Journal of Pharmacy and Pharmacology 8 (2020) 7-12
doi: 10.17265/2328-2150/2020.01.002

Spectrophotometric Evaluation of Tannin Content in Domestic Beer Samples with Fe(III) and 1,10-Phenanthroline

Crnkic Mirsad and Klepo Lejla

Department of chemistry, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33, 71 000 Sarajevo, Bosnia and Herzegovina

Abstract: Tannins are polyphenolic compounds that can be divided into two groups by hydrolyzing and condensing. Tannins are produced in organisms of vascular plants called tannosomes and sequestered in vacuoles. The essential uses of tannins are in leather production, as adhesives, additives for wine, beer and fruit juices, etc. They are most present in the growing tissues of the plant as a crust, so beer that ripens in new oak barrels will contain a higher concentration of tannins. Tannins are extracted from malt during grinding and from the hops during cooking. The objective of this research is to determine the tannins content in twelve domestic beer samples (three samples of dark beer and nine samples of light beer) by spectrophotometric method. The method is based on the reduction of Fe(III) to Fe(II) by tannins. The iron(II) reacts with 1,10-phenanthroline at pH 4.4 to form a color complex. The absorbance measurements were made at 540 nm. As a standard tannic acid was used, tannin content was in range 15.49-1722.05 µg/mL. Tannins are present in all beers, above the threshold of detection. When tannins are present in excess, they negatively impact beer by causing astringency but beer completely devoid of tannins does not taste right.

Key words: Tannins, domestic beer, spectrophotometry, 1,10-phenanthroline.

1. Introduction

Polyphenols are a large group of compounds that contain in their structure more than one phenolic hydroxyl group. The phenolic compounds found in plants are mostly polyphenols and can then be defined as various groups of biological molecules of natural origin that contain more phenolic groups in their structure. These compounds are mostly present in taller plants. They are responsible for the organoleptic properties of food products derived from the processing of plants. Polyphenols are widespread in various types of seeds, fruits, and other organs of the plant, primarily in the free form or attached to sugars as glycosides or esters. Polyphenols can be divided into three main groups: flavonoids, lignin carbohydrate complexes and tannins [1]. Tannins are water-soluble plant polyphenols and a group of compounds that differ significantly in their structure. Their common property is that they bind and precipitate proteins. The name tannins came from the word “tanna” (Old German word for oak or fir tree). The tannins obtained from these herbs were used for tanning animal skin and fur. Tannins are most prevalent in plants such as oak, oak bark (*Quercus* spp.), Chestnut (*Castanea* spp.), Pickle or rush (*Rhus typhina*) and bodies (*Tellima grandiflora*), mangroves. The dark colour and astringent taste of food are often ascribed to tannins. They can have a large influence on the nutritive value of many foods consumed by humans such as vegetables, fruits, chocolate, tea, alcoholic and non-alcoholic beverages, etc. [2, 3]. The essential uses of tannins are in leather production, as adhesives, especially wood adhesives, additives for wine, beer and fruit juices, cement superplasticizers, medical and pharmaceutical applications. The ability of tannins to complex with proteins is largely responsible for the production of leather from hide and

Corresponding author: Lejla Klepo, Ph.D, assistant professor, research fields: organic chemistry, phytochemistry.

in that way they become an integral part of the final product [4].

There are numerous classifications of tannins, but the most common classification is:

- Hydrolyzing tannins (Galotanins and ellagitannins)
- Condensed tannins or proanthocyanidins.

Some authors also introduce a third group of tannins, the so-called complex tannins [3].

Galotanins and ellagitannins, unlike proanthocyanidins, are much less abundant in nature. Galotanins and ellagitannins are present in fruits, pods, and cecidia (and in some cases in wood and bark) dicotyledons such as oak, chestnut, rust, and others, but not detected in monocotyledons [5]. Tannins are most prevalent in growing tissues of plants as a bark, so the beer that ripens in the new oak barrels will contain a higher concentration of tannin. Tannins are extracted from malt during milling and from hops during cooking. They react with proteins in the wort during warm boiling (coagulation of proteins and their precipitation and precipitation). Tannins also react with proteins, as the wort cools during cold boiling. In addition, tannins in beer can react with proteins to form foggy complexes. The tannins are brown and may be responsible for the color in the wort or beer. The products containing a relatively high amount of tannins such as tea, beer and wine are the most widely consumed non-alcoholic and alcoholic beverages not only in Turkey but also all over the world. Tannins in beer can be reduced by the addition of polyvinylpyrrolidone. Tannic acid binds to proteins that create opacity but does not have a strong effect on proteins that cause foaming. Each beer contains tannins. In fact, a certain amount of tannin is required to make the beer taste acceptable. Beers containing tannins at concentrations well below normal limits were considered less palatable than normal beers. However, if tannins are present in excess, they cause pungency in the beer. Pungency can be described as drying, crackling caused by tannins that react with saliva proteins on the tongue. Pungency in beer is sometimes replaced by a taste of bitterness. The reason is that some tannins and bitters (they activate the response in the taste buds in the palate) are responsible for bitterness [2, 6].

Beer is the third most popular beverage behind water and tea, and the most consumed alcoholic drink in the world. Beer is typically brewed using four main ingredients: a starch source, yeast, hops, and water. Beer is made by fermentation, where yeast is added to consume sugars from the wort and to produce carbon dioxide, ethanol, and volatile phenolic compounds. After anywhere from a few days to several weeks of fermentation, the yeast is removed, and the beer is matured to develop more flavor before being filtered and bottled for sale [7]. Depending on the condition of fermentation (like temperature) and type of yeast that was used there are several kinds of beer like Lager, Ale, Porter, Stout, Pilsner, etc. [8]. Lager beers are generally lighter and light beers, but of course, there are all shades of color. Yeasts used in lager have a lower tolerance for alcohol, ferment at lower temperatures 5-13 °C, and after fermentation, they are lowered to the bottom. Lager belongs to the category of lower boiling beer. Due to their higher alcohol resistance, ale beers also need a higher boiling point, 15-25 °C. The fermentation takes place on the surface, the so-called upper fermentation beer. They have high alcohol content 8%-12%. Most varieties of beers are considered as types of Ales or Lagers. Stout and Porter are two different styles of Ale beer with very little difference. Stout is darker and denser than porter, has a creamy texture and strong flavors. Pilsner is most similar to our “ordinary” beers, but in principle, it is a light lager. It is characterized by a golden color, light but pungent taste due to its higher CO_2 content. Its taste may contain weak notes of bitterness, which it gets from hops [6, 8].

The aim of this paper is to evaluate the content of tannins in different types of beer of domestic manufacturers by the spectrophotometric method with iron(III) and 1,10-phenanthroline according to the method of Lau et al. [9].

2. Materials and Methods

2.1 Materials

Chemicals that were used were all analytical grade and did not require further purification. Tannic acid ($C_{76}H_{52}O_{46}$), 1,10-phenanthroline ($C_{12}H_8N_2$), iron(III) chloride hexahydrate ($FeCl_3 \cdot x6H_2O$), hydrochloric acid (HCl, 36%), acetic acid (CH_3COOH, 96%), sodium acetate (CH_3COONa), gelatine and sulphuric acid (H_2SO_4, 98%) were from Semikemd. o.o. Bosnia and Herzegovina (BiH); sodium chloride (NaCl) was from Solana d.d., Tuzla, BiH and kaolinite ($Al_2Si_2O_5(OH)_4$) was from Sedlecký kaolin a.s. The Czech Republic. All measurements were done on UV/Vis Spectrophotometer (Perkin Elmer Lambda 25), for preparing samples an ultrasonic bath (Branson) was used and an analytical balance (E. Metler, type AB 104) for weighting of substances.

2.2 Reagents

Tannic acid stock solution (100 μg/mL) was prepared fresh by dissolving 100 mg of tannic acid in 1 L of distilled water. The 1,10-phenanthroline solution (0.015 mol/L) was prepared by dissolving 2.970 g of 1,10-phenanthroline powder in 1 L of distilled water. Iron(III) chloride hexahydrate solution (0.01 mol/L) was prepared by adding 1.350 g iron(III) chloride hexahydrate in distilled water and adding 1 mL of hydrochloric acid (36%) and diluting with distilled water to 500 mL. Ethylenediaminetetraacetic acid (EDTA) solution (0.05 mol/L) was prepared by dissolving 1.850 g of EDTA in 100 mL distilled water. Acetate buffer pH 4.4 was prepared by dissolving 20.00 g of sodium acetate and 14.30 mL of acetic acid in 250 mL of distilled water. The pH was adjusted by the addition of concentrated acetic acid. Gelatine solution (0.3% m/V) was prepared by dissolving 3.00 g of gelatine in saturated sodium chloride solution and diluting to 1 L with a saturated sodium chloride solution. Acidic sodium chloride solution was prepared by adding 25 mL of sulfuric acid (98%) in 374 mL of saturated sodium chloride solution [7, 9].

2.3 Samples

For the evaluation of tannin content by the spectrophotometric method with iron(III) and 1,10-phenanthroline in beer from Bosnian manufacturers, all twelve beer samples were commercially available. Of the twelve beer samples, nine were light and three were dark. According to the manufacturing process, eight samples were Lager beer, two were Ale beer, one Porter and one Pilsner.

2.4 Methods

2.4.1 Making Calibration Curve

Aliquots of standard tannic acid (100 μg/mL) were pipetted to 25.00 mL flasks so that the final concentrations of tannic acid were 0.5, 1.0, 2.0, 3.0 and 4.0 μg/mL. Each flash was added 2.50 mL of iron(III) chloride hexahydrate solution (0.01 mol/L) and the mixture was incubated in a water bath at 80 °C for 20 minutes. Mixture of 2.50 mL of acetic buffer (pH 4.4), 5.0 mL of 1,10-phenanthroline solution (0.015 mol/L) and 0.50 mL of EDTA (0.05 mol/L) were added to each flask. When the solution is cooled, the volumetric flask is made up with distilled water to the mark. The absorbance of the solution is measured at a wavelength of 540 nm relative to the blank. The blank contains all the components as well as the standards except the standard solution of tannic acid. These measurements of the sample absorbance were used for the plotting of the calibration curve [9].

2.4.2 Preparing of Samples for the Analysis

Before the use, each beer sample was de-gassed to remove the carbon dioxide that was present. Aliquots of the corresponding beer samples were treated as previously mentioned for the standards of tannic acid.

2.4.3 Preparation of Blank for Beer Samples

The blank samples containing 10.00 mL aliquots of the corresponding beer samples were added in separate 100 mL calibrated flasks which were previously added 0.50 mL of gelatine solution (0.3% m/V). After the addition of 10.00 mL acidic sodium chloride solution and 2.00 g of kaolinite, the mixture was shaken and filtrated after the participate settled. The 10.0 mL of the filtrate was added in a 100.00 mL flask which was pipetted 10.00 mL of acidic sodium chloride solution, 3.00 mL of gelatin solution, 6.00 mL of distilled water. After the addition of 2.00 g of kaolinite and shaking, the mixture was allowed to settle and then filtrated. The filtrate after the second filtration was treated like the previously mentioned standard of tannic acid and samples of beer; addition of iron(III) chloride hexahydrate solution, incubation at 80 °C, acetic buffer (pH 4.4), 1,10-phenanthroline solution (0.015 mol/L) and EDTA and measured [9].

2.4.4 Preparing the Gelatin Blank

To evaluate the tannin content in the samples, gelatine blank samples were made to the corresponding blank samples. The gelatine blank samples were made with the same procedure as the blank beer samples except distilled water was used instead of the sample solution, filtrated twice and prepared like the samples for the standard of tannic acid and beer sample. The difference between the blank beer sample and the gelatine blank sample gave the net blank sample. The tannin content was determined from the difference in absorbance in the beer samples and net blank samples and their concentration deduced from the calibration graph. The corresponding equations are listed below:

$$A_{nbs} = A_{bbs} - A_{gbs}$$

where A_{nbs} is the absorbance value of the net blank samples, A_{bbs} value of the blank beer samples and A_{gbs} values of the gelatine blank sample for the corresponding sample.

$$A_t = A_s - A_{nbs}$$

where A_t represents the absorbance value of the tannins in the sample and A_s the value of the beer sample [9].

3. Results and Discussion

The calibration curve of standard solutions of tannic acid in a range of concentrations from 0.5 to 4.0 μg/mL was recorded at a wavelength of 540 nm. Good linearity was achieved with a correlation coefficient of $R = 0.9943$. The obtained direction equation $y = 0.2781x + 0.5286$ was used to calculate the tannin content of the samples tested.

The objective of this research is to determine the tannins content in twelve domestic beer samples (three samples of dark beer and nine samples of light beer) by the spectrophotometric method with iron(III) and 1,10-phenanthroline. The method is based on the reduction of Fe(III) to Fe(II) by tannins. The iron(II) reacts with 1,10-phenanthroline at pH 4.4 to form a color complex whose absorbance was measured at the wavelength of 540 nm. All measurements were done in triplicates.

The calibration curve was constructed by plotting the concentration-dependent absorbance for different concentrations of standard tannic acid (from 0.5 to 4.0 μg/mL). Good linearity was achieved with a correlation coefficient of $R = 0.9943$. The obtained equation $y = 0.2781x + 0.5286$ was used to calculate the tannin content of the samples tested.

The tannin content was calculated from the equation of the direction $y = 0.2781x + 0.5286$ and the results of the analyzed beer samples of the Bosnian manufacturers are shown in Table 1.

Tannin concentration in the average finished beer is usually not higher than 150 to 330 μg/mL. Depending on the area where the barley was grown, these values vary. About two-thirds of this is obtained from the barley husk and about one-third from the hops. The exact proportions vary, of course, with the original weight of the beer and the amount of hops used in the beer [10, 11].

In all twelve beer samples (light and dark) the content of tannins was from 15.49 ± 6.30 to 1,722.05 ± 5.06 μg/mL. Samples of dark beer showed higher content of tannins than light beers which are in according to the literature values.

Table 1 The tannin content in tested domestic beer samples.

#	Sample	City of manufacturing	Data on the label		Content of tannins (μg/mL) (expressed as tannic acid)
			Extract content (%)	Alcohol content (%)	
Light beers					
1	Pale Ale beer	Doboj	12.8	5.2	360.86 ± 6.87
2	Kastel lager beer	Banja Luka	11.4	5.0	192.74 ± 1.03
3	Pilsner light beer	Tuzla	11.2	4.7	112.22 ± 8.33
4	Oetiger lager beer	Sarajevo	12.2	5.4	73.00 ± 3.96
5	Erster lager beer	Tuzla	10.2	4.3	66.73 ± 6.67
6	Lager light beer	Sarajevo	11.8	4.9	64.15 ± 4.74
7	Preminger lager beer	Bihac	11.4	4.8	61.40 ± 3.10
8	Pale ale light beer	Livno	12.2	5.0	39.03 ± 1.20
9	Lager light premium beer	Sarajevo	12.0	4.9	15.49 ± 6.30
Dark beers					
10	Porter dark beer	Livno	13.0	5.5	1,722.05 ± 5.06
11	Lager dark beer	Tuzla	13.0	4.7	1,316.82 ± 6.32
12	Lager dark beer	Sarajevo	12.2	4.9	1,084.63 ± 2.38

The lowest content of tannins among dark beers was in the beer of Sarajevo brewery (1,084.63 ± 2.38 μg/mL), followed by the dark beer of Tuzla brewery (1,316.82 ± 6.32 μg/mL), while the highest tannin content was in the dark beer of Livno brewery (1,722.05 ± 5.06 μg/mL). For the samples of light beer, the content of tannins was from 15.49 ± 6.30 μg/mL to 360.86 ± 6.87 μg/mL. The lowest tannin content in light beer samples was in Lager light premium beer form Sarajevo brewery (15.49 ± 6.30 μg/mL), followed by Livno brewery Pal ale light beer (39.03 ± 1.20 μg/mL), Preminger lager beer, Bihac brewery (61.40 ± 3.10 μg/mL), while the highest tannin content was in light pal ale beer of Doboj brewery (360.86 ± 6.87 μg/mL). This beer sample had also a very strong fruity aroma hence, probably, the high tannin content. Obtained results for domestic beer are in correlation with the tannin content of analyzed Turkish beers (65.37 ± 2.61~74.73 ± 4.10 μg/mL) [2]. Comparing to the results of Debebe et al. [12] for tella (a malt beverage like beer) where tannin content is in range of 0.18 ± 0.11 to 8.84 ± 1.89 μg/mL GAE (gallic acid), results for our domestic beer are higher. Reason for lower content of tannins may be because malt beers have a different process of making and a different method for the determination of the tannin content was used. Also comparing the results of five analyzed beers of Lau et al. [9] where tannin content was in a range from 90.00 ± 4.8 to 101.0 ± 0.10 μg/mL (expressed as tannic acid) with obtained results we can conclude that our results are in correlation.

As far as color is concerned, most beers are translucent light yellow to dark yellow. Dark beers contained tannins at concentrations above 1,000 μg/mL. All dark beers exhibited a more intense odor than light beers. Polyphenols are the most oxidizing molecules in beer so they act as a protector but too much can produce complexes that cause turbidity. Beers containing tannins at concentrations well below normal limits were considered less palatable than normal beers, on the contrary, if the tannin concentration is too high, it can cause pungency [8].

4. Conclusions

Water-soluble polyphenols, particularly tannins, are important to the flavour, colour and mouth feel of almost all kinds of beverages and food. The spectrophotometric method using iron(III) and 1,10-phenanthroline was used to evaluate tannin content in domestic beer samples.

The tannin content of all twelve analyzed samples ranged from 15.49 ± 6.30 to 360.86 ± 6.87 μg/mL for light beers and up to 1,722.05 ± 5.06 μg/mL (expressed as tannic acid) for dark beers. Light domestic beers showed that the content of tannins is similar to the beers made in Turkey. Also, our result showed that dark beers contain tannins at concentrations above 1,000 μg/mL which can be attributed to the higher content of the extract.

The concentration of tannin in domestic beer samples was in the interval of concentrations for average beer.

References

[1] Baruah, J. B. 2011. *Chemistry Research and Application, Chemistry of Phenolic Compounds—State of the Art*. New York: Nova Science Publishers, Inc.

[2] Tinkiliç, N., and Uyanik, A. 2001. "Spectrophotometric Determination of the Tannin Contents of Various Turkish Black Tea, Beer and Wine Samples." *International Journal of Food Sciences and Nutrition* 52: 289-94.

[3] Vermerris, W., and Nicholson, R. 2007. *Phenolic Compound Biochemistry*. Dordrecht: Springer

[4] Elgailani, I. E. H., and Ishak, C. Y. 2014. "Determination of Tannins of Three Common *Acacia* Species of Sudan." *Advances in Chemistry* http://dx.doi.org/10.1155/2014/192708.

[5] Hagerman, A. E. 2002. *Tannin handbook*, Department of Chemistry and Biochemistry, Miami University Oxford.

[6] Colby, C. 2014. "Tannins for brewers (The basics)." *Beer and Wine Journal*, Accessed December 11, 2019, https://beerandwinejournal.com/tannins/.

[7] Anderson, H. E., Santos, I. C., Hildenbrand, Z. L., and Schug, K. A. 2019. "A Review of the Analytical Methods Used for Beer Ingredient and Finished Product Analysis and Quality Control." *Analytica Chimica Acta* 1085: 1-20.

[8] Bamforth, C. W. 2004. *Beer—Health and Nutrition.* Oxford: Blackwell Science Ltd.

[9] Lau, O. W., Luk, S. F., and Huang, H. L. 1989. "Spectrophotometric Determination of Tannins in Tea and Beer Samples with Iron(III) and 1,10-Phenanthroline as Reagents." *Analyst* 114 (5): 631-3.

[10] Palmer, J. J. 2006. "How the Mash Works." In *How to Brew: Everything You Need to Know to Brew Beer Right the First Time*. Boulder, CO: Brewers association.

[11] Palmer, J. J. 2017. *How To Brew, Everything You Need to Know to Brew Great Beer Every Time*. Brewers Publications

[12] Debebe, A., Chandravanshi, B. S., and Redi-Abshiro, M. 2016. "Total Content of Phenolics, Flavonoids and Antioxidant Capacity of Selected Traditional Ethiopian Alcoholic Beverages." *Bulletin of the Chemical Society of Ethiopia* 30 (1): 27-37.