



OPTICAL PROPERTIES AND CHEMICAL CHARACTERIZATION OF WOODY SYRUP BY USING METHODS OF APPLIED PHOTONICS

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ABSTRACT:

Introduction: The natural woody syrup is a typical product from the Canary Islands and America. It is often used for health food supplement for children, athletes and elderly because it has well-pronounced antioxidant properties. The interest in this syrup is increasing because it is often used in homeopathic medicine and consists of phenolic content. Antioxidants reduce cell damaging effects of free radicals. They are more effective *in vitro* antioxidants than vitamins D and C and have a significant effect towards protection of unwanted *in vivo* oxidation of proteins and lipids.

Objective: The natural woody syrup is prepared from Canadian maple syrup and syrup from 5 types of palms from Southeast Asia. The nutritional properties (such as the content of sugars) were analyzed. The antioxidant activity, total phenolic content and optical properties have been investigated.

Purpose: The aim of this study is to explore the relations between fluorescence in natural woody syrup and its total phenolic content and total antioxidant activity in view of the usefulness of this product for human health and to determine their nutrient composition by the physico-chemical properties.

Materials and methods: The natural woody syrup will be investigated by using methods of applied photonic, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) methods for antioxidant activity, Folin-Ciocalteu reagent for total phenols and High-Performance Liquid Chromatography Refractive Index Detector HPLC-RID analysis for determination of the sugar content.

Results: The dependence between antioxidant activity and total phenolic content was established. The sample has a small quantity of flavonoids, and for this reason, there is not the distinct peak at 380 nm. Excitation in the visible region is suitable for distinguishing the riboflavin

and pigments.

Conclusions: The fluorescence of the syrup was correlated with their antioxidant activity. Two emission fluorescence maxima have been observed:

- The first one is attributed to the riboflavin in the region 550 nm - 580 nm
- The second one is connected with the pigments similar to the chlorophyll at about 690 nm

Keywords: natural woody syrup, fluorescence spectroscopy, phenolic content, antioxidant activity, sugar content

INTRODUCTION:

The natural woody syrup is a typical product from the Canary Islands and America [1]. It is made from maple sap and palms syrup, which contain some minerals as Mn, Fe, K, Na, Ca and Zn [2]. The purpose of this investigation is to determinate the nutrition composition of the syrup and relation between antioxidant activity and some substances with phenolic character by using the parameters of applied photonics. These properties of the syrup are important because the latter is used with great success in the cases:

- Edema degradation (haematomas)
- Improved limited mobility of the joints
- Reducing chronic pain.

MATERIALS AND METHODS:

Samples:

Natural woody syrup from Switzerland has been investigated. It consists of Canadian maple syrup and syrup from 5 types of palms from Southeast Asia. The syrup is obtained from trees growing in natural conditions.

The natural Canadian maple syrup is obtained from 40 years old trees and must be concentrated to be protected from fermentation. It is from "class C" and has high mineral content. The palm syrup comprises coconut palms, palms

from the rain forests, palms of swamps places and palms trees from Sri Lanka.

Methods:

Total phenolic content (TPC) was measured using a Folin-Ciocalteu reagent [3] with some modifications. Briefly, Folin-Ciocalteu reagent (1 mL) diluted five times was mixed with 0.2 ml sample and 0.8 ml 7.5% Na₂CO₃. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against a blank, prepared with 70 % methanol. The results were expressed as mg equivalent of gallic acid (GAE) according to the calibration curve, linear in the range of 0.02 - 0.10 mg gallic acid used as a standard [3].

The total flavonoids content was analyzed by Al(NO₃)₃ reagents. The absorbance was measured at 415 nm against blank. The results were presented as mg equivalents quercetin (QE) per g dry weight (dw) [4].

The antioxidant activities were evaluated by two methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical based on mixed hydrogen atom transfer (HAT) and single electron transfer mechanisms and FRAP (ferric reducing antioxidant power) based only on single electron transfer mechanism.

The DPPH radical-scavenging ability

The analyzed sample (0.15 ml) was mixed with 2.85 ml freshly prepared 0.1mM solution of DPPH in methanol. The sample was incubated for 15 min at 37°C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol and % inhibition was calculated [4]. A standard curve was built with 6-hydroxy-2,5,7,8-tetramethyl-chroman- 2- carboxylic acid (Trolox) in concentration between 0.005 and 1.0 mM. The results were expressed in mM Trolox® equivalents (TE).

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to [5] with slight modification. The FRAP reagent was freshly by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10

mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM FeCl₃.6H₂O in d. H₂O. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness, and the absorbance was measured at 593 nm against blank prepared with methanol. Antioxidant activity was expressed as mM Trolox® equivalents (TE).

Determination of total soluble carbohydrates

The total soluble carbohydrate content was estimated by the phenol-sulphuric acid method. The reducing sugars were estimated by PAHBAH method at 410 nm. Chromatographic separations and determination of presented sugars were performed on an HPLC instrument Elite Chrome Hitachi, coupled with refractive index detector (RID) Chromaster 5450. The separation was done on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with Pb²⁺ and a guard column Shodex SP - G (5 µm , 6 × 50 mm) operating at 85°C, mobile phase d. H₂O with flow rate of 1.0 ml/min and the injection volume of properly diluted sample 20 µl [6].

Fluorescence measurement

The sources used to measure the fluorescence spectra are 295 nm, 395 nm, 405 nm, 410 nm, 415 nm light emitting diodes (LEDs). A fiber optic spectrometer (Brolight, Avantes) with sensitivity in the (200-1100) nm range and a resolution of about 8 nm was used to measure the fluorescence spectra. The syrup was placed in a cuvette 1mm × 1mm and irradiated by laser diodes (LDs) or light emitting diodes (LEDs).

Statistical procedures

All results are obtained in triplicate. The standard deviation was given in the table. The excitation –emission matrix was obtained by using MATLAB 9.0.

RESULTS:

The content of total soluble carbohydrate in natural woody syrup was summarized in Table 1.

Table 1. Soluble carbohydrate content in woody syrup, g/100 g

Total soluble carbohydrates	Reducing sugars	Sucrose	Glucose	Fructose
58,98±2,12	20,07±0.2	38,57±0,12	11,43±2,09	6,22±0,96

The dependence between the parameters of applied photonics and total phenolic content exists. The content of biologically active substances and antioxidant activity was presented in Table 2. FRAP assay gave higher results

than DPPH assay. This showed that antioxidant activity was due mainly to a phenolic substance that activates a single electron transfer mechanism.

Table 2. Total phenols, flavonoids and antioxidant activity of woody syrup

Total phenolic content, mg GAE/g	Total flavonoids, mg QE/g	Antioxidant activity, mM TE/g	
		DPPH	FRAP
1,97±0,23	0,27±0,08	19,61±0,24	24,99±0,85

The fluorescence spectra are determined with illumination the sample with light with wavelength into UV and visible region.

The obtained spectra in the UV region were not presented, because of the appearance of some noises. But they are used for determination of the finger print for the natural woody syrup. On the Fig. 2 the excitation emission matrix has been drawn, and two dimensional counter map was also presented.

Fig. 1. Fluorescence spectra in the visible region for natural woody syrup

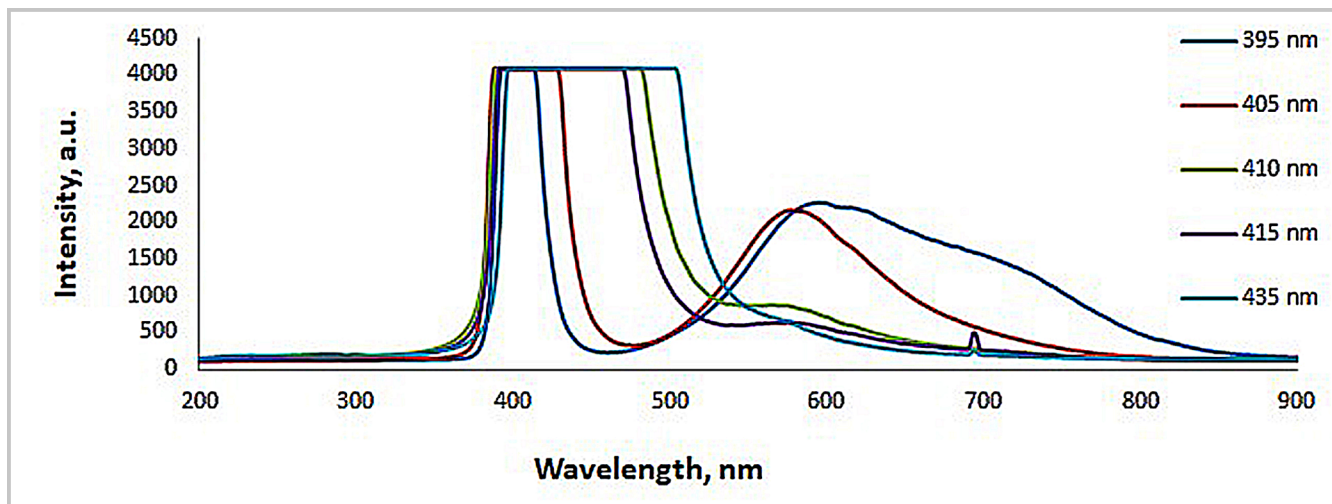
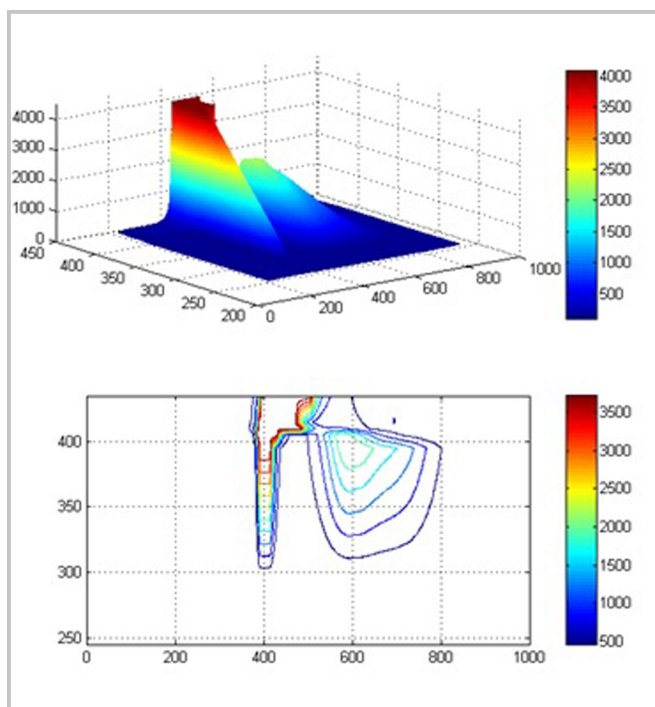


Fig. 2. Excitation emission matrix and counter map for a natural woody syrup



DISCUSSION:

According to the measurement of fluorescence in the UV region, there is one weak peak at 280 nm. One possibility for the source of fluorescence signal was some phenolic compounds. The similar conclusion is made for southwestern Maple syrup from Kermashla and Dumont [7]. There are two fluorescence maxima in the visible region:

- The first emission peak of syrup is observed between 550 nm - 580 nm when the sample is excited with light at the range of 340 to 410 nm. This peak can be related with the presence of riboflavin. The similar peak is reported from Sikorovska and co-authors [8].

- The second fluorescence maximum is around to 690 nm, and it is connected with pigments similar to the chlorophyll. It is observed only for excitation wavelength 415 nm.

From the spectra obtained by using methods of applied photonic can be concluded that the natural woody syrup contains the riboflavin, a small quantity of chlorophyll pigments and phenolic compounds.

It is expressed in Trolox equivalent. The values determined by the used methods are similar. A connection between total phenol content and AOA exists. As known, the phenolic compounds effectively capture free radicals [9-11]. The low content of total flavonoids was found. For this reason, an emission peak in fluorescence spectra in UV region is not observed at 380 nm.

The significant content of polyphenols is determined in the investigated syrup. They are organic compounds from different chemical nature including phenolic acids, flavonoids, glucosides. In combination with vitamins and carotenoids, they protect tissues of the body from oxidative stress.

In general, investigated natural woody syrup contained a significant amount of soluble carbohydrates (58.98 g/100 g). The sample is rich in mono- and disaccharides. HPLC-RID analysis revealed in details the presence only of sucrose, glucose and fructose as individual sugars in this sample. Moreover, the values of sucrose (38, 57 g/100 g) significantly dominated above

monosaccharide. The similar tendency was observed with carob flour and syrup repapered form it husks and pods [6]. The content of investigated sugars is similar to this of the palm tree syrup reported [12]. This fact can be explained by climate and harvest conditions. There was a slight tendency for decreasing of sucrose and increasing the concentration of glucose and fructose. It depends on the climate, humidity and acidity of syrup. Fructose was in lower values in comparison of other sugars. Therefore, the natural woody syrup was evaluated as a natural source of energy, due to high sucrose content in it.

CONCLUSION

The natural woody syrup is a supersaturated solution of sugars and water, it is rich of phenolic components. It

can be suggested as a natural product, which could be suitable raw materials of production of natural sweeteners. The biologically active substances and related with them antioxidant activity contribute to the interaction with many basic cellular activities, acts with the free radicals and exhibit cardio-health protection.

The optical properties as absorbance and fluorescence can be used for the qualitative determination of pigments and some vitamins. The phenolic compounds are the influential factors of the color and flavour of the syrup. Therefore, this complex of phytochemical active substances in natural woody syrup offers various fields of prospective applications in medicine and nutrition for healthy food production.

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Please cite this article as: Radusheva P, Nikolova K, Petkova N, Gabrova R, Naydenova D. Optical properties and chemical characterization of woody syrup by using methods of applied photonics. *J of IMAB.* 2018 Oct-Dec;24(4):2250-2253. DOI: <https://doi.org/10.5272/jimab.2018244.2250>

Received: 22/05/2018; Published online: 23/11/2018



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