=20), but the separation at 210 nm is better, peaks are sharper and it is specific for peptide bond.

Wieser (2000) determined gluten proteins qualitatively and quantitatively in the framework of comparative studies of different wheat varieties. Glutenins were extracted with 50% (v/v) 1-propanol and dithioerythritol (DTE). The detection wavelength was 210 nm. Based on the obtained results, the retention time of LMW glutenin was > 27.

Horvat et al. (2006) analyzed gluten proteins in wheat by RP-HPLC chromatography. Different wheat varieties were used for protein extraction. The column temperature was 50°C and the detection wavelength was 210 nm. Based on the obtained results, retention time for LMW glutenins was 15-30 minutes (16-19 subunits).

Qian et al. (2008) characterized wheat gluten protein by high-pressure liquid chromatography (HPLC) and MALDI TOF mass spectrometry. The column temperature was 60 °C and the detection wavelength was 210 nm. Based on the obtained results, LMW glutenins identified 22 peaks.

Scherf (2016) investigated how the method of preparation of gliadin, glutenin and gluten from wheat starch samples affects their content when determining by high-pressure reverse phase liquid chromatography (RP-HPLC) and enzyme-linked immunosorbent assay (ELISA). Glutenins were extracted with 50% (v/v) 1-propanol containing Tris-HCl (pH=7.5), 0.06 mol/l (v/v) dithioerythritol and 2 mol/l urea. Samples were analyzed by high pressure reverse phase liquid chromatography (RP-HPLC), at a column temperature of 60 °C and at a wavelength of 210 nm. Separation of glutenin proteins was performed for 22 minutes.

Figure 2. shows selected proteins of LMW glutenins. They extracted with 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) and separated at a column Zorbax 300 SB-C3, Agilent, dimensions 4.6 x 150 mm, particle size 5µm, at a column temperature of 40 ºC and a pressure of 80 bar. Glutenin protein separation time was 21.0 min and at a wavelength of 210 nm.

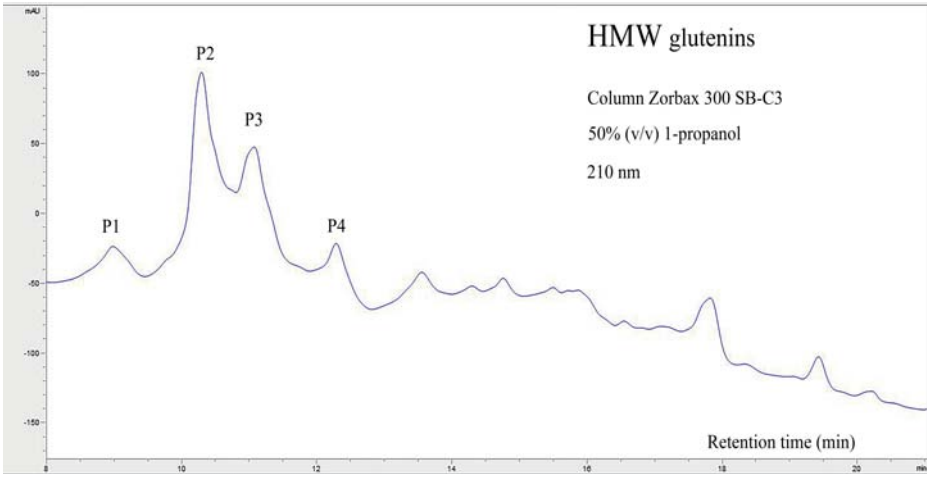


Figure 2. Chromatogram of selected proteins of LMW glutenins

Table 5. shows average values of retention time and relative concentration of selected proteins of LMW glutenins.

Table 5. Average values of retention time and relative concentration of selected LMW glutenins

Proteins	Retention time ± SD	Relative concentration ± SD
P1	8.94 ± 0.10	20.94 ± 0.95
P2	10.29 ± 0.15	26.01 ± 1.20
P3	11.08 ± 0.12	6.84 ± 0.55
P4	12.28 ± 0.08	4.25 ± 0.61

Based on the obtained results it can be seen that repeatability of these proteins is good, the standard deviations is small. Six repetitions were done.

In this work, the influence of solvent type was investigated (50% v/v ethanol, 1-propanol and isopropanol) to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and column temperature on the separation efficiency and identification of LMW glutenin, in two wavelengths. Separation was performed on column C3. The most effective solvent was 50% (v/v) 1-propanol and a column temperature of 40 °C (Xav=20). The wavelength of 210 nm is more specific because it indicates the presence of a peptide bond. The results obtained in this paper are in agreement with the authors Horvat et al. (2006), Qian et al. (2008) and Scherf (2016).

CONCLUSION

After the LMW glutenins were chromatographically separated on an HPLC chromatograph, and after extraction with different solvents and at different column temperatures, the following conclusions were reached.

After extraction of LMW glutenin with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added, at different column temperatures (40, 45 and 50 °C) and by measuring the absorbance at 210 nm, the highest number of proteins was obtained by extraction of 50% (v/v) with 1-propanol and at a column temperature of 40 °C (Xav=20, respectively RC=69.56%), and the lowest number at a column temperature of 45 °C (Xav=14.17, respec-

tively RC=66.42%). When measuring the absorbance at 280 nm, and by extraction and separation under the same conditions as previously mentioned, the highest number of proteins was obtained by extraction of 50% (v/v) isopropanol and at a column temperature of 50 °C (Xav=24.17, respectively RC=56.47%), and the lowest number by extraction with 50% (v/v) ethanol and at a column temperature of 50 °C (Xav=12.17, respectively RC=56.45%).

ACKNOWLEDGMENTS

This work is a part of the research project Analysis gluten proteins holders of allergic reactions by high performance liquid chromatography, number 19/6-020/96123/18, financed by the Ministry of Science and Technology, Higher Education and Information Society Republic of Srpska.

REFERENCES

Beom, H.R., Kim, J.S., Jang, Y-R., Lim, S.H., Kim, C.K., Lee, C.K., Lee, J.Y. (2018). Proteomic analysis of low-molecular-weight glutenin subunits and relationship with their genes in a common wheat variety. 3 Biotech 8(1), 56, doi: 10.1007/s13205-017-1081-2.

Bonilla, J.C., Erturk, M.Y., Schaber, J.A, Kokini, J.L. (2020). Distribution and function of LMW glutenins, HMW glutenins, and gliadins in wheat doughs analyzed with '*in situ*' detection and quantitative imaging techniques. Journal of Cereal Science 93, https://doi.org/10.1016/j.jcs.2020.102931.

Delcour, J.A., Joye, I.J., Pareyt, B., Wilderjans, E., Brijs, K., Lagrain, B. (2012). Wheat gluten functionality as a quality determinant in cereal-based food products. Annual Review of Food Science and Technology, 3, 469–492.

Gao, Y., Janes, M., Chaiya, B., Brennan, M., Brennan, C., Prinyawiwatkul, W. (2017). Gluten-free bakery and pasta products: prevalence and quality improvement. International Journal of Food Science and Technology, https://doi.org/10.1111/ijfs.13505.

Gojković V., Marjanović-Balaban Ž., Grujić R., Vujadinović D., Vukić M. (2019). The influence of extraction conditions and chromatographic separation on the ability of identifying gliadins from wheat flour. Journal of Hygienic Engineering and Design 26, 118-126.

Grosch, W., Wieser, H. (1999). Redox reactions in wheat dough as affected by ascorbic acid. Journal of Cereal Science, 29(1), 1–16.

Horvat, D., Drezner, G., Šimić, G., Dvojković, K. (2006). Rezervne bjelančevine pšenice analizirane RP-HPLC metodom. Poljoprivreda, 12(2), 42-47.

Huang, L., He, Y., Jin, Y., Wang, F., He, J. (2018). Characterization of novel LMW glutenin subunit genes at the Glu-M3 locus from Aegilops comosa. 3 Biotech, 8(9), 379, doi: 10.1007/s13205-018-1407-8.

Scherf, K.A. (2016). Impact of the preparation procedure on gliadin, glutenin and gluten contents of wheat starches determined by RP-HPLC and ELISA. European Food Research and Technology, doi 10.1007/s00217-016-2683-3.

Urade, R., Sato, N., Sugiyama, M. (2018). Gliadins from wheat grain: an overview, from primary structure to nanostructures of aggregates. Biophysical Reviews, 10(2), 435–443.

Qian, Y., Preston, K., Krokhin, O., Mellish, J., Ens, W. (2008). Characterization of Wheat Gluten Proteins by HPLC and MALDI TOF Mass Spectrometry. Journal of the American Society for Mass Spectrometry, 19, 1542–1550.

Wang, D., Li, F., Cao, Shuanghe, Zhang, K. (2020). Genomic and functional genomics analyses of gluten proteins and prospect for simultaneous improvement of end-use and health-related traits in wheat. Theoretical and Applied Genetics, 133, 1521-1539.

Wieser H., Antes S., Seilmeier W. (1998). Quantitative Determination of Gluten Protein Types in Wheat Flour by Reversed-Phase High-Performance Liquid Chromatography. Cereal Chemistry 75(5), 644-650.

Wieser, H. (2000). Comparative investigations of gluten proteins from different wheat species I. Qualitative and quantitative composition of gluten protein types. European Food Research and Technology, 211(4), 262–268.

Wieser, H., Kieffer, R. (2001). Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. Journal of Cereal Science, 34(1), 19–27.

Wieser, H. (2003). The use of redox agents. In: Cauvain, S.P. (Ed.), Bread Making - Improving Quality, Woodhead Publishing Ltd., Cambridge, 424–446.

Wieser, H., Bushuk, W., MacRitchie, F. (2006). The polymeric glutenins. In: Wrigley, C., Bekes, F., Bushuk, W. (Eds.), Gliadin and Glutenin: the Unique Balance of Wheat Quality, St. Paul American Association of Cereal Chemistry, 213–240.

Wieser, H. (2007). Chemistry of gluten proteins. Food Microbiology, 24(2), 115-119.

Recived: July 18, 2020
Accepted: July 29, 2020

DOI: 10.7251/QOL2003094M **UDC:** 615.065:[615.012:66.013.8
Professional Paper

Importance of Public Health Control of Metals as Chemical Risks in Dietary Supplements

Marija Milosavljević[1], Ljiljana Stojanović Bjelić[1], Vesna Petković[2], Mirjana Đermanović[2], Marijana Vicanović[1], Borka Kotur[2]
[1]*Pan-Europan University APEIRON, Banjaluka, Faculty of Health Sciences, kozmomarija@gmail.com*
[2]*PHI Public Health Institute, Republic of Srpska, Banja Luka*

Abstract: Popularity and use of dietary supplements are constantly growing. Dietary supplements are food products intended to supplement the usual diet and are concentrated source of nutrients or other substances with nutritional or physiological effecst. The purpose of the Paper is to determine frequency of presence of cadmium, lead and mercury metals in dietary supplements based on protein and amino acids that were analyzed during 2018 and 2019 at the Public Health Institute of Republic of Srpska in Banja Luka. Content of metal was determined by the Atomic Absorption Spectrophotometry method. No health defective samples were identified by public health control, but due to modern frequent use of dietary supplements in various population groups (children, adolescents, pregnant women, athletes, etc.), the aim of the Paper is to raise people's awareness of the risks, such as heavy metals and artificial sweeteners, colors, prohormones and other chemical risks from dietary supplements since they may be associated with chronic health risks.

Keywords: public health control, chemical risks, dietary supplements, health risks.

INTRODUCTION

Dietary supplements are food products intended to supplement the usual diet and are concentrated source of nutrients (vitamins and minerals) or other substances with nutritional or physiological effects, individually or in combination - amino acids, essential fatty acids, fibers, microorganisms, edible fungi, algae, bees products, raw materials of plant origin - bioflavonoids, carotenoids, isoflavones, glucosinolates and the like. (Food Additive Regulations, 2018). Dietary supplements are marketed in a dosage form such as capsules, lozenges, tablets, pills, ampoules with liquid, dropper vials and other similar forms for use in metered small quantities.

The beginning of dietary supplements use is associated with Japan, when, in the eighties of the 20th century, began the use of food products for special nutritional purposes (Eng. Foods for special dietary uses - FOSHU). By using dietary supplements, consumers want to improve their overall health, "neutralize" the impact of malnutrition, or delay the onset of disease. Expertly justified reasons for use of dietary supplements are prevention of nutritional deficits in age-specific population groups, persons with poor absorption syndrome, hepato-biliary and metabolic disorders, after surgical interventions in the gastrointestinal tract and long-term medicamentous therapy with corticosteroids and other medications (Torović et al., 2014).

Food is harmful to human health if it contains chemical risks above the maximum approved levels (Food Law, 2008; Regulation on maximum levels for certain contaminants in food, 2012), and presence of metals can occur as by-product of operations during production, processing, transport or storing food.

The goals of the paper are:

1. Determine the incidence of cadmium, lead and mercury in dietary supplements in Republika Srpska analyzed during 2018 and 2019;

2. Indicate the public health importance of the presence of heavy metals in dietary supplementation patterns due to possible health consequences;

3. Point out the need for monitoring and other chemical risks potentially present in dietary supplements, ie artificial sweeteners, colors and prohormones as health risks.

Lead, cadmium and mercury represent persistent chemical risks to human health due to acute toxic and chronic cumulative effects on human body (Enviromnental Protection Agency US, 2005). Mercury shows teratogenic, spermiotoxic and neurotoxic effects. Cadmium, due to its incorporation into bone instead of calcium, leads to osteomalacia and bone fractures, and, by affecting zinc metabolism, it represents risk for heart disease (Norberg et al., 2007). The International Agency for Research on Cancer (IARC) classifies lead in Group 2B of human carcinogens and cadmium in Group 1 in human carcinogens because there is sufficient evidence of carcinogenic effect on humans (WHO/IARC, 2006).

MATERIAL AND METHODS

The study was conducted as retrospective study on 90 samples of dietary supplements based on protein and amino acids submitted for registration to the Public Health Institute in Republic of Srpska in 2018 and 2019. The metal content was determined by the Atomic Absorption Spectrophotometry method (Atomic Absorption Spectrophotometry; AAS) on the "UNICAM" England device, a flame technique for lead and cadmium analysis. The mercury content of the samples was determined by method of amalgamation by atomic absorption spectrophotometry using direct mercury analyzer (DMA-80). Elemental mercury analyzers, also known as automatic and direct analyzers, with atomic absorption and atomic fluorescence detection methods are very sensitive and are designed for direct mercury detection in solid and liquid samples without previous sample preparation. Working principle of the instrument is based on thermal degradation, catalytic conversion, amalgamation and atomic absorption spectrophotometry. Descriptive statistics indicators (number of samples, minimum and maximum concentrations) were used for lead, cadmium and mercury content. Statistical analysis of mercury content of dietary supplementation samples (arithmetic mean, standard deviation) was performed. Determination of health safety of the samples was carried out in accordance with food safety regulations that wre in force during the study period (Food Law, 2008).

RESULTS AND DISCUSSION

Rezults regarding number of dietary supplements samples analyzed for metals in 2018 and 2019 are shown in the Table 1.

Table 1: Number of dietary supplements samples analyzed for metals in 2018 and 2019

Year	2018	2019	TOTAL
Total	62	28	90
Contaminated	0	0	0

Table 2 shows data on types of metals that were analyzed in dietary supplements (lead, cadmium and mercury) in 2018 and 2019, as well as their minimum and maximum concentrations. In all dietary supplements samples, lead concentrations were found to be <0.01mg/kg and cadmium <0.03mg/kg. Mercury concentrations ranged from 0.008- <0.10 mg/kg (Table 2). The reference allowed values for lead content in dietary supplements are ≤ 3.0mg/kg, for cadmium ≤ 1.0mg/kg and for mercury ≤ 0.10mg/kg.

Table 2: Types and concentrations of analyzed metals in dietary supplements in 2018 and 2019

Type of metal	No. of Analyzed samples	Unit measure	Minimum concentration	Maximum concentration
Lead	90	mg/kg	< 0.01	< 0.01
Cadmium	90	mg/kg	< 0.03	< 0.03
Mercury	90	mg/kg	0.008	< 0.10

The results of the study of lead, cadmium and mercury at the Institute of Public Health in 2018 and 2019 indicate that no health defective food samples were determined for the tested characteristics - lead, cadmium and mercury, that is, all determined concentrations of metals were below the legally prescribed maximum allowed concentration (MDC). The lead and cadmium concentrations found in all dietary supplement samples were below the detection limit. A statistical analysis of the mercury content is presented in a table in dietary supplementation samples, ie concentration range, arithmetic mean and standard deviation(Table 3).

Table 3: Mercury concentrations in protein and amino acid based dietary supplementation samples- sports supplements

Type of metal	N	Unit measure	Range (a)	Mean (b)	SD (c)
Mercury	90	mg/kg	0.008 - 0.010	0.0095	0.0007

N –Number of samples
(a) Range concentration
(b) Arithmetic mean
(c) Standard deviation

The mercury content (as well as lead and cadmium) were analyzed in protein and amino acid based dietary supplements, which were submitted for registration to the Institute of Public Health of Republika Srpska during 2018 and 2019, and due to the representativeness of the sample (N = 90 samples). this type of dietary supplement.

The mercury study reduced the number of samples compared to the study presented, in West Africa (Ghana), consensus results were obtained because no health-safe dietary supplementation patterns were identified. All established concentrations of mercury in ten omega-3 acid supplement products from different manufacturers were within acceptable limits. There are frequent use of omega-3 acid supplements in cardiovascular patients and pregnant women as these supplements are recommended as a measure to prevent cardiovascular disease and enhance intrauterine fetal development (Akwasi et al, 2014).

Consistent results as in the present study were confirmed by studying of small number of dietary supplements samples (n = 10) in BiH (Bosnia and Herzegovina) during 2019 and no health defective food samples were identified (Food Safety Agency, 2019).

The authors (Costa et al, 2019) investigate metals but also other contaminants (toxins, pesticides, dioxins and PCBs) as chemical risks in dietary supplements, highlighting the frequent preventive use of dietary supplements as a strategy to combat oxidative stress and aging, but despite the popularity of dietary supplements, there are concerns about their safety. The variety of nutritional supplements and the presence of potential metal contaminants (lead, mercury, arsenic and cadmium), each of which has a variety of toxic effects, undoubtedly adds to the complexity of the safety issues to be considered given the additional harmful chemicals from other products and their possible toxicological interactions with toxic metals. Although

the presence of a contaminant does not necessarily mean that their concentrations exceed the maximum allowable concentrations or that the intake of dietary supplements poses a risk to human health, it does warn of the need to continue monitoring the safety of dietary supplements.

There are studies where health defective dietary supplements samples were identified when analyzed for metals. The content of arsenic, cadmium, mercury and lead was analyzed in 95 dietary supplements and the measured concentrations of arsenic and cadmium were below the permitted limits, with only one dietary supplement having unacceptable mercury values. Nonetheless, the authors argued that this concern could be reduced in some way if part of the total determined concentration of mercury corresponds to the inorganic form of mercury rather than methyl mercury (Dolan et al. 2003).

The dietary supplements market in Poland is growing rapidly and number of registered products and their consumption is constantly increasing. Among the most popular supplements and those that are easily available are herbal supplements, available at any supermarket. During the study, 24 dietary supplements available on the Polish market and containing one or more herbal ingredients were tested. The mercury content in concentration higher than allowed was found in preparations - the bamboo shoots and in algae Chlorella pyrenoidosa. The studies have shown that mercury is present in every herbal supplement tested, and its content in two preparations (with bamboo and algae) exceeds the allowed limit of 0.10 mg/kg. Statistically significant differences were found in occurrence of mercury depending on plant ingredient in the supplement. The lowest content was found in preparation with Tanacetum parthenia, and the highest in bamboo shoots. The mercury content of the tested herbal supplements was statistically significant in the form of supplements - tablets and capsules. Calculation of daily, weekly, monthly and annual consumption of mercury with the tested dietary supplements was performed - results did not exceed PTWI - temporary intolerable weekly mercury intake (Brodziak-Dopierała et al., 2018).

Study in Lebanon shows that consumption of dietary supplements is widespread and on the rise. Dietary supplements are generally used without prescription, proper counseling or awareness of their health risk. The study aimed to analyze metals in 33 samples of imported dietary supplements, which were frequently consumed by the Lebanese population, to ensure safety and increase citizens' awareness of dietary supplements. It was found that all dietary supplements contained mercury and lead concentrations below the allowed limits, as well as daily exposure, while 30% of the analyzed samples had cadmium levels above the allowed limits but they statistically correlated with essential minerals calcium and zinc. For dietary supplements consumed as basic nutrients, it has been emphasized that their calcium, zinc, iron and manganese content should be monitored for the level of toxic metals and their natural geochemical association with these parent metals to ensure safe allowed levels for consumers (Korfali et al ., 2013).

Public health control of harmful substances in dietary supplements is important for consumer safety given the increasing frequency of use of dietary supplements in all population groups, especially specific age groups such as infants, young children, pregnant and lactating women, menopausal women and elderly persons. Many studies show the frequent use of dietary supplements in various countries.

In the United States, a study conducted by the FDA (Food and drug administration) in 2002 found that as many as 73% of the population used dietary supplements and considered them natural and safe to use (Timbo et al., 2006). According to the study conducted by the NHANES (National Health and Nutrition Examination Survey) in 1999-2002 period, over 60% of the population with coronary artery disease, hypertension, or hypercholesterolemia take one or more dietary supplements (Buettner et al., 2007). The study on use of non-vitamin /non-mineral dietary supplements such as amino acids, herbs and herbal products, conducted in early 2000, shows that 6.0% of respondents use these dietary supplements every day (Millen et al., 2004).

Study of the Canadian Institute has shown that more than 50% of people combine the use of at least one dietary supplement with medications they use, where an interaction was noticed in 28% of people, one-third of whom had moderate or severe clinical consequences (Singh and Levine, 2004).

Risk control in dietary supplements is important especially in sensitive population groups such as children, as exposure of children to chemical risks in food can have adverse effects on children's health (WHO, 2009).

Due to the use of additives in the production of dietary supplements, colors and artificial sweeteners (acesulfame K, Na-saccharin, aspartame and Na-cyclamate) as chemical risks, there is possibility of chronic health risks (EFSA, 2006).

The widespread use of dietary supplements also points to other types of chemical and biological risks. The European Food Safety Authority (EFSA) has been asked for scientific advice on safety of hydroxanthracene derivatives and on daily intake that does not raise concerns about adverse health effects. Based on currently available data, the EFSA commission concluded that hydroxanthracene derivatives such as emodin, aloe-emodin and structurally related substance danthron showed in vitro genotoxicity. Aloe extracts have also shown to be genotoxic in vitro, possibly due to the presence of hydroxanthracene derivatives in the extract, as well as that aloe-emodin is genotoxic in vivo and that aloe extract and structural analog danthron are carcinogenic. Epidemiological data suggest an increased risk of colorectal cancer associated with general use of laxatives, some of which contain hydroxanthracene derivatives. Considering possible presence of aloe-emodin and emodin in extracts, the commission concluded that there were concerns about extracts containing hydroxanthracene derivatives that could be considered genotoxic and carcinogenic unless the opposite specific data existed. The Commission has been unable to advise on daily intake of hydroxanthracene derivatives that would not raise concerns about adverse health effects (EFSA, 2018).

EFSA was asked to provide scientific advice on safety of green tea catechins from food prodcts sources, including dietary supplements. Most polyphenols in green tea are made up of catechins. The EFSA Commission considered the possible link between (-) - epigallocatechin-3-gallate (EGCG) consumption, the most relevant catechin in green tea, and hepatotoxicity. The Commission concluded that catechins from green tea infusions prepared in traditional way and reconstituted beverages of the same composition as traditional green tea infusions, are generally considered safe under presumption of safety approach, provided that intake corresponds to the reported intakes in the European member states. However, rare cases of the liver damage have been reported after consumption of green tea infusion, most likely due to an idiosyncratic reaction. On the basis of available data on potential adverse effects of catechins from green tea to the liver, the EFSA Commission concluded that there was evidence from clinical trials showing that intake of doses equal to or greater than 800 mg EGCG daily from dietary supplements induces statistically significant increase of serum transaminase in treated persons in comparison with control group (EFSA, 2018).

Lately, concerns have been growing regarding the use of dietary supplements, especially when used for treating certain disease. There is increasing number of reported side effects and interactions that are associated with them. Unfortunately, credibility and amount of clinical evidence continues to vary, and these data are largely based on reported side effects during administration of these preparations. One extreme example of side effect is the risk of heart attack, arrhythmia, and even death associated with use of dietary supplements containing ephedra, which led to the fact that those products lost the license in the United States territory in 2004 (Rogers et al ., 2001).

EFSA delivers scientific opinion on assessing safety of using the ephedra plant and its preparations when used in dietary supplements. The ephedra plant contains biological alkaloids: ephedrine, pseu-

doephedrine, norephedrine, norpseudoephedrine (cathine), methylphedrine and methylpseudoephedrine. These alkaloids have a sympathomimetic effect, and some of them are also used as active ingredients in medicinal products. Use of ephedra plant in dietary supplements is banned in several European countries; however, supplements containing ephedra plant in combination with caffeine are marketed online. There are large differences in concentration of individual ephedra alkaloids in different dietary supplements containing ephedra. Due to the lack of adequate data on genotoxicity and reproductive toxicity, the EFSA Commission did not advise on daily intake of ephedra and its preparations that would not cause concern for adverse health effects. Consumption of ephedra dietary supplements may result in exposure to total ephedra alkaloids or ephedrine, which may exceed therapeutic doses for individual ephedra alkaloids or ephedrine in medications. Such exposure can lead to serious adverse effects that can be enhanced when combined with caffeine. The EFSA Commission concluded that the ephedra plant and its alkaloids used as dietary supplements represent significant concern for human safety at the level of estimated use (EFSA, 2013).

The five-year study conducted by the "National Institute of Health" (NIH) in the USA, which included 295 344 men who did not suffer from cancer, examined association of prostate cancer with use of multivitamines as dietary supplements. These studies have shown higher incidence of moderate and severe prostate cancer forms in men who used the multivitamins seven or more times per week compared to men who did not take the multivitamins (Wood et al., 2003). In addition to this case, harmfulness of excessive intake of dietary supplements was also determined on an example of use of antioxidants for primary and secondary protection of the body against disease. Studies have shown that persons who have been taking beta-carotene, vitamin C, vitamin A, vitamin E, and selenium in excess quantities increased their mortality rate (by various factors which led to such outcome) compared with persons who did not take dietary supplements with antioxidant (Lanski et al., 2003).

Dietary supplements may contain substances that are not indicated on a declaration and may be due to expiration of the preparation or subsequent contamination of the product. According to the Food Law, declaration is segment of product quality and an inadequately declared product is food of inadequate quality.

Frequent use of dietary supplements in adolescents and athletes draws additional attention. The International Olympic Committee conducted a study which showed that 14.6% of dietary supplements intended for athletes contain prehormones which are not listed on a declaration (Geyer et al., 2006). The tennis player Guillermo Coria filed a lawsuit in 2003 against a company that produced dietary supplements, claiming that the multivitamin he used contained substances that led to his doping test being positive (Johnson, 2007). Two cases of severe liver damage in 2007 were reported by patients using dietary supplements containing anabolic androgenic steroids as adulterant (Kafrouni et al., 2007). Also in the USA, Joel Romero, a mixed martial arts fighter, filed a lawsuit in 2016 against the "Gold Star Performance Products", which produced dietary supplements, claiming that he had failed the doping test because the dietary supplements he used contained ibutamoren. Romero received monetary compensation in May 2019 as he won a lawsuit against the said company (Bohn, 2019).

Dietary supplements should be taken as recommended by a healthcare professional. Data from the survey conducted by the "Health and Diet Survey" in the United States in 2002 indicated that among dietary supplement users who reported an adverse effect, 33% of them were taking these preparations on their own instead of medical device that they were prescribed for treatment of relevant disease, where only 54% of respondents discussed possible replacement of therapy with physician or other healthcare professional (Timbo et al., 2006).

Use of dietary supplements is becoming more frequent in persons with no nutritional deficiency who use dietary supplements to maintain health. An additional problem is that in public, through the media,

large amount of information about dietary supplements is disseminated, and there are many unsubstantiated claims about their action. Dietary supplements, for the purpose of informing consumers, labeling, advertising and presenting, in addition to requirements of the dietary supplement rulebook, should also meet requirements in terms of providing information to consumers about food and regulations on nutrition and health claims (Nutrition and Health Claims Regulation, 2018).

The following are examples of health claims that appear on dietary supplement declaration and which are not in compliance with applicable regulations because they are not on the approved health claims list:

- *"During exercise, it replaces lost water, electrolytes, carbohydrates and vitamins-minerals, necessary for proper metabolism during intense or prolonged training."*
- *"L-carnitine stimulates and accelerates higher consumption of calories during exercise"*
- *"L-glutamine will ensure more effective and faster regeneration after exercise."*
- *"Isotonic drinks are much better for adding energy, water and electrolytes than other beverages, and in addition they contribute to better durability. The drink is intended for athletes and other persons for long and enduring trainings."*

In everyday laboratory practice, the original dietary supplement declaration in English language often contains health claims approved by the European Food Safety Authority (EFSA) which may accordingly be quoted on declaration in one of the languages which are in official use in RS.

CONCLUSION

- During 2018 and 2019, a total of 90 samples of dietary supplements submitted for registration to the Public Health Institute in the Republic of Srpska were analyzed for lead, cadmium and mercury presence.
- No health defective samples were identified during the study in terms of metal content, but early identification of chemical risks (metals, artificial sweeteners, colors, prohormones...) and other risks from dietary supplements has the public health significance for maintaining health of population considering the increasing frequency of use of dietary supplements in all population groups, especially specific age groups such as infants, young children, pregnant and lactating women, menopausal women and the elderly persons.
- Long-term exposure even to the allowed concentrations of chemical risks can lead to chronic health risks (allergic reactions, potentially toxic effects and other adverse effects).
- On the dietary supplement declaration, only approved health claims shall be stated.
- - In order to increase the consumer safety, it is necessary to conduct further public health studies on dietary supplements and exercise stricter dietary supplements control.

REFERENCES

Akwasi, A., Godfred, D., Joseph, A., Adolf, O-B. (2014). Assessment of Mercury Levels in

Omega-3 Food Supplements Available on the Ghanaian Market, Asian Journal of Applied Sciences, 2(5), 658-662.

Bohn, M. (2019). UFC's Yoel Romero awarded $27 million in tainted supplement lawsuit, MMA Junkie <https://www.usatoday.com/story/sports/mma/2019/05/29/ufcs-yoel-romero-wins-tainted- supplement-lawsuit-awarded-27-million-in-damages/39523171/ > Accessed 25.9.2019.

Brodziak-Dopierała, B., Fischer, A.,Szczelina, W., Stojko J. (2018). The Content of Mercury in Herbal Dietary Supplements. Biol Trace Elem Res, 185(1), 236–243.

Buettner, C., Phillips, RS., Davis, RB. (2007). Use of dietary supplements among US adults with coronary artery disease and atherosclerosis risk, Am J Cardiol, 99, 661-6.

Costa, J G., Vidovic, B., Saraiva, N., do Céu Costa, M., Del Favero, G., Marko, D., Oliviara G. N., Fernandes, A. S. (2019). Contaminants: a dark side of food supplements**?** FREE RADICAL RESEARCH, 53(51),1113-1135. < https://doi.org/10.1080/10715762.2019.1636045> Accessed 16.03.20.

Dolan, SP. Nortrup, DA., Bolger, PM., et al. (2003). Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry. J Agric Food Chem., 51(5),1307–1312.

Enviromnental Protection Agency US. (2005). Guidelines for carcinogen risk assessment, Risk Assessment. Washington, DC. <http://epa.gov/cancerguidelines/> Accessed 21.02.20. European Food Safety Authority. (2018). Safety of hydroxyanthracene derivatives for use in food <https://www.efsa.europa.eu/en/efsajournal/pub/5090 > Accessed 18.02.20. European Food Safety Authority. (2018.) Scientific opinion on the safety of green tea catechins <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5239 > Accessed 19.02.20.

European Food Safety Authority. (2013). Scientific Opinion on safety evaluation of Ephedra species for use in food, 2013) < https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3467> Accessed 19.02.20.

European Food Safety Authority. (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to a new long-term carcinogenicity study on aspartame <https://www.efsa.europa.eu/en/efsajournal/pub/356> Accessed 20.02.20.

Geyer, H., Parr, M., Mareck, U. (2006). Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids-results of an international study, Int J Sports Med, 6, 15.

International Agency for Research on Cancer, IARC/WHO (2006). Monographs on the evaluation of carcinogenic risks to humans, vol 87. Inorganic and Organic Lead Compaunds. Lyon, France.37.

Izvještaj o procjeni rizika iz oblasti sigurnosti hrane (Agencija za sigurnost hrane Bosne i Hercegovine, 2019).

Johnson, L. (2006). Tennis pro sues vitamin marker over suspension, lost endorsements, MSNBC.com< http://msnbc.msn.com/id*19194545/ > Accessed 18.06.07.

Kafrouni, MI., Anders, RA., Verma, S. (2007). Hepatotoxicity associated with dietary supplemets containing anabolic steroids, Clin Gastroenteroal Hepatol, 5, 809-12.

Korfali, I. S., Hawi, T., Mroueh, M. (2013). Evaluation of heavy metals content in dietary supplements in Lebanon. Chem Cent J, 7(10).

Lanski, SL., Greenwald, M., Perkins, A., Simon, HK (2003). Herbal therapy use in a pediatric emergency department population: expect the unexpected, Pediatrics, 111, 981-985.

Millen, AE., Dodd, KW., Subar, AF. (2004). Use of vitamin, mineral, nonvitamin and nonmineral supplements in United States: The 1987, 1992, 2000 National Health Interview Survey results, J Am Diet Assoc, 104, 447-54.

Nordberg, F., Fowler, A., Nordberg, M., Friberg, L. (2007). Handbook on the toxicology of metal, Salt Lake City (USA): Academic Press Inc.

Pravilnik o maksimalno dozvoljenim količinama za određene kontaminante u hrani (Službeni glasnik Bosne i Hercegovine broj 39/12).

Pravilnik o prehrambenim i zdravstvenim tvrdnjama (Službeni glasnik Republike Srpske broj 19/18).

Pravilnik o dodacima ishrani (Službeni glasnik Republike Srpske broj 10/18.).

Rogers, EA., Gough, JE., Brewer, KL. (2001). Are emergency department patients at risk for herb-drug interactions?, Acad Emerg Med, 8, 932-394.

Singh, SR., Levine, M. (2004). Natural health product use in Canada: patterns of use and the risk of interactions with pharmaceuticals. Clin Pharmacol Ther, Canada, 10, 81-86.

Timbo, BB., Ross, MP., McCarthy, PV., Lin, CT. (2006). Dietary supplements in a national survey: prevalence of use and reports of adverse events. J Am Diet Assoc, 106, 1966-74.

Torović, LJ., Budimka, N. (2014). Bromatologija, Novi Sad: Medicinski fakultet.

Zakon o hrani (Službeni glasnik Republike Srpske broj 49/08).

WHO's work on estimating disease burden from chemicals World Health Organisation.. Department of Public Health and Environment, 2011).

Wood, MJ., Stewart, RL., Merry, H., Johnstone, DE., Cox, JL. (2003). Use of complementary and alternative medical therapies in patients with cardiovascular disease, Am Heart J, 145, 806-812.

World Health Organization. (2009). Monitoring for chemicals in foods. <http://www.euro.who.int/en/what-we-do/health-topics/disease-prevention/food/safety/activities/monitoring-for-chemicals-in-foods > Accessed 20.02.20.

Recived: February 26, 2020
Accepted: June 1, 2020

DOI: 10.7251/QOL2003102K **UDC:** 615.012:66.013.8]:615.965

Professional Paper

Risk Assessment and Adoption of Foodstuffs Sampling Plans for Microbiological Safety Based on the Results of Multiyear Food Sampling

Marin Kvaternik, Milena Todorović
Public Health Institute of the Republic of Srpska – Regional Centre Doboj

Abstract: The goal of this paper is to point at the possibility of risk assessment and adoption of foodstuffs sampling plans for microbiological safety based on the results of multiyear food sampling. The results of microbiological food analysis performed in the Public Health Institute of the Republic of Srpska – Regional centre Doboj in the period 2015 – 2019 were used as a sample.

Keywords: foodstuffs, microbiological safety, risk assessment.

INTRODUCTION

In the daily diet, people use various types of food. Many of these foods are a suitable environment for the development and reproduction of microorganisms which, when introduced into the human body, can be the cause of many infectious diseases. Biological contamination of food most often occurs due to improper food storage. Saprophytic microorganisms do not pose a danger to human health, but by breaking down proteins, fats and carbohydrates, they reduce the nutritional value of food. Pathogenic microorganisms present in food are danger to human health.

The primary goal of food hygiene is to eliminate or reduce the risk of exposure to food borne diseases.[1] It is necessary to monitor the hygiene of the food production process, storage, transport, as well as food distribution, which can be achieved by applying the applicable legislation for microbiological cleanliness of the facility.[2] That is, in order to protect food from contamination, it is necessary to implement legal regulations in primary production, which relate to the prevention of infectious diseases transmitted by food, in secondary production and processing, to control hygienic conditions and temperature, in the transport phase to control packaging, means of transport and food storage. The importance of maintaining personal hygiene of employees working on food preparation (wearing jewellery, rings, and bracelets) was confirmed by research which proved the presence of enterobacteria and staphylococci, and even some *E.coli* colonies as indicators of faecal contamination.[3]

Food monitoring calls for microbiological analysis which show which microorganisms are present, i.e. whether food meets microbiological standards that each country adopts individually. Application of applicable legislation in food control should ensure food safety at the time of production and at the time of placing it at market during its declared shelf life. Microbiological analysis and interpretation of the obtained results in Bosnia and Herzegovina are performed according to the standards prescribed by the applicable legislation at the level of Bosnia and Herzegovina, i.e. its entities. It is important to monitor sampling results over several years, to make risk assessments based on them and to adopt a sampling plan or adjust the existing plan for the next period in order to achieve even greater reliability in the production process.[4]

PAPER GOALS

1. To point at the importance of risk assessment and adoption of food sampling plans for microbiological safety based on analysis of the results of multiyear food sampling.
2. To present the results of food analysis for microbiological safety performed at the Public Health Institute of the Republic of Srpska - Regional Centre Doboj in the period 2015-2019.

MATERIALS AND METHODS

The research was conducted as a retrospective study that included the results of food analysis for microbiological safety submitted to the Public Health Institute of the Republic of Srpska - Regional Centre Doboj in the period 2015-2019. The Central Protocol of the Laboratory of the Public Health Institute of the Republic of Srpska - Regional Centre Doboj was used as data source. The results of the analysis of food samples are presented by individual years in relation to 10 food groups: milk and dairy products, meat and meat products, eggs, biscuits and related products, vegetables and vegetable products, fruits and fruit products, grains and grain products, oil, margarine, mayonnaise, non-alcoholic drinks and beer. Determination of microbiological safety of food samples was performed in accordance with the relevant regulations in the field of food safety applicable during the research period (Rulebook on microbiological criteria for food - Official Gazette of RS 109/12, Rulebook on microbiological criteria for food of animal origin - Official Gazette of RS 69/19)). The results of the analysis are presented in the form of tables and graphs. Methods of descriptive (tables and graphs) and inferential (chi-square test) statistics were used in data processing.

RESULTS

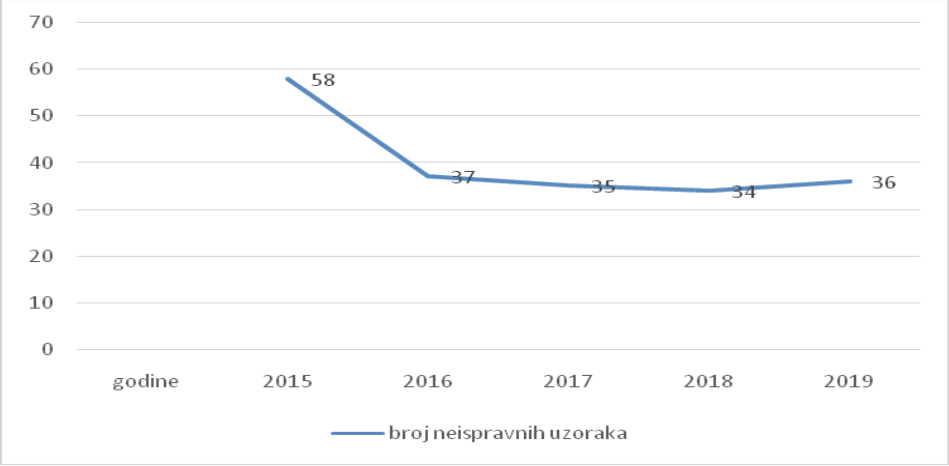
In the period 2015-2019, a total of 14,784 food samples were analysed for microbiological safety at the Public Health Institute of the Republic of Srpska - Regional Centre Doboj with 200 samples or 1.35% marked as contaminated. (Table 1). In relation to the origin of the analysed foods, the lowest percentage of contaminated samples was recorded for imported foods, at a level of high significance (χ^2=17.184; p=0.0017).

Table 1. Review of food analysis for microbiological safety in the period 2015- 2019

		Total	Imported	Off-the-shelf	From production
2015	Total	2645	733	497	1415
	Contaminated	58	2	23	33
	%	2.19	0.27	4.63	2.33
2016	Total	3155	446	469	2240
	Contaminated	37	1	6	30
	%	1.17	0.22	1.28	1.34
2017	Total	2987	270	594	2123
	Contaminated	35	1	9	25
	%	1.17	0.37	1.51	1.18
2018	Total	3062	302	655	2105
	Contaminated	34	0	3	31
	%	1.11	0	0,46	1.47
2019	Total	2935	260	668	2007
	Contaminated	36	0	1	25
	%	1.23	0	1.15	1.25

Total	Total	14.784	2.011	2.883	9.890
	Contaminated	200	4	42	144
	%	1.35	0.20	1.46	1.46

Table 1 shows the total number of analysis, and the number or percentage of contaminated foods by year. The highest level of irregular samples compared to other years was in 2015, and this difference was highly significant. (χ^2 =20.542; p=0.0003). Graph 1, in a more obvious way in the form of a curve, shows previous descriptive difference in terms of contaminated samples in the first year of the observed period compared to other years.



Graph 1. Graphic representation of the number of contaminated food samples by years

In the period 2015-2019 200 samples were found contaminated and most commonly only one cause of contamination was recorded per analysed food sample, more precisely 1.04 on average.

Table 2. Causes of microbiological contamination of analysed foodstuffs in the period 2015-2019

		Total of samples	1	2	3	4	5	6	7	8
2015	No	58	0	0	15	15	3	0	16	9
	%	2.19	0	0	25.86	25.86	5.17	0	27.59	15.52
2016	No	37	0	0	0	5	5	0	24	4
	%	1.17	0	0	0	13.51	13.51	0	64.86	10.81
2017	No	35	2	0	0	4	9	0	15	6
	%	1.17	5.71	0	0	11.42	25.71	0	42.85	17.14
2018	No	34	2	0	0	7	2	0	21	2
	%	1.11	5.88	0	0	20.59	5.88	0	61.76	5.88
2019	No	36	3	0	1	14	0	0	15	10
	%	1.23	8.33	0	2.78	38.89	0	0	41.67	27.78
TOTAL	No	200	7	0	16	45	19	0	91	31
	%	1.35	3.35	0.00	7.65	21.53	9.09	0.00	43.54	14.83

Legend: 1. *Staphylococcus 2.* Staphylococcal enterotoxin *3. Escherichia coli 4.* Yeasts and molds *5. Salmonella 6.Listeria monocitogenes 7. Enterobacteriaceae* 8. Total number of microorganisms

Three most common causes of contamination are *Enetrobacteriaceae* (43.54%), followed by the presence of yeasts and molds (21.53%), and an increased total number of bacteria (14.83%) (Table 2). No malformations for staphylococcus and listeria monocytogenes were found in any of the samples.

Table 3. Overview of the number of analysis and the share of contaminated findings in relation to foodstuffs groups in the period 2015-2019

Foodstuffs		2015	2016	1017	2018	2019	Total
Milk and dairy products	Number of analysis	156	229	204	188	128	905
	% of contaminated	10.90	10.04	6.86	10.64	6.25	9.06
Meat and meat products	Number of analysis	381	305	424	518	576	2204
	% of contaminated	5.77	1.96	1.89	0.39	0.00	1.72
Eggs	Number of analysis	11	15	14	15	13	68
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00
Biscuits and related products	Number of analysis	130	196	193	206	164	889
	% of contaminated	1.54	0.00	0.00	0.48	1.22	0.56
Vegetables and vegetable products	Number of analysis	22	41	41	40	29	173
	% of contaminated	0.00	0.00	2.44	5.00	0.00	2.31
Fruits and fruit products	Number of analysis	1	8	13	7	3	32
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00
Oils, margarine, mayonnaise	Number of analysis	25	35	36	38	38	172
	% of contaminated	4.00	0.00	0.00	0.00	2.63	1.16
Grains and products	Number of analysis	560	922	818	725	624	3.649
	% of contaminated	0.89	0.65	0.49	0.83	2.56	1.01
Refreshing non-alcoholic drinks	Number of analysis	170	123	101	83	99	576
	% of contaminated	1.76	0.00	4.95	0.00	1.01	1.56
Beer	Number of analysis	532	167	135	142	131	1107
	% of contaminated	0.00	0.00	0.00	0.00	0.00	0.00

Among food groups, microbiological contamination was most often recorded in milk and milk products, i.e. almost every tenth product (9.06%) was microbiologically contaminated (Table 3). During the analysis of eggs, fruits and fruit products, and beer, no positive case was recorded. The percentage of contaminated samples in other food groups ranged from 0.56% (biscuits and related products) to 2.31% (vegetables and vegetable products).

Table 4 Causes of microbiological contamination of analyzed foodstuffs for the period 2015-2019 in relation to food groups

Foodstuffs		1	2	3	4	5	6	7	8
Milk and dairy products	Number of contaminated samples	1	0	1	1	1	0	76	3
	% of contaminated	1.20	0	1.20	1.20	1.20	0	91.57	3.61
Meat and meat products	Number of contaminated samples	0	0	15	0	18	0	0	5
	% of contaminated	0.00	0.00	39.47	0.00	47.37	0.00	0.00	13.16
Biscuits and related products	Number of contaminated samples	1	0	0	2	0	0	2	0
	% of contaminated	20.00	0.00	0.00	40.00	0.00	0.00	40.00	0.00
Vegetables and vegetable products	Number of contaminated samples	0	0	0	2	0	0	0	1
	% of contaminated	0.00	0.00	0.00	66.67	0.00	0	0.00	33.33
Oils, margarine, mayonnaise	Number of contaminated samples	0	0	0	1	0	0	1	1
	% of contaminated	0.00	0.00	0.00	33.33	0.00	0.00	33.33	33.33
Grains and products	Number of contaminated samples	1	0	0	30	0	0	7	1
	% of contaminated	2.56	0.00	0.00	76.92	0.00	0.00	17.95	2.56
Refreshing non-alcoholic drinks	Number of contaminated samples	0	0	0	1	0	0	0	8
	% of contaminated	0.00	0.00	0.00	11.11	0.00	0.00	0.00	88.89
	Total % of contaminated samples	3	0	16	37	19	0	86	19

Legend: 1. Staphylococcus 2. Staphylococcal enterotoxin 3. Escherichia coli 4. Yeasts and molds 5. Salmonella 6.Listeria monocitogenes 7. Enterobacteriaceae 8. Total number of microorganisms

Table 4 shows the causes of microbiological contamination of the analysed foodstuffs by food groups. In the case of milk and dairy products, the total number of bacteria was stated as the cause of the contamination in as much as 91.57%. In meat and meat products, the most common causes of contamination were *Salmonella* (47.37%) and *Escherichia colli* (39.47%), while in grains and products, slightly more than ¾ of contaminated samples (76.02%) were yeasts and molds.

DISCUSSION

As there is no so-called concept of zero risk of contaminants in food, it is a tendency in all developed countries around the world to apply modern scientific methods to identify certain risks and reduce them to a minimum.[2] one way is to analyze the results of multi-year sampling.

In the period of five years, 2015-2019, in the microbiological laboratory of the Regional Centre Doboj a total of 14,784 food samples were analysed for microbiological safety, where in 200 foods or 1.35% some kind of microbiological contamination was detected (Table 1). During the first year of the observed period lowest number of samples was analysed but it resulted in the highest number of contaminated samples. Data for the entire territory of the Republic of Srpska show a steady increase in the number of analysed samples, from 14,498 in 2015 to 17,228 in 2019. [5-9] The total number of samples for microbiologi-

cal safety was 82,661out of which 719 or 0.87% were contaminated, which is evidently a lower percentage of contaminated samples compared to the share of contaminated samples analysed in the Regional Centre Doboj in the same period. Such a 'low' percentage of contaminated samples in the Republic of Srpska was primarily conditioned by the participation of contaminated samples in 2016 (0.61%) and 2017 (0.59%). The causes of this phenomenon should be specifically examined. If we transfer the analysis to the entire territory of Bosnia and Herzegovina, then we notice almost twice as many samples in 2018 (66,035) compared to 2015 (34,469).[11] Percentage of contaminated samples in the period 2015-2018 in Bosnia and Herzegovina was 1.19%.

Highest level of contaminated samples was recorded in 2015. Similar results were obtained based on the collected data on the results of laboratory analysis of food samples for microbiological safety for the entire territory of Bosnia and Herzegovina, where the percentage of contaminated samples in 2015 was 1.50%, and in 2018 (for 2019 no results have been published yet) only 1.20%. The results for the entire territory of the Republic of Srpska show lower level of contaminated samples, with the lowest percentages recorded in the first three years of the observed period, and in 2018 and 2019 the share of contaminated samples was similar to those recorded in the Regional Centre Doboj, i.e. for the whole Bosnia and Herzegovina.

Differences in the share of contaminated food samples in relation to their origin (Table 1), with lowest level of contamination of imported samples compared to off-the-shelf and food from production, can be explained primarily by quality controls in countries of origin. The three most common causes of food contamination in the five-year period were *Enetrobacteriaceae* (43.54%), followed by the presence of yeasts and molds (21.53%), and an increased total number of bacteria (14.83%) (Table 2). If we add that *Escherichia coli* were also a very frequent cause of contamination, we can make a conclusion that causes of contamination were hygienic failures. It is these, the so-called hygiene indicators, especially in the case of their increased number, that should be taken as a warning about possible appearance of pathogens and the need to perform additional analysis to exclude pathogens. Right behind these causes of contamination stands *Salmonella*. Very similar causes of food contamination were recorded for the entire territory of Bosnia and Herzegovina: *Enterobacteriaceae, Escherichia coli*, aerobic mesophilic bacteria, yeasts and molds, *Salmonella*. [10 - 13] The three-year expert report of the Croatian Food Agency registered the presence of an increased number of aerobic mesophilic bacteria in most food categories, followed by enterobacteria, yeasts and molds. Reports from the European Food Safety Authority and Centres for Disease Control from different countries show some similarities, but also differences in terms of the most common food contaminants: *Campylobacter spp., Salmonella spp. (especially Salmonella enteritidis), Listeria monocytogenes, Escherichia coli, Yersinia enterocolitica*. [14,15] While *Listeria monocytogenes* is one of the commonest causes of contamination here, at the same time, for a five-year period, Doboj Regional Centre did not record this type of contamination in any foodstuffs. At the Republic of Srpska level only individual cases have been reported. [5 - 9]

Food of animal origin (meat, poultry, fish, eggs, milk) is most often contaminated, while food of plant origin is less suitable for the reproduction of microorganisms, and thus less dangerous to human health. So, milk and milk products proved to be the ' riskiest' food group followed by meat and meat products (Table 3). Of particular concern is the fact that almost every tenth sample of milk and milk products was contaminated, having 9 out of 10 causes of contamination related to an increased number of total bacteria. The most common causes of contamination in meat and meat products, *Salmonella and Escherichia coli*, indicate poor hygiene, as well as the risk of transmitting salmonella to humans, i.e. causing food poisoning. It is indicative that, for example only in 2019, two major epidemics of food poisoning were registered in the

area under the jurisdiction of the Doboj Regional Centre, where salmonella was undeniably confirmed by laboratory analysis as the causative agent, and the epidemiological survey among patients showed that the route of transmission was through mayonnaise. However, the mayonnaise samples from the places where the incriminated food was consumed were negative because they were probably 'prepared' for sampling in the meantime. In this, but also in similar cases in previous years, it was a 'homemade' mayonnaise which was not properly stored before use, primarily referring to the air temperature during summer months. This indicates the importance of preventive analysis of this type of food, i.e. raw materials (primarily eggs) from which these foods are obtained. However, the number of analysis shows a low level of eggs control, i.e. egg products, primarily mayonnaise. Dominant cause of contamination in the group of grains and grain products was in the form of yeasts and molds. By its activity mold creates compounds that can be useful, e.g. antibiotics, but also harmful, e.g. mycotoxins. And it is the molds in grains that create the fear of the appearance of mycotoxins, i.e. increased control of this group of foods for mycotoxins is required, whether they are imported or domestically produced..[16] An additional problem, a potential danger to human health, is the diet of domestic animals with foods in which mycotoxins are present, which may result in the presence of mycotoxins in milk and meat. In the whole of Bosnia and Herzegovina, meat and meat products, as well as grains, proved to be the riskiest food groups.

CONCLUSION

The lack of so-called zero risk of contaminants in foodstuffs imposes the need for constant monitoring of microbiological food safety. In the five-year period, 2015-2019, in the microbiological laboratory of the Regional Centre Doboj a total of 14,784 food samples were analysed for microbiological safety, where in 200 foods or 1.35% some kind of microbiological contamination was detected. Hygiene indicators, as the most common causes of contamination, indicate the need for higher hygiene control throughout the food production process, from raw materials to the final product, as well as the need to conduct additional analysis to exclude pathogens. Milk and milk products, and meat and meat products, have proven to be groups of foods whose consumption carries the highest risk of transmitting the infection through food. Given that mayonnaise has proven to be the most common way of transmitting infectious diseases through food in the area of jurisdiction of the Regional Centre Doboj in epidemic form, the control of this food, i.e. eggs as a basic raw material for its production, in addition to the already mentioned two groups of foods, should be one of the priorities in food control, especially during summer months. Complete absence of listeria monocytogenes and staphylococci in food indicates the need to additionally examine this and to conduct additional tests.

Based on the multi-year continuous monitoring of test results, it is possible to make a risk assessment and adopt plans for food sampling for microbiological safety. Accordingly, we propose that in Bosnia and Herzegovina, in addition to previous annual reports for local levels, entity levels, level of Bosnia and Herzegovina, which are often stereotypical, analysis of results of foodstuffs control, should be done for a period of several years e.g. three –year period. Application of the proposed method would improve food safety in Bosnia and Herzegovina.

REFERENCES:

[1] Jugović Z, Pecarski D, Jordović B, Bugunović M. Bezbednost hrane U: Zbornik radova Prve konferencije sa međunarodnim učešćem „Bezbednost hrane i zdravlje". Čačak, 2017. Udruženje Klaster komora za zaštitu životne sredine i održivi razvoj, 7-15

[2] Okpala COR, Ezeonu I. Food Hygiene/Microbiological Safety in the Typical Household Kitchen: Some Basic 'Must Knows' for the General Public, J Pure Appl Microbiol., 2019; 13(2): 697-713.

[3] Mengual LM, Gamez NM, Carcedo I, Lopez MA, Alava JI. Accessories of Food Handlers and Restaurant Staff as a Source for Food Contamination. J Food Microbiol Saf Hyg. 2016; 1(1):1-5.

[4] Kozačinski L, Petrić J, Hengl B, Gross Bošković A. Pregled dostupnih rezultata mikrobiološke pretrage hrane koju prouzrokuju subjekti u poslovanju hranom u okviru svojih planova samokontrole – stručno izvješće. Hrvatska agencija za hranu. Internet Dostupno na http://www.hah.hr (pristupljeno 01.07.2020.)

[5] Šiljak S, Štrkić D, Jandrić Lj, Bratić R, Danojević D, Grujić Rudić V, i dr. Zdravstveno stanje stanovništva Republike Srpske, 2015. Institut za javno zdravstvo Republike Srpske. Internet Dostupno na http://www.phi.rs.ba (pristupljeno 03.07.2020.)

[6] Šiljak S, Štrkić D, Janjić B, Jandrić LJ, Bratić R, Danojević D, i dr. Zdravstveno stanje stanovništva Republike Srpske, 2016. Institut za javno zdravstvo Republike Srpske. Internet Dostupno na http://www.phi.rs.ba (pristupljeno 03.07.2020.)

[7] Šiljak S, Štrkić D, Jandrić LJ, Bratić R, Danojević D, Petković V, i dr. Zdravstveno stanje stanovništva Republike Srpske, 2017. Institut za javno zdravstvo Republike Srpske. Internet Dostupno na http://www.phi.rs.ba (pristupljeno 04.07.2020.)

[8] Šiljak S, Štrkić D, Janjić B, Vranješ S, Rodić Vukmir N, Jandrić LJ i dr. Zdravstveno stanje stanovništva Republike Srpske, 2018. Institut za javno zdravstvo Republike Srpske. Internet Dostupno na http://www.phi.rs.ba (pristupljeno 04.07.2020.)

[9] Šiljak S, Štrkić D, Janjić B, Vranješ S, Rodić Vukmir N, Jandrić LJ i dr. Zdravstveno stanje stanovništva Republike Srpske, 2019. Institut za javno zdravstvo Republike Srpske. U pripremi za štampanje

[10] Izvještaj o stanju u oblasti sigurnosti hrane u Bosni i Hercegovini za 2018. godinu. Agencija za bezbjednost hrane Bosne i Hercegovine. Mostar, 2019. Internet Dostupno na http://www.fsa.gov.ba (pristupljeno 02.07.2020.)

[11] Izvještaj o stanju u oblasti sigurnosti hrane u Bosni i Hercegovini za 2015. godinu. Agencija za bezbjednost hrane Bosne i Hercegovine. Mostar, 2016. Internet Dostupno na http://www.fsa.gov.ba (pristupljeno 01.07.2020.)

[12] Izvještaj o stanju u oblasti sigurnosti hrane u Bosni i Hercegovini za 2016. godinu. Agencija za bezbjednost hrane Bosne i Hercegovine. Mostar, 2017. Internet Dostupno na http://www.fsa.gov.ba (pristupljeno 01.07.2020.)

[13] Izvještaj o stanju u oblasti sigurnosti hrane u Bosni i Hercegovini za 2017. godinu. Agencija za bezbjednost hrane Bosne i Hercegovine. Mostar, 2018. Internet Dostupno na http://www.fsa.gov.ba (pristupljeno 02.07.2020.)

[14] Kozak GK, J. Crichton, J Farber. Control of Pathogens at Retail. Chapter 2. In: Retail Food Safety. Jeffrey Farber, Jackie Crichton, O. Peter Snyder, Jr. (Eds). Springer Science+Business Media, LLC 2014. DOI 10.1007/978-1-4939-1550-7_2

[15] Anonimno. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2016. Scientific report of EFSA and ECDC. EFSA Journal 2017;15(12),5077. doi: 10.2903/j.efsa.2017.507

[16] Kvaternik M, Todorović M, Nikolić M, Protić M. **Aflatoxins as Food Contaminants.** Quality Of Life, 2019; vol 19:33-40

Recived: July 23, 2020
Accepted: July 30, 2020

DOI: 10.7251/QOL2003110L **UDC:** 663/664:[621.798.1:66.017/.018

Review Article

Phthalates in Food Packaging-Impact on Human Health

Mirjana Lovrenović[1], Nevena Mirjanić[2], Slaven Grbić[2]
[1]*Republic Administration for Inspection Activities*
[2]*Veterinary Institute - SLAVEN doo*

Abstract: Phthalates are esters of phthalic acid and aliphatic alcohols. It is widely used in everyday life, and can be used as a plasticizer, solvents and additives in many products, from food packaging to items of general use. Plastic materials are widely used in food industry and potentially can be source of phtalates in food. Phtalates can be present in food as a result of contamination of food. Phtalates in food, as a result of contamination of food or migration from packaging can jeopardiye human health. This work provides an overview of the presence of phthalates in food packaging, its migration into food, as well as the negative impact on human health by consuming and inhaling them.

Keywords: Phtalates, food, packaging, contamination, human health.

INTRODUCTION

Phthalates are esters of phthalic acid and aliphatic alcohols. It is widely used in everyday life, and can be used as a plasticizer, solvents and additives in many products, from food packaging to items of general use. High molecular weight phthalates, of which DEHP is the most common, are used as a plasticizer in food-grade plastic packaging material, while low molecular weight phthalates, as DEP and DnBP, are used as solvents and plasticizers in the production of items of general use.

The use of plastic materials is generally present in the food industry, so they are used as packaging material for packaging finished products, and in the process of processing and production. Since phthalates are not chemically bounded to the PVC polymer, they migrate very easily from the packaging material to the food product, but migration can occur during the technological process of food production and processing from plants in industrial facilities. Because of their lipophilic character, phthalates most often migrate to foods that are high in fat such as milk and dairy products, meat and meat products, fish, vegetable oils and fats.

Phthalates are, also, detected in food products that are not packed in packaging material containing phthalates, from which it can be concluded that the contamination of the product occurred due to environmental pollution such as water, air, soil as well as due to widespread use and its disposal to landfills.

Based on available scientific research, it has been proven that there is no bioaccumulation of phthalates and their esters in the human body. Because of the increasing use of phthalate, as well as its presence in the environment there is a justified concern for human health, especially because it leads to disorders in the reproductive and endocrine systems of human. Considering the high exposure of human to phthalates, the question of the justification of the use of phthalates arises, both in food packaging and in items of general use. This work provides an overview of the presence of phthalates in food packaging, its migration into food, as well as the negative impact on human health by consuming and inhaling them.

DIVISION, PROPERTIES AND METHABOLISM OF PHTHALATES

Phthalates are compounds of synthetic origin that are most often added to plastics to improve their mechanical properties, especially softness, flexibility and extensibility. As plasticizing additives, they are present in a number of items of general use, such as children's toys, cosmetics, solvents and insecticides, food packaging, medical devices, transfusion accessories and home improvement products, as well as in technological processes in the food and similar industries. (David and associates, 2003; ATDSR, 2001; ATDSR, 2002; Heudorf and associates, 2007). According to their chemical composition, phthalates are esters of phthalic acid and aliphatic alcohols (diesters of phthalic acid). These are volatile liquids that are added to plastic to increase its mobility.

Phthalates are obtained by esterification of phthalic anhydrides with long-chain alcohols (C7-C10) (Earls amd associates, 2003). If two ester groups are attached in the meta and para positions on the aromatic ring, they are called isophthalates or teraphthalates. In case the two ester groups are in the ortho position on the aromatic nucleus of 1,2-benzenedicarboxylic acid, they are called phthalic acid esters. The two alkaline groups may be similar or different; they may be branched or unbranched; may contain aromatic substitutes, for example butyl benyl phthalate (BBP) or other functional groups (Croatian Food Agency, 2014)

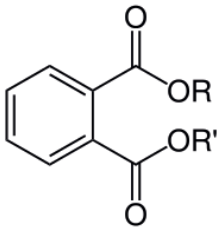


FIGURE 1: General chemical structural formula of phthalate

The proportion of phthalate in a plastic product can be up to 45% of its total weight, depending on the type and purpose of the plastic product (Peakall, 1975; Brooke and associates, 1991; WHO, 1997; Bouma and Schakel, 2002). The most common DEHP that adds to plastics to provide elasticity and softness (Croatian Food Agency, 2014). Every year, about a million tons of phthalate are produced in Western Europe, where the most common are: di- (2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) (Fierens and associates, 2012), di metli phthalate (DMP), di ethyl phthalate (DEP), di n butyl phthalate (DBP), butyl benzyl phthalate (BBP) and polyethylene ether phthalate (PET) which in addition to being used as an intermolecular lubricant tend to be excreted from the medium (Karen and Wright, 2006).

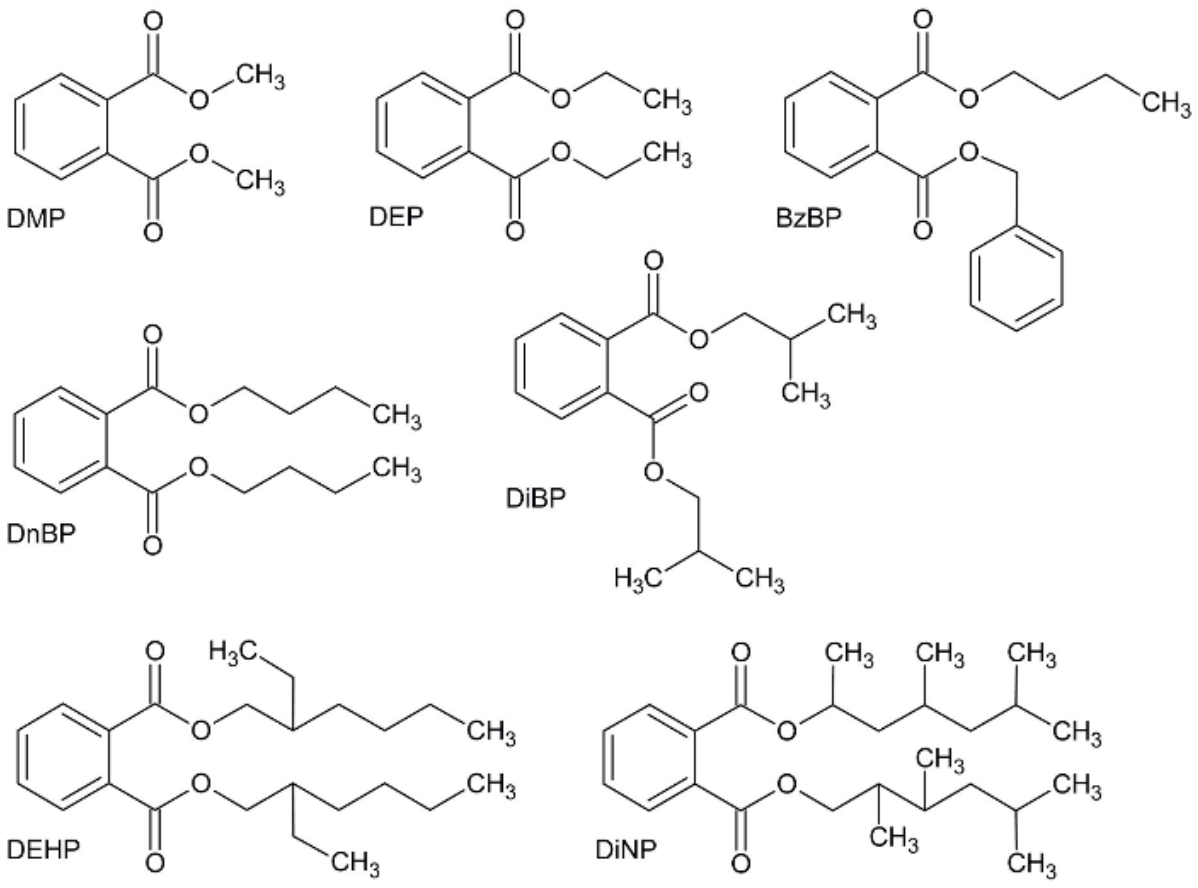


FIGURE 2: Structural formulas of phthalates (Frederiksen and associates, 2007)

Phthalates which have slightly short alkyl groups in their structure, such as methyl and butyl groups, have the feature of solubility in water, and phthalates with long alkyl chains or aromatic structures on the end chains are poorly soluble in water. High molecular weight phthalates, such as DEHP, are mainly used as a plasticizer in the production of PVC polyvinyl chloride, which is used in consumer products, such as food packaging (plastic packaging film), medical devices, vinyl foils (ATSDR , 2002). Lower molecular weight phthalates, such as DEP and DnBP, are used as solvents and plasticizers for cellulose acetate, in the manufacture of varnishes, personal care products (e.g. perfumes, lotions), as well as medicine packaging (ATSDR, 1995, 2001) such as prolonged-release medications (Hauser and associates, 2004).

Because of its lipophilicity, the largest amount of phthalates is found in fatty foods, such as milk and dairy products, fish, meat, and vegetable oils (David and associates, 2003). If short alkyl groups such as e.g. methyl or ethyl group, are at the end of chains, the volatility of phthalates is stronger, so it is possible that we will inhale phthalates without even being aware of it, so in the cosmetics industry these compounds are used in the production of perfumes and fragrances.

Phthalates are not chemically bound to polymers, so they can migrate freely from packaging as well as during the production process into food, beverages and drinking water (Serôdio and Nogueira, 2006). Phthalate migration is accelerated by aging and cracking of plastic packaging or plastic parts used in the food and beverage production process (Harris and Sumpter, 2001).

Since they are not chemically bound to plastic material, phthalates are easily washed away, evaporate quickly into the air and easily migrate into food, beverages and drinking water, most often from pack-

aging material, but other sources of food contamination with phthalates are also possible, e.g. from parts of the technological process of food production (European Chemical Bureau, 2008; European Union Council, 2001; Balafas and associates, 1999; Chou and Wright, 2006). Thanks to this, phthalates contaminate the environment and the food chain and are now one of the ubiquitous environmental contaminants. As a result, the general population is extensively and continuously exposed to phthalates (Sioen and associates, 2012). Since its first use as a plasticizer in 1930, phthalates have become one of the most widespread contaminants in the modern world. Unlike persistent organic pollutants such as organochlorine pesticides e.g. DDT, phthalates and its metabolites do not accumulate in the environment and have a short half-life in living organisms. Thus, monoester metabolites were detected in 90–100% of urine samples of men and women of the general population (Duty and associates, 2005a; Hoppin and associates, 2002; Kato and associates , 2005; Swan and associates, 2005).

One of the essential properties of phthalates, which determines their application, is the molecular weight. High molecular weight phthalates DEHP, DINP and DIDP are mainly used to soften PVC. Low molecular weight phthalates DEP, DBP and BBP have solvent function in consumer items (Cao XuL, 2010). They are mostly used in the cosmetic and pharmaceutical industries. DEP is used as an odor fixative or carrier in the production of fragrances, perfumes and in prolonged-release medications. Among other low molecular weight phthalates, dimethyl phthalate (DMP) should be mentioned. DMP is most commonly used to stabilize and dilute organic peroxides during transport and storage (Clark and associates, 2011).

Table 1: Properties of phthalates (Cao XuL, 2010)

Phthalates	CAS number	Formula	Density (g/ml)	Boiling point (°C)	Melting point (°C)	Water solubility (mg/l) at 25°C
DEHP (di-2-ethylhexyl phthalate)	117-81-7	C24H38O4	0,985	384	-47	0,195
DBP (di-n-butyl phthalate)	84-74-2	C16H22O4	1,043	340	-35	9,9
BBP (benzyl butyl phthalate)	85-68-7	C16H22O4	1,119	370	-35	3,8
DINP (di-iso-nonyl phthalate)	68515-48-0; 28553-12-0	C26H42O4	0,972	370	-50	3,08 x 10-4
DIDP (di-iso-decyl phthalate)	68515-49-1; 26761-40-0	C28H46O4	0,966	400	-50	3,81 x 10-5
DNOP (di-n-octyl phthalate)	117-84-0	C24H30O4	0,985	390	-25	2,49 x 10-3
DEP (diethyl phthalate)	84-66-2	C12H14O4	1,232	295	-40,5	591

PHTHALATES IN FOOD PRODUCTS

Progress in the science of packaging materials in recent decades have led to the widespread and diverse use of plastics to provide cheaper, lighter, stronger, safer, more durable and versatile products and consumer goods that serve to improve quality of life. Plastics can be designed to keep food fresher for longer, and can provide therapeutic benefits through time-release medications and other medical applications (Andrady and Neal. 2009; Thompson and associates, 2009 a, b).

The presence of phthalates in food can be the result of their migration from packaging materials and objects in direct contact with food, and food contamination from the environment or food contamination caused during the production or processing process. The most commonly observed phthalate in food

is DEHP, which is not surprising given the fact that 50% of total annual phthalate production is DEHP (Wenzl, 2009). Phthalates identified in food are mainly higher molecular weight phthalates such as DIDP, BBP and DIBP. The amount of phthalates that will migrate into food, and especially into fatty foods, will be greatly influenced by their initial concentration in the material, lipophilic character, temperature, storage and storage conditions, and the fat content in the food. Phthalates are not covalently bound within the PVC molecule, in principle similarly dissolve similarly, small amounts of fatty foods or oils are sufficient for complete extraction of lipophilic softeners into food. As the release of phthalates from PVC is mainly related to direct contact with fatty foods, manufacturers of packaging materials have changed the recipes. Diisononyl adipate (DINA) and di-2-ethylhexyl adipate (DEHA), which until recently were used as a substitute for DEHP (Cao XuL, 2010), are slowly being replaced by citrates. The most commonly used citrate as a softener for PVC but also for other types of packaging materials in contact with fatty and non-fatty foods is acetyl tributyl citrate (ATBC). Except ATBC, there are a significant number of other substitute softeners for phthalates, but there is insufficient scientific evidence of their adverse effects on human health. Phthalates are most often present in multilayer packaging, while in smaller quantities they are found in varnishes and printing inks. Phthalates can be present in the coatings of dishes, utensils and food preparation equipment from which they can be released into food during thermal food preparation (Fierens and associates, 2012). Choosing the type of packaging in which a particular type of food will be packaged can reduce or increase the specific migration of phthalates.

Food products are contaminated with phthalates by unintended migration during the technological process of production, processing and packaging of finished products through packaging materials in which the finished products are packed. Fresh agricultural products contain minimal concentrations of phthalates. The total concentration of phthalate in fresh meat (muscle) and raw milk is 120 - 280 micrograms / kg, rarely exceeding 500 micrograms / kg (Casajuana and Lacorte, 2004; Rhind and associates., 2005; Sharman and associates, 1994). Elevated phthalate values up to 53000 micrograms / kg have been detected in finished food products that are considered to be most likely contaminated during processing or in the packaging process (Castle and associates, 1989). Studies have shown an increase in DEHP from 80 micrograms / kg in fresh chicken meat to 13100 micrograms / kg after frying in a Teflon-lined pan, and 16100 micrograms / kg after packaging (Tsumura and associates, 2001a).

Hot fats and food products packed in PVC packaging, significantly raise the level of phthalates. High temperatures and fatty hot food in contact with PVC products can cause high levels of phthalate contamination. Many of us buy and store cooking oil in plastic bottles, eat butter, margarine, cheese spreads from plastic containers and it never occurs to us to take in some foreign substances that "dissolve from plastic". Phthalates are released especially quickly from plastic bottles that contain mineral water and carbonated drinks. Contamination of the land with phthalates near the waste recycling site was also observed. So waste recycling places have become a threat to human health. The main pollutants found in the soil are DEHP and DnBP.

The plastic film used in greenhouses has become another source of phthalate in food, with an elevated concentration of phthalate in vegetables ranging from 790 ± 630 to 3010 ± 2130 mg / kg on Nanjing agricultural land where vegetables are grown. Ma and associates, 2015).

EFSA (2005) reported a tolerant daily intake (TDI) of 50 μg / kg body weight for DEHP and 10 μg / kg body weight for DnBP. A regulation issued by EFSA to prevent food contamination by phthalates considers that phthalate concentrations in food greater than or equal to a specific migration limit (SML) of 300 μg / kg are high (Petersen and Jensen, 2010). Concentrations in food between 0 and 50 μg / kg were considered low, because in the opinion of EFSE, the migration in these concentrations reflects the low exposure potential. Concentrations between 50 and 300 μg / kg are marked as mean (Engel and associates,

2012). Thus, when classifying food, it is necessary to apply the criteria for average, and not for maximum measurements, so as not to misclassify food as food with a high phthalate concentration. To protect human health, the European Food Safety Authority (EFSA) has established a total daily intake (TDI) for some of these food-contaminating substances, in particular for DBP 0.01 mg / kg body weight, for BBP 0.5 mg / kg body weight, for DiNP and DiDP 0.15 mg / kg body weight, for DEHP 0.05 mg / kg body weight (EFSA, 2005a, b, c, d, e). Due to toxicity and widespread use, European Regulation no. 10/2011 established a specific migration limit (SML) for DEHP of 1.5 mg / kg.

Serrano and associates (2014) in their study compared the association of a group of foods with high (≥300 μg / kg) and low (<50 μg / kg) phthalate concentrations, as well as their relationship with the load they represent for the human body. Based on the data obtained in the study, they found high concentrations of DEHP in poultry meat, roasting oil and cream-based dairy products (≥300 μg / kg). Also, the presence of DEP in small concentrations in all food groups was determined. According to the data of this research, epidemiological studies have shown a positive connection between the consumption of meat, fat, dairy products and DEHP. The estimated DEHP exposure based on typical diet was 5.7; 8.1 and 42.1 μg / kg per day for women of reproductive age, adolescents and newborns, with dairy products as the largest source of exposure. A diet rich in meat and milk resulted in a twofold increase in exposure. Estimates for infants based on a typical diet exceeded the Environmental Protection Agency's reference values of 20 μg / kg per day, while a diet rich in milk and meat in adolescents also exceeded this threshold.

Fierens and associates (2012) investigated the effect of cooking (cooking, steaming, frying or grilling) at home on the level of phthalates in different types of food (starch products, vegetables, meat and fish). In general, phthalate concentrations in food were reduced after cooking, except in vegetables, where there were almost no changes. DEHP, which was present in all raw foods, decreased to 65.4% after cooking. Sioen and associates (2012) in their study, found cooked food in kindergartens and primary schools has a higher concentration of DEHP and DBP after packaging in aluminum-lined polyethylene containers and stored in warm electric isothermal serving carts compared to before, which implies migration from packaging (Cirrilo and associates, 2011). The influence of cooking and heating in phthalate-containing containers must be taken into account when calculating the exposure assessment in order to determine the exact phthalate content in food directly consumed. Also, it is necessary to determine the difference between food products when calculating daily intakes (milk, meat, cereals), and take into account which of the products are most exposed to phthalates, this is especially important when making dietary recommendations for foods with high phthalate exposure that should be avoided.

PLASTIC WRAPPING

Today's way of life is difficult to imagine without the use of plastic products, which, as such, accompany a person throughout his life, starting from birth (Brooke and associates, 1991; WHO, 1997; Peterson and Breindahl, 2000).

Food packaging is very important for storing food at different temperatures, prolongs the shelf life of the food product itself, and also protects foods from natural agents such as air, which can reduce or change the quality of the product itself. In practice, plastic is used as a safe and suitable packaging for primary food packaging. There are several different types of plastics, each of thoose has unique properties and applications in the food sector, e.g. polycarbonate, high and low density polyethylene, styrene, polypropylene. Plastic packaging is made of various polymers, and additives are used to improve elasticity, flexibility, color, resistance, durability, etc. Both plastics and additives can migrate over time from packaging to food or beverages as a result of increased product temperatures or mechanical stress.

According to Cao (2010) research, phthalates can migrate into foods made from PVC materials, such as pipes commonly used in the milking process, lid seals, food packaging foils, gloves used in food preparation, and conveyor belts. Phthalals are also found in printing inks and adhesives on food wrappers, as well as container coatings used in food packaging (NTP-CERHR, 2003; Indirect food additives, 2014). In the United States, phthalates are approved by the Food and Drug Administration (FDA) as plasticizers in food packaging and food contact materials used during processing and storage, while the European Commission and Chinese authorities have restricted phthalates in contact with food (Petersen and Jensen, 2008). There may be large variability in phthalate concentrations in food groups depending on the food production process, processing process, presence and type of packaging, and lipid content (Wittassek and associates, 2011; Schecter and associates, 2013). The assessment of phthalate exposure in food has become a topic of great interest given the importance of the dietary pathway and the health impacts associated with specific phthalate species found in food.

Increased phthalate content has been reported in drinking water packaged in polyethylene bottles (Criado and associates, 2005). The results of the research (Bošnir and associates, 2007) show that the values of phthalate migration from plastic packaging into soft drinks are many times higher (5 to 40 times) than the migration of phthalate from the same packaging into mineral water. As one of the possible reasons, the authors state a difference in pH value that is less than 3 in soft drinks and greater than 5 in all mineral waters (Jurica and associates, 2013). The World Health Organization has defined the maximum permissible concentration of the most used phthalate DEHP in drinking water and it is 8 micrograms / l (WHO, 2011).

Polyvinyl chloride (PVC) polymer without the addition of softener would be very heavy, brittle and practically useless for technical application, so that with the addition of softener it becomes flexible and elastic. Softeners are mostly added to polymers used in the food industry. These are foils for wrapping food and the like. Contamination of food with softeners can occur. The best known softeners to be added are high molecular weight phthalates, such as butylbenzyl phthalate (BBzP), di-2-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP) used as plasticizers in PVC materials, such as are food packaging and medical products. In recent years, di-nonyl phthalate (DiNP) and di-decyl phthalate (DiDP) have increasingly replaced DEHP in these products (Zotta and associates, 2014). Due to non-covalent bonds between phthalates and their parent substances, there can be significant migration of phthalates not only into the food product but also into the environment which can lead to environmental pollution and thus ubiquitous exposure in the population.

Castle and associates (1988a) proposed two ways to reduce the migration of phthalates from PVC food films:

- production of thinner films with a reduced level of DEHA that is normally present in PVC films
- partial or complete replacement of DEHA with higher molecular weight plasticizers.

The migration of plasticizers from DEHA-coated PVC packaging films increases with prolonged contact time between the food product and the PVC packaging film (Down and Gilbert, 1977). It was observed that when using thinner PVC films containing 13.3% DEHA compared to conventional PVC film with 18.30% there was a decrease in the level of DEHA migration from 41% to 53%. The migration level of plasticizer from PVC foil with 23% plasticizer was 3 to 21 times lower than the level of DEHA migrating from conventional PVC film with 18% DEHA. Also, DEP was detected in baked food products, which were packed in cardboard boxes with cellulose-acetate openings plasticized with 16 to 17% DEP, where DEP is present in the product in the range of 1.7 to 4.5 mg / kg. From which it can be concluded that DEP evaporated, and thus DEP migrated into food, although there was no direct contact of DEP with food.

Tsumura and associates (2001b) investigated the contamination of food with phthalates derived

from PVC gloves in retail outlets serving ready-to-eat foods. PVC gloves used in food preparation contain up to 41% DEHP, 60.2% DEHA, 74.8% DiNO, 27.9% BBzP. They came to the conclusion that the level of phthalates in ready meals (in contact with PVC gloves) was increased in relation to the raw materials from which the finished dish was prepared (not yet in contact with PVC gloves). The authors also noted lower phthalate levels in ready meals in which PVC gloves were not used in the preparation.

Different plasticizers such as epoxidized soybean oil (ESBO), phthalates, adipates, etc. can be used as PVC sealants in different countries. In order to reduce the migration of plasticizers from PVC lid sealants into foods, especially fatty foods, the use of polyadipates as high molecular weight plasticizers was investigated (Biedermannand associates, 2008). Although polyadipates have been used successfully as PVC interrogators, they are not easy to work with because they create a viscous plastisol that makes it difficult to place a uniform ring in the lid, so viscose plastisol needs to be diluted with less viscous plasticizers (Castle and associates, 1988a). The results of the research showed that the migration of polyladipates was below the legal limits in 11 foods that were stored for two years in packaging containing polyadipates. Thus, di (2-ethylhexyl) terephthalate (DEHT) is used as a new plasticizer in Canada, as a substitute for DEHP in PVC sealants used to close beverage bottles (Parent, 2009), indicating a declining trend in the use of DEHP in packaging materials in food products in North America.

Polystyrene (PS) is a styrene polymer that in its pure state is a hard, colorless plastic with limited flexibility. It can be poured into molds with fine details and thus used for packaging yogurt and dairy products. The packaging for the yoghurt packaging contained traces of DMP, but also high amounts of DEHA. These compounds can migrate into fatty foods at high temperatures during production. On the other hand, expanded polystyrene can be used as a base for packaging meat, fish, cheese, fruit and the like. Polystyrene substrates can release some compounds such as DMP, DEHP, OP, NP and DEHA (Fasano and associates, 2012).

PET is a product formed by the reaction of terephthalic acid and ethylene glycol. Because of the properties such as strength and clarity, this polymer is used for the production of PET bottles for water packaging, soft drinks and other food products. Comonomers such as isophthalic acid and dimethyl terephthalate can be used to produce polymers that provide thicker-walled PET bottles, which allows them to be used for packaging large-volume liquids (Park and associates, 2008). Due to incomplete reaction, residues of monomers (terephthalic acid, ethylene glycol, isophthalic acid, dimethyl terephthalate) in the polymer can migrate from PET packaging to food. PET degradation products (e.g., terephthalate) and polymeric additives such as Tinuvin P or Tinuvin 234 can migrate into food (Monteiro and associates, 1999; Begley and associates, 2004; Choodum and associates, 2007). Due to the name "phthalate" in the name of the polymer, it gives the wrong impression that phthalates and DEHA can be migrated from PET packaging to food (Biscard and associates, 2003; Farhoodi and associates, 2008; Montuori and associates, 2008). It should be emphasized that PET polymers have no chemical or physical bonds with phthalates, even their chemical structures are different. Phthalates are esters of orthophthalic acid, while paraphthalic acid (terephthalic acid) or metaphthalic acid (isophthalic acid) is used to produce PET polymers. Unlike PVC packaging, which needs to be plasticized with phthalates in order to be more flexible, PET packaging should be as strong and rigid as possible, for that reason it is important to emphasize that phthalates are not used in the production of PET polymers.

Plasticizers, such as DBP, DCHP and DEHP are ingredients in printing inks (2-8%) for food packaging, and are used to improve the adhesion of paint to the packaging surface, flexibility and resistance to crease formation (Castle and associates, 1988). Regardless of the fact that the ink is on the outside of food packaging (film, cardboard), it can be one of the sources of phthalates in food. The ink used to print declarations on confectionery and snack products is one source of phthalate contamination of these products

(Castle and associates, 1989). Confectionery and snack products were stored for 90 to 180 days at 20 ℃ in polypropylene packaging with printing ink. Considering propylene itself does not contain plasticizers, the migration of plasticizers into food came from the printing ink on the packaging. Castle and co-workers found in their research that there was an increase in DBP migration from 0.2 to 6.7 mg / kg over a period of 180 days. The presence of one or more plasticizers has been observed in a number of confectionery and snack products packaged in printed polypropylene packaging. Balafas and associates (1999) discovered the presence of phthalates and adipates in food products packaged in printed PE materials, which led to the conclusion that printing inks are the ultimate source of food contamination. To solve these problems, new technologies are being introduced where packaging material manufacturers are focusing on minimizing the use of ink and introducing new ink curing techniques such as fast UV systems that reduce ink evaporation. Phthalates were also detected in food packaged in paper and cardboard packaging, e.g., the level of DiBP and DBP in sugar packaged in paper packaging (packaging contains 95 - 98 mg / kg DiBP and 56 - 64 mg / kg DBP) after storage at room temperature after 4 months it was 2.2 - 2.6 mg / kg DiBP, or 0.50 - 1.00 mg / kg. The main source of phthalates in paper and cardboard packaging was from printing inks and adhesives. Also, if recycled materials are used to make paper and cardboard packaging, phthalates may be transferred from the ink and glue if the ink and glue have not been completely removed during the recycling process.

PVC pipes are commonly used in the process of milking and transferring milk between tanks and reservoirs in dairy farms and milk processing plants. Phthalates are used as plasticizers that give PVC pipes flexibility, of which DEHP is used the most with as much as 40% in pipes (Ruuska and associates, 1987; Tsumura and associates, 2002a). Because they are not chemically bound to the polymer, plasticizers can migrate from PVC pipes to milk, especially at higher temperatures during the milking process. In dairy products, more than 80% of the total phthalate concentration, found in an amount of 50 to 200 micrograms / kg, may come from milking equipment (Casajuana and Lacorte, 2004; Castle and associates, 1990). Additional processing, packaging, and condensation can lead to a 5 to 100-fold increase in DEHP concentration in dairy products, such as cheese and cream (Casajuana and Lacorte, 2004; Mortensen and associates, 2005; Petersen, 1991; Sharman and associates , 1994). Due to concern for human health, some countries have banned the use of DEHP in the production of PVC pipes for milking, e.g. Denmark banned the use of DEHP in the production of milking tubes in 1989, while in Norway DEHP is replaced by other plasticizers in the production of milking tubes (Petersen, 1991). The UK uses plasticizer-free pipes, except in the center of gravity between tankers and tanks where DiDP-coated PVC pipes are used rather than DEHP (Castle and associates, 1990). In their study, Feng and associates (2005) found that the level of DEHP in cow's milk collected using PVC tubes plasticized with 28% DEHP ranged from 111.7 to 282.9 ng / g, which is on average 15 times more than milk hand-collected without the use of PVC pipes (8.4 to 23.7 ng / g), indicating that migration from PVC pipes could be a major source of DEHP in milk and dairy products . The increase in the concentration of DEHP to 11000 micrograms / kg in food products can also be attributed to rinsing from PVC gloves used during food preparation (Tsumura and associates, 2001a; Tsumura and associates, 2001b).

Food that is prepared by heating in microwave ovens can be contaminated with phthalates, so that heating facilitates the migration of phthalates from the packaging material in which the food is packaged to the food product. In the case of food products packed in cardboard boxes with cellulose acetate windows plasticized with 16 - 17% DEP, the presence of DEP in the range of 1.70 to 4.5 mg / kg was detected during heating. It is concluded that there is a possibility that DEP has evaporated from the film to the food without direct contact of the food packaging (Cao, 2010).

HEALTH IMPACT

Phthalates, in addition to affecting the organoleptic properties of food (Wagner and Oehlmann, 2009), are considered by many to be endocrine distortions (EDs), and affect the human reproductive system and may have a carcinogenic effect on the human body. In the public, phthalates have been associated with adverse health effects, particularly with regard to early life exposure (Serranoand associates, 2014).

The main source of exposure of the human body to phthalates is the consumption of food that is contaminated during production, processing and packaging. Other important sources of exposure include inhalation of contaminated air, unintentional inhalation of dust, and the use of general use items such as personal care products and cosmetics (Koo and Lee, 2004; Kavlock and associates, 2006). So we can conclude that human exposure to phthalates can be oral (by consuming food and water), inhalation (by inhaling dust particles) and dermal (using cosmetic products and personal hygiene products). Exposure of the human body to phthalates through food is mainly possible through the accumulation of phthalates in the food chain and the use of PVC in food packaging (Chai and associates, 2008; Li and associates, 2012; Spillmann and associates, 2009; Wormuth and associates, 2006).

Because DEHP is a lipophilic molecule, it dissolves immediately in whole blood, plasma, platelet concentrate, lipid-containing infusion solutions, parenteral solutions, or other substances that dissolve intravenous drugs. This is the reason for phthalate exposure to medical procedures such as: hemodialysis, whole blood, platelet or plasma transfusion, extracorporeal membrane oxygenation, cardiopulmonary bypass, intravenous infusion solutions, enteral and parenteral nutrition (Vidić, 2008).

In humans, phthalates e.g. DEHP, are intensively hydrolyzed to monoesters, then further metabolized by enzymatic oxidation of the alkyl chain to more hydrophilic metabolites (MEHP), which are further oxidized to mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP, 5OH-MEHP) and mono- 2-ethyl-5-oxohexyl phthalate (MEOHP). In contrast, monoethyl phthalate (MEP), the DEP hydrolysis monoester, is mainly excreted in its free form (Gomez and Gallart, 2018).

The major metabolite of DEHP, mono-2-ethylhexyl phthalate, is excreted in the urine along with urine and faeces, unchanged or conjugated as glucuronide (Itoh and associates, 2005) but has been detected in breast serum and breast milk (Ghisari and Bonefeld-Jorgensen , 2009; Albro and Lavenhar, 1989; Koch and associates, 2005; Frederiksen and associates, 2007; Gomez and Gallart, 2018). After exposure, phthalates are rapidly metabolized and excreted in urine and feces (ATSDR 1995, 2001, 2002).

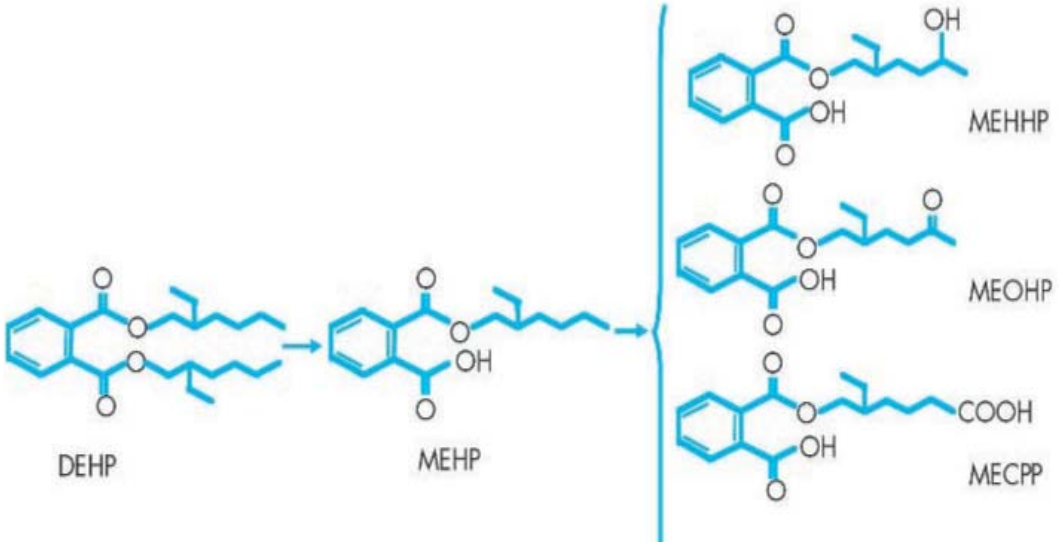


FIGURE 3: DEHP metabolism in the human body (Hauser R., Calafat A.M., 2005)

The most common approach to researching human exposure to phthalates is to measure urinary concentrations (biomarkers) of phthalate metabolites such as mono-ethyl phthalate (MEP), mono-2-ethylhexyl phthalate (MEHP), mono-butyl phthalate (MBP) and mono-benzyl phthalate. (MBzP). Two oxidative metabolites of DEHP, mono- (2-ethyl-5-hydroxylhexyl) phthalate (MEHHP) and mono- (2-ethyl-5-oxohexyl) phthalate (MEOHP) were present in most subjects at urinary concentrations higher than those in MEHP , which is a hydrolytic metabolite of DEHP (CDC 2005).

The metabolites DMP, DEP, DiBP, DnBP, BBzp, DEHP, DiNP, and DiDP have been identified as biomarkers of current human exposure to phthalates (Latini and associates, 2005).

TABLE 2: Phthalates and their metabolites (Serrano and associates, 2014)

NAME OF PHTHALATE	CODE	URINARY METABOTYL	CODE
Dimethyl phthalate	DMP	Mono-n-methyl phthalate	MmMP
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP
Di-isobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP
Di-n-octyl phthalate	DnOP	Mono- (3-carboxypropyl) phthalate	MCPP
Di-n-isononyl phthalate	DiNP	Mono-carboxyoctyl phthalate	MCOP
Di-n-isodecyl phthalate	DiDP	Mono-carboxynonyl phthalate	MCNP
Benzylbutyl phthalate	BzBP	Mono-benzyl phthalate	MBzP
Di-2-ethylhexyl phthalate	DEHP	Mono-2-etilheksil ftalat	MEHP
		Mono- (2-ethyl-5-hydroxyhexyl) phthalate	MEHHP
		Mono- (2-ethyl-5-oxyhexyl) phthalate	MEOHP
		Mono-(2-etil-5-karboksipentil) ftalat	MECPP

Koch and associates (2013) monitored urinary phthalate excretion in individuals who fasted for 48 hours, found that diet was the most important route of DEHP, DiNP, and DiDP exposure, while DMP, DEP, DiBP, DnBP, and BBzP were primarily associated with exposure to other items of general use and surroundings.

Phthalates have also been identified in the placenta, and their presence has also been found in breast milk. From which it can be concluded that fetal exposure is directly related to maternal exposure to phthalates (Latini and associates, 2003; Lin and associates, 2008). Increased concentration of metabolites DEP, DiBP, DnBP, DEHP in the mother also affects the shortening of anogenital gap (AGD) in male newborns, a marker of andorgenization (Swan and associates , 2005; Swan, 2008). Prenatal exposure to phthalates has been associated with changes in labor time, neonatal hormone levels, and mental behavior in children and infants (Sathyanarayana, 2008).

In the adult population, various epidemiological studies support an association between phthalate exposure and testicular markers in men, particularly with reduced semen quality (Joensen and associates, 2012). Some phthalates act as antiandrogens and can seriously disrupt the development of the male reproductive system, although details of the mechanism are still incomplete. Toxicity of some phthalates to testes and ovaries, including DEHP, is due in part to interference with follicle-stimulating hormone (FSH) function at the level of sertoli cells in the testis and granulosa cells in the ovary. Some phthalates are weak estrogens, as evidenced by binding to estrogen receptors (WHO, 2012). Phthalates are considered endocrine

distortions due to their complex effects on several hormonal systems, including the estrogenic androgenic system. Some phthalates, including BBP and DBP, act as weak estrogens in cell culture systems. They can bind to estrogen receptors, elicit estrogen-appropriate cellular responses, and act as an adjunct to natural estrogen, estradiol, in altering these systems (Joblind and associates, 1995; Kang and associates, 2005). Phthalates also bind less to androgen receptors, disrupting cellular activities that are otherwise initiated by androgens (Borch and associates, 2006). DBP, DiBP, and BBP bind most strongly to androgen receptors, and could therefore be expected to act most extensively through this cellular pathway (Fang and associates, 2003). In addition to their direct effects prescribed through interactions with steroid hormone receptors, phthalates can also cause proliferation, malignant invasions, and breast tumor formation in carcinogenic cell cultures that have low hormone deficiency or deficiency, indicating that at least some effects of these compounds are independent of their direct estrogenic or androgenic effects (Hsieh and associates, 2012).

Stahlhut and associates (2007) link endometriosis in women with high levels of phthalate metabolites. Cobellis and associates (2003) published a study examining the association between DEHP levels in plasma, peritoneal fluid, and endometriosis. Plasma samples were obtained the day before surgery or immediately before anesthesia for laparoscopy. Peritoneal fluid was obtained during laparoscopy. Concentrations of DEHP and MEHP in plasma and peritoneal fluid were measured using HPLC-UV. The results showed that women with endometriosis show higher plasma DEHP concentrations. An association between plasma DEHP concentration and endometriosis was observed, suggesting a possible role of phthalate esters in disease development.

Duty and associates (2005) examined the relationship between urinary phthalate levels, sperm quality and phthalate exposure, as well as reproductive hormone levels in adult men using LC-MS / MS analysis. They found that some phthalate monoesters were associated with lower sperm concentration, lower motility, and increased sperm percentage with abnormal morphology in humans. In particular, an association between monobutyl phthalate (MBP), a metabolite of DBP, with motility and sperm concentration was observed.

CONCLUSION

Many chemicals, including phthalates, which are in commercial use, can damage human health. The presence of phthalates in the human body can cause endocrine distortions that can negatively affect the human reproductive system, and cause carcinogenic effects in the human body. Recently, the rate of patients with endocrine-related cancers and the number of people with genital malformations, as well as complications during pregnancy, have increased. Thus, it is concluded that genetic factors can not be the only ones responsible, other factors must be taken into account, such as diet, lifestyle, environment with chemicals, and the influence of the external environment.

Since phthalates enter the human body mostly from packaging material, the aim of this work is to point out its harmfulness, as well as to point out the need to find packaging material that is not harmful to the human body. Research into the effects caused by phthalates in the human body is very important to provide evidence that would help in the adoption of adequate legislation in this area, and thus reduce the risk of diseases that may arise from the possible use of these materials.

LITERATURA:

ATSDR, 1995., Toxicological profile for diethyl phthalate (DEP) Atlanta, GA: Agency for Toxic Substances and Disease Registry

ATSDR, 2001., Toxicological profile for di-n-butyl phthalate (DBP) Atlanta, GA: Agency for Toxic Substances and Disease Registry

ATSDR, 2002., Toxicological profile for di(2-ethylhexyl)phthalate (DEHP) Atlanta, GA: Agency for Toxic Substances and Disease Registry

Albro P.W., Lavenhar, S.R., 1989., Metabolism of Di(2-ethylhexyl)phthalate, Drug Metab. Rev. 21(1), 13–34, doi: 10.3109/03602538909029953

Andrady A. L., Neal M. A., 2009., Applications and societal benefits of plastics, Phil. Trans. R. Soc. B 364, 1977–1984

Aurela B., Kulmala H., Soderhjelm L., 1999., Phthalates in paper and board packaging and theirmigration into Tenax and sugar, Food Addit Contam 16(12), 571 – 7

Balafas D., Shaw K.J., Whitfield F.B., 1999., Phthalate and adipateesters in Australian packaging materials, Food Chem. 65,279 – 87, doi: 10.1016/S0308-8146(98)00240-4

Begley T.H., Biles J.E., Cunningham C., 2004., Migration of a UV stabilizer from polyethyleneterephthalate (PET) into food simulants, Food Addit Contam 21(10),1007 – 14

Biscardi D., Monarca S., De Fusco R., Senatore F., Poli P., Buschini A., Rossi C., Zani C., 2003.,Evaluation of the migration of mutagens/carcinogens from PET bottles into mineral waterby Tradescantia/micronuclei test, comet assay on leukoctes and GC/MS, Sci Total Environ. 302, 101 – 8

Biedermann M., Fiselier K., Marmiroli G., Avanzini G., Rutschmann E., Pfenninger S., Grob K., 2008., Migration from the gaskets of lids into oily foods: first results on polyadipates, EurFood Res Technol 226, 1399 – 1407

Borch J., Axelstad M., Vinggaard A., Metzdorff S., Brokken L., Dalgaard M., 2006., Mechanisms under lying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis, Toxicol 223, 144 – 155

Bouma K., Schakel D.J., 2002., Migration of phthalates from PVC toys into saliva simulant by dynamic extraction, Food Additives and Contaminants 19, 602 – 10

Bošnir J., Puntarić D., Galić A., Škes I., Dijanić T., Klarić M.,Grgić M., Čurković M., Šmit Z. 2007., Migration of phthalates fromplastic containers into soft drinks and mineral water, FoodTechnol Biotechnol 45, 91 – 5

Brooke D.N., Dobson S., Howe P.D., Nielsen J.R., 1991., Environmental hazard assessment: di-(2-ethylhexyl) phthalate, United Kingdom Department of the Environment, Toxic Substances Division, Report TSD/2, London

Cao X.L., 2010., Phthalate ndergrou Foods: Sources, Occurrence, and Analytical Methods, Comprehensive Reviews in Food Science and Food Safety 9(1), 21 – 43

Casajuana N., Lacorte S., 2004., New methodology forthe determination of phthalate esters, bisphenol A, diglycidyl ether and nonylphenol in commercial whole milk samples, J Agric Food Chem 52, 3702 – 3707

Castle L., Gilbert J., Eklund T., 1990., Migration of plasticizerfrom poly (vinyl chloride) milk tubing, Food Addit Contam 7, 591 – 596

Castle L., Mayo A., Gilbert J., 1989., Migration of plasticizers fromprinting inks into foods, Food Addit Contam 6, 437 – 443

Castle L., Mercer A.J., Startin J.R., Gilbert J., 1988., Migration fromplasticized films into foods. 3. Migration of phthalate, sebacate,citrate and phosphate esters from films used for retail food pack-aging., Food Addit Contam 5, 9 – 20

CDC, 2005., Third National Report on human exposure to environmental chemicals Washington, DC: Centers for Disease Control and Prevention

Chai W., Suzuki M., Handa Y., Murakami M., Utsukihara T., Honma Y., Nakajima K., Saito M., Horiuchi C., 2008., Biodegradation of di-(2ethylhexyl) phtalate by fungi, Research Gate, 72, 83 – 87

Choodum A., Thavarungkul P., Kanatharana P., 2007., Acetaldehyde residue in polyethylen eter phthalate, J Environ Sci Health Part B 42, 577 – 585

Chou K., Wright R.O., 2006., Phthalates in food and medical devices, J Med Toxicol 2, 126 – 35, doi: 10.1007/BF03161027

Cirillo T., Fasano E., Castaldi E., Montouri P., Amodio Cocchieri R., 2011., Children's exposure to di (2-ethylhexyl) phthalate and dibutylphthalate plasticizers from school meals, J Agric Food Chem 59, 10532 – 10538, doi: 10.1021/jf2020446

Clark K.E., David R.M., Guinn R., Kramarz K.W., Lampi M.A., Staples C.A., 2011., Modeling Human Exposure to Phthalate Esters: A Comparison of Indirect and Biomonitoring Estimation Methods, Human and Ecological Risk Assessment: An International Journal 17(4), 923 – 965

Cobellis L., Latini G., DeFelice C., Razzi S., Paris I., Ruggieri F., Mazzeo P., Petraglia F., 2003., High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis., Hum. Reprod. 18(7), 1512 – 1515, doi: 10.1093/humrep/deg254

Criado M. V., Fernandez P. V. E., Badessari A., Cabral D., 2005., Conditions that regulate the growth of moulds inoc-ulated into bottled mineral water, Int J Food Microbiol 99, 343 – 349

Daun H., Gilbert S.G., 1977., Migration of plasticizers from polyvinylchloride packaging filmsto meat, J Food Sci 42, 561 – 2,

David F., Sandra P., Tienpont B., Vanwalleghem F., Ikonomou M., 2003., Analytical methods review, TheHandbook of Environmental Chemistry. Part Q, PhthalateEsters. Vol. 3. Berlin: Springer, 9 – 56

Duty S.M., Ackerman R.M., Calafat A.M., Hauser R., 2005a., Personal care product use predicts urinary concentrations of somephthalate monoesters, Environ Health Perspect 113, 1530 – 1535

Earls A.O., Axford I.P., Braybrook J.H.,2003., Gas chromatography–mass spectrometry determination of the migration of phthalate plasticisers

from polyvinyl chloride toys and childcare articles, Journal of Chromatography A, 983(1–2), 237 - 246

Engel K.H., Feigenbaum A., Lhuguenot J.C., Mennes W., Nielson K.R., Pratt I., Jany K.D., Spyropoulos D., Theobald A., 2012., Food contact materials, flavouring substances and smoke flavourings. Eur Food Safety Authority J. 10, 1007

European Chemical Bureau, 2008., European Union Risk Assessment Report; bis (2-ethylhexyl) phthalate (DEHP), Luxembourg, Office for Official Publications of the European Communities, http://echa.europa.eu/d o c u m e n t s

European Union Council, 2000., Decision No. 2455/2001/ECestablishing the list of priority substances in the field of waterpolicy and amending Directive 2000/60/EC. Off J EurCommun 2001, L 331, 1 – 5

Fang H., Tong W., Branham W.S., Moland C.L., Dial S. L., Hong H., Sheehan D.M., 2003., Study of 202 Natural, Synthetic, and Environmental Chemicals for Binding to the Androgen Receptor, ChemRes Toxicol 16(10), 1338 – 1358

Farhoodi M., Emam-Djomeh Z., Ehsani M.R., Oromiehie A., 2008., Migration of model con-taminants (ethylene glycol, DEHA and DEHP) from PET bottles into Iranian yogurt drink, E-Polymers 37, 1 – 9

Fasano E., Bono-Blay F., Cirillo T., Montuori P., Lacorte S., 2012., Migration of phthalates, alkylphenols, bisphenol A and di (2-ethylhexyl) adipate fro food packing, Food Control 27, 132 – 138

Feng Y.L., Zhu J., Sensenstein R., 2005., Development of a headspace solid phase microextraction method combined with gas chromatography mass spectrometry for the determinationof phthalate esters in cow milk, Anal Chim Acta 538, 41 – 8

Fierens T., Servaes K., Holderbeke M.V., Geerts L., De Henauw S., Sioen I., Vanermen G., 2012., Analysis of phthalates in food products and packaging materials sold on the Belgian market, Food and Chemical Toxicology 50, 2575 – 2583

Fierens T., Vanermen G., Van Holderbeke M.V., Henauw S.D., Sioen I., 2012., Effect of cooking at home on the levels of eight phthalates in foods, Food Chem Toxicol. 50, 4428 – 4435, doi: 10.1016/j.fct.2012.09.004

Frederiksen H., Skakkebak N.E., Andersson A.M., 2007., Metabolism of phtalates in human, Mol. Nutr. Food Res 51, 899 – 911

Ghisari M., Bonefeld-Jorgensen, E. C., 2009., Effects of plasticizers and theirmixtures on estrogen receptor and thyroid hormone functions, ToxicologyLetters 189, 67 – 77

Gomez C., Gallart-Ayala H., 2018., Metabolomics: a tool to characterize the effect of Phalates and Bisphenol A, Enviromental Reviews

Harris C.A., Sumpter J.P., 2001., The endocrine disrupting potential of phthalates, in: The Handbook of Environmental Chemistry, vol. 3, Part L, Springer-Verlag, Berlin, Heidelberg, 169 – 200

Hatch E.E., Nelson J.W., Qureshi M.M., Weinberg J., Moore L.L., Singer M., Webster T.F., 2008., Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ Health. 7, 7 – 27, doi: 10.1186/1476-069X-7-27

Hauser R., Calafat A.M., 2005., Phthalates and human healt, Occup Environ Med 62, 806 – 818

Heudorf U., Mersch-Sundermann V., Angerer J., 2007., Phthalates:toxicology and exposure, Int J Hyg Env Health 210, 623 – 34, doi: 10.1016/j.ijheh.2007.07.011

He L., Gielen G., Bolan N.S., Zhang X., Qin H., Huang H., Wang H., 2015., Contamination and remediation phthalic acid esters in agricultural soils in China : a review, Agronomy for Sustainable Development, vol. 35, 519 – 534

Hoppin J.A., Brock J.W., Davis B.J., Baird D.D., 2002., Reproducibility of urinary phthalate metabolites in first morning urine samples, Environ Health Perspect 110, 515 – 518

Hsieh T.H., Tsai C.F., Hsu, C.Y., Kuo P.L., Lee J.N., Chai C.Y., Tsai E.M., 2012., Phthalates induce proliferation and invasiveness of estrogen receptor-negative breast cancer throughthe AhR/HDAC6/c-Myc signaling pathway, FASEB Journal 26(2), 778 – 787

Indirect food additives: adhesives and components of coatings", 2014., Title 21 Code of Federal Regulations, Part 175

Indirect food additives: polymers: Indirect food additives: polymers", 2014., Title 21 Code of Federal Regulations, Part 177

Itoh H., Yoshida K., Masunaga S., 2005., Evaluation of the effect of governmentalcontrol of human exposure to two phthalates in Japan using a urinarybiomarker approach, International Journal of Hygiene and Environmental Health 208, 237 – 245

Jobling S., Reynolds T.,White R., Parker M.G., Sumpter J.P., 1995., A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic, Environ Health Perspect 103, 582 – 587

Joensen U.N., Fredericksen H., Jensen M.B., Lauritsen M.P., Olesen I.A., Lassen T.H., Andersson A.M., Jorgensen N., 2012., Phthalate excretion pattern and testicular function: a study of 881 healthy Danish men, Environ Health Perspect. 120, 1397 – 1403, doi: 10.1289/ehp.1205113

Jurica K.,Uršulin-Trstenjak N., Vukić-Lušić D., Lušić D., Šmit Z., 2013., Izloženost ftalatima i njihova pojavnost u alkoholnim pićima, Arh Hig Rada Toksikol 64, 317 – 325

Kang S.C., Lee B.M., 2005., DNA methylation of estrogen receptor α gene by phthalates.J Toxicol Environ Health, 68, 1995 – 2003

Karen C., Wright R.O., 2006., Phthalates ih food and medical devices, Journal of medical toxicology 3, vol.2

Kavlock R., Barr D., Boekelheide K., Breslin W.,Breysse P., Chapin R., Gaido K., Hodgson E., Marcus M., Shea K., Williams P., 2006., NTP-CERHR expert panel update on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate, ReprodToxicol 22, 291 – 399

Kato K., Silva M.J., Needham L.L., Calafat A.M., 2005., Determination of 16 phthalate metabolites in urine using automated sample preparation and online preconcentration/high-performance liquid chromatography/tandem mass spectrometry, AnalChem 77, 2985 – 2991

Kazazić M., Đapo M., 2013., Štetnost ftalata i bisfeonola A i njihov uticaj na zdravlje, 9th International Scientific Conference on Production Engineering DEVELOPMENT AND MODERNIZATION OF PRODUCTION, 651 – 656

Koch H.M., Lorber M., Christensen K.L.Y., Palmke C., Koslitz S., Bruning T., 2013., Identifying sources of phthalate exposure with human biomonitoring: Results of a 48 h fasting study with urine collection and personal activity patterns, Int J Hyg Environ Health. 216 (6), 672 – 681, doi: 10.1016/j.ijheh.2012.12.002

Koch H.M., Bolt H.M., Preuss R. I., Angerer J., 2005., New metabolites of di(2-ethylhexyl) phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP, Arch. Toxicol. 79(7), 367 – 376, doi: 10.1007/s00204-004-0642-4

Koo H.J., Lee B.M., 2004., Estimated exposure to phthalates in cosmeticsand risk assessment, J Toxicol Environ Health 67, 1901 – 1914

Latini G., 2005., Monitoring phthalate exposure in humans, Clin. Chim. Acta. 361 (1-2), 20 – 29

Lin L., Zheng L.X., Gu Y.P., Wang J.Y., Zhang Y.H., Song W.M., 2008., Levels of environmental endocrine disruptors in umbilical cord blood and maternal blood of low-birth-weight infants, Zhonghua Yu Fang Yi Xue Za Zhi 42, 177 – 180

Li J.H., Ko Y.C., 2012., Plasticizer incident and its health effects inTaiwan, Kaohsiung J.Med.Sci. 28, 17 – 21

Ma T.T., Wu L.H., Chen L., Zhang H.B., Teng Y., Luo Y.M., 2015., Phthalate esters contamination in soils and vegetables of plastic film greenhouses of suburbnanjing, China and the potential human health risk. Environ. Sci. Pollut. Res., doi: 10.1007/s11356-015-4401-2

Mortensen G.K., Main K.M., Andersson A.M., Leffers H.,Skakkebaek N.E., 2005., Determination of phthalate monoesters in human milk, consumer milk and infant formula by tandem massspectrometry (LC-MS-MS), Anal Bioanal Chem 382, 1084 – 1092

Monteiro M., Nerin C., Reyes F.G., 1999., Migration of Tinuvin P, a UV stablizer, from PET bottles into fatty-food simulants, Pack Technol Sci 12, 241 – 8

Montuori P., Jover E., Morgantini M., Nayona J.M., Triassi M., 2008., Assessing human exposure tophthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles, Food Addit Contam 25(4), 511 – 8

National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR): Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP)., 2003., NIH Publication No. 03–4487

Park H.J., Lee Y.J., Kim M.R., Kim K.M., 2008., Safety of polyethylene terephthalate food containers evaluated by HPLC, migration test, and estimated daily intake, J Food Sci 73(6), 83 – 9

Peakall D.B., 1975., Phthalate esters: Occurrence and biological effects, Residue Reviews, 54,1 – 41

Petersen J. H., 1991., Survey of di-(2-ethylhexyl)phthalate plasticizer contamination of retail Danish milks, Food Addit Contam 8, 701–705

Peterson J.H., Breindahl T., 2000., Plasticizers in total diet samples, baby food and infant formulae, Food Additives & Contaminants 17, 133 – 41

Petersen J.H., Jensen L.K., 2010., Phthalates and food-contact materials: enforcing the 2008 European Union plastics legislation. Food Addit ContamPart A Chem Anal Control Expo Risk Assess. 27, 1608 – 1616, doi: 10.1080/19440049.2010.501825

Rhind S.M., Kyle C.E., Telfer G., Duff E.I., Smith A., 2005., Alkylphenols and diethylhexyl phthalate in tissues of sheep grazingpastures fertilized with sewage sludge or inorganic fertilizer, Environ Health Perspect 113, 447 – 453

Rudel R.A., Gray J.M., Engel C.L., Rawsthorne T.W., Dodson R.E., Ackerman J.M., Rizzo J., Nudelman J.L., Brody J.G., 2011., Food packaging and bisphenol A and bis(2-ethyhexyl) phthalate exposure: findings from a dietary intervention, Environ Health Perspect. 119, 914 – 920, doi: 10.1289/ehp.1003170

Ruuska R.M., Korkeala H., Liukkonen-Lilja H., Suortti T., Salminen K., 1987., Migration of contaminants from milk tubes and teat liners, J Food Protection 50, 316 – 20

Sathyanarayana S., 2008., Phthalates and children's health, Curr Probl Pediatr Adolesc Health Care. 38, 34 – 49, doi: 10.1016/j.cppeds.2007.001

Serôdio P., Nogueira M.F., 2006., Considerations on ultratrace analysis of phthalates in drinking water, Water Res. 40, 2572 – 2582

Serrano S.E., Braun J., Trasande L., Dills R., Sathyanarayana S., 2014., Phtalates and diet: a review of the food monitoring and epidemilogy data, Environmental Healthvolume 13, 43

Schecter A., Lorber M., Guo Y., Wu Q., Yun S.H., Kannan K., Hommel M., Imran N., Hynan L.S., Cheng D., Colacino J.A., Birnbaum L.S., 2013., Phthalate concentrations and dietary exposure from food purchased in New York state. Environ Health Perspect. 121, 473 – 494

Sharman M., Read W.A., Castle L., Gilbert J., 1994., Levels of di-(2-ethylhexyl)phthalate and total phthalate esters in milk,cream, butter and cheese, Food Addit Contam 11, 375 – 385

Sioen I., Fierens T., Holderbeke M.V., Geerts L., Bellemans M., Maeyer M.D., Servaes K., Vanermen G., Boon P.E., De Henauw S., 2012., Phthalates dietary exposure and food sources for Belgian preschool children and adults, Environmental International 48, 102 – 108

Spillmann M.D., Siegrist M., Keller C., Wormuth M., 2009., Phthalate exposure trought food and consumers risk perception of chemicals in food, Risk Analysis. 29 (8) 1170 – 81

Stahlhut R.W., van Wijngaarden E.,Dye T.D., Cook S., Swan S.H., 2007., Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult US males, Environ Health Perspect. 115, 876 – 882, doi: 10.1289/ehp.9882

Swan S.H.,2008., Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans, Environ Res. 108, 177 – 184, doi: 10.1016/j.envres.2008.08.007

Swan S.H., Main K.M., Liu F., Stewart S.L., Kruse R.L., Calafat A.M.,Mao C.S., Redmon J.B., Ternand C.L., Sullivan S., Teague J.L., 2005., Decrease in anogenital distance among male infants with prenatal phthalate exposure, Environ Health Perspect 113, 1056 – 1061

Thompson R. C., Swan S. H., Moore C. J., vom Saal F. S., 2009a., Our plastic age. Phil. Trans. R. Soc. B 364, 1973 – 1976

Thompson R. C., Moore C. J., vom Saal F. S., Swan S. H., 2009b, Plastics, the environment and human health: current consensus and future trends, Phil. Trans. R. Soc. B 364, 2153 – 2166

Tsumura Y., Ishimitsu S., Kaihara A., Yoshii K., Nakamura Y., Tonogai Y., 2001a., Di(2-ethylhexyl) phthalate contamination of retail packed lunches caused by PVC gloves used in thepreparation of foods, Food Addit Contam 18, 569 – 579

Tsumura Y., Ishimitsu S., Saito I., Sakai H., Kobayashi Y., Tonogai Y., 2001b., Eleven phthalate esters and di(2-ethylhexyl) adipatein one-week duplicate diet samples obtained from hospitals andtheir estimated daily intake, Food Addit Contam 18, 449 – 460

Tsumura Y., Ishimitsu S., Hirayama K., Fujimaki T., Nakazawa H., Tonogai Y., 2002a., Migrationof di(2-ethylhexyl) phthalate from polyvinyl chloride tubes used in preparation of foods, Shokuhin Eiseigaku Zasshi 43(4), 254 – 9

Vidić Š., 2008., Nazočnost estera ftalne kiseline (ftalati) u infuzijskim otopinama, Magistarski rad, Prirodoslovno - matematički fakultet, Zagreb

Zota A.R., Calafat A.M., Woodruff T.J., 2014., Temporal trends in phthalate exposures: Findings from the National Health and Nutrition Examination Survey 2001–2010. Environ Health Perspect. 122(3), 235 – 241, doi: 10.1289/ehp.1306681

Wagner M., Oehlmann J., 2009., Endocrine disruptors in bottled mineral water:total estrogenic burden and migration from plastic bottles, EnvironmentalScience and Pollution Research 16, 278 – 286

Wenzl T., 2009., Methods for the determination of phthalates in food-Outcome of a survey conducted among European food control laboratories JRC Scientific and Technical reports. http://www.bezpecnostpotravin.cz/UserFiles/File/Publikace/ftalaty.pdf (accessed November 2014)

WHO, World Health Organization: Di-n-Butyl Phthalate. International Programme on Chemical Safety, WHO Environmental Health Criteria 189, Ženeva

World Health Organization (WHO)., 2011., Guidelines for DrinkingWater Quality. Chapter 8: Chemical Aspects. 4th ed.Geneva: World Health Organization

WHO, State of the science of endocrine disrupting chemicals, 2012.,An assessment of the state of thescience of endocrine disruptors prepared by a group of experts for the United Nations EnvironmentProgramme (UNEP)

Wittassek M., Koch H.M., Angerer J., Bruning T., 2011., Assessing exposure to phthalates – the human biomonitoring approach. Mol Nutr Food Res. 55, 7 – 31, doi: 10.1002/mnfr.201000121

Wormuth M., Scheringer M., Vollenweider M., Hungerbuhler K., 2006., What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?, Risk Anal. 26, 803 – 82, doi: 10.1111/j.1539-6924.2006.00770.x

Recived: June 17, 2020
Accepted: July 12, 2020

Quality of Life (2020) 11(3-4):126-130

DOI: 10.7251/QOL2003126B **UDC:** 616.71-007.234:618.173

Review Article

Association of Urolithiasis With Osteoporosis in Postmenopausal Women: Risk Predictors for the Disease

Radojka Bijelić[1], Snježana Miličević[2]
[1]*Health Center Banja Luka, BiH*
[2]*Urology Clinic, RS University Clinical Center, BiH*

Abstract: Urolithiasis and osteoporosis are two significant multifactorial diseases that cause the constant increase in the number of affected persons due to the increased age of population and negative effects of environmental factors, i.e. unhealthy lifestyle. Those affected by urolithiasis have an increased risk for osteoporosis. Association of urolithiasis and osteoporosis in postmenopausal women is still not completely understood, but it is certain that those diseases may cause serious consequences leading to the permanent disability and even death due to osteoporotic hip fractures. This is why those disorders remain very significant social, economic and health problems not only for those affected but for the whole society, due to very high treatment costs. Identification of risk factors for menopausal women aims at decreasing the rate of disease and improving of preventive measures. Since both disorders are preventable, preventive measures should be applied from young age, with identification of risk factors being extremely important for significant decrease of morbidity rate.

Key words: osteoporosis, urolithiasis, postmenopause, risk factors.

INTRODUCTION

Urolithiasis and osteoporosis are two pathological entities that represent serious metabolic disorders and significantly contribute to increased disability rate of affected persons. Osteoporosis is metabolic bone disease causing very significant public health problems worldwide, with postmenopausal women being especially affected. Also, urolithiasis remains one of the biggest health problems, with its prevalence constantly increasing in the last two decades and its increased rate among female population; studies have shown that more than 7% of women had an episode of symptomatic urolithiasis in their middle age (1, 2).

This review article discusses different pathophysiological mechanisms contributing to development of those diseases, their age and sex distribution, overweight and obesity as important segments of metabolic syndrome, as well as bad lifestyle habits (smoking, drinking coffee and alcohol, insufficient physical activity) as strong predictors of those diseases. Timely preventive measures and educating population on risk factors may greatly reduce mortality and morbidity rates of those diseases.

PATHOPHYSIOLOGICAL MECHANISMS

Association of urolithiasis and osteoporosis in postmenopausal women may be caused by several reasons. Clinical research revealed increased destruction and reduced bone mass in patients with calcium urolithiasis. Some studies indicated that hypercalciuria and increased excretion of potassium, which are present in majority of urolithiasis patients, turned out to be major risk factors for osteoporosis and bone fracture in postmenopausal women (3-7). In their study, Denburg et al. also concluded that urolithiasis is associated with high incidence of bone fracture, especially in women older than 70 (8). On the other hand, some studies found reduced bone density in patients with normal calciuria (9, 10).

Bone mass is reduced in all patients with urolithiasis, i.e. there is a reciprocal relationship between

urolithiasis and loss of bone mass, regardless of the value of some serum parameters (11). Patients with recurrent calcium urolithiasis and idiopathic hypercalciuria have reduced bone mineral density (BMD), i.e. they have increased incidence of osteopenia and osteoporosis measured by DEXA method (12). In postmenopausal women there may be some additional problems present: negative balance of calcium, which is doubled during menopause, increased values of parathyroid hormone (PTH) and low level of estrogen. All that may result in lower levels of serum calcium, with consequent formation of calcium calculi, reduced bone mineral density (BMD) and development of disease (13,14).

AGE AND SEX

Regardless of numerous scientific studies, the association of urolithiasis and osteoporosis in women remains controversial (15). It should be emphasized that although urolithiasis and osteoporosis are two different pathological entities, both are characterized with high risk of complications (fracture of hip, spine), especially in older population, which may have severe consequences for the overall health of patients, leading to disability and even death (16). Scientific studies show increased presence of osteopenia and osteoporosis in older population, meaning that patient's age (over 60) represent important risk factor for urolithiasis and osteoporosis. Prevalence of osteoporosis increases with age; 2-8% of men and 9-38% of women over 50 who live in industrialized countries are affected by this disease. Results of numerous studies show that osteoporosis incidence increases with age, approximately by 20% (16, 17). Normal DEXA findings decrease with age, i.e. the percentage of those with abnormal findings is increased in older population (18). New studies by multiple regression show significant impact of age and daily calcium intake on bone mass loss in urolithiasis patients. Significantly reduced bone mass was found in urolithiasis patients compared to control group, and it was more prevalent in older population, especially women in menopause (19).

Urolithiasis prevalence is increased worldwide, including both sexes and different age groups. Women are especially affected in USA. Overweight is important risk factor in development of this disease, as well as insulin resistance and hypertension, conditions met in metabolic syndrome, and they contribute to this phenomenon (20). Recent scientific research revealed higher percentage of urolithiasis patients who also have osteopenia to be women in older age (over 60). Bone mineral density analysis by DEXA method show significantly higher percentage of women with osteopenia and urolithiasis (36.1%) compared to men (2.1%), which represent statistically very significant difference (21).

OVERWEIGHT AND OBESITY

Numerous studies indicated overweight as significant factor contributing to development of urolithiasis. According to the estimate of World Health Organization (WHO), 1.7 billion people worldwide are affected by obesity (BMI > 30 kg/m^2) and overweight (BMI 25-29 kg/m^2), and increased urolithiasis incidence -- higher than 75% was found in overweight and obese patients compared to persons of normal body weight (22-24). Numerous studies revealed that overweight and increasing body mass directly correlated with increased risk of kidney disease like urolithiasis, where increased risk had the tendency of being higher in women than in men (25). Wrobel et al. indicated association of overweight and kidney disease, like calcium-oxalate calculi, with significant results related to BMI, urine pH and urine citrates, but overweight was not found to be risk predictor for recurrent calcium-oxalate calculi (26). Recent studies have shown association between increased BMI and urolithiasis by age groups, i.e. there is statistically significant correlation between older age (over 60) and urolithiasis (27).

For years overweight and obesity have been considered protective factors for bones and osteoporosis development. Nevertheless, new studies show that obesity, being one of main components of metabolic

syndrome, has negative effects on bones despite normal values of bone mineral density (BMD) measured by DEXA method, which is considered to be the consequence of increased mechanical pressure on the bone (28, 29). Ong et al. think that higher BMD is not protective factor for osteoporotic fractures, for significant number of such fractures occurs in obese women due to body habitus or mechanism of injury (30). Research in this area found obesity to be very significant risk factor affecting reduction of bone mineral density and occurrence of vertebral fractures (27, 31). Adipose tissue functions as endocrine organ and releases more adipokines that modulate metabolism, inflammatory reactions, insulin resistance and disrupts normal homeostasis of bone cells, which may cause reduced bone mineral density and disease (32-34). Reference data indicate that overweight and obesity are more prevalent in men with urolithiasis than in women, while the results of linear regressive analysis show significant positive relationship between increased BMI among men and women with urolithiasis ($p = 0.015$) (25, 35).

Data on longitudinal correlation between BMI and bone mineral density from early childhood (early adolescence), being the period in life when bone density is mostly formed, and during its later variations, are very scarce. Also, association of adipose and muscle tissues with bone mineral density in postmenopausal women may be the result of many factors working together, mainly genetic components and different effects of environmental risk factors, which should be within the scope of some future studies.

GENETIC PREDISPOSITION AND ENVIRONMENTAL FACTORS

There are numerous factors that increase the risk of urolithiasis and osteoporosis. The data indicate increase of urolithiasis prevalence by 70% in the last 15 years, affecting all age and ethnic groups, especially women (36), where it is associated with osteoporosis. Scientists agree that those two diseases represent the consequence of different factors working together, including genetic predisposition, environmental factors (dietary habits, different lifestyles, bad habits), lack of physical activity and sedentary lifestyle.

Despite all the efforts, *genetic predisposition* for urolithiasis has not been clearly identified so far. Genome-wide association studies are widely used for identification of genetic risk factors for different diseases, for they lighten the load of DNA sequence examination with the aim of finding mutations, variants and single nucleotide polymorphisms (SNPs) (37). Such polymorphisms play very important role in determining the genes associated with urolithiasis, and understanding ways of their association might aid in interpretation of genomic initiators of lithogenesis. Also, understanding responsible genes might lead to more effective and directed genetic therapy and better prevention of this disease in the future (38).

The reference data indicate that different environmental factors, together with adopted bad lifestyle habits, may be important predictors of development of diseases like urolithiasis and osteoporosis in postmenopausal women. Increased intake of salt (6, 39), calories (40), animal proteins (41), lower calcium intake (4) and decreased intake of fluids (41) are significant risk factors that individually or in synergism may lead to stone formation. *Smoking* is an independent risk factor for osteoporosis in postmenopausal women (42) and many studies indicate much higher osteoporosis prevalence in group of smokers (31.3%) compared to former smokers (28.6%) or non-smokers (7.5%) (43-45). Also, *excessive consumption of alcohol and coffee* may lead to rapid bone loss in lumbar spine in older postmenopausal women (46-48), which is related to increased risk of fractures. Research revealed that daily consumption of large amounts of alcohol has negative effects on mechanisms of bone remodeling, i.e. has direct negative effects on bone homeostasis (49, 50), thus representing significant risk factor for osteoporosis and bone fracture (51, 52).

Physical activity is significant protective factor for bones; more active women are more rarely affected by osteoporosis. It is a known fact that increased bone density results from increased mechanical load on bones and increased activity of bone cells osteoblasts (53). Studies show that recreational sports or

active walking for 30-60 minutes more than twice a week reduces the risk of osteoporosis in women (54). Exercising improve the balance and movement coordination, as well as functional muscle activity, which reduces the risk of falls and fractures (55, 56). That is why regular exercises are required for healthy bones, but physical activities should be adjusted individually.

REFERENCES

[1] L. D. Carbone, K.M .Hovey, C.A. Andrews et al. Urinary Tract Stones and Osteoporosis: Findings from the Women's Health Initiative. Journal of Bone and Mineral Resarch. Vol 30 No.11 Nov 2015. pp 2096-2102.

[2] Brauer CA, Coca- Perraillon M, Cutler DM et al. Incidence and mortality of hip fractures in the United States.JAMA, 2009; 302(14):1573-9.

[3] Giannini S, Nobile M, Daile Carbonate l et al. Hypercalciuria is a common and important finding in postmenopausal women with osteoporosis. Eur J Endocrinol. 2003; 149(3): 20-13.

[4] Weaver CM. Potassium and health. Adv Nutr 2013; 4(3):3685-775.

[5] Liern M, Bohorquez M, Vallejo G. Treatment of idiopathic hypercalciuria and its impact on associated disease. Arch Argent Pediatr.2013; 111(2): 110-4.

[6] Aruga S, Honma Y. Renal calcium excretion and urolithiasis. Clin Calcium. 2011; 21(10): 1465-72.

[7] Taylor EN, Feskanich D, Paik M et al. Nephrolithiasis and risk of incident bone fracture. J Urol 2016; 195: 1482-6.

[8] Denburg MR, Leonard MB, Haynes K et al. Risk of Fracture in Urolithiasis: A Population-Based Cohort Study Using the Health Improvement Network. Clin J Am Soc Nephrol 2014; 9: 2133–2140.

[9] Tsuji H, Umekawa T, Kurita T, et all. Analysis of bone mineral density in urolithiasis patients. Int urol 2005; 12: 335-9.

[10] Peris P, Martinez- Ferrer A, Monegal A, et all. Aetiology and clinical characteristics of male osteoporosis. Have they changed in the last few years? Clinin Exp Rheumatol 2008; 26: 582-8.

[11] Jaeger P, Lippuner K, Casez JP, Hess B, Ackermann D, Hug C. Low bone mass in

[12] idiopathic renal stone formers: magnitude and significance. J bone Miner Res 1994; 9: 1525-32.

[13] Arrabal –Polo, Miguel A, del Carmen Cano- Garcia et al. Calcium nephrolithiasis and bone demineralization: pathophysiology, diagnosis, and medical management. Curr Opin. In Urology: Nov 2014; vol. 24(6): 633-638.

[14] Heilberg IP, Weisinger JR. Bone disease in idiopathic hypercalciuria. Curr Opin Nephrol Hypertens 2006; 15: 394-402.

[15] Caudarella R, Vescini F, Buffa A, et all. Bone mass loss in calcium stone disease: focus on hypercalciuria and metabolic factors. J Nephrol 2003; 16: 260-6.

[16] Keller JJ, Lin CC, Kang JH et al. Association between osteoporosis and urinary calculus: evidence from a population- based study. Osteoporosis Int. 2013; 24(2): 651-7.

[17] Yen-Man Lu, Tsu-Ming Chien, Ching-Chia Li et al. Urolithiasis is associated with the increased risk for osteoporosis: A nationwide 9-year follow-up study. Urological Science XXX.2017; 1-5.

[18] Wade SW, Strader C, Fitzpatrick LA et al. Estimating prevalence of osteoporosis: examples from industrialized countries. Arch Osteoporosis 2014; 9: 182.

[19] Bijelic R, Milicevic S, Balaban J. Correlation of osteoporosis and calcium urolithiasis in adult population. Med Arch 2016; 70: 66-8.

[20] Trinchieri A. Bone mineral content in calcium renal stone formers. Urol Res 2005; 33: 247-53.

[21] Seitz, C. & Fajkovic, H. Epidemiological gender-specific aspects in urolithiasis. World J Urol. Oct. 2013; 31(5): 1087-1092.

[22] Bijelic R. Balaban M. Milicevic S. Gender Representation of Osteoporosis in Patients with Urolithiasis. Med Arh. Oct.2015; 69(5): 331-333.

[23] YeeV. Wong, Cook P, Somani BK. The association of Metabolic Syndrome and Urolithiasis. Int. Journal of End. 2015; p.9.

[24] James PT, Leach R, Kalamara E, Shayeghi M. The world-wide obesity epidemic. Obesity Res. 2001; 9 (4): 228- 233.

[25] Antonelli JA, Maalouf NM, Perale MS. et al. Use of the national health and nutrition examination survey to calculate the impact of obesity and diabetes on cost and prevalence of urolithiasis in 2030. Europ.Urology 2014; 66(4): 724-729.

[26] Taylor EN, Stampfer, Curhan. Obesity, weight gain, and the risk of kidney stones. Journal of american Med Association. 2005; 293(4): 455-462.

[27] Wrobel BM, Wrobel B, Hormann wt al. Overweight and obesity: risk factors in calcium oxalate stone disease?. Adv in Urology 2012; p. 4.

[28] Milicevic S, Bijelic R, Krivokuca V at all. Correlation of the Body Mass Index and Calcium Nephrolithiasis in Adult Population. Med Arh. Dec 2013;67(6): 393-396.

[29] Migliaccio S, Greco EA, Fornari R, Donini LM, Luigi LG, et al. Skeletal alterations in women affected by obesity. Aging Clin Exp Res 2013; 25 (1): 35-37.

[30] Caffareli C, Alessi C, Nuti R, Gonnelli S. Divergent effects of obesity on fragility fractures. Clin Interv Aging 2014; 9: 1629- 1636.

[31] Ong T, Opinder S, Tan W, Marshall L. A United Kingdom perceptive on the relationship between body mass index (BMI) and bone health: A cross sectional analysis of data from of Nottingham Fracture Liaison Service. Bone 2014; vol 59: 207-210.

[32] Kim KC, Shin DH, Lee SY, Im JA, Lee DC. Relation between obesity and bone mineral density and vertebral fractures in Korean postmenopausal women. Yonsei Med J 2010; 51: 857.

[33] Gomez-Ambrosi J, Rodriguez A. The bone-adipose axis in obesity and weight loss. Obes Surg 2008; 18(9):1134–1143.

[34] Gimble JM, Zvonic S. Playing with bone and fat. J Cellular Biochem 2006; 98:251–266.

[35] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. JCEM 2004; 89 (6): 2548–2556

[36] Daudon M, Lacour B, Jungers P. Influence of body size on urinary stone composition in men and women. Urol Res 2006; 34(3): 193-9.

[37] Scales CD Jr, Hanley JM, Saigal CS. Urologic Disease in America Project. Prevalence of kidney stones in the United States. Eur Urol.2012; 62(1) 160-5.

[38] Yasui T, Okada A, Hamamoto S et al. Pathophysiology- based treatment of urolithiasis. Int J Urol, 2017; 24: 32-38.

[39] Taguchi K, Yasui T, Milliner DS et al. Genetic risk factors for urolithiasis: A systematic review of the literature and causal network analysis. Europ.Urology Focus 2017; 3(1): 72-81.

[40] **Caudarella R, VesciniP, Rizzoli E et al. Salt intake, hypertension and osteoporosis. J Endocrinol. Invest. 2009; 32(4): 15-20.**

[41] **Daudon M. Epidemiology of nephrolithiasis in France. Ann Urol.2005; 39(6): 209-31.**

[42] **Sorensen MD, Kahn AJ, Reiner AP et al. Impact of nutrition factors on incident kidney stone formation: a report from the WHI OS. J Urol 2012; 187(5): 1645-9.**

[43] Bijelic R, Milicevic S, Balaban J. Risk Factors for Osteoporosis in Postmenopausal Women. Med Arch. Feb 2017; 71(1): 26-29.

[44] Bosić-Živanović D, Markov Z. Faktori rizika koji su povezani sa smanjenjem mineralne gustine kosti (Risk factors related to reduced bone mineral density). Opšta medicina 2011;17(1- 2): 28-35.

[45] Baheiraei A, Pocock NA, Eisman JA, Nguyen DN. Bone mineral density, body mass index and cigarette smoking among Iranian women: Implications for prevention. Musculoskelet Disord 2005; 24: 6-34.

[46] Øyin J, Gjesdal CG, Nyagård OK, Lie SA, Mayer HE et al. Smoking and Body Fat Mass in Relation to Bone Mineral Density and Hip Fractures: The Hordaland Health Study. PloS ONE 2014; 9(6): e101335 doi

[47] Yang P, Zhang XZ, Zhang K, Tang Z. Associations between frequency of coffee consumption and osteoporosis in Chinese postmenopausal women. Int J Clin Exp Med 2015; 8(9): 15958- 15966

[48] Shuai L, Zhipeng D, Qiang W. Effect of coffee intake on hip fracture: a meta- analysis of prospective cohort. Nutr J 2015; 14:38.

[49] Wolk A, Glynn A, Warensjö E, Byberg L, Michaëlsson K. Coffee consumption in relation to osteoporotic fracture risk and bone mineral density: a prospective longitudinal cohort study. Bone 2013; 50: 36.

[50] Diamond T, Stiel D, Lunzer M, Wilkinson M, Posen S. Ethanol reduces bone formation and may cause osteoporosis. Am J Med 1989; 86: 282-288.

[51] Samson HW. Alcohol's harmful effects on bone. Alcohol Health Res World 1998; 22: 190-19.

[52] Kanis JA, Johanssen H, Johnell O. Alcohol intake as a risk factor for fracture. Osteoporosis Int 2005; 16: 737-42.

[53] Zhang X, Yu Z, Yu M, Qu X. Alcohol consumption and hip fracture risk. Osteoporosis Int 2015; 26: 531- 542.

[54] Frost HM. Vital biomechanics: proposed general concepts for skeletal adaptations to mechanical usage. Calcification Tissue International 1988; 42: 145-56.

[55] Schmitt NM, Schmitt J, Doren M. The role of physical activity in the prevention of osteoporosis in postmenopausal women - An update. Maturitas 2009; 63: 34-8.

[56] Dušek T, Kaštelan D, Pećina M. Tjelesna aktivnost u prevenciji osteoporoze (Physical activity in osteoporosis prevention). Arh Hig Rada Toksikol 2012; 63(3): 41-46.

[57] Carter ND, Kannus P, Khan KM. Exercise in the prevention of falls in older people: a systematic literature review examining the rationale and the evidence. Sports Med 2001; 31: 427- 38.

Recived: July 7, 2020
Accepted: July 17, 2020

Instructions to Authors

Quality of Life publishes original research papers and reviews and aims to provide a forum for the rapid dissemination of significant novel research in the various disciplines encompassing the Science and Technology of Food, Public health engineering, Sanitary inspection and control, Environmental and Public Health.

Quality of Life **publishes** original scientific papers, preliminary communications, professional papers, scientific notes and reviews:

Original scientific papers report unpublished results of original research. They must contain significant and original observations to be critically evaluated. Experimental data should be presented in a way that enables reproduction and verification of analyses and deductions on which the conclusions are based.

Preliminary communications include short information on the results of scientific research which require immediate publication.

Scientific notes include reports on shorter but completed research or descriptions of original laboratory techniques (methods, apparatus etc.) and should be concise.

Reviews are original, critical and up-to-date surveys of an area in which, preferably, the author himself/herself is active. They should include recent references from international publications.

Professional papers present new possibilities of improvement within areas of science. The emphasis is on the application of known methods and facts as well as on broadening the knowledge in the particular area. The acquired knowledge is applied to the object of research.

Difference between scientific and professional papers is in their original results and conclusions as well as method used. Although professional paper may be more useful for the application it is not considered as a new scientific contribution.

Papers previously reported at a congress, symposium etc. will be published only if they have not previously been published in proceedings. However, these papers are subject to regular editorial procedure, i.e. evaluation by referees and revision.

Submission

Only the manuscripts that conform to the following instructions will be considered:

The manuscript should be submitted in duplicate printouts of 10–15 typewritten pages with 1.5 spacing on one side of the paper (A4 format) accompanied by the identical file by e-mail (preferably Microsoft Word compatible formats). Normal plain font should be used (Times New Roman, font size 12) for both text and figures.

All papers must be written in English. If English is not the authors' first language, the manuscript should be given to a native speaker of English for proofreading.

The cover letter should contain full names (with underlined surnames) of all authors, their titles and their signatures confirming that manuscript or part of it was not accepted for publication or being considered for publication or published somewhere else.

The manuscript must contain full names and business addresses of all authors with asterisk next to the name of the corresponding author. Footnote at the bottom of the first page should contain information about the corresponding author (phone, fax and e-mail).

Latin words, phrases and abbreviations, including generic and specific names, should be written in italic. The references should be cited with ordinal numbers of the references in round brackets, with only the number written in italic.

Figures, tables and their legends should be included at the end of the document and their position marked in the text.

The manuscripts should be sent to the following address:

Quality of Life, Editorial Board,
Pan-European University APEIRON
Pere Krece 13,
78000 Banja Luka, Bosnia and Hercegovina,
phone: +38451 247 910 and fax: +387 51 247921,
e-mail: info@qol-au.com, sekretar@qol-au.com, redakcija@qol-au.com
URL: http://www.qol-au.com

Potential referees

All papers will be peer reviewed. Authors are asked to submit full contact details, including e-mail addresses, for three potential referees. Referees should be experts in the field of the paper, and not associated with the institution with which the authors are affiliated. The final choice of referees will remain entirely with the Editor.

General format

For clearness the paper should be divided into the following sections: **Title Page**, **Abstract**, *Key words*, **Introduction**, **Materials and Methods**, **Results**, **Discussion** (**Results and Discussion**), **Conclusions**, *Acknowledgements*, and **References**.

Title Page

The title page should be devoted to the title (in caps), the full name(s) of the author(s), and the full postal addresses for all co-authors. In multi-authored texts indicate author affiliation by superscript Arabic numbers placed after author's name and before the appropriate address. Clearly indicate who is willing to handle correspondence at all stages of refereeing, publication and post-publication. The corresponding author should be identified with an asterisk. A footnote should contain an e-mail address, telephone number and fax number for the corresponding author. Title should be concise and explanatory of the content of the paper.

Abstract

Abstract (not longer than 250 words) should explain the aim of the paper and include the most relevant results and conclusions. Directly below the summary, authors should provide the key words.

Key words

Key words should list the main topic of the paper and should not contain more than 6 words or phrases, which should be separated by commas.

Introduction

It is particularly important that the introductory part be as brief as possible and clear in description of the aims of investigation. Previous relevant work regarding the topic of the manuscript should be included with references.

Materials and Methods

Experimental part should be written clearly and in sufficient detail to allow the work to be repeated. Detailed description is required only for new techniques

and procedures, while the known methods must be cited in the references. For chemicals and apparatus used full data should be given including the name, company/manufacturer and country of origin. Statistical analysis should also be included. All unnecessary details should be omitted from the experimental part. Spectra, chromatograms and similar will not be published if their only purpose is to additionally characterize particular compounds.

Results and Discussion

Results and Discussion can be written as two separate or one combined section. Discussion should not be merely the repetition of the obtained results. Combining the results with discussion can simplify the presentation.

Each table and illustration must have all necessary information to be understood independently of the text. The same data should not be reproduced in both diagrams and tables. Whenever, possible formulae and equations are to be written in one line.

All figures (graphs, photographs, diagrams, etc.) and tables should be cited in the text and numbered consecutively throughout. Preferred program for writing figures and tables is Excel. The placement of figures and tables should be indicated. The size of letters and other symbols on diagrams and figures should be such as to allow reduction to column width without loss in legibility. Several figures should be grouped in a plate on one page. Unmounted figures are preferred. Figures and other illustrations should be of good quality, well-contrasted and black and white. If authors insist on color prints, they are requested to cover the additional cost of printing.

Figure legends should be placed at the bottom of each figure, while table headings should appear above the tables. The values on the x- and y-axes must be clearly and precisely defined, decimal numbers must have decimal points, not commas. Footnotes to tables should be indicated by superscript letters or symbols. Experimental error and statistical significance should be stated clearly.

Nomenclature

If symbols, letters and abbreviations are used in the text they should be listed with their explanations. **SI (Système International) units** should be used. **Nomenclature** of inorganic and organic compounds should conform to the rules of the International Union of Pure and Applied Chemistry (IUPAC).

Conclusion

It should indicate the significant contribution of the manuscript with its applications.

Acknowledgements

Acknowledgements to colleagues or institutions or companies for donations or any other assistance are recommended to be put at the end of the manuscript, before references, rather than in the text.

References

All publications cited in the text should be presented in a list of references following the text of the manuscript. No more than 30 references should be cited in your manuscript. In the text refer to the author's name (without initials) and year of publication (e.g. "Martinez, Fearne, Caswell and Henson (2007) studied the effects..." or "...similar to values reported by others (Soares, 1998)..."). For 2-6 authors all authors are to be listed at first citation. At subsequent citations use first author et al. When there are more than 6 authors, first author et al. should be used throughout the text. The list of references should be arranged alphabetically by authors' names and should be as full as possible, listing all authors, the full title of articles and journals, publisher and year. The manuscript should be carefully checked to ensure that the spelling of authors' names and dates are exactly the same in the text as in the reference list.

References should be given in the following form:

References to a journal publication:

Scollan, N., Hocquette, J., Nuernberg, K., Dannenberger, D., Richardson, I. & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science, 74(1), 17–33.

Dransfield, E., Martin, J. F., Fisher, A., Nute, G. R., Zygyiannis, D., Stamataris, C., et al. (2000). Home placement testing of lamb conducted in six countries. Journal of Sensory Studies, 15(4), 421–436.

Beltran, E., Pla, R., Yuste, M., & Mor-Mur, M. (2003). Lipid oxidation of pressurized and cooked chicken: Role of sodium chloride and mechanical processing on TBARS and hexanal values. Meat Science, 64(1), 19–25.

Mann, N. (2000). Dietary lean red meat and human evolution. European Journal of Clinical Nutrition, 39, 71–79.

Johansson, J. K. (1989). Determinants and effects of the use of 'made in' labels. International Marketing Review, 6, 47–58.

Scott, J. M. (1999). Folate and vitamin B12. Proceedings of the Nutrition Society, 58, 441–448.

Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: A review. Journal of Muscle Foods, 1, 217–243.

Ramanathan, R., Konda, M. K. R., Mancini, R. A., & Faustman, C. (2009). Speciesspecific effects of sarcoplasmic extracts on lipid oxidation in vitro. Journal of Food Science, 74, C73–C76.

References to a conference:

Savell, J. W., & Shackelford, S. D. (1992). The significance of tenderness to the meat industry. In Proceedings of the 45th Reciprocal Meat Conference (pp. 43–46). Chicago, IL.

Joseph, P., Suman, S. P., Li, S., Beach, C. M., & Claus, J. R. (2008). Mass spectrometric characterization and thermostability of turkey myoglobin. In Proceedings of 61st annual reciprocal meat conference, Gainesville, FL. Abstract no. 87.

References to a book:

Meilgaard M., Civille G.V., & Carr T.B. (1999). Sensory Evaluation Techniques. (3rd ed.). CRC Press, Printed in USA.

Strunk, W., Jr., & White, E. B. (1979). The elements of style. (3rd ed.). New York: Macmillan, (Chapter 4).

Morgan, J. B., Cannon, J. B., McKeith, F. K., Meeker, D., & Smith, G. C. (1993). National pork chain quality audit (packer-processor-distributor). Final Report to the National Pork Producers Council.

USDA. (1997). USDA advises consumers to use a meat thermometer when cooking hamburger. FSIS News and Information Bulletin. FSIS, USDA. Washington, DC.

USDA-FSIS (2005). Federal register notice: HACCP plan reassessment for mechanically tenderized beef products. Federal Register, 70, 30331–30334.

References to a chapter in an edited book:

Gudmundsson, M., & Hafsteinsson, H. (2002). New non-thermal techniques for processing seafood. In H. A. Bremner (Ed.), Safety and quality issues in fish processing (pp. 308–329). Cambridge, England: CRC Press, Woodhead Publishing Ltd.

Olson, J. C. (1977). Price as an informational cue: Effects on product evaluations. In A. G. Woodside, J. N. Sheth, & P. D. Bennett (Eds.), Consumer and industrial buying behavior (pp. 267–286). New York: Elsevier.

Monroe, K. B., & Krishnan, R. (1985). The effect of price on subjective product evaluations. In J. Jacoby & J. C. Olson (Eds.), Perceived quality: How consumers view stores and merchandise (pp. 209–232). Toronto: Lexington.

References to a internet:

World Health Organisation. (1990). Diet, nutrition and the prevention of chronic disease. Technical Report Series No. 797. Geneva: WHO. Available at <http://www.who.int/dietphysicalactivity/publications/trs916/en/gsfao_global.pdf>. Accessed 10.06.10

National Bison Association. (2009). <http://www.bisoncentral.com>. Accessed 22.06.10

Bowater, F. J. (2001). Rapid carcass chilling plants compared to conventional systems. International Institute of Refrigeration. <www.fjb.co.uk>. Accessed 12.05.10.

Soares, N. F. F. (1998). Bitterness reduction in citrus juice through nariginase immobilized into polymer film. New York: Cornell University. 130 p. (PhD Dissertation).

Citing and listing of web references. As a minimum, the full URL should be given. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.(example: Abbott, A., Basurto, M., Daley, C.A., Nader, G. and Larsen, S. (2004). Enhanced nutrient content of grass-fed beef: justification for health benefit label claim. Available at: http://www.csuchico.edu/agr/grassfedbeef/health-benefits/index.html (Accessed: 11 July, 2007). Anonymous. (2007). 2006 International Beef Quality Perceptions Survey. Canadian Beef Export Federation (http://www.cbef.com, Accessed 10 October 2007).

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly “Articles in Press” because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (example taken from a document in the journal *Physics Letters B:* doi:10.1016/jphysletb.2003.10.071

When you use the DOI to create URL hyperlinks to documents on the web, they are guaranteed never to change.

Additional Information

Review Process

All manuscripts are sent to at least two independent referees who will be asked to complete the refereeing job within 4 to 6 weeks. The final decision regarding acceptance will be made by the Editors. Manuscripts may be sent back to authors for revision if necessary. Revised manuscript submissions should be made as soon as possible (within 4 weeks) after the receipt of the referees’ comments.

Proofs

One set of page proofs in PDF format will be sent by e-mail to the corresponding Author. The author may list the corrections and return to the journal in an e-mail. Please list the corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments on a printout of your proof and return by fax, or scan the pages and e-mail, or by post.

This proof should only be used for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will not be considered at this stage. It is important to ensure that all corrections are sent back to the journal in one communication. The publication of the article may be proceeded if no response is received.

Proofs must be corrected and returned to the publishers within 48 hours of receipt.

Offprints: The corresponding author, at no cost, will be provided with a PDF file of the article (e-offprints) via e-mail and 10 free paper offprints.

Copyright

The authors bear the sole responsibility for the content of the contributions. The Editorial Board assumes that by submitting their papers the authors have not violated any internal rules or regulations of their institutions related to the content of the contributions and that they have not submitted the paper somewhere else. The acceptance of the paper obliges the authors not to publish the same material elsewhere. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

Contents

I SSN 1986- 6020