REDISCOVERY OF REFRESHING DRINK - BRAGA
AND CONTRIBUTIONS TO ITS ADVANCED CHARACTERISATION

Ioana-Andreia Afilipoie*, Ioan Gontariu
Stefan cel Mare University of Suceava, Faculty of Food Engineering
13 University Street, 720229 Suceava, Romania
*E-mail: ioana.afilipoie@student.usv.ro

Abstract
This paper proposes the rediscovery and characterization by modern means of a soft drink, named braga, made from a mixture of cereals, obtained through double fermentation: the process is initiated by a short alcoholic fermentation, followed by a lactic fermentation during which forms the majority of flavour compounds. Braga is a traditional Turkish fermented beverages most appreciated in our country in the past. In the first part of the paper shall be submitted an improved recipe and are specified nutritional benefits of this drink, and in the second part of the paper shall be submitted the appropriate investigations relating to the identification and dosage of specific flavors drink, as well as analyzes that aim to identify and assay trace elements and heavy metals in braga. The aroma compounds were determined via chromatographic analysis, using a gas chromatograph mass spectrometer equipped with a Shimadzu GC-MS. In this beverage have been identified a number of 64 compounds, responsible for the flavor being most part of esters. This aroma compounds derived from cereals – raw material, but the majority of flavour compound resulting from alcoholic and acetic fermentation. This beverage was analysed in term of minerals and heavy metal content using an inductively coupled plasma mass spectrometer equipped with AGILENT 7500 ICP-MS.

Keywords: fermentative beverage, aromatic profile, trace elements, heavy metals

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1.INTRODUCTION

Appreciated in the past in large “bragagerii” (the shops where is selling braga) of the royal avenues until the last slum, braga is an alternative to carbonated drinks, which are most valued by consumers, but have negative effect on the body. The drinks very popular in the past, with oriental specific, seems to be in danger of extinction in today's Romania. Braga, in terms authentic “boza”, is a refreshing drink with sweet-sour taste and characteristic odor, a pronounced flavor, with a slightly cloudy/turbid and nectar consistency, prepared from a mixture of cereals (millet, barley, oats, rye, wheat bran and corn meal) subject to mechanical and fermentative processes. (Vasudha S., Mishra H.N., 2013) 8000 - 9000 years ago, braga is consumed fresh in Mesopotamia and in Thrace region, where it was prepared by a traditional technical, with specific utensils and tanks. Since the 18th century, braga became a specific drink for nomad-turks from Central Asia, spreading later in the Balkans, while the diversified its preparation. (Alan J. Et al., 2014)

This study proposes that recipe for obtaining braga a mixture of equal quantities of cereals (200g): barley, rye, wheat bran and corn meal and 600g of millet. Cereals were immersed in water and left for three days to germinate. Subsequently this have elapsed, let them be to dry and proceeded to their grinding. From their mixture with wheat bran and cornmeal has been made a batter/dough of which have shaped some palm sized cakes which have been baked. After cooling, they were broken in pieces, were placed into a container with a capacity of 10 liters, it was poured six liters of hot water over them and were allowed to ferment for three days. The liquid obtained was filtered, it was then divided into glass vessels and were given cold for consumption.
Braga contains about 0.50–1.61% protein, 12.3% carbohydrate and 75–85% moisture. In general, the pH of the boza samples ranges from 3.16 to 4.02, and the average alcohol content is 0.13% (w/v). (Todorov S.D., Holzopfel W.H., 2014)

Due to its nutritional content noticed by a large quantity of vitamins, as well as vitamins A, B1, B2, B6, B12, C, D, E, niacin, biotin, pantothenic acid, folic acid, minerals, such as zinc and magnesium, enzymes and phytonutrients necessary for the functioning of the human body, this drink is considered to be a tonic for both body as well as for mental stress. Braga is recommended for all, less diabetes. (Alan J. et al., 2014; Todorov S.D., Holzopfel W.H., 2014)

Cereal seed which is the raw material for obtaining braga have a high content of enzymes and phytonutrients which are then found in braga. The composition, the taste and flavors resulting from fermentation are higher since the water used is pure. (Todorov S.D., Holzopfel W.H., 2014)

The flavor of food is due to the presence of volatile compounds which may stimulate the olfactory receptors. Structurally, these compounds are separated and determined with difficulty and in quantitative terms can be found in a small percentage of the product, but they are of a great chemical diversity, especially if foods have been undergone fermentation or thermal processes. Studies have shown that from all of these compounds, less than 5% contribute to product flavor compounds being characterized as compound of the impact. (Iordache A.M., 2011; Maarse H. et. al., 1992)

Despite piercingly smell, braga is an appreciated drink owing to its character refreshing and energizing, but especially the beneficial effects on the body.

Specific flavor which prints in braga particular odorant characteristics, originate in large proportion of malt used in its preparation, but also after fermentative processes and transformations that occur during evolution. Their concentration is influenced by the conditions to which it is undergoes during the fermentation process. Among volatile compounds that define the flavor of product it list esters, alcohols, aldehydes and ketones, volatile phenols, lactones, free fatty acids and various chemical compounds. (Giuliano Drogone et al., 2009; José M. Oliveira, 2009)

Two different types of fermentation occur simultaneously during boza fermentation. The first is the alcoholic fermentation that produces carbon dioxide bubbles and increases the volume; the second, lactic acid fermentation, produces lactic acid and gives the acidic character to boza. Due to the increase in volume during fermentation, the wooden barrels should not be fully filled. After production, boza should be consumed within a couple of days to prevent an excessively sour taste. In practice, the fermentation rate is reduced by cold storage to extend the shelf life of boza. (Todorov S.D., Holzopfel W.H., 2014)

During fermentation, in braga is accumulated a quantity of organic acids (0.5 - 0.6 %), a major importance taking a lactic acid, which prints of drink in fermentation a pleasant and fresh flavor. Moreover, the lactic acid has a bactericidal action and occurs positively in the digestive processes. Is also recommended because has stimulant property of milk secretion for women after birth, but also is a source of vitamins and minerals in the diet of athletes. (Alan J. et al., 2014)

Studies on this drink have demonstrated that in a cubic centimeter of sweet braga was found 784500 germs, and in a cubic centimeter of sour braga, which is obtained from sweet braga after 5 - 8 days, was found 585000 germs which can restore intestinal saprophytic flora of the one who has used antibiotic treatment. (Alan J. et al., 2014; Vasudha S., Mihra H.N., 2013)

The presence of various microflora in braga, particularly bakery yeasts and lactic bacteria, has a beneficial effect on the digestive system. (Todorov S.D., Holzopfel W.H., 2014)

Of great importance is the quality of the food, this issue being considered a right of consumers with direct effects on their life, guaranteeing their safety as well as protection on their interests. (Iordache A.M., 2011)
Knowing that living matter is composed of macro and micronutrients whose amount varies from the order of grams parts per million (ppm) or billion (ppb), it has been demonstrated that some of these are absolutely necessary, for example, Mg, Na, Co, Ni, Cu, Zn, Cr, Mo, Mn, Se, other are essential such as Al, Ba, Sr, B, Li, and some non-essential or tolerable within certain limits: As, Hg, Cd, Pb, Au, Ag, U. (Iordache A.M., 2011, Rusu T.E., 2011)

Along with minerals considered to be liable for beneficial actions to the body, in braga may appear less desirable trace elements, which may have as a source raw materials used and which in a certain concentration may cause some disturbances on consumer health.

The aim of this paper is to present a refreshing, natural and healthy drink - braga, in terms of qualitative and quantitative parameters, which define both aromatic profile and the composition in trace elements and heavy metals.

The objectives which have been the basis of study were:
- the identification of volatile compounds responsible for flavor printed in product by using gas-chromatography analysis coupled with mass spectrometry detection (GC-MS);
- the determinations of the parameters that characterize the quality and safety of this beverages applying inductively coupled plasma mass spectrometry (ICP-MS) for the determination of heavy metals;
- the determinations of trace elements as part of the composition of braga using inductively coupled plasma mass spectrometry (ICP-MS).

2. MATERIAL AND METHODS

Of all methods of separation of different substances from a mixture, used in in analytical chemistry and technology, the chromatographic method is considered the most efficient. Chromatography is a method of separation of multicomponent mixtures which is based on the distribution of different components of a mixture between a mobile phase and a stationary phase, and hence moving with different speeds of the components worn mobile phase along stationary phase. (Gutt Gh., Gutt S., 2005) The technique combining gas-chromatography with mass spectrometry (GC-MS) is very useful for the identification and the study of volatile compounds present in various alcoholic and non-alcoholic beverages, after generating a specific chromatograms. (Iordache A.M., 2011) In the research of braga has used a gas-chromatograph coupled with mass spectrometry GC-MS QP2010 Plus, Shimadzu within Instrumental Analysis Laboratory of the Faculty of Food Engineering, Suceava.

The flavor compounds were separated using a capillary chromatographic columns CP-sil88 with the following dimensions: length – 50 m, outer diameter - 0.32 mm, internal diameter - 0.2 µm Stationary phase has been siloxane, the flow rate of helium - 1 ml/min. Gas chromatograph has been connected to a computer with program for the registration of data, their processing occurs using soft GC-MS Postrun Analysis. Compounds have been identified using the database of mass spectrometry NIST.

In order to identify of volatile compounds in braga via gas-chromatographic technique combined with the mass spectrometry, the sample was processed as follows: have been introduced in a glass bottle covered with a rubber stopper a mixture of 5 ml of the sample with 2 g of NaCl and was thermostated for one hour at a temperature of 85°C for separating volatile compounds. Using a syringe were extracted the resulting vapors and have been inject in the chromatography column after have been specify working specific parameters of computer program. The duration of the analysis was 50 minutes, during which time on the computer monitor has been generated specific chromatogram, its interpretation being achieved through software GC-MS Postrun Analysis from the software of the computer.

The mass spectroscopy is the most sensitive method of structural analysis, using a microanalytical technique that allows to measurement of relative molecular masses of unitary compounds, as well as highlighting certain atomics and functional species existing in the compound analyzed. (Gutt Gh., Gutt S.,
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This analytical method allows to getting the ions, their separation on the basis of the mass-charge ratio \((m/z)\) and their registration, having been used for quantitative determination and structural atoms and molecules. (Culea M., 2008)

The mass spectroscopy has allowed the development of methods for determining of the content of heavy metals in drinking water and beverages, using inductively coupled plasma mass spectrometry (ICP-MS), technique used for the quantitative determination multielement at the level of traces \((\text{mg/l})\). A solution can be analyzed in a minute allowing the analysis of a large number of samples in a very short time, at low detection limits, to the level of parts per trillion \((\text{ppt})\). (Iordache A.M., 2011, Rusu T.E., 2011)

For analysis of heavy metals and trace elements in braga has been used a mass spectrometer with inductively coupled plasma ISP-MS Agilent 7500, within Instrumental Analysis Laboratory of the Faculty of Food Engineering, Suceava. The parameters of ICP-MS have been: nebulizers - 0.9 ml/min, RF power 1500W, carrier gas - argon \((0.92 \text{L/min})\), the mass-range 7-205, integration time 0.1 seconds, the acquisition 22.7 seconds.

For the analysis of trace elements and heavy metals in braga whit the aid ISP-MS technique were extracted in a crucible 5 ml of the sample, using a syringe, the mixture having a neutral-slightly acidic pH (between 5.5 - 6) and has been introduced in an oven at a temperature of 200°C, maintaining up to completely evaporation on the liquid. The method is based on the principle of calcination of the sample at 450°C with gradual increase of the temperature and the dissolving of ash with 2 ml \(\text{HNO}_3\) 65%. The mixture has been brought to the mark with distilled water in a 25 ml graduated flask, after which the sample has been subjected to analysis at the ISP-MS, electrical component generating corresponding results which have been processed by specific calculation techniques. The calibration has been carried out with a solution of multielement \((\text{Li, Be, B, Na, MG, Al, K, as well as, V, Cr, Mn, Fe, Fe, Co, Ni, Cu, Zn, GA, As, se, Rb, Sr, Ag, Cd, Cs, BA, are you, Pb, U})\) purchased from a specialized company and was correlated the concentration of each component with the 10 \(\text{mg/l}\) and it was determined quantity of multielement in the distilled water sample used for dilution.

3. RESULTS AND DISCUSSION

Having as main objective the aromatic profile of natural drink braga, following gas-chromatographic analysis have been identified a number of 64 compounds. Only a part of this compounds have been responsible for the flavor, highlighting the mainly esters.

In Fig.1 is shown the chromatogram generated after the analysis and the identification of compounds, red points representing each of the components identified, including those who belong to the column. The highest peaks are specific compounds in the column, such as siloxane and naphthalene.

![Fig.1. Gas chromatogram of volatile compounds in braga](image-url)
Among the identified compounds were found: 20 esters, 19 alkanes, 12 acids, 5 amines, 4 alcohols, 2 aldehydes, ketones and other 4 the organic compounds containing chlorine and iodine. Of these, the compounds that give specific flavor of braga are esters, for example pentaphluoropropyl undecyl ester; methoxiacetyl,4-hexadecyl ester; propionyl, 2-methyl, 2,2-dimethyl propyl ester; octyl benzyl ester; trimethyl-4-oxo, 3,4-hydroxy- 2-piran ester; benzendicarboxyl mono ester; some of the acids, such as trichloroacetic acid and ascorbic acid which print and sweet-sour taste, and some of alkanes, such as hexadecane, octadecane, eicosane.

Following the identification of the flavor compounds, the chromatogram recorded and other volatile compounds that does not print special properties in product, such as phthalates (diethyl phthalate, dibutyl phthalate) or amines (diphenylamine, caprolactam), whose source is the raw material - cereals, the soil in which they have been grown or the most likely of the treatments applied in agriculture (pesticides, insecticides).

In figures 2.a.-2.j. are shown details of gas-chromatogram specific for braga. Generating of specific peaks of volatile compounds was achieved after 8 minutes at the sample injection.
Fig. 2.C. Detail of Figure 1 for the minutes 16.2 - 20.2

Fig. 2.D. Detail of Figure 1 for the minutes 20.3 - 24.3

Fig. 2.E. Detail of Figure 1 for the minutes 24.4 - 28.0
Fig. 2.F. Detail of Figure 1 for the minutes 28.1 - 32.0

Fig. 2.G. Detail of Figure 1 for the minutes 32.1 - 36.2

Fig. 2.H. Detail of Figure 1 for the minutes 36.3 - 40
According to Article 63 of Order No. 611 of 3 April 1995 for the approval of hygiene norms of food and sanitary protection thereof, published in M.O. No. 59bis of 22th March 1996 braga must meet the following quality requirements: dry matter in refractometric degrees at 20°C min. 7, acidity in lactic acid not exceeding 0.65g / 100ml of the product.

If using altered raw materials, synthetic sweetening substances, emulsifiers or preservatives, is not permitted for human consumption.

Having as objective to identify trace elements and heavy metals in braga, after interpretation of the results obtained from the analysis of ICP-MS and the calculations, has been generated the graph in Figure 3, representing the variation in concentration of trace elements. Thus, it has been demonstrated that in braga are present minerals such as: Mg, Na, K, As, Mn, Zn, the highest concentration having a magnesium, respectively 61,2055 µg/l and the lowest zinc, respectively 0,0285 mg/l.

As regards the content of heavy metals, data obtained from the analysis have demonstrated the presence of Al, Ni, Pb, Cu, Cr, U, I, increased values of concentrations recorded among aluminum and nickel.
Analyzing the results obtained with those present in current rules on food safety (Law 311/2004 and STAS 6362/85) shall reflect the fact that braga is a properly product, heavy metals identified having a concentration value does not exceed the maximum permissible concentration.

In view of the fact that in the preparation of beverage have not been used dishes and utensils which contain metals identified, the most likely origin of these traces of heavy metals would be raw material or soil where grown cereals.

4. CONCLUSIONS

To obtain a high quality finish product with a very fine flavour, decisive is the quality of the raw material used in the process.

Due to the characteristics that satisfies the nutritional point of view but also in term of quality and safety of food, braga represents an efficient and healthy alternative among soft drinks.

Although it has been identified a wide range of volatile compounds, those who give the specific aroma and taste are in a smaller number, being represented especially by some substances of category esters.

From the point of view of food safety, braga is a beverage falling within the relevant rules, being found only traces of heavy metals which do not have any direct adverse effects on the body.

5. REFERENCES


[9] Rusu T.E., **Comparative study of biomarkers of quality, safety and authenticity of traditional Romanian distilled beverages – abstract thesis**, University of Agricultural Science and Veterinary Medicine, Cluj Napoca, 2011;
