EFFECT OF DISTILLED BEVERAGES ON THE ANTIOXIDANT STATUS AND ON THE STABILITY OF POLYPHENOLIC COMPOSITION AFTER THE GASTROINTESTINAL DIGESTION, *IN VITRO* STUDY

INFLUENȚA DISTILATELOR ALCOOLICE ASUPRA STATUSULUI ANTIOXIDANT ŞI A STABILITĂȚII COMPOZIȚIEI POLIFENOLICE ÎN URMA DIGESTIEI GASTROINTESTINALE, STUDIU *IN VITRO*

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Abstract. *The in vitro effect of digestion on the quantity of phenols and on the antioxidant status after consuming three types of alcoholic beverages (bilberry brandy from commerce, cherry brandy from craft production and a topinambur distillate supplemented with natural extracts) was determined. There were identified a high stability of the total polyphenolic content and a relatively constant value of ABTS scavenging activity and of cupric reducing antioxidant capacity. This study also indicated a high stability related to the action of the pancreatin and of bile salts in the case of the artisanal sample. If the effect of alcohol intake was eliminated, the results have shown a reduced stability of the beverages containing bilberry and sour cherries, but also a reduced action of the distillate supplemented with spices – Sample 3.*
Key words: polyphenols, *in vitro* gastrointestinal digestion, antioxidant activity

Rezumat. *Efectul digestiei in vitro asupra cantității de fenoli şi a statusului antioxidant în urma consumului a trei tipuri de băuturi alcoolice (afinată din comerț, vişinată din producție artizanală şi un distilat din topinambur suplimentat cu extracte naturale) a fost determinat. S-a observat o stabilitate ridicată la nivel gastric a conținutului polifenolic total şi o valoare relativ constantă a inhibării radicalului ABTS şi a capacității antioxidante de reducere a cuprului. În acest studiu, a rezultat o stabilitate ridicată şi la acțiunea pancreatinei şi a sărurilor biliare a probei obținute artizanal. Dacă se elimină efectul ingestiei de alcool, rezultatele au demonstrat o stabilitate redusă a băuturilor ce conțin afin şi vişine, dar şi o acțiune mai redusă a distilatului suplimentat cu mirodenii – Proba 3.*
Cuvinte cheie: polifenoli, digestie gastrointestinală *in vitro*, activitate antioxidantă

INTRODUCTION

Fruits are the fermentable substrate most used to make natural distillates. In rural areas, from these, apples, plums and grapes are the most common sources of

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sugars, omnipresent in every peasant household. To improve the taste and to increase the palatability, the distillate obtained is supplemented with various natural juices from: cherries, sour cherries, blueberries etc. Beside the first two, the main juices are prepared from berries (Bermudez-Soto *et al.,* 2007). Excluding the ethanol and/ or sugar content, the consumptions of these beverages may be a beneficial one due to the content of active biological compounds in natural juices.

Among the bioactive compounds present, we distinguish mainly those which are part of the flavonoids category (for example, anthocyanins), which are found in high amount in *Vaccinium myrtillus* fruits. These compounds, identified in most of the fruits, are responsible for the antioxidant status which they express, which is significantly important against pathologies determined by the oxidative stress. Beside these, a strong antimicrobial effect can be added (Benzie *et al.,* 2011).Therefore, the action of gastrointestinal digestion on the antioxidant status and on total polyphenol stability was determined, following the consumption of two natural beverages (distillate of fruits supplemented with cherries syrup and distillate of topinambur supplemented with cumin, cinnamon and ginger). The antioxidant status was determined by the inhibition of two free radical species and of cupric reducing antioxidant capacity. *In vitro* simulation tests aimed at the pH and pepsin at gastric level, as well as that of bile salts and pancreatin for the small intestine.

MATERIAL AND METHOD

Samples. The following samples were used: bilberry brandy from commerce – Sample 1 (Angelli …), cherry brandy from craft production – Sample 2 (provided from Claudiu Raicu master student) and a topinambur distillate supplemented with natural extracts – Sample 3 (provided from SC Hypericum Impex SRL) – Sample 3.

ABTS scavenging activity. ABTS radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) on incubating the mixture at room temperature in darkness for 16 h. The solution thus obtained was further diluted with phosphate buffered saline to give an absorbance of 1.000. 50 µL sample was added to 950 µL of the ABTS working solution to give a final volume of 1 mL. The absorbance was recorded immediately at 734 nm with the Helios λ spectrophotometer. The percentage of inhibition was calculated with the following equation: % inhibition = [(Absorbance of control –Absorbance of test sample)/Absorbance control] x100 (Vamanu, 2013).

Reducing power. Cupric ion reducing power was evaluated by the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent, measuring the absorbance at 450 nm with a Helios spectrophotometer (Thermo Fisher Scientific, Inc., USA) (Pop *et al.,* 2016).

Total phenolic content. The reaction mixture was made from 0.5mL of the sample, 2.5 mL of Folin-Ciocalteu reagent in a dilution ratio of 1:10, and a saturated solution of sodium carbonate (75 g/L, 2 mL on average) was added 4 min later. The reaction mixture's absorbance was identified at 760 nm after a 2h incubation period, at room temperature. The reference standard engaged was Gallic acid, with the outcome provided as mg GAE (Gallic acid equivalent) of the extract (Vamanu, 2017).

***In vitro* simulation.** It was realized after the technology presented in the following patent request (Vamanu *et al*., 2011) by using sterile Duran screw thread tubes, GL with silicone seal for samples obtaining. The samples were taken with a seringe, 2 mL volume.

RESULTS AND DISCUSSIONS

The effect of gastric digestion

The antioxidant status and the total phenol level determined following the gastric transit is presented in table 1. Considering also the action of the lysozyme in the simulated saliva solution, it was determined that the total phenol content has increased a bit, mainly after one hour of action of the digestion determined by the pepsin. Results have been confirmed by the product Sample 1 (alcoholic beverage from commerce). Sample 2 was an exception, as it had an increase of the total phenols quantity which exceeded 50%. The proteolytic action of the pepsin combined with the low pH leads to an additional release of phenol compounds from the molecules resulted from the fruits juice added to the final formula.

The antioxidant status was determined after the consumption of Sample 2, which has maintained its ABTS scavenging activity. *In vitro* tests of evaluation of the antioxidant status for Sample 1 have decreased in time. For Sample 3, stability was noticed regarding the power of reduction, at the gastric transit, which might be interpreted as a constant presence of reductones.

Table 1

Total phenolic content and antioxidant status after *in vitro* digestion

Level of digestion		Total phenolic content (µg/mL gallic acid)			ABTS scavenging activity (%)			CUPRAC (450 nm)		
		Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Gastric level	0 h	18.25	22.25	13.85	75.47	94.91	29.21	1.14	1.31	0.69
	1 h	21.10	26.25	18.60	68.36	93.00	45.62	1.09	1.00	0.72
	2 h	20.00	30.00	20.00	53.00	72.75	50.00	1.05	2.10	0.67
Small intestine level	3 h	10.00	20.00	11.00	15.80	54.35	11.42	0.72	1.82	0.43
	4 h	10.00	18.76	9.69	14.68	53.87	10.67	0.68	1.74	0.45
	5 h	9.58	21.57	3.68	14.77	54.00	9.40	0.60	1.76	0.45
	6 h	7.65	19.67	4.78	15.04	54.24	8.75	0.51	1.67	0.48
	7 h	7.00	19.87	3.00	15.12	54.06	8.25	0.52	1.70	0.47

The effect of digestion in the small intestine

Following the digestion suffered by the pancreatic juice and the bile salts (tab. 1) it has resulted that Sample 2 was the most stable from the point of view of

the phenol composition. The differences were of maximum 6%. In exchange, the rest of the samples have manifested a low resistance after minimum two hours of digestion at this level. Sample 3 has showed a loss of over 60% of the total phenols quantity. This compared to the product traded – Sample 1, approximately 3 times higher. Results have demonstrated a low stability at this level, compared to the gastric digestion. An exception was represented by Sample 2 which showed a mix of stable phenol compounds under these conditions. Results obtained have demonstrated the presence of some similar phenol compounds in Sample 1 and Sample 3, which do not resist to the pH change, to enzymes and bile salts of the human digestive tube.

The antioxidant activity determined after the pancreatic digestion was high only for Sample 2, being of over 50%. For the other two samples, the ABTS scavenging activity did not exceed an average of 15%. These findings were directly correlated with the polyphenol level of table 1. The degradation suffered by auto-oxidation by these compounds during the digestion at neutral pH is a known aspect and corresponds with the presence of some derivates of the catechin.

Results of the value of the reduction power for Sample 3 might demonstrate an increase in time of the pH value due to the entire composition. The low pH value determines, normally, a chemical instability of the compound, which is compensated by the composition in bioactive compounds of the products (Lee *et al.*, 2003).

The same trend was also determined for CUPRAC, the maximum loss being calculated for Sample 1 (approximately 50%). The differences between the three samples are due to the conditions of products and to the fruit species from which they have been obtained, which directly affect the composition and stability of the bioactive compounds. Some of the compounds of Sample 1, which can be preserve the stability of the product in time, are added to all these.

According to some previous studies, following the gastric digestion, the derivates of the caffeic and cholorogenic acids are not affected by the pepsin attach and by that of the low pH. These compounds are found in the composition of extracts from species of plants used in making these drinks (Mechikova et al., 2010). Results obtained confirm the stability in various food products of the polyphenol acids. In addition, the slight increase of the total quantity (tab. 1) of polyphenols can be interpreted as a degradation of some compounds with higher molecular weight binding these molecules. Retention of these compounds is also confirmed by studies which showed absorption of the anthocyanins at gastric level (Bermudez-Soto *et al.,* 2007).

According to some previous studies there are important differences, of approximately 50%, between the quantities of polyphenols which shall be available and which can be absorbed, by studies *in vitro* in a static simulation system. For this research, a protocol similar to that of the in vitro testing of some microbial strains was used, but normally, there are changes in protocol. In

exchange, no other enzymes were added, for the digestion in the small intestine, as there is data in the literature that support the stability of various phenol compounds directly. If there was a direct consumption of fruits, the enzyme composition is important for the bioavailability of these compounds (for example, catechin) (Hur *et al*., 2011).

An important aspect is the estimation of results *in vivo*, as well as the comparison with other results obtained in other models of simulation *in vitro*. Most of the times, a compromise will be made between the constructive type (static or dynamic) of the simulator *in vitro* and the physiological requirements which need to be complied with. These types of results are valuable and fully accepted in case of extreme pathological conditions, such as gastric hypo or hyperacidity and/or pancreatic insufficiency (Guerra *et al*., 2012).

CONCLUSIONS

The results thus obtained demonstrate that the three types of beverages determine, after consumption, an important contribution of active biological compounds. Within reasonable limits, the beverages can be consumed without problems for the health, with the purpose to act as functional products. The beverage obtained from sour cherries presented the highest antioxidant status after the gastrointestinal digestion (expressed as ABTS scavenging activity and reduction power). The other two samples have shown a low stability, mainly after the action of the pancreatin and bile salts.

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