American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AkiNik

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2015; 3(2): 30-34

Received: 20-08-2015
Accepted: 22-09-2015

Sophie Demchik
Wisconsin Institute for Sustainable Technology, University of Wisconsin - Stevens Point, Stevens Point, WI 54481-3897, USA.

Alex Rajangam
Wisconsin Institute for Sustainable Technology, University of Wisconsin - Stevens Point, Stevens Point, WI 54481-3897, USA.

Justin Hall
Wisconsin Institute for Sustainable Technology, University of Wisconsin - Stevens Point, Stevens Point, WI 54481-3897, USA.

Eric Singsaas
Wisconsin Institute for Sustainable Technology, University of Wisconsin - Stevens Point, Stevens Point, WI 54481-3897, USA.

Correspondence:
Sophie Demchik
Wisconsin Institute for Sustainable Technology, University of Wisconsin - Stevens Point, Stevens Point, WI 54481-3897, USA.

Fatty Acids, Carbohydrates and Total Proteins of Wild Sumac (*Rhus typhina* L.) Drupes from the Upper Midwest of the United States

Sophie Demchik, Alex Rajangam, Justin Hall, Eric Singsaas

Abstract
We assessed staghorn sumac (*Rhus typhina* L.) drupes growing in the Upper Midwest of the United States for content of fatty acids, carbohydrates and total extractable proteins. Drupes were less than 5% fatty acids by dry weight, composed of predominately palmitic, oleic and linoleic acids. The predominant carbohydrate was xylose (22.1% of the dry weight). This quantity is exciting, as it is comparable to that of current commercial sources, and indicates that sumac can be a feasible source of xylose for the production of xylitol. Total extractable protein was relatively low, at 6.8%, and could possibly find a market as a sustainably produced vegetarian feed additive.

Keywords: *Rhus typhina*, Fatty acids, Carbohydrates, Protein.

1. Introduction
Use of plants as industrial raw materials has a long history and many plants have been extensively explored for their bioactive components. The Upper Midwest of the United States is a region with a long dormant season and weather extremes which require plants to have adaptations to these extremes. These adaptations can lead to plants with novel characteristics, making the Upper Midwest of the United States an area with unrealized potential for research on native and naturalized plants as sources of raw materials. Concerns about the long-term viability of fossil fuels have led to a continued interest in sources of renewable raw materials from plants. This interest comes at a time when the sustainability of agricultural systems has been questioned. Development of alternative agricultural crops with more limited deleterious environmental impacts could increase the sustainability of agriculture. Use of plants which are strongly adapted to the growing area can provide new sources of commercially valuable raw materials for the pharmaceutical, chemical, agricultural and food industries, as well as new opportunities for farmers. Staghorn sumac (*Rhus typhina* L., syn. *Rhus hirta* L. Sudworth) is native to the Upper Midwest of the United States. Work on the characteristics of oil in other species or other locations of sumac [6, 9, 1, 2], as well as the ability of *R. typhina* to adapt to extreme, highly-disturbed environments, suggested that it may be a good source of bioactive components, including oils, carbohydrates, and proteins. Because many agricultural crops originated from disturbance-mediated species, staghorn sumac may have potential as an agricultural crop. It has the ability to grow on marginal/sub-marginal sites, and therefore would only compete with commodity crops on the poorest and least sustainably cropped sites. As a perennial, sumac forms large colonies from roots and can re-sprout after coppicing. Both of these characteristics are advantageous for renewably produced materials, and would reduce the need for repeated tillage on these marginal lands. The small number of studies to date show that various *Rhus* species contain bioactive components which could be of interest to the pharmaceutical, chemical, agricultural and food industries, and which could be commercializable. To our knowledge there are no oil, carbohydrate or protein studies that utilize *R. typhina* grown in the Upper Midwest of the United States. However, there are a few studies that have examined fatty acids and/or proteins in *R. typhina* grown elsewhere, or in other species. Kossah *et al*. [9, 2] found that the total amount of unsaturated fatty acids was higher in *R. typhina* grown in China compared to *R. coriaria* grown in Syria. Dogan and Akgul [6, 1] found differences in fatty acid composition among four *R. coriaria* cultivars grown in Turkey. Protein content of *R. typhina* seeds varied from 1.3% in seeds grown in Iraq to 4.3% in seeds grown in China [1, 9, 3, 2]. We were unable to find any literature on carbohydrate content of sumac. Our objective in this study was to assess the content of fatty acids, carbohydrate sand

total extractable proteins of *R. typhina* grown in the Upper Midwest of the United States for potential uses in the natural products industry.

2. Materials and Methods

2.1 Plant Material

Drupes from *R. typhina* L. were collected in August and September 2014 from three Portage County, Wisconsin, USA locations: *Belmont II* samples were collected in the town of Belmont, *Schmeeckle* samples were collected within the Schmeeckle Reserve on the campus of the University of Wisconsin-Stevens Point and *Ellis* samples were collected in the city of Stevens Point. Samples were verified by Dr. Emmet Judziewicz of the University of Wisconsin-Stevens Point.

2.2 Sample Preparation

For carbohydrate and protein content, collected plant parts were oven-dried at 40 ˚C for 72 hours. Individual drupes were separated from any foreign material (soil, insects and stems) by hand. Drupes, including seeds and husks, were finely ground using a coffee/spice grinder.

2.3 Fatty Acid Composition

We converted fatty acids to methyl esters (FAME) according to the following method, adapted from the method of Dowd and Farve [7, 4]. Dried seeds (40 ˚C for 72 h) were finely ground using a coffee/spice mill. Ground seeds of known weight (approximately 0.5 g) were placed in each of 3 glass screw-cap vials, 1.0 ml of 1 N MeOH/HCL was added, and the mixture was vortexed briefly, then incubated at 85 ˚C for 24 h to ensure complete derivatization. After removal from the oven, vials were cooled to room temperature, then 250 µl of 0.9% KCl was added, followed by 1600 µl of hexane. The mixture was briefly vortexed, and after phase separation, an aliquot of the upper phase which contained FAME was collected for analysis. FAME were separated using gas chromatography (Agilent Technologies 7890 A GC system, Santa Clara, CA) equipped with flame-ionization detector (GC/FID). Injection sample volume was 5 µl. The column used was DB-23 60 µm X 250 µm X 0.15 µm; carrier gas was hydrogen (30 ml/min), using split injection mode (split ratio 50:1). Column temperature was held at 100 ˚C for 5 min, increased to 240 ˚C at 4 ˚C/min and maintained at 240 ˚C for 30 min. The injection port temperature was 270 ˚C and the detector temperature was 260 ˚C. The chromatographic peaks corresponding to each fatty acid were identified by comparing the retention times and responses with those of known samples; Figure 2).

2.4 Carbohydrates

Sugar content of acid-hydrolyzed sumac drupes was determined using high-performance anion-exchange chromatography (Thermo Scientific Dionex ICS-3000, Sunnyvale, CA) following the method of Sluiter *et al*. [13, 5]. For acid hydrolysis, 0.3 g of ground sumac drupes were hydrolyzed with 3 ml sulfuric acid (72%), and then incubated in a water bath at 30 ˚C for 1 h. After 1 h, 87 ml of distilled water was added and samples were autoclaved for 1 h at 121 ˚C. After cooling to room temperature (22 ˚C), the samples were filtered to remove lignin, and frozen at -20 ˚C until analysis. Thawed samples were filtered using Millipore 0.20 µm syringe filters; afterward, 20 µl fucose internal standard, 50 µl of sample solution and 930 µl of deionized water were added to screw-cap vials immediately before analysis. Analytical conditions were as follows: the column used was 4 x 250 mm Carbo Pac PA1 (Dionex); temperature was 25 °C; the eluent gradient was 100% 3mM NaOH from -3 to 0 min, 100% 3 mM NaOH from 0 to 3 min, 100% 3 mM NaOH to 80% 3 mM NaOH and 20% ultrapure water 3 to 45 min, 100% 300 mM NaOH from 45 to 50 min.

2.5 Total Extractable Protein

Total extractable protein of drupes was determined following the Bradford method with adaptations [3, 6]. Buffer consisting of 100 mM3-(N-morpholino) propanesulfonic acid (MOPS), and 2 mM EDTA in deionized water was prepared. A single oven-dried drupe (three replicates from each of three locations were assessed for a total of 9 samples) was weighed and then ground under liquid nitrogen with a mortar and pestle. One ml of buffer was added to the mortar and the resulting solution was transferred to each 1.5 ml micro centrifuge tube. The tubes were inverted several times to mix and incubated on ice for 10 minutes. After incubation, 10 µl of 6 M urea was added to aid in protein recovery and the solution was incubated on ice for 30 minutes. After 30 minutes, 50 µl of protein reagent (Bio-Rad, Hercules, CA) was added and the solution was allowed to react for 10 minutes. The absorbance at 595 nm was measured using a UV-visible spectrophotometer (Thermo Scientific Evolution 60S, Waltham, MA). A standard curve was created with 2, 4, 6, 8 and 10 µg/ml (r^2=0.99), using bovine serum albumin (Pierce, Rockford, IL).

2.6 Statistical Analysis

Means were compared using one-way ANOVA. Values expressed are the mean of three replicates. Error bars are represented as ± 1 standard error.

3. Results and Discussion

3.1 Fatty Acids

Interest in sumac as an oilseed crop can be traced to Brubaker [5, 7], who found that *R. glabra* contains approximately 11% oil, has the consistency of petroleum jelly at 16 ˚C, and has an iodine value of 126.8, indicating that it has good drying characteristics. A consistency of petroleum jelly at 16 ˚C strongly suggests high levels of either saturated fats or waxes. This seems to contrast with our results for *R. typhina*, as well as results from Kossah *et al*. [9, 2], which showed most of the fatty acids in both *R. coriaria* and R. *typhina* to be unsaturated. We found significant differences between sites for palmitic and linoleic fatty acids from the three sources of sumac that we tested (Table 2). There was no difference in total percent fatty acid between sites, and fatty acid level was relatively low (Table 2). Fatty acid profile was not dissimilar from corn oil and several other commercially available cooking oils [8, 11, 8, 9]. While the fatty acid profile is acceptable for use as edible or industrial oil, the relatively low levels of fatty acids show sumac to be an unlikely oilseed crop unless plant breeding could increase fatty acid content. There are several smaller peaks on the chromatogram; at this time, no attempt to identify these peaks was made, as the very low quantities are not likely to be of commercial interest. Fatty acids, carbohydrates and acid insoluble lignin, and total extractable proteins account for approximately 82.5% of the dry biomass (Table 1). The remaining (approximately 17.5%) dry matter likely consists of minor components such as soluble tannins, phenolic acids, and others [2, 10].

3.2 Carbohydrates

Using lignocellulosic sources as industrial feed stocks is appealing. Because most of these sources are not digestible to humans, there is little competition with food markets and fewer philosophical debates on "food versus fuel". However, economic viability has presented issues with many lignocellulosic biomass projects in the past [15, 11, 12]. Most lignocellulosic materials available in any large quantity have been tested for distribution of the sugars produced by hydrolysis [4, 16, 13, 14, 15] for some examples). Sumac is an exception to this, as it grows abundantly in areas of the northeastern United States, yet we were unable to locate studies on sumac sugars produced by hydrolysis. For the sugars resulting from hydrolysis in this study, xylose represents 22.1±3.2% of the dry weight of the drupes, suggesting a potential use for this high-value product (Figure 1). Xylose is an industrial feedstock used to produce xylitol. Although xylitol is found as a naturally occurring sugar alcohol in a range of fruits and vegetables (up to 1 percent of dry weight in yellow plums; [17, 16]) commercially available xylitol comes from extracted xylan. Ucar and Balaban [14] found xylose to represent from 0% (in cotton linters) to 20.6% (in carob heartwood) of the total sugars generated by hydrolysis when testing 14 potential sources. The current commercial sources for xylans are corncobs and birch chips, which contain 20-35% xylose [17, 16]. Xylitol is used in a number of foods and other industrial products with a global market demand of 125,000 tons [12, 11]. Total carbohydrates and acid insoluble lignin extracted from the drupes represent 71 ± 5.0% of the dry weight (Table 1). Acid insoluble lignin is relatively high (30.1 ± 0.1%).

3.3 Total Extractable Protein

While not the main focus of this study, the residual protein of the sumac drupes was found to be 6.8 ± 1.7%.Sumac fruits are utilized by several species of wildlife [10, 17]. The amino acid distribution is unknown; however, this co-product could likely find a market in livestock feeds or other food products. Overall, *R. typhina* is an interesting crop. The plant itself is a highly aggressive colonizer and is easy to grow. However, in its wild form, without selection, its fatty acid level is simply not high enough to be of commercial interest. Whether the plant could respond to selection for higher fatty acid content of the seeds is unknown; further research may be warranted. While the use of sumac as an oilseed currently seems unlikely, its composition of lignocellulosic sugars is intriguing, especially the high levels of xylose in the sugars generated from hydrolysis, which are comparable to the current commercial sources. Finally, the residual protein meal may have a livestock feed outlet.

3.4 Tables and Figures

Table 1: Fatty acid, carbohydrate and total extractable protein content of *R. typhina* drupes (based on percent of total dry biomass)*.

	% of total dry biomass
Fatty Acids	
Palmitic acid	0.7 ± 0.1
Oleic acid	1.0 ± 0.3
Linoleic acid	2.5 ± 0.2
All others	0.5 ± 0.1
Total	4.7 ± 0.4
Carbohydrates	
Glucan	14.9 ± 1.8
Xylan	22.1 ± 3.2
Arabinan	0.7 ± 0.1
Galactan	3.2 ± 0.5
Acid Insoluble Lignin	30.1 ± 0.1
Total	71.0 ± 5.0
Total Extractable Proteins	6.8 ± 1.7

*Fatty acids and carbohydrates that were less than 0.05 percent of biomass were not included. Data are means of three replicates ± SE.

Table 2: Fatty acid, carbohydrate and total protein composition of *R. typhina* drupes growing in three central Wisconsin locations (% of total biomass) including total fatty acids, ratio of saturated to unsaturated fatty acids, ratio of omega 6 to omega 3 fatty acids, total carbohydrates, acid insoluble lignin and total extractable proteins.

	Belmont II	**Ellis**	**Schmeeckle**
Fatty Acids			
Palmitic	0.71±0.02*	0.46±0.06*	0.81±0.05*
Oleic	1.20±0.55	1.02±0.23	0.89±0.84
Linoleic	2.06±0.04*	2.99±0.23*	2.54±0.11*
All others	0.46±0.04	0.42±0.04	0.55±0.03
Total	4.43±0.53	4.89±0.45	4.80±0.97
Sat. to Unsat.	0.25±0.03	0.15±0.01	0.27±0.04
ω 6 to ω 3	9.40±0.06*	16.76±0.62*	9.25±0.13*
Carbohydrates			
Glucan	16.73±1.66	11.27±1.73	16.67±6.06
Xylan	21.20±2.42	17.07±2.73	27.90±10.90
Arabinan	0.90±0.10	0.80±0.23	0.50±0.21
Galactan	4.13±0.35	2.43±0.54	2.93±0.50
Total carb (no lignin)	42.97±4.30	31.57±4.55	48.00±16.70
Acid Insoluble Lignin	30.18±0.38	29.97±0.03	30.23±1.02
Total Extractable Proteins	10.24±2.58	5.16±0.60	4.88±0.57

* Indicates significant difference at $P<0.05$. Data are means of three replicates ± SE.

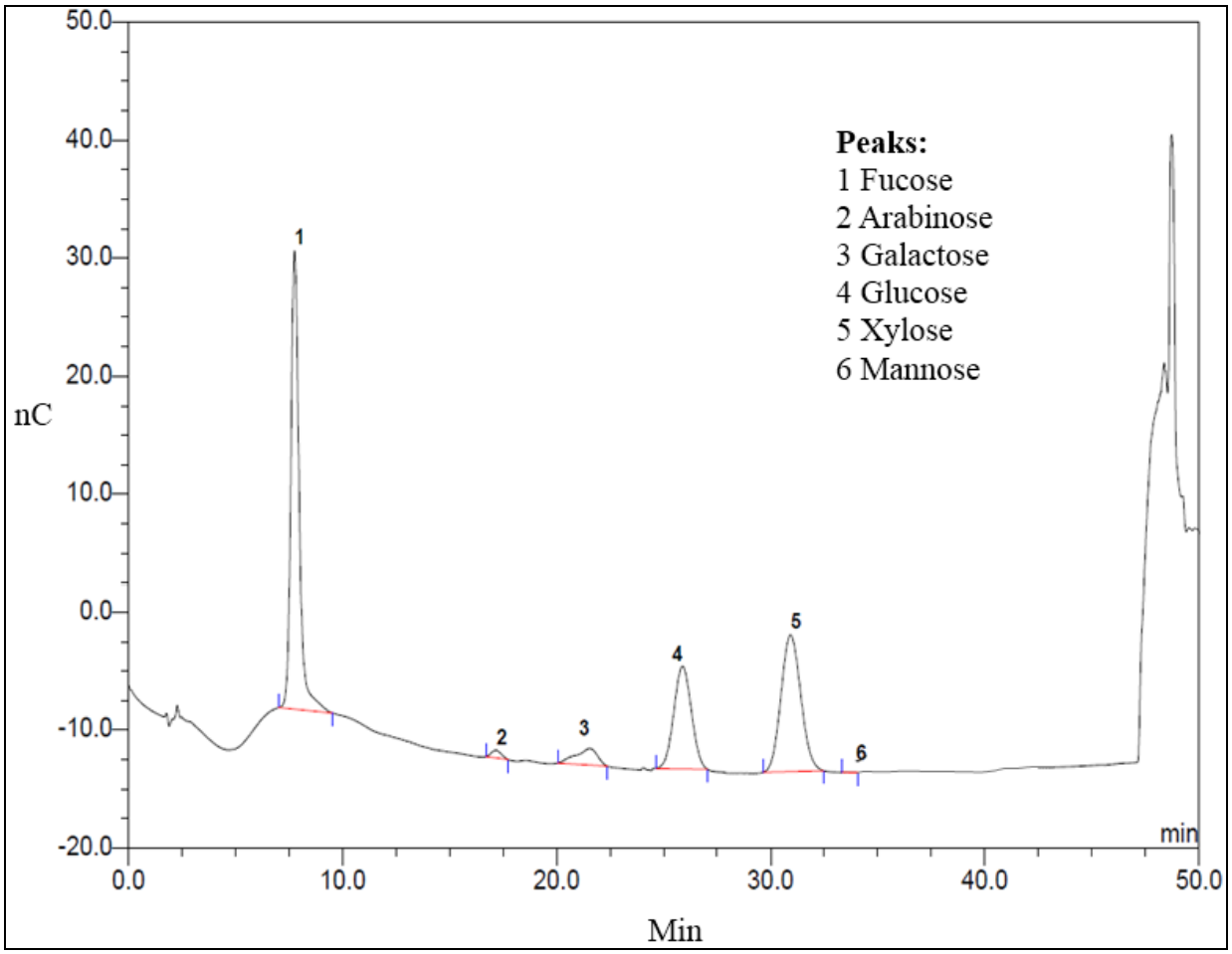

Fig 1: Chromatogram of carbohydrate analysis of *R. typhina* drupes using high-performance anion-exchange chromatography.

Note: Fucose is the internal standard.

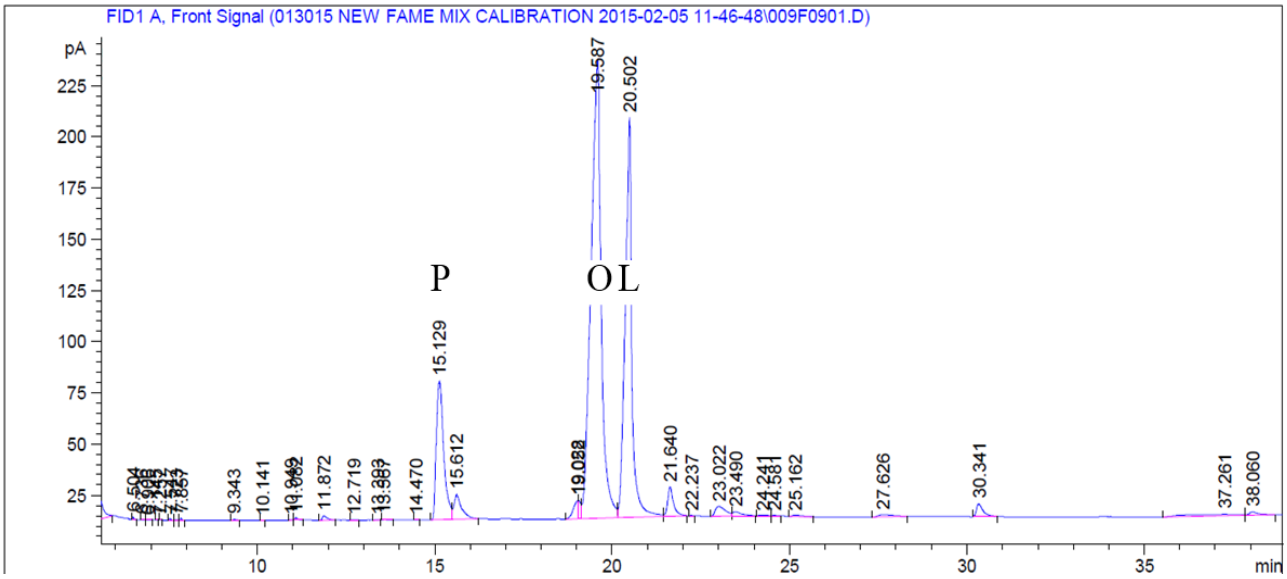

Fig 2: Representative chromatogram of FAME analysis using gas chromatography: FAME extraction from *R. typhina* from Schmeeckle. P- Palmitic acid, O- Oleic, L- Linoleic.

4. Conclusions

Native populations of *R. typhina* collected in the Upper Midwest of the United States were sampled for content of fatty acids, carbohydrates and proteins. Fatty acid levels in the wild form of sumac, without selection, are not likely high enough to be of commercial interest. However, the plant may be able to respond to selection for higher fatty acid content of the seeds; further research may be warranted. The high levels of xylose in the sugars generated from hydrolysis are comparable to the current commercial sources; this suggests potential for commercial production of xylitol. Finally, as a co-product, the residual protein meal may have a livestock feed outlet.

5. Acknowledgments

We thank Mike Demchik for plant material and comments on the manuscript and Brad Gorzek for supplying plant material. We also thank Shona Duncan, Raghu Gurram and Malek Alkasrawi for their review of the manuscript. The Wisconsin Institute for Sustainable Technology (WIST) is an institute within the College of Natural Resources at the University of Wisconsin-Stevens Point, USA. Major funding for WIST operations has been provided by the US Department of Agriculture National Institute of Food and Agriculture.

6. References

1. Dogan M, Akgul A. Characteristics and Fatty Acid Compositions of *Rhuscoriaria* Cultivars from Southeast Turkey. Chem. Nat. Compd., 2005; 41(6):724-725.
2. Kossah R, Nsabimana C, Zhao J, Chen H, Tian F, Zhang H, *et al*. Comparative Study on the Chemical Composition of Syrian Sumac (*Rhuscoriaria* L.) and Chinese Sumac (*Rhus typhina* L.) Fruits. Pak. J. Nutr., 2009; 8(10):1570-1574.
3. Al-Shabibi MMA, Siddiqi AMS, Kassim S, Haddad BA. Studies on the Sumach of Iraq. I. Proximate Analysis and Characterization of Seed Coat Lipids. Can. Inst. Food Sci. Technol. J., 1982; 15(1):065-067.
4. Dowd MK, Farve MC. Fatty acid composition of Tilia spp. seed oils. Grasas y Aceites, 2013; 64(3):243-249.
5. Sluiter A, Hames R, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. Determination of Structural Carbohydrates and Lignin in Biomass. Technical Report NREL/TP-510-42618 for National Renewable Energy Laboratory: July 2011.
6. Bradford MM. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, 1976; 72(1-2):248-254.
7. Brubaker HW. A Study of the Oil from Sumac (*Rhusglabra*). Ind. Eng. Chem, 1919; 11, 950.
8. Dubois V, Breton S, Linder M, Fanni J, Parmentier M. Fatty acid profiles of 80 vegetable oils with regard to their nutritional profile. Eur. J. Lipid Sci. Technol., 2007; 109(7):710-732.
9. Princen LH. New Oilseed Crops on the Horizon. Econ. Bot., 1983; 37(4):478-492.
10. Bell JM. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci., 1993; 73(4):679-697.
11. Ravella SR, Gallagher J, Fish S, Prakasham RS. Overview on Commercial Production of Xylitol, Economic Analysis and Market Trends. In D-Xylitol; da Silva, S.S. and Chandel, A.K., Eds.; Springer, Berlin Heidelberg, Germany, 2012; 291-306.
12. Viikari L, Vehmaanperä J, Koivula A. Lignocellulosic ethanol: from science to industry. Biomass Bioenerg, 2012; 46:13-24.
13. Brown DE. Lignocellulose Hydrolysis. Philos. Trans. R. Soc. London, 1983; 300:305-322.
14. Ucar G, Balaban M. Hydrolysis of polysaccharides with 77% sulfuric acid for quantitative saccharification. Turk. J. Agric. For., 2003; 27(6):361-365.
15. Wyman CE. Ethanol from Lignocellulosic Biomass: Technology, Economics, and Opportunities. Bioresource. Technol., 1994; 50(1):3-16.
16. Zacharis C. Xylitol. In Sweeteners and Sugar Alternatives in Food Technology, Second Edition; O'Donnell, K and Kearsley MW, Eds.; John Wiley and Sons, Ltd, Hoboken, NJ, USA, 2012, 347-382.
17. Martin AC, Zim HS, Nelson AL. American Wildlife and Plants: A Guide to Wildlife Food Habits. Dover Publications, New York, USA, 2010, 500.