# THE TOTAL CONTENT OF POLYPHENOLS AND FLAVONOIDS OF SOME PLANTS EXTRACTS, AS POTENTIAL ADDITIONS FOR CIDER OBTAINING

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*This article contains information concerning the realization of a comparative study between the content of antioxidants (poly phenols and flavonoids) of some liquid extracts obtained from plant sources (e.g. berries), which can be used in the preparation of weak alcoholic beverages, referring directly to cider. Together with experimental data, including here total polyphenol content (TPC) and total flavonoids content (TFC) measurements in these liquid extracts, it will try to develop aspects of industrial product in the form of cider, insisting on the implications of technological nature.*

**Keywords**: liquid extracts, natural antioxidants, antioxidant activity, cider

## 1. Introduction

The main organoleptic characteristics in the case of the majority of the plants and also of the product of their transformation are determined by the phenolic compounds. They contribute in particular to color, bitterness and, also, to astringency. Usually, beverage phenol composition depends on the nature of the raw material used, but not only. An important factor is represented by the way of the processing them, which ultimately influences their transfer into the finished product. This will be explained below, when will be detailed the way of obtaining the cider in laboratory.

Based on these considerations, the topic exposed on this path, wants to participate in the diversification of the range of food, namely weak alcoholic drinks branch, referring directly to cider, by incorporating previously mentioned constituents (natural antioxidants as polyphenols and flavonoids) in form of liquid extracts obtained from vegetable sources.

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## 2. Natural antioxidants sources

There are studies that refer to various associations, between herbs/plants and natural antioxidants, being known that in product development is taken into consideration the addition of extracts from such sources, for replacement of the synthetic ones. Therefore, this part will highlight the general classes of what antioxidants are found in certain sources, which can be used as alternative natural antioxidants for quality improvement of cider [1]. Methods for the isolation and characterization of natural antioxidants, extracted from the sources mentioned below, will be discussed in the following including the result of work for getting certain extracts.

- ***Antioxidants found in various vegetables & fruits (application directly to the cider)***

Table 1 comprises information regarding what type of antioxidant compounds can be found in the a few sources, the one mentioned as the possible source for obtaining an extract below, being *Solanum tuberosum* L and specifically berries.

The antioxidants activity of fruit (including berries), juice and "wine" made from these, vary partially due to different oxidation systems and due to methods of analysis of antioxidants. Recent literature has been widespread focus on the study of the effect of antioxidant polyphenols (flavonoids and phenolic acids) isolated from the fruit. Table 1 also shows the main antioxidant compounds from different fruits.

*Table 1*

**Antioxidant compounds found in different vegetables and fruits [3, 6]**

| Vegetable/fruit type | Antioxidant compounds |
|---|---|
| potato | coffey acid derivates, chlorogenic acids, patatina |
| sweet purple potato | peonidin-glicozide |
| apple | chlorogenic acid |
| apple juice | chlorogenic acid, ascorbic acid, phloretin glycosides |
| berries | anthocyanins, hydroxyl cinnamates, flavones |

- ***Antioxidants found in plants/herbs***

Phenolic compounds are extremely diverse, being naturally present in plants, and not just. In the following being presented an overview of the flowers of *Hibiscus Sabdariffa* L., the practical part including the role of antioxidants contained in them, for coloring of cider.

The plant is cultivated especially for its flowers, characterized by the presence of pigments responsible for spreading their dark red color, being mostly

common in areas which presents tropical climate. From the point of view of "pharmacology" context, the main relevant constituents to this purpose are organic acids, flavonoids (with emphasis on the anthocyanins) and polysaccharides.

A considerable share of the extract components of *Hibiscus Sabdariffa* L. is occupied by the organic acids, being included into this category citric and hydroxyl citric acid, hibiscus acid and minor components such as ascorbic acid [7].

*Hibiscus Sabdariffa* L. (H.S.L.) contains polyphenols, more exactly the subclass of flavonoids, from which flavonoids and flavones in simple of polymerized form, are the most common. In the category of flavonoids, have been identified during the time in extracts of H.S.L. the following: hibiscitrin (hibiscetin-3-glucoside), sabdaritrin, gossypitrin, quercitin and Luteolin, as well such as chlorogenic acid, protocatechuic acid, eugenol or β-sitosterol [7].

Anthocyanins represent a subgroup of flavonoids, being natural pigments present in dried flowers of H.S.L., their color ranging depending on the pH. The first anthocyanin isolated from H.S.L. was "hibiscin", also known later under the name of delphinidin-3-sambubioside, renamed eventually delphinidin-pentoside-glucoside [7].

## 3. Material and method

The main objective of this research work was to measure the TPC and TFC in extracts obtained from cranberries, raspberries, blackberries fruits and *Hibiscus Sabdariffa* L. in order to use them for the cider production.

### 3.1. Extracts obtaining

Extracts (potential additions for experimental variants of cider enriched with bioactive compounds) have been obtained from the following sources: plant berries from the spontaneous flora (e.g. Cyanococcus within the genus *Vaccinium, Rubus fruticosus, Rubus idaeus*) and rose petals of Japanese *Hibiscus sabdarrifa.*

The extraction was performed using as solvent, the following liquid mediums: ethanol 96%, 2% acidified ethanol, distilled water, 2% acidulated water. For acidification was used the citric acid.

All samples were freeze dried with a 0.12 mBa vacuum at -50$^{o}$C (ScanVac CoolSafe 55-9 Pro Freeze Dryer, Denmark), ground to a fine powder (using a coffee grinder) and stored in plastic zipper bags at -20$^{o}$C until analysis.

The samples were dosed and then were mixed with the solvent (report sample: solvent = 1 : 20) for 30 min. at a temperature of 30 ° C, using the extraction solid-liquid. After extraction carrying out, the plant extracts were kept at a temperature of 4-8 $^{o}$C in the refrigerator.

### 3.2. Extracts samples preparing for analysis

One ml of extract (obtained and stored as described above) was weight into a centrifuge tube (50 ml) and 10 ml of aqueous 96% ethanol was added. The mixture was homogenized for 5 min. (Vortex), centrifuged at 10000 rpm for 10 min. The supernatant was filtered through Whatman (Number 400) filter paper. The preparation of the sample is presented in Fig. 1 .

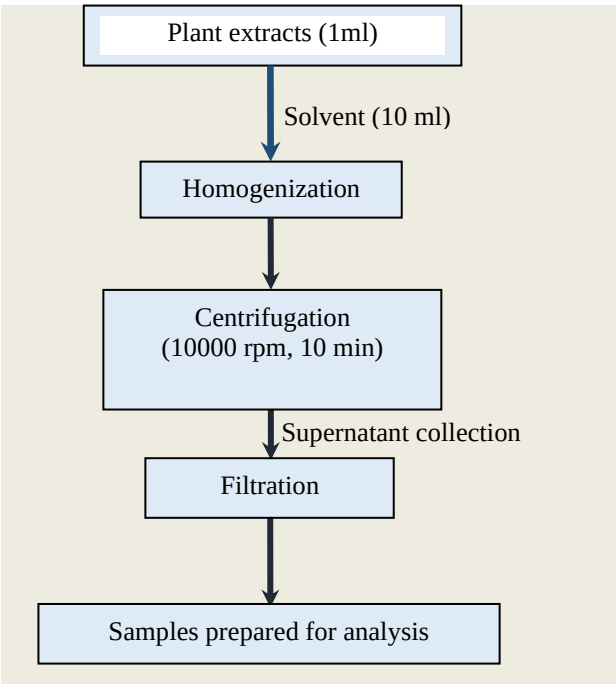


Fig 1. Extraction procedure for poliphenols and flavonoids content of the extracts.

#### 3.2.1. Total Phenol Content (TPC) analysis

The TPC was determined spectrophotometrically by Folin Ciocâlteu method [2] with several modifications [4]. 20 μl of skin extracts and 50 μl of flesh extracts were mixed with 50μl, respectively 100μl pure water in a 96 well flat bottom assay plate (NUNC, Denmark). 50ml Folin Ciocâlteu reagent were added and mixed for 1 min. After 5 min., 80 μl of a 20% solution (w/v) of Na2CO3 were added and mixed with a pipette; the microplates were shaken for 5 min. in the plate reader. After that, the plates were incubated at room temperature in the dark, agitating at 150 rpm on a Micro Plate Shaker (Biosan PST-60HL-4, Latvia) for 90 min. The absorbance of the samples was determined at 725 nm (TecanSun Rise,

software Magellan). Gallic acid was used as standard and total phenolic content was expressed as milligrams GAE (Gallic acid equivalents) per gram of dry weight (DW) materials.

### 3.2.2. Total flavonoids content (TFC) analysis

TFC analysis is based on the nitration of any aromatic ring bearing a catechol group (two contiguous hydroxyls in the aromatic ring) with its three or four positions unsubstituted or not sterically blocked, followed by the formation of an aluminum complex which turns to red in basic medium [5].

Aliquots of 150μl of extracts were also transferred to 1.5ml tubes. Volumes of 600μl distillated water and 45μl of a 7.5% solution sodium nitrite were added to each tube, mixed by inversion and left to react for 5 min. A volume of 45μl aluminum chloride 10% solution was pipetted onto the tubes, mixed by inversion and allowed to react for 1min. Finally, 300μl of a 1N sodium hydroxide solution and 360 μl distillated water were added and the tubes vortex mixed. The absorbance of every solution was measured at 510nm against the blank using a spectrophotometer DR2800 (Hach Lange, USA).

Values of the absorbance samples were interpolated into a minimum squares' regression equation (a 5-point calibration curve with an $R^2$ value of 0.998), which was calculated with the absorbance and the corresponding concentration of each quercetin standard. Quercetin hydrate in ethanol was used as the standard. Final results were calculated taking into account sample weight, extraction volumes and dilution factors applied and were expressed as mg quercetin equivalents (QE) per gram of dry weight (DW) of sample.
The experiments were carried out in triplicates.

## 4. Results

The TPC of obtained extracts is presented in Table 2.

*Table 2*

**TPC (mg GAE/100ml) of extracts obtained from some berries and *Hibiscus***

| Type of plant source | Solvent | | | |
|---|---|---|---|---|
| | Ethanol | Ethanol acid citric (2 %) | Citric acid 2% water solution | Water |
| Cranberries | 82.95±1.04 | 103.71±1.36 | 59.40±1.18 | 40.37±1.28 |
| Raspberries | 80.40±0.92 | 92.25±2.73 | 41.11±1.47 | 28.97±1.39 |
| Blackberries | 74.36±1.63 | 88.96±2.10 | 60.67±0.91 | 37.66±1.72 |
| Hibiscus | 81.69±2.63 | 101.22±2.51 | 83.93±1.56 | 53.14±2.51 |

Extracts were prepared using fruits from spontaneous flora
Values of TPC ± standard deviation

The influences of plant source (type of the extract that can be used for cider obtaining) and of the solvent used on TPC are presented in Fig. 2-3.

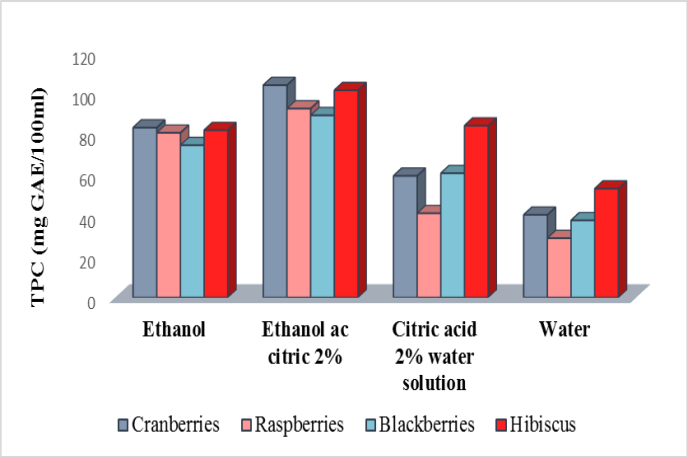


Fig. 2. Influence of solvent and plant source on TPC of extracts.

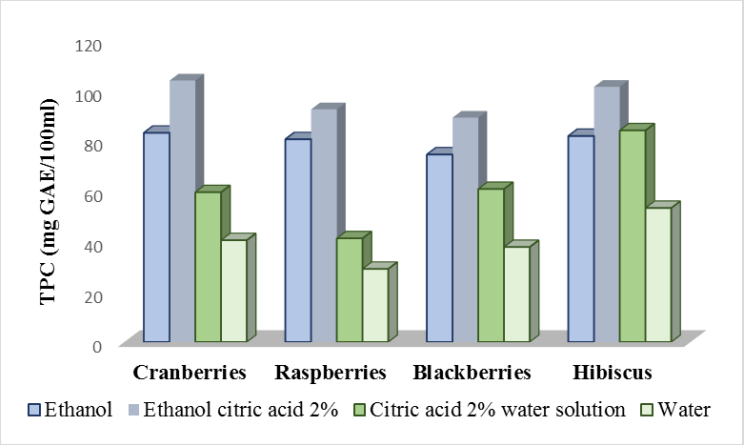


Fig. 3. Influence of plant source and solvent on the TPC of the extracts.

From Table 1, and Figs. 2-3, it can be noticed that irrespective of the vegetable source used, the highest content of polyphenols had the extracts obtained with acidified ethanol (2% citric acid).

The used plant source significantly influenced TPC, the highest values of this parameter being recorded for Hibiscus samples (Fig. 3). The total flavonoids content (TFC) of obtained extracts is presented in Table 3.

*Table 3*

**Total flavonoids content (mg QE/100ml) of extracts obtained from some berries and *Hibiscus***

| Type of plant source | Solvent | | | |
|---|---|---|---|---|
| | Ethanol | Ethanol acid citric (2%) | Citric acid 2% water solution | Water |

| | | | | |
|---|---|---|---|---|
| Cranberries | 33.44±1.61 | 34.45±0.73 | 12.68±1.45 | 13.55±0.61 |
| Raspberries | 15.35±0.52 | 19.29±0.24 | 8.38±0.39 | 7.69±0.56 |
| Blackberries | 34.17±1.58 | 15.86±0.90 | 12.66±1.84 | 10.16±1.12 |
| Hibiscus | 24.59±1.07 | 41.80±1.69 | 26.54±1.69 | 16.85±2.47 |

Extracts were prepared using fruits from spontaneous flora
Values of TPC ± standard deviation

The influences of solvent and plant source on TPC and TFC of extracts are presented in Figs. 4 -5.

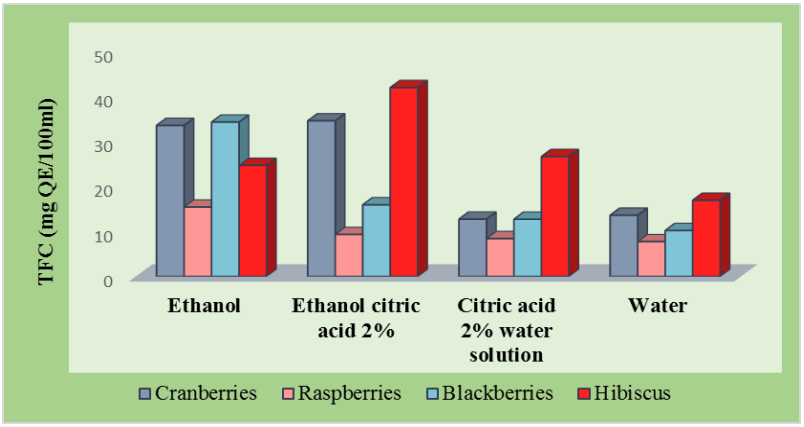


Fig. 4. Influence of solvent and plant source on TPC of extracts.

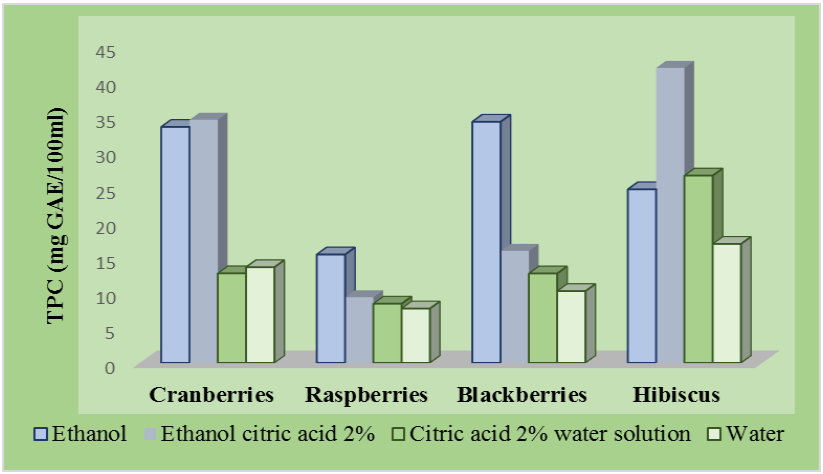


Fig. 5. Influence of solvent and plant source on TFC of extracts.

The highest flavonoid content was in the case of Hibiscus extracts, using as solvent ethanol citric acid 2%. TFC was particularly influenced by the type of vegetable source used to obtain the extracts. The solvent that led to extracts with high TFC values was all ethanol, but the addition of citric acid had differently influenced its values.

## 5. Conclusions

The highest polyphenol content was found in case of *Hibiscus* extracts, for an extraction time of 30 min. with ethanol acidified 2% (citric acid) as solvent. The lowest TPC values were found in case of extraction with water for the raspberry's extracts. The citric acid used for acidified the solvent (ethanol or water) had a beneficial influence on the TPC of the extracts obtained from the all plant source (berries and *Hibiscus*). The TPC and TFC values for all the extracts were lower when the water is used as solvent.

The recovery of antioxidants from berries, in order to be used for cider enrichment, must offer a few new opportunities for cider studying, as long as industrial practices have been already included different categories of additives, such as colorants from natural sources, and so on.

The objective of the study was to find a new niche, considering plants/berries extract usage for cider manufacturing. Such nutritional elements can be added during the technological flow, due to these solid-liquid phenolic extracts.

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