DETERMINATION OF THE FEEDING VALUE OF FOOD INDUSTRY BY-PRODUCTS

Tatiana Dumitra Panaite[1*], Rodica Diana Criste[1], Mariana Ropota[1], V. Criste[2], Gabriela Vasile[2], Margareta Olteanu[1], Monica Mitoi[3], Raluca Socoliuc[1], Al. Vlaicu[1]

[1]*National Research-Development Institute for Animal Biology and Nutrition (IBNA), Balotesti, Ilfov, Romania*
[2]*The National Research - Development Institute for Industrial Ecology-ECOIND, Bucharest, Romania*
[3]*Institute of Biology Bucharest, Romanian Academy*

Abstract

The use of plant by-products from the food industry in animal feeding is a way of reusing resources with a high feeding potential. With the view of their potential use in poultry diets, 5 by-products (rapeseeds meal, grape seeds meal, flax meal, buckthorn meal and pumpkin meal) were characterized chemically and physically to determine their feeding value. The chemical determinations revealed a variable content of protein, ranging between 11.91% (grape seeds meal) and 34.88% (pumpkin meal). The rape seeds meal has 33.15% protein, with high concentrations of limiting amino acids (1.85% lysine, 0.71% cystine and 0.71% methionine), compared to the grape seeds meal (0.42% lysine) or the buckthorn meal (0.16% cystine; 0,17% methionine). The flax meal has a high concentration of α linolenic acid (42.93 g/100 g total fatty acids), and the grape seeds meal has a high antioxidant capacity (493.074 mMTrolox/g). The grape seeds meal has a high concentration of Fe (362 mg/kg), while the rapeseed meal has a high concentration of manganese (82.9 mg/kg), selenium (1.2 mg/kg) and zinc (95 mg/kg). The pumpkin meal has high concentrations (mg/kg) of Co (0.78), Cu (19.2), Ni (447) and Mo (116). The gross energy level (MJ/kg) was 16.94 (rapeseeds meal); 18.07 (grape seeds meal); 18.94 (buckthorn meal); 19.31 (flax meal); 20.10 (pumpkin meal). These results show that the analysed by-products meet the feeding requirements to be used as feed ingredients in layer diets.

Key words: meals, rapeseed, grape seeds, flax, pumpkin, buckthorn, chemical composition

INTRODUCTION

In Romania the production of oils, vegetable and animal fats increases year by year, resulting in large and constant a mounts of organic residues and by-products, whose economic value is rather low. On the other hand, feeding costs are the main cost factor for animal farmers, with up to 80% of the production costs for the poultry farmers. Within this context, the proper use of low input plant materials, such as the by-products, is a solution for cost efficiency. Among the oil extraction industry by-products, meals are vegetable raw materials that can be used in animal feeding. These are highly diverse products, with complex composition and rich in particular nutrients. Rapeseeds meal, grape seeds meal, flax meal, buckthorn meal and pumpkin meal, are just a few examples. Thus, the *rapeseeds meal* is an important protein source used in animal feeding, with a balanced amino acids composition [3]. *Grape seeds meal* has antioxidant properties and high levels of essential amino acids [9]. Used in layer feeding, it can improve their performance and lower egg cholesterol [12; 27]. *Flax meal* is a rich source of omega-3 fatty acids and of amino acids, fibre, lecithin, vitamins and minerals [1; 2]. The ratio of Ω6/Ω3 fatty acids is smaller than the unit in the flax meal [8]. The *buckthorn meal* too, has over 200 bioactive components [16; 24]. This meal has a high antioxidant capacity mare [11; 23] because it is rich in carotenoids, xanthophylls and flavonoids [13]. Research reports show that buckthorn is beneficial for poultry performance [4; 12; 14]. *Pumpkin meal* has

*Corresponding author: tatiana_panaite@yahoo.com
The manuscript was received: 03.09.2016
Accepted for publication: 17.10.2016

high levels of β-carotene (precursor of vitamin A) and antioxidant properties. The pumpkin and pumpkin seeds contain polysaccharides, essential amino acids, carotenoids, minerals and fatty acids.

Purpose of the experiment: Starting from the necessity to use low input forages in poultry feeding, we aimed to determine the feeding value of food industry by-products (meals) by ascertaining their chemical composition.

MATERIAL AND METHOD

We analysed five types of meals (rapeseeds, grape seeds, buckthorn, flax and pumpkin) in terms of their chemical composition. All five meals come from the same producer of edible oils. Standardized methods complying with *Regulation (CE) 152/2009* and ISO standards were used to determine the nutrient concentration (dry matter, protein, amino acids, fat, fibre, ash, phosphor, arsenic, cadmium, chrome, copper, iron, manganese, nickel, lead, selenium and zinc):

- The dry matter (DM) was determined using the gravimetric method, whose working principle involves the determination of sample mass by drying at 103°C, according to We used a Sartorius (Gottingen, Germany) scale and BMT drying closet, ECOCELL Blueline Comfort (Neuremberg, Germany);

- The crude protein (CP) was determined using the Kjeldahl method, using a semiautomatic KJELTEC auto 2300 system – Tecator (Sweden);

- The amino acids were determined by high performance liquid chromatography (HPLC) after sample derivation with OPA reagent OPA (ortho-phthalaldehyde) and detection at 338 nm. We used a chromatographic *HPLC Surveyor Plus system fitted with* PDA *detector*, Hypersil BDS C18 column with silicagel, dimensions 250 4.6 mm, particle size 5 µm, with inverse phase, rotavapor R-205;

- *Ether extractives* (EE) were determined by extraction in organic solvents using a SOXTEC-2055 FOSS – Tecator;

- The crude fibre (CF) was determined with the method with intermediary using a FIBERTEC 2010 system – Tecator (Sweden)

- The ash (Ash) was determined by the gravimetric method using a Caloris CL 1206 furnace;

-The phosphorus was determined by spectrophotometry (reading at 420 nm) with a molecular absorption spectrophotometer ABLE JASCO Romania V 530;

- The *arsenic, cadmium, total chrome, copper, iron, manganese, nickel, lead, selenium and zinc* were determined by inductively coupled plasma optical emission spectrometry using a ICP-EOS Optima 5300 DV Perkin Elmer spectrometer

- Calcium was determined by atomic absorption spectrometry using an atomic absorption spectrophotometer Thermo Electron tip SOLAAR M;

- The fatty acids were determined using the chromatographic method [21], which involved the transformation of the fatty acids from the sample in methyl esters, followed by the separation of the compounds in a chromatographic column and their identification by comparison with standard chromatograms. The method complies with *Regulation (CE) 152/2009 and standard SR CEN ISO/TS 17764 -2: 2008*. We used a Perkin Elmer-Clarus 500 chromatograph, fitted with a system for injection into the capillary column, with high polarity stationary phase (BPX70: 60m x 0.25mm inner diameters and 0.25µm thick film); or high polarity cyanopril phases, which have similar resolution for different geometric isomers (THERMO TR-Fame: 120m x 0.25mm ID x 0.25µm film).

- The polyphenol content of the methanol extracts has been determined according to the method described by [18], modified. We used a UV-VIS Thermo Scientific spectrophotometer.

- The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method. The antioxidant capacity has been estimated by calculating the difference between the control and the sample, compared to a standard curve which used Trolox (synthetic antioxidant analogue to α-tocopherol), as standard antioxidant. We used a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel.

- The gross energy was determined by calculation using the equations of [5].

RESULTS AND DISCUSSIONS

The basic chemical composition of the meals (Table 1) shows that they all have an average protein content ranging between 11.91 (grape seeds meal) and 34.88 (pumpkin meal). The protein level of the rapeseeds meal is 33.15%, which ranks it second to the soybean meal as source of protein for animal diets. On the other hand, the high fibre level, 10.00 to 35.68%, is the limiting factor for the inclusion of these meals in compound feeds for poultry. The literature on rapeseeds meal gives values of 94% DM, 38% CP, 12.8% fibre (NRC; 1988). For the grape seeds meal some researchers like [20] reported 88.44% DM, 10.64% CP and 40.66% fibre and other researchers [22] determined for the flax meal: 90.8% DM, 24% CP and 10.5% fibre. These values reflect the limiting factor for the compound feeds formulation with these by-products, stability of the chemical composition. The amount of essential amino acids was determined as marker of protein quality (Table 2).

Table 1 Basic chemical composition of the studied by-products

Item	Rapeseeds meal	Grape seeds meal	Buckthorn meal	Flax meal	Pumpkin meal
DM real, %	89.60	89.16	88.97	89.25	87.67
GE, MJ/kg	16.94	18.07	18.94	19.31	20.10
CP, %	33.15	11.91	12.83	32.99	34.88
EE, %	1.04	5.96	12.19	9.42	12.38
Fibre, %	12.40	35.68	13.89	11.99	27.54
Ash, %	8.02	2.93	2.84	4.65	5.34
NFE, %	35.00	32.68	47.21	30.20	7.53
Ca, %	0.77	0.54	0.05	0.26	0.16
P, %	1.30	0.47	0.27	0.87	1.28

Table 2 Concentration of essential amino acids in the studied by-products

Item	Rapeseeds meal	Grape seeds meal	Buckthorn meal	Flax meal	Pumpkin meal
	g/100 g dry matter				
threonine	2.24	0.63	0.61	1.58	1.90
arginine	2.07	0.90	1.09	3.53	4.61
valine	2.45	0.64	0.61	1.87	1.85
phenylalanine	1.54	0.52	0.56	1.55	1.76
isoleucine	1.46	0.48	0.50	1.26	1.40
leucine	2.59	0.85	0.84	2.39	2.53
lysine	1.85	0.42	0.49	1.22	1.42
methionine	0.72	0.23	0.17	0.48	0.51

Of the eight essential amino acids determined in the studied products, two are limiting amino acids (lysine and methionine), because they limit the use of the other ones. It was noticed (Table 2) that the rapeseeds meal has a significant level of lysine (1.85%) and methionine (0.72%) compared to the grape seeds meal, which had the lowest level of lysine (0.42%), or with the buckthorn meal, which had the lowest level of methionine (0.17%). The amino acids profile influence the quality of the dietary protein, which reflects in egg quality, when layer s are concerned. The total amount of albumen depends on the balance of the dietary amino acids. The deficit of lysine or threonine affects egg yolk weight, while the deficit of methionine influences albumen weight and its feeding value (higher water/protein ratio). The data shown in Table 2 are comparable with the literature data for the rapeseeds meal [10]; *flax meal* [8]; *grape seeds meal* [9; 20]; *buckthorn meal* [31] and pumpkin meal [17].

Table 3 shows that the flax meal has the highest level of linolenic acid (Ω3) and the lowest ration of polyunsaturated fatty acids (PUFA) Ω6/Ω3 (0.64). Researchers like [30] who investigated the fatty acids content from two subspecies of buckthorn, found that they have a high level of PUFA. They also noticed that the palmitoleic acid is virtually missing in the buckthorn seeds oil, but was found in amounts of 12.1-39.0% in the pulp/skin oil. A high dietary level of Ω3 PUFA enriches the yolk in these amino acids which are important for human health [7].

Table 3 Fatty acids profile of the studied by-products

Items		Rapeseeds meal	Grape seeds meal	Buckthorn meal	Flax meal	Pumpkin meal
		g/100 g dry matter				
Lauric	C 12:0	0.32	0.08	-	-	-
Myristic	C 14:0	0.50	0.16	0.32	0.10	0.15
Palmitic	C 16:0	11.92	9.68	21.56	7. 70	11.06
Palmitoleic	C 16:1	1.84	0.30	14.69	0.16	0.16
Heptadecanoic	C17:0	1.36	-	-	0.06	-
Stearic	C 18:0	2.73	3.56	1.80	3.07	4.18
Oleic cis	C 18:1	41.06	21.04	30.70	18.54	31.72
Linoleic	C 18:2	35.03	62.39	24.26	26.64	50.61
Arachidic	C 20:0	-	-	-	0.14	-
Linolenic	C 18:3n6	-	-	-	0.18	-
Linolenic α	C 18:3n3	4.42	1.33	4.84	42.93	0.32
Eicosadienoic	C20(2n6)	0.58	0.16	0.17	0.17	0.29
Eicosatrienoic	C20(3n6)	0.24	0.29	0.48	0.17	0.10
Eicosatrienoic	C20(3n3)	-	-	0.20	-	-
Arachidonic	C20(4n6)	-	0.40	0.49	0.14	0.34
Eicosapentaenoic	C20(5n3)	-	0.14	-	-	0.15
Docosatetraenoic	C22(4n6)	-	-	-	-	0.37
Other fatt acids		-	0.47	0.48	-	0.49
Fatty acids profile						
SFA		16.83	13.48	23.69	11.07	15.43
MUFA		42.90	21.34	45.39	18.71	31.89
UFA		83.17	86.05	75.83	88.93	84.08
PUFA, of which:		40.26	64.71	30.44	70.23	52.19
Ω3		4.42	1.47	5.04	42.93	0.47
Ω6		35.85	63.23	25.40	27.30	51.72
Ω6/Ω3		8.12	42.91	5.04	0.64	109.65

Table 4 Mineral content of the studied by-products

Item	Rapeseeds meal	Grape seeds meal	Buckthorn meal	Flax meal	Pumpkin meal
	g/100 g dry matter				
Iron	291	362	111	72.1	199
Manganese	82.9	40.6	15	40.1	47
Cobalt	0.21	-	0.05	0.29	0.78
Arsenic	< 0.13	-	< 0.13	0.63	<0.13
Cadmium	< 0.02	0.06	< 0.02	0.18	0.13
Chrome	< 0.03	2.76	< 0.03	< 0.03	119
Copper	8.62	9.52	9.04	16	19.2
Lead	0.51	0.18	0.46	0.18	2.15
Nickel	< 0.03	17.9	2.18	< 0.03	447
Selenium	1.2	< 0.30	< 0.30	< 0.30	1.04
Molybdenum	< 0.03	-	0.38	< 0.03	116
Zinc	95	41.5	17	52.1	75.8
Mercury	< 0.05	-	< 0.05	< 0.05	< 0.05

It can be noticed from Table 4 that the grape seeds meal is very rich in iron, while the rapeseeds meal has a high level of manganese, lead, selenium and zinc. The rapeseeds meal has a high concentration of calcium, iron, selenium and phosphorus. Some researchers [28] identified concentrations of: 10.4 mg/ kg copper, 159.0 mg/ kg iron, 1.0 mg/ kg selenium and 71.4 mg/ kg zinc. Selenium availability is high in the rapeseed meal [15]. Pumpkin meal has the highest level of Pb (2.15 mg/kg). Some one [25] determined in the flax meal the following levels of minerals: 6.01±0.23 mg/100 g iron; 4.43±0.18 mg/100g zinc; 1.90±0.09 mg/100g copper; 236.40±7.26 mg/100g calcium and 2.73±0.10 mg/100g manganese; Other researchers [29] analysed the buckthorn fruits and reported the following levels of minerals: 30.9 mg/kg iron; 1.4 mg/kg zinc; 0.7 mg/kg copper an 1.1 mg/kg manganese. Others [6] determined the minerals content of the grape seeds meal and reported 18.08±0.03 mg/100g iron; 0.817±0.550 mg/100g manganese; and 0.98±0.702 mg/100g zinc.

Table 5 Antioxidant capacity of the analysed by-products (average values)

Item	Polyphenols concentration (mg/g)	Antioxidant capacity (mMTrolox/g)
Rapeseeds meal	7.948 ± 0.37	24.571 ± 0.48
Grape seeds meal	90.415 ± 8.53	493.074 ± 49.26
Buckthorn meal	10.392 ± 1.29	56.784 ± 3.59
Flax meal	3.328 ± 0.05	9.896 ± 0.18
Pumpkin meal	2.501 ± 0.14	14.802 ± 1.53

The data on the antioxidant capacity of the analysed by-products (Table 5) show that the grape seeds meal has the highest level of polyphenols (90.415 mg/g) and the highest antioxidant capacity (493.074 mMTrolox/g). The polyphenols concentrations reported by [26] for winery by-products are comparable with the results for the flax seeds (5.09mg equivalent gallic acid/g sample) and wheat germs (3.49mg equivalent gallic acid/g sample).

CONCLUSIONS

- The analysed by-products meet the feeding requirements for inclusion as dietary ingredients because of their high level of essential nutrients (protein, amino acids, fatty acids and minerals) and to their high antioxidant capacity.
- Two of the studied meals are particularly rich sources of PUFA (rapeseeds and flax meals), while two have antioxidant properties (grape seeds and buckthorn meals).
- The use of the analysed by-products in animal feeding can help changing the mentality and perception about them, i.e. that they are resources, not problems.

ACKNOWLEDGEMENTS

This paper was done within project financed through MADR PROGRAM– ADER/ 6.1.2./01.10.2015.

REFERENCES

[1] Alzuetaa C., RodrÍgueza M.L., Cutulib M.T., RebolÉa A., Ortiza L.T., Centenoc C., TreviÑoa J., 2003: Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilisation and intestinal microflora, British Poultry Science Volume 44 (1):67-74.

[2] Aziza A.E., Panda A.K, Quezada N., Cherian G., 2013: Nutrient digestibility, egg quality, and fatty acid composition of brown laying hens fed camelina or flaxseed meal-Department of Animal and Rangeland Sciences, Oregon State University, Corvallis 97331

[3] Bell J.M., Rakow G., Downey R.K., 2000: Comparisons of amino acid and protein levels in oil-extracted seeds of *Brassica*and *Sinapis* species, with observations on environmental effects. *Canadian J. of Anim. Sci.*, 80(1):169-174

[4] Biswas, A., Bharti V.K., charya S.A., Pawar D.D and Singh S.B. (2010). Sea buckthorn: new feed oportunity for poultry in cold arid Ladakh region of India.World,s Poultry Science of Journal. 66(04):707-714.

[5] Burlacu Gh., Cavache A., Burlacu R., Surdu I., 1999: Ghid pentru alcatuirea ratiilor de hrana la pasari. - ISBN: 973-8036-00-3

[6] Castro S.E., Uchôa-Thomaz A.M., Carioca J.O.B, de Morais S.M., de Lima A., Martins C.G., Alexandrino C.D., Ferreira P.A.T, Rodrigues A.L.M, Rodrigues S.P., Silva J.N., Rodrigues L., 2014: Chemical composition and bioactive compounds of grape pomace (Vitis vinifera L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Sci. Technol (Campinas) vol.34 (1)

[7] Criste R.D., Panaite T.D., Ciurescu C., Ropota M., Rachieru D., 2009: Effects of moderate (5%) levels of linseed in layer diets, Archiva Zootehnica 12:3, 11-21

[8] Criste R.D., Panaite T.D., Olteanu M., Varzaru I., Untea A., Zarug T., Panaite C., 2015: Oul cu continut scazut de colesterol, aliment functional cu efecte benefice in mentinerea starii de sanatate - Solutii nutritionale inovative pentru gaini ouatoare in vederea scaderii nivelului de colesterol in ou, Editura MarLink, 2015; ISBN: 978-973-8411-89-0

[9] El-Shami S.M., El-Mallah M.H., Mohamed SS., 1992: Studies on the lipid constituents of grape seeds recovered from pomace resulting from white grape processing, Fats and Oils Dept., National Research Centre, Dokki, Cairo, Egypt, Vol. 43 (3)

[10] Feng D., Zuo J., 2003: Nutritional and anti-nutritional composition of rapeseed meal and its utilization as a feed ingredient for animal. Feed and industrial raw material

[11] Geetha S., Sai Ram M., Sharma S.K., Ilavazhagan G., Banerjee P.K., Sawhney R.C., 2009: Cytoprotective and antioxidant activity of seabuckthorn (Hippophae rhamnoides L.) flavones

against tert-butyl hydroperoxideinduced cytotoxicity in lymphocytes. Journal of Medicinal Food. 12:151-158.
[12] Hu J.Z., Guo, X.F., 2006: Evaluation of nutrient value of seabuckthorn in north China. Forestrystudies in China.8:50-52.
[13] Jung E.K., Clark R.M., Park Y., Lee J., Fernandez M.L., 2012: Lutein decreases oxidative stress and inflammation in liver and eyes of guinea pigs fed a hypercholesterolemic diet. Nutrition Research Practice, 6:113-119.
[14] Kaushal M., Sharma P.C., 2011: Nutritional and antimicrobial property of sea buckthorn seed oil. Journal of Scientific and Industrial Research. pp 1033-1036.
[15] Keith M.O, Bell J. M., 1987: Feeding value for pigs of canola meal derived from westar and triazine-tolerant cultivars. Can. J. Anim. Sci. 67: 811-819.
[16] Luhua Z., Ying T., Zhengyu Z., Guangji W., 2004: Determination of alpha-tocopherol in the Traditional Chinese Medicinal preparation Sea buckthorn oil capsule by non- supplementation can alleviate negative effects of heat stress on egg production, egg quality, and digestibility of nutrients and egg yolk mineral concentrations of Japanese quails. Research in Veterinary Science. 73:307-312.
[17] Martínez Y., Valdivié M., Martínez O., Estarrón M., Córdova J., 2010: Utilization of pumpkin (Cucurbita moschata) seed in broiler chicken diets. Cuban Journal of Agricultural Science, Volume 44(4)
[18] Mihailović V., Matić S., Mišić D., Solujić S., Stanić S., Katanić J., Stanković N., 2013: Chemical composition, antioxidant and antigenotoxic activities of different fractions of Gentiana asclepiadea L. roots extract. EXCLI Journal, 12:807-823
[19] National Research Council, 1988: Nutrient Requirement s of Domestics Animals, No 2. Nutrient Requirements of Swine. 9th end. National Academy of Sciences, Washington D.C.
[20] Olteanu M., Criste R.D., Panaite T.D., Ropota M., Mitoi M., Varzaru I., Untea A.E., 2014: Study of the feeding value and antioxidant capacity of winery by-products, potential natural antioxidants for farm animal diet formulations, Archiva Zootechnica 17(2), 55-71;
[21] Panaite T., Criste R.D., Ropota M., Cornescu G.M., Alexandrescu D.C., Criste V., Vasile G., Olteanu M., Untea A., 2016: Effect of layer diets enriched in omega-3 fatty acids supplemented with Cu on the feeding value of the eggs. Romanian Biotechnological Letters, Vol. 21, No. 4: 11652-11660
[22] Petit H.V., 2003: Digestion, Milk Production, Milk Composition, and Blood Composition of Dairy Cows Fed Formaldehyde Treated Flaxseed or Sunflower Seed. J. Dairy Sci. 86:2637–2646
[23] Püssa T., Pällin R., Raudsepp P., Soidla R., Rei M., 2007: Inhibition of lipid oxidation and dynamics of polyphenol content in mechanically deboned meat supplemented with sea buckthorn (Hippophae rhamnoides L.) berry residues. Journal of Food Chemistry. 107:714-721.
[24] Ranjith A., SarinKumar K., Venugopalan V.V., Arumughan C., Sawhney R.C., Singh V., 2006: Fatty acids, tocols, and carotenoids in pulp oil of three sea buckthorn species (Hippophae rhamnoides, H. salicifolia, and H. tibetana) grown in the Indian himalayas. Journal of the American Oil Chemists' Society. 83:359-364.
[25] Shahzad H., Faquir M.A., Masood S.B., Munir A.S., 2008: Chemical composition and functional properties of flaxseed flour. Sarhad J. Agric. Vol.24, No.4.
[26] Shi J., Yu J., Pohorly J,J., Young C., Bryan M., Wu Y., 2003: Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Food, Agriculture & Environment Vol.1(2) : 42-47
[27] Su K.P., Huang S.Y., Chiu T.H., Huang K.C., Huang C.L., Chang H.C., Pariante C.M., 2008: Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial, Journal Clinique Psychiatry., pp. 644-651.
[28] Thacker P.A., Kirkwood R.N., 1992: Non-Traditional Feeds for Use in Swine Production, CRC Press.
[29] Tsering S., Chaurasia O.P., Korekar G., Mundra S., Zulfikar A, Yadav A., Singh S.B., 2010: Attributes of Seabuckthorn (Hippophae rhamnoides L.) to Meet Nutritional Requirements in High Altitude. Defence Science Journal, Vol. 60(2):226-230
[30] Yang B., Kallio H.P., 2001: Fatty Acid Composition of Lipids in Sea Buckthorn (Hippophaë rhamnoides L.) Berries of Different Origins. J. Agric. Food Chem.,, 49 (4):1939–1947.
[31] Zhang W., Yan J., Duo J., Ren B., Guo J., 1989: Preliminary study of biochemical constitutions of berry of sea buckthorn growing in Shanxi province and their changing trend. Proceedings of International Symposium on Sea Buckthorn (H. rhamnoides L.), Xian, China; pp 96105.