საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 12, №3, 2018
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 12, no.3, 2018

Biochemistry

Phenolic Compounds and Antioxidant Properties of some White Varieties of Grape Wines Spread in Western Georgia

Maia Kharadze*, Indira Djaparidze*, Armaz Shalashvili, Maia Vanidze*, Aleko Kalandia***

** Georgian Batumi Shota Rustaveli State University, Batumi, Georgia*
*** Georgian Agrarian University, Tbilisi, Georgia*

(Presented by Academy Member Tengiz Beridze)

ABSTRACT. The content of phenolic compounds and antioxidant properties of wines made of five varieties of white grapes were studied in three regions of Western Georgia (Adjara, Imereti and Samegrelo). Using the UPLC-MS method the (-) Epicatechine (m/z-289), Quercetin-3-glucoside (m/z-463), Quercetin-3-ramnoside (m/z-447), Quercitrin-3-glucuronide (m/z-477) and Procyanidin B_2 (m/z-577) were identified. With the help of the spectral methods the total amount of phenols, catechines and flavonoids was defined in the wines of all five varieties of grapes and their antioxidant properties were established. It was found that wines produced from Tsolikouri and Tsitska grapes are characterized by phenolic compound content and antioxidant action. *© 2018 Bull. Georg. Natl. Acad. Sci.*

Key words: wine, phenolic compounds, catechins, flavonoids, antioxidant activity

Culture of the Georgian wine was developed and created in wine-making regions of Western Georgia (Imereti, Adjara and Samegrelo) for centuries [1]. Phenolic compounds play a significant role in the organoleptic characteristics of wine. The color, taste, aroma and antimicrobial activity of wine depends on the phenolic compounds. The versatile biological activity of phenolic compounds including cardioprotective, anti-inflammatory and anti-cancerous action determined by their antioxidant properties is especially noteworthy [2-6].

The goal of the present work is to identify some compounds, to study the total number of phenolic compounds, catechins, flavonoids and their antioxidant activity in the wines produced from the vine varieties of (*Vitis vinifera* L.) Tsolikouri, Tsitska, Klarjuli, Krakhuna and Kutaturi grapes cultivated in three regions of western Georgia (Adjara, Imereti, Samegrelo).

Tsolikouri is a local Imeretian variety spread in almost every district of Western Georgia. There are various types of table wines produced from Tsolikouri, which are characterized by good taste and rich chemical composition. Apart from relatively high-alcohol content Tsolikouri wine has a rich body and contains an ample amount of acids

Table 1. Samples of wines obtained for analysis

№	variety	Region	Region	Village	Wine
1	Colikouri	Adjara	Keda	Kokotauri	W.1
2	Colikouri	Adjara	Kobuleti	Gvara	W.2
3	Cicka	Adjara	Kobuleti	Gvara	W.3
4	Klarjuli	Adjara	Kobuleti	Gvara	W.4
5	Krachuna	Adjara	Kobuleti	Gvara	W.5
6	Qutaturi	Adjara	Kobuleti	Gvara	W.6
7	Colikouri	Samegrelo	Martvili	Bandza	W.7
8	Colikouri	Samegrelo	Martvili	Najachao	W.8
9	Colikouri	Samegrelo	Martvili	Muchurcha	W.9
10	Colikouri	Samegrelo	Martvili	Lechaidravo	W.10
11	Colikouri	Samegrelo	Martvili	Nagvazao	W.11
12	Colikouri	Samegrelo	Martvili	Vedidkari	W.12
13	Colikouri	Imereti	Bagdadi	Ofcha	W.13
14	Cicka	Imereti	Bagdadi	Ofcha	W.14

that ultimately improves the wine aging and its preservation. Tsitska is a local grape variety of high quality widespread in Imereti region. It gives the best table wine and the quality material for sparkling wines. The Tsitska table wine is of a light beige color with greenish tone. It is characterized by a full body, energy and pleasure with delicious and harmonious taste. In aging, it develops a very pleasant gentle bouquet. Krakhuna gives a high quality table wine. The wine made in a European way is yellowish beige characterized by maturity, energy and pleasant taste. Imeretian wine is darker and is characterized by a peculiar varietal aroma, energy and full body. Klarjuli belongs to a group of white grape varieties. Klarjuli is one of the best varieties of table grapes spread in Georgia for its excellent taste, transportability, storage capacity (is kept until almost spring), the external beauty of bunches and grape and also for the abundant yield [13,14].

Materials and Methods

Study was conducted in the Department of Chemical Analysis and Food Safety of the Agrarian and Membrane Technology Institute, Batumi Shota Rustaveli State University. Also, at West Georgia Chromatography Center. (Grant AP/96/13 Georgia National Science Foundation).

Biochemical and chemical analyses were carried out by different physico-chemical and instrumental methods. Separation, identification and quantative analyses were carried out using UPLC-MS (Waters Acquity QDa detector), pH-meters (Mettler Toledo); refractrometer: Misco, Spectrometer – Cuvette Changer (Mettler Toledo UV5A); chemicals: radical stability - 2,2-Diphenil-1-picrilhydrazyl (Aldrich-Germany), AlCl3, Folin Ciocalteu reagent (preparation); standads – Gallic acid (Sigma), + Catechin (Teodor Schuchard), Quercetin (Loba-chemie); C18 Cartridge Solid Phase Extraction (SPE) Waters Sep-Pak C18 (500 mg); Waters Acrodisc LC PVDF Filter 13 mm 0.45 μm.

The samples of five types of grapes were obtained for analysis in October and November 2016-2017 thereof: Tsolikouri in Adjara, Imereti and Samegrelo; Tsitska in Adjara and Imereti, and Krakhuna, Klarjuli and Kutaturi from the nursery of the vine and fruit trees of the Agroservice Center (Adjara) (Table 1). All five varieties of grape samples (each 10 kg) were pressed in a grape crusher machine after removing the stem material. The grape juice was placed in a glass vessel and added by the yeast (10 CB 2000 per 25 g/hL). At the end of alcoholic fermentation the wine was clarified and placed in the refrigerator. Analyses were carried out after 5 months of wine making (Table 1).

The samples were filtered by Cartridge Solid Phase Extraction (SPE) of Waters Sep-Pak C18 (500 mg) was in conditions of 2 ml methanol,

equilibration – 2 ml water, load of 2 ml samples, wash – 2ml water + 0.1 % formic acid and elute – 4 x 1 ml acetonitrile+0.1 % formic acid. Before chromatography all the samples were filtered (0.45 μm).

Antioxidant action was determined using DPPH (2,2-Diphenyl-1-pic-rylhydrazil) methods [7,8]. For determination of radical antioxidant action the 1 ml of the sample is added by 3 ml of DPPH extract (0.1 mM DPPH - 0.004 g/100mL ethil alcohol) and after 30 minutes the optical density was evaluated on spectrophotometer. DPPH and 96% ethyl alcohol were used as blanks. For determination of action of free radical inhibition (DPPH) the following formula was used: In % = A_C – A_S/A_C*100%, where A_c indicates absorbtion of DPPH/Alcohol solution and A_s indicates absorption of the extract.

Total Phenolic compounds were defined using Folin-Ciocalteus spectral methods [9]. Extraction of the samples was conducted using 80% ethil alcohole; 0.5 or 1.0 ml of the extract was transferred to 25 ml volumetric flask, and 5.0 ml of water was added, with 1.0 ml of Folin-Ciocalteu reagent. After 8 minutes of delay at 25^0C it was added by 10.0 ml of 7% Na_2CO_3, the flask was then filled with water and left at room temperature for 2 hours. Determination was conducted at 750 nm. For control, 1 ml of extragent was used. The calculations of obtained values were performed using the calibration curve of Gallic Acid. For determination of the phenols the following formula was used: X = (D K V F)*1000 /m, where X is the amount of phenols (mg/kg); D – optical density; K – coefficient; F – factor of dilution ; V – volume of extract in ml; m – mass of the raw material used for extraction (gr).

Catechins and Procyanidine contents were determined by Swain and Hill spectral method [10]: 1 ml of samples were added 3 ml of 1 % vanillin reagents (1g vanillin added by 70 % -sulfur acid solution). All the solutions except the samples were used as blank. After 15 min, spectral adsorption was determined at 750 nm. Total amount of flavonoid content (TFC) was determined by the aluminum chloride colorimetric method as previously described [11]. Wine samples (0.5 mL) were mixed with 2 mL of distilled water and 150 μL of 5% NaNO2 solution. After 5 min, it was added by 150 μL of 10% AlCl3 and, after 6 min, by 2 mL of 1 mol/L NaOH solution. The end volume was increased to 5 mL with distilled water. Finally, the absorbance was measured at 510 nm. Results were expressed in mg/l of catechin (or Ruthin) .

Individual compounds were identified by UPLC-PDA-MS analysis methods: column BEN C18, 1.7μm, BEN Amide1.7μm; eluents acetonitrile 0.1 % formic acid, 0.1 % formic acid (gradient); flow 0.4 ml/min, column temperature 40^0C; samplers temperature 10^0C; MS- scan 100-1100 da; probe 600^0C; negative 0.8 kV, Capilarity 1.5 kV, C -20V) [12].

Results and Discussion

The study of individual compounds of white grapes made it possible to obtain individual fractions of the dominant phenolic HPLC-Prep column and to investigate their spectral characteristics UPLC-MS-PDA. By the use of UPLC-MS method the folowing compounds were identified: Procyanidin B_2 –Epicatechin-(4) –Epicatechin (retention time 2.315 Min; MW -578, m/z-577, fragments 289, λmax 280 nm); (-)-Epicatechine (retention time 2.426 Min; MW -290, m/z-289,(fragments 245) λmax 280 nm); out of flavonoids: Quercitrin-3-glucuronide(retention time 2.828 Min; MW -478, m/z-477, fragments 301, λmax 256, 354 nm); Quercetin-3- glucoside (retention time 2.833 Min; MW -464, m/z-463, fragments 301, λmax 256, 356 nm); Quercetin-3- ramnoside (ret ention time 2,949 Min; MW -448, m/z-447, fragments 301, λmax 256. 354 nm); (Fig.1), (Table 2).

Table 3 presents the data on quantitative contents and antioxidant activity of 14 samples of phenolic compounds of wine produced from five

Table 2. UPLC-PDA-MS spectral characteristics of white wines

Compound names	RT (min)	MW	[M-H]- (Fragment m/z)	UV band (nm)
(-)-Epicatechin	2.426	290	289 (245)	280
Quercitrin-3-ramnoside	2.949	448	447 (301)	256 (max), 352
Quercitrin-3-glucoside	2.833	464	463 (301)	256 (max), 356
Quercitrin-3-glucuronide	2.828	478	477 (301)	256 (max), 354
Procyanidin B_2	2.315	578	577 (289)	280

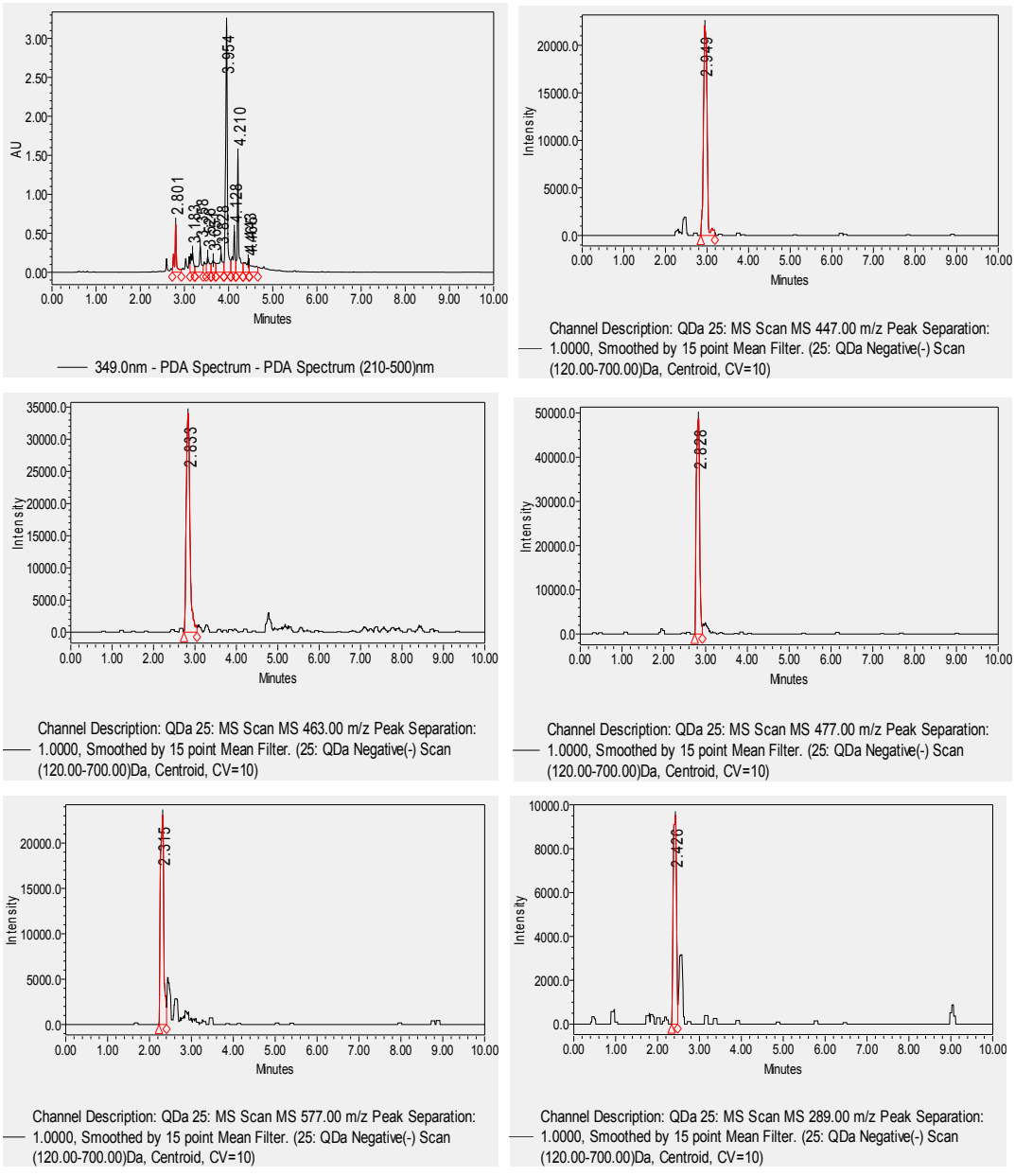

Fig. 1. Wine compounds UPLC-PDA-MS chromatograms

white varieties of grapes (Tsolikouri, Tsitska, Klarjuri and Kutaturi) in three regions of western Georgia (Adjara, Samegrelo and Imereti). In Adjara, the greatest total number of phenolic

compounds is in two wines made from Tsolikouri: W_1 (686mg /l) and W_2 (633.4mg /l). Then they are followed by Tsitska (W_3), Klarjuri (W_4) and Krakuna (W_5) with 611, 488.88, 405.8 and 386.68 mg /l, respectively.

In the wines made from every five species the amount of catechines ranges from 32.5 to 42.53 mg/l, and the amount of flavonols from 105mg to 272 mg/l. It should be noted that the wine (W_1) made from Tsolikouri grapes in the mountainous Adjara (Keda Municipality) contains greater total amount of phenols (686 mg/l) and catechines (42.35 mg / l) than the wine (W_2) made from the same variety of grapes in Kobuleti containing 633.4 and 37.96 mg /l of phenols and catechines, respectively, while flavonoids are almost equal in both wines (220.2 and 220.8 mg/l, respectively). Also, in the wines made from the Tsitska (W_{14}) and Tsolikouri (W_{13}) grapes picked in Imereti (Baghdadi Municipality) the total amount of phenols (653.22, 845.0 mg/l), catechines (44.85, 45.25 mg/l) and flavonols (392,380 mg/l) exceeds the phenolic compounds (633.4 and 611.0 mg / l), catechines (37.96 and 40. 0 mg/l) and flavonoids (220.8 and 272 mg/l) in the wines (W_2, W_3,) made from the same variety of grapes in Adjara (Kobuleti).

In the wines (W_{12}, W_9) made from Tsolikouri grapes picked in six villages of Martvili Municipality, Samegrelo Region (Table 3) the total amount of phenols ranges from 476.6 mg/l (W_{12}) to 504.2 mg/l (W_9), catechines from 45.25 mg/l (W_{12}) to 38.96 mg/l (W_9), and flavonoids from 145.9 mg/l (W_{12}) to 162.5 mg/l (W_9).

Table 3. Phenolic compounds and antioxidant action of the wines made from the Tsolikouri, Tsitska, Klarjuli, Krakhuna and Kutaturi grapes

№ Wine	Total phenols, mg/kg	Catechine, mg/kg	Flavonoids mg/kg	Antioxidant activity dilution factor 1:50 %
W.1	686.0	42.35	220.2	42.35
W.2	633.4	37.96	220.8	39.96
W.3	611.0	40.0	272	36.6
W.4	488.88	33.81	170	33.81
W.5	405.8	32.5	109	32.5
W.6	386.68	35.78	105	35.78
W.7	499.7	37.8	161.3	38.56
W.8	488.7	35.5	151.9	33.0
W.9	504.2	38.96	162.5	38.96
W.10	497.8	36.9	159.7	37.5
W.11	490.5	36.4	155.6	35.5
W.12	476.6	33.0	145.9	27.0
W.13	845.0	45.25	380	45.25
W.14	653.22	44.85	392	44.85

Out of the samples taken from all three regions the wines W_1, W_2, W_{13}, W_{14} produced from Adjara and Imereti Tsolikouri grapes and Imereti Tsitka grapes are characterized by antioxidant action: 42.35%, 39.96%, 45.25%, 44.85%.

The amount of phenolic compounds content in wines made from white varieties of vine spread in other countries is of special interest. For example, in 24 wines produced from different varieties spread in the Czech Republic the total amount of phenols ranges from 292 mg to 858 mg/l [15]. Also, in 8 white wines produced in the Czech Republic the total amount of phenols varies from 90 mg/l to 166 mg/l [16] and in the white wine produced in Greece from 450 mg/l and 267 mg/l [17].

Conclusion

Using the UPLC-MS method the Epicatechine (m/z-289), Quercetin-3 glucoside (m/z-463), Quercetin-3- ramnoside (m / z-447), Quercitrin-3- glucuronide (m / z-477) and Procyanidin B1 (m / z-577) are identified in the wines produced from five white varieties of grapes (Tsolikouri, Tsitskha, Klarjula, Krakhuna and Kutaturi) in three regions of Western Georgia (Adjara, Imereti and Samegrelo), and with the help of spectral methods, the total number of phenols, catechins and flavonoids were estimated in all five varieties of grapes. The wines from Tsolikouri and Tsitska grapes contain phenolic compounds and are characterized by antioxidant activity.

The above project was fulfilled by financial support of the Georgina National Science Foundation (Grant AP/96/13, Grant 216816). Any idea in this publication is possessed by the authors and may not represent the opinion of the Georgian National Science Foundation.

ბიოქიმია

დასავლეთ საქართველოში გავრცელებული ზოგიერთი თეთრი ჯიშის ყურძნის ღვინოების ფენოლური ნაერთები და ანტიოქსიდანტური თვისებები

მ. ხარაძე*, ი. ჯაფარიძე*, ა. შალაშვილი**, მ. ვანიძე*, ა. კალანდია*

*ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტი
**საქართველოს აგრარული უნივერსიტეტი

(წარმოდგენილია აკადემიის წევრის თ. ბერიძის მიერ)

შესწავლილია დასავლეთ საქართველოს სამი რეგიონის (აჭარა, იმერეთი და სამეგრელო) ხუთი თეთრი ჯიშის ყურძნიდან დაყენებული ღვინოების ფენოლური ნაერთები და ანტიოქსიდანტური თვისებები. UPLC-MS მეთოდით. იდენტიფიცირებულია (-) Epicatechine (m/z-289), Quercetin-3- glucoside (m/z-463), Quercetin-3- ramnoside (m/z-447), Quercitrin-3-glucuronide (m/z-477) and Procyanidin B_2 (m/z-577). სპექტრალური მეთოდების დახმარებით ხუთივე ჯიშის ყურძნის ღვინოში რაოდენობრივადაა განსაზღვრული ფენოლების საერთო რაოდენობა, კატექინები, ფლავონოლები და დადგენილოა ანტიოქსიდანტური თვისებები. ფენოლური ნაერთების შემცველობით და ანტიოქსიდანტური აქტიურობით გამოირჩევა ცოლიკოურისა და ციცქას ყურძნიდან დაყენებული ღვინოები.

REFERENCES

1. Tsertsvadze N. (2012) In: Caucasus and Northern Black Sea Region Ampelography, Siebeldingen, Germany, JKI, p. 177-239.
2. Shalashvili A., Tsutskiridze E., Beridze N., Targamadze I., Chankvetadze B., (2015) Phenolic compounds in grape bunch and wine of Georgian autocthonal vine variety Tsolikauri. *Bulletin of the Georg. Natl. Academy of Sciences,* **9**, 2 :74-78
3. Shalashvili A., Ugrekhelidze D., Mitaishvili T., Targamadze I., Zambakhidze N. (2012) Phenolic compounds of wines from Georgian autochthonous grapes, Rkatsiteli and Saperavi, prepared by Georgian (Kakhetian) technology. *Bull.Georg. Natl. Acad.Sci*. **6**, 3: 99-103.
4. Ali K., Maltese F., Choi Y. H., Verpoorte R. (2010) *Phytochem. Rev.* **9**: 357-378.
5. Iriti M., Faoro F. (2010) In: Bioactive Foods in Promoting Health. Academic Press, p. 581-620.
6. Fernandez-Marin M. I., Guerro R. F., Puertas B., Garcia-Parrila M. C., Cantos-Villar E. (2013) In: Natural Products: Phytochemistry, Botany and Metabolizm of Alkaloids, Phenolics and Terpenes. Berlin, Springer-Verlag, pp. 2581-2615.
7. Okawa M, Kinjo J., Nohara T., Ono M. (2001) DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medical plants, *Biological and Pharmaceutical Bulletin*, **24,** 10 : 1202-1204.
8. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, **15**, 2: 127-130
9. Compendium of International Methods of Wine and Must Analysis Edition (2016)- International Organization of Vine and Wine - Method OIV-MA-AS2-10 - Resolutions adopted in Mainz (Germany) 13th A.G.
10. González-Rodríguez J., Pérez-Juan P., Luque de Castro M.D. (2002) Flow injection determination of total catechins and procyanidins in white and red wines, *Innov. Food Sci.E.Technol.*, **3, 3** : 289-291.
11. Jara-Palacios M. J., Hernanz D., Escudero-Gilete M. L., Francisco J. Heredia, (2016) The use of grape seed byproducts rich in flavonoids to improve the antioxidant potential of red wines. *Molecules*, **21**, 11: 1526.
12. Flamini R., Traldi P. (2010) Mass spectrometry in grape and wine chemistry. WILEY publ., Padova, Italy, p.163.
13. Tsertsvadze N. (2012) in: Caucasus and Northen Black Sea Region Ampelography. Siebeldingen, Germany, p.177
14. Ketskhoveli N., Ramishvili M., Tabidze D. (1960) Georgian Ampelographia, Tbilisi p.357,364 (in Georgian).
15. Pazouzek J., Gajdosova D., Spanila M., Farkova M., Novotna K., Hovol J., (2005) *Journal of chromatography* A,1081,48.
16. Stratil P., Kuban V., Fojtova J. (2008) Czech J. Food Sci. **26**,4: 242-253
17. Roussis G.I., Lambropoulos I., Soulti K (2005) Food Technol. Biotechnol, **43**, 4 : 351
18. Monagas. M, Bartolome B., Gomez-Cordoves C. (2005) Critical Reviews in Food Science and Nutrition; **45**, 2: 85-118

Received January, 2018