

# Total Polyphenols Estimation from Berries

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## Abstract

The improved procedures and methodology for obtaining berry matrices represent an issue that is part of the existing national and international concerns of enriching the range of nutraceutical products with a high content of antioxidants, in this case resveratrol, through scale production wide range of alternatives designed to both help allopathic medication and prevent the occurrence of diseases. Our study aims to test different solutions for extraction of polyphenols from studied berries from the Cluj County in order to emphasize the best extraction solvent/solvents. The highest values of total polyphenols were obtained with the ethanol/water binary extraction system (80 : 20, v/v).

**Keywords:** binary system, ethanol solvent, ternary system.

## 1. Introduction

The improved procedures and methodology for obtaining berry matrices represent an issue that is part of the existing national and international concerns of enriching the range of nutraceutical products with a high content of antioxidants, in this case resveratrol, through scale production wide range of alternatives designed to both help allopathic medication and prevent the occurrence of diseases [2, 4].

Obtaining food supplements falls within the field of potential for intelligent specialization of that component of the pharmaceutical industry focused on the production of phytotherapeutic food supplements [1, 7].

The national and international interest in food supplements is based on the fact that an adequate and varied diet could provide, under normal conditions, all the nutrients necessary for normal development and ensuring a healthy life, but due to the changes produced in the last decades in the style of life, many consumers choose to supplement their diet with food

supplements, easy to administer and procure (free access, without medical prescription), often from the same food outlets in specialized stores, retail/supermarket networks [1, 3, 5, 6].

The purpose of the present study is to highlight some partial results regarding the valorization of forest fruit production in Cluj County, based on a qualitative and quantitative estimation methodology of their nutritional/healing potential.

## 2. Material and Method

The samples of forest fruits used in the study consist of the species: lingonberries (*Vaccinium vitis-idaea* L.) and bilberries (*Vaccinium myrtillus* L.). They were collected from various regions belonging to the mountainous area of Cluj County.

The plant material was dehydrated and properly stored in vials for crude chemical analysis. The content in total polyphenols was determined by specific methodologies. The determination of the content in total polyphenols

was achieved by measuring the optical density of the primary extract which, by complexing with the Folin-Ciocalteu reagent, absorbs in the UV-Vis range at the wavelength  $\lambda = 750$  nm.

In order to test the effectiveness of resveratrol extraction systems from the berry species studied, with solvents or mixtures (binary or tertiary) of solvents (methanol, ethanol, ethyl acetate, water) in different proportions, the total content was identified in polyphenols of the obtained extracts, expressed in gallic acid equivalents/g (GAE/g).

To prepare the standard, 25 mg of gallic acid was weighed on the analytical balance and introduced into a 25 mL volumetric flask.

Then, 15 mL of 40% ethanol was added, and the mixture was sonicated until complete dissolution. The room temperature solution was made up to 25 mL with solvent.

The result was a solution with a concentration of 1mg/mL.

This represented the mother solution, standard, from which 5 dilutions were prepared:

- 1 mg/100 mL;
- 0.5 mg/100 mL;
- 0.25 mg/100 mL;
- 0.125 mg/mL and
- 0.0625 mg/mL.

Preparation of the standard solution: 1 mL of the standard stock solution was introduced into a 100 mL volumetric flask, 60 mL of distilled water was added, stirred, then 5 mL of Folin-Ciocalteu reagent was added and the mixture was homogenized.

After 1 minute and before 8 minutes, 15 mL of 7.5% sodium carbonate solution were added. This moment was considered "time 0".

A new homogenization was performed, and the reagent mixture was brought to the volume of 100 mL with distilled water.

After 2 hours, the absorbance was read at  $\lambda = 750$  nm compared to the control (blank). The control was prepared in the same way, replacing 1 mL of standard solution with 1 mL of 40% ethanol. For the samples, the procedure was similar, but by replacing the standard with the obtained extracts followed by reading the absorbance at  $\lambda = 750$  nm for each sample.

The total amount of polyphenols was expressed according to the calibration curve drawn with the help of the gallic acid standard. Based on the absorbances read at the different concentrations of the standard solution, the calibration curve (mg/100mL) was constructed.

Based on the equation of the calibration curve ( $Y = aX + b$ ), where:

W is the absorbance read by the spectrophotometer,

X is the sample concentration, and a and b are the constants of the line), the concentrations of polyphenols in the samples were determined.

### 3. Results and Discussions

The analysis of the aqueous extracts from the berry matrices led to polyphenolic content values of 0.162 mg GAE/g for lingonberries and 1.95 mg GAE/g for bilberries. Methanolic extracts led to polyphenol content values of 1.91 mg GAE/g for lingonberries and 3.21 mg GAE/g for bilberries. The analysis of the ethanolic extracts from the berry matrices led to polyphenolic content values of 1.87 mg GAE/g for lingonberries and 3.19 mg GAE/g for bilberries.

The analysis of the extracts from the berry matrices made with ethyl acetate led to polyphenolic content values of 1.85 mg GAE/g for lingonberry and 2.08 mg GAE/g for bilberry, respectively (Table 1).

**Table 1.** The polyphenolic content (GAE, mg/g) of single solvent extractions of wild berries

Nr crt.	Specia	Solvent	GAE (mg/g)
1	Lingonberries	Water	1.62
	Bilberries		1.95
2	Lingonberries	Methanol	1.91
	Bilberries		3.21
3	Lingonberries	Ethanol	1.87
	Bilberries		3.19
4	Lingonberries	Ethyl acetate	1.85
	Bilberries		2.08

The analysis of the extracts from the berry arrays made with binary systems led to specific results. Thus, the methanol/water system (80 : 20, v/v) led to values equal to 2.12 mg GAE/g for lingonberries and 3.75 mg GAE/g for bilberries,

the ethanol/water system to .17 mg GAE /g for lingonberries and 3.78 mg GAE/g for bilberries, the ethyl acetate/water system at 1.76 mg GAE/g for lingonberries, and 3.11 mg GAE/g for bilberries (Table 2).

**Table 2.** The polyphenolic content (GAE, mg/g) of binary solvent mixtures (80:20, v/v) extractions of wild berries

No. crt.	Species	Binary solvent	GAE (mg/g)
1	Lingonberries	Methanol/water	2.12
	Bilberries		3.75
2	Lingonberries	Ethanol/water	2.17
	Bilberries		3.78
3	Lingonberries	Ethyl acetate/water	1.76
	Bilberries		3.11

The analysis of the extracts from the berry matrices made with the ternary systems (25 : 25 : 50, v/v) also determined different values for the two broad species under study.

The methanol/ethyl acetate/water system led to polyphenol content values of 1.85 mg GAE/g for lingonberries and 2.56 mg GAE/g for

bilberries, the ternary ethanol/ethyl acetate/water system to 1.82 mg GAE/g for lingonberries and 2.52 mg GAE/g for bilberries, and the ternary methanol/ethanol/water system (to 1.81 mg GAE/g for lingonberries - *Vaccinium vitis-idaea* L. and 2.49 mg GAE/g for bilberries - *Vaccinium myrtillus* L. (Table 3).

**Table 3.** The polyphenolic content (GAE, mg/g) of ternary solvent mixtures (25:25:50, v/v) extractions of wild berries

No. crt.	Species	Ternary solvent	GAE (mg/g)
1	Lingonberries	Methanol/ethyl acetate/water	1.85
	Bilberries		2.56
2	Lingonberries	Ethanol/ethyl acetate/water	1.82
	Bilberries		2.52
3	Lingonberries	Methanol/Ethanol/water	1.81
	Bilberries		2.49

#### 4. Conclusions

The highest values of total polyphenols were obtained with the extraction system consisting of the binary ethanol/water mixture (80 : 20, v/v). Thus, the analysis of the extracts from the matrices of the two species of forest fruits taken in the study made with the binary system ethanol/water (80 : 20, v/v) led to the following values of the polyphenolic content equal to 2.17 mg GAE/g for lingonberries and 3.78 mg GAE/g for bilberries.

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