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Determining total and bio-available concentrations in Fe, Mn, Zn and Cu in grapes and assessing grape mineral supply

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Abstract

The paper supplies data regarding total and bio-available concentrations in four essential minerals – Fe, Me, Zn and Cu in grape samples from pollution-free areas in Caras-Severin County, Romania. Mean values of total and bio-accessible concentrations – 8.42 mg/kg Fe, 0.82 mg/kg Mn, 1.58 mg/kg Zn and 1.98 mg/kg Cu, and 3.90 mg/kg Fe, 0.49 mg/kg Mn, 1.28 ¬mg/kg Zn and 1.46 mg/kg Cu, respectively – determined experimentally show that the grapes analysed can be a supplementary source of micro-elements.

Bio-accessibility of micro-elements determined by calculus was 81.01% in Zn, 73.74% in Cu, 59.76 in Mn and 46.32% in Fe.

Under the conditions of this trial, mineral supply, i.e. the degree of coverage of the necessary daily intakes of bio-accessible micro-elements, reached 64.89% in Cu for both men and women, 19.5% in Fe in men and 8.67% in Fe for women, 8.52% in Mn for men and 10.89% in Mn for women, and 4.65% in Zn for men and 6.40% in Zn for women.

Keywords: grapes, micro-elements, bio-accessibility, mineral supply

1. Introduction

Grapes are among the most important fruits used in human diet. The nutritional qualities of this fruit are due to its rich content in vitamins such as A, B1, B2, B6, C, PP and K, and in numerous essential minerals such as K, P, Mg, Ca, Fe, Mn, Zn, Cu, etc. [1,2]. Grapes are also very rich in amino acids, tartric and citric acids, and they contain important amounts of resveratrol, an antioxidant that protects against cancer, heart diseases and degenerative diseases [3].

Given the important content in bio-elements of grapes, this fruit can be a supplementary, alternative source of micro-elements.

But we should take into account the fact that grape total amounts of minerals cannot be entirely used by or stored in the human body. Therefore, just knowing the total content of minerals is not enough to assess whether this fruit is or not an important source of minerals. To assess the mineral supply of a food, in general, and to determine its contribution with essential elements, we need to determine the concentration of bio-accessible minerals [4]. Bio-accessibility is generally defined as the amount of nutrients absorbed by a living organism from foods that can be used for the body's physiological functions [5].

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In this paper, the bio-accessible concentration of a mineral micro-element is used to define the amount of micro-element that can be released from grapes after the process of gastro-intestinal digestion that can be used by the organism for its functions [6].

This paper aims at presenting total and bio-accessible concentrations in Fe, Mn, Zn and Cu of grape samples from private producers from a pollution-free area in Caras-Severin County, Romania, to assess mineral bio-accessibility and supply to the daily recommended intake.

2. Materials and Methods

2.1. Materials

To carry on the trial, we sampled table grapes (black grapes) from private producers in Caransebes, Caras-Severin County. These samples were used to prepare two trial sets: three samples to determine total mineral concentrations and three samples to determine bio-accessible mineral concentrations.

2.2. Reagents

- Super-pure nitric acid 65% - Merck (d = 1.39 g/cm^3) to prepare a solution of 0.5 n HNO_3;
- Concentrated standard solution of Merck purity to prepare calibration solutions;
- Work standard solutions to dilute the concentrated standard solution: for each element analysed, we prepared six sets of standard solutions to cover the concentration intervals of the elements analysed;
- Distilled water;
- Pepsin, Merck;
- Bile salt, SIGMA ALDRICH;
- Pancreatine, ACROS ORGANICS;
- Amylase, SIGMA ALDRICH;
- Solution of gastric juice used to simulate gastric digestion, containing 12.5 g/L pepsin in 0.15 mol/L NaCl, pH = 2.5;
- NaOH solution 1 mol/L;
- Solution of intestinal juice used to simulate intestinal digestion, containing 37.5 g/L pancreatine, 12.5 g/L amylase and 1.875 g/L bile salts.

2.3. Apparatus

- Spectrophotometer with flame atomic absorption Varian AA 240 FS;
- Stir thermostat GFL 33031;
- Centrifuge HETTICH Universal 32 R (10.000 rpm);
- Thermal regulation electric stove Raypa;
- Calcination oven Nabertherm (max 3000^{o}C);
- Drying stove Memert (max. 200^{o}C).

2.4. Procedure

2.4.1. Determining Total Concentration

Total concentrations in Fe, Mn, Zn and Cu were determined through atomic absorption according to a protocol described by Gogoașă et al., 2015 [7]. Practically, 20 g of sample were dried (for 6 h in an electric drying stove), and calcinated at 550^{0}C in two sessions of 4 h each.

After calcination, the solubilisation of inorganic matter was done in a solution of HNO_3 0.5 N. To do so, ash samples were treated with 20 mL solution of HNO_3 0.5 N, evaporated on the electric stove until almost dry, added another 20 mL solution of HNO_3 0.5 N and the again evaporated; this was done twice, after which the sample was brought up to 50 mL. The clear solution thus obtained was used to determine total content in Fe, Mn, Zn and Cu through the FAAS method.

2.4.2. Determining Bio-accessible concentration

Bio-accessible concentrations in Fe, Mn, Zn and Cu were determined using an *in vitro* gastro-intestinal digestion after two protocols used by Pawel Pohl et al., 2011 and 2012, adapted to the equipment of our laboratory [8, 9].

Practically, about 20-30 g of grape berries were crushed in a porcelain capsule 10 cm diameter; we then sampled three medium samples of 1.5 g each and incubated them with 20 mL gastric solution under stirring (in a Thermostat GFL 33031) for 4 h, at 37.5^{0}C. after the time for gastric digestion simulation was over, the mixture of fruit and gastric solution was neutralised with a solution of natrium hydroxide to a pH = 7.5; then we added 20 mL solution of intestinal juice and we continued the incubation (under the same conditions) for another 4 h. finally, the incubated obtained after the simulation of gastro-intestinal digestion was centrifuged at 5000 rpm for 15 min). The supernatant thus obtained was used to determine Fe, Mn, Zn and C through FAAS.

2.4.3. Determining bio-accessibility of Fe, Mn, Zn and Cu

Bio-accessibility or the bio-accessible fraction of minerals in the analysed grapes was determined with the formula:

$$FB(\%) = \frac{C_B}{C_T}$$

where: FB – bio-accessible fraction (%);

C_B–bio-accessible concentration of analysed element (mg/kg);

C_T – total concentration of analysed element (m/kg).

2.4.4. Determining Mineral Supply in Daily Recommended Intake

Mineral supply (MS), i.e. the coverage degree of the necessary Fe, Mn, Zn and Cu in the daily recommended intake was determined with the formula:

$$MS(\%) = \frac{m}{m_r}$$

where:

MS – mineral supply (%); m – bio-accessible amount of element in consumed grapes (g); m_r – amount of element recommended for the daily intake (g).

3. Results and Discussion

3.1. Total Concentrations in Fe, Mn, Zn and Cu

Total concentration in essential elements is shown in Table 1 below.

Table 1. Total concentration in Fe, Mn, Zn and Cu in grapes

Values	**Mineral element, mg/kg of fresh produce**			
	Fe	**Mn**	**Zn**	**Cu**
Minimum	9.34	1.02	1.86	2.31
Maximum	6.12	0.68	1.35	1.63
Medium	*8.42*	*0.82*	*1.58*	*1.98*

As shown in Table 1, analysed grapes contain appreciable amounts of total essential micro-elements. Among the micro-elements analysed, Fe ranked first with a medium concentration value of 8.42 mg/kg. Smaller, yet close concentrations were in Cu and Zn, whose medium concentrations were 1.98 mg/kg in Cu and 1.58 mg/kg in Zn. Manganese was identified in smaller amounts than Cu and Zn, and much smaller than in Fe; total concentration of Mn in the grapes analysed was 0.82 mg/kg. Total medium concentrations of the micro-elements determined in fresh grapes show the following descending trend: Fe> Cu≈ Zn> Mn.

3.2. Bio-accessible concentrations and bio-accessibilities of Fe, Mn, Zn and Cu

Bio-accessible concentrations of the micro-elements Fe, Mn, Zn and Cu in grapes determined through *in vitro* gastro-intestinal digestion are shown in Table 2 below.

Table 2. Bio-accessible concentrations in Fe, Mn, Zn and Cu in grapes

Values	**Mineral element, mg/kg of fresh produce**			
	Fe	**Mn**	**Zn**	**Cu**
Minimum	3.64	0.31	1.09	1.52
Maximum	4.35	0.76	1.2	1.31
Medium	*3.90*	*0.49*	*1.28*	*1.46*

These data show the fact that the grapes analysed contain appreciable bio-accessible amounts of Fe, Mn, Zn and Cu with bio-accessible concentration levels between 0.49-3.90 mg/kg. Among bio-accessible elements, Fe is the best represented (3.90 mg/kg), followed by Cu and Zn in smaller but close concentrations (1.46 mg/kg and 1.28 mg/kg, respectively) and Mn identified with the lowest concentration (0.49 mg/kg).

It is worth mentioning that these bio-accessible concentrations (determined through *in vitro* gastro-intestinal digestion) are concentrations of micro-elements released from the fruit matrix after gastro-intestinal digestion.

Comparing the values of total and bio-accessible concentrations, we can see that the values of bio-accessible concentrations are lower than those of total concentrations. This proves that only a certain percentage of the total concentration can be released after gastro-intestinal digestion.

The bio-accessible fraction or the bio-accessibility of Fe, Mn, Cu and Zn in the studied grapes was determined through calculus. Their values are shown in Table 3 below.

Table 3. Values of bio-accessibility of Fe, Mn, Zn and Cu in grapes

Element	**C_T**	**C_B**	**FB**
	mg/kg	**mg/kg**	**%**
Iron	8.42	3.90	46.32
Manganese	0.82	0.49	59.76
Zinc	1.58	1.28	81.01
Copper	1.98	1.46	73.74

CT – total concentration; CT – bio-accessible concentration; FB – bio-accessible fraction.

Bio-accessibility of analysed micro-elements, i.e. their accessible fraction, had values between 46.32% in Fe and 81.01% in Zn. Among the elements analysed, Zn is the most available (81.01%), followed by Cu (73.74%), Mn (59.76%) and Fe (46.32%).

Comparing the experimental values of bio-accessibility of Fe, Mn, Zn and Cu with the values obtained by Ramos et al. (2012) and by Pohl et al. (1011 and 2012) from other foods, we see that there are no notable differences [8, 9, 10].

3.3. Assessing Mineral Supply in Grapes

Nutritive value of the studied grapes can also be assessed by evaluating their mineral supply in the daily recommended intake. Given that simply knowing total concentrations of mineral elements is not enough to establish whether this fruit can be an important source of minerals, we calculated the concentrations of bio-accessible minerals to assess the mineral supply of grapes [11,12]. Thus, taking into account the bio-accessible concentrations of Fe, Mn, Zn and Cu (Table 2) and the recommendations of nutrition specialists [13] regarding the necessary minerals in the daily recommended intake (Table 4), we determined the mineral supply of essential micro-elements in the daily intake.

Table 4. Reference values of necessary Fe, Mn, Zn and Cu in the daily recommended intake

People sample	Element (mg)			
	Fe	Mn	Zn	Cu
Men aged 19-50	8	2.3	11	0.9
Women aged 19-50	18	1.8	8	0.9

Under the conditions of this trial, the supply of bio-accessible Fe, Mn, Zn and Cu in the daily recommended intake for 400 g of grapes containing 1.56 mg Fe, 0.196 mg Mn, 0.512 mg Zn and 0.585 mg Cu is shown in Table 5 below.

Table 5. Supply of bio-accessible Fe, Mn, Zn and Cu in the daily recommended intake for 400 g of grapes

People sample	Bio-accessible mineral supply (%)			
	Fe	Mn	Zn	Cu
Men aged 19-50	19.50	8.52	4.65	64.89
Women aged 19-50	8.67	10.89	6.40	64.89

The mineral supply in the daily recommended intake calculated based on the bio-accessibility of Fe, Mn, Zn and Cu (Table 5) has values between 4.65% in Zn and 64.89% in Cu in men and between 6.40 in Zn and 64.89% in Cu in women.

In these conditions, the degree of coverage of the daily necessary intake of bio-accessible micro-elements ranged between 64.89% in Cu in men and women, 19.5% in Fe in men and 8.67% in Fe in women, 8.52% in Mn in men and 10.89% in Mn in women, and 4.65% in Zn in men and 6.40% in Zn in women.

Mineral bio-accessible supply has the descending trend Cu > Fe > Mn > Zn in men and Cu > Mn > Fe > Zn in women.

4. Conclusion

Total concentration of minerals in grapes has mean values between 0.82-8.42 mg/kg. The best represented of the micro-elements is Fe (842 mg/kg), followed by Cu and Zn with smaller yet close values (1.98 mg/kg and 1.58 mg/kg, respectively); Mn was identified in smaller amounts (0.82 mg/kg).

Bio-accessible concentrations in Fe, Mn, Zn and Cu range between 0.49 and 3.90 mg/kg. Iron is the best represented bio-accessible element (3.90 mg/kg), followed by Cu and Zn in smaller, yet close concentrations (1.46 mg/kg and 1.28 mg/kg, respectively) and Mn with the lowest concentration (0.49 mg /kg).

Bio-accessibility of micro-elements analysed has values between 46.32% in Fe and 81.01% in Zn. Zinc is the most bio-accessible (81.01%), followed by Cu (73.74%), Mn (59.76%) and Fe (46.32%).

Mineral supply in the daily recommended intake based on bio-accessibility of Fe, Mn, Zn and Cu ranges between 4.65% in Zn and 64.89 in Cu in men and between 6.40 in Zn and 64.89% in Cu in women.

In the current trial, the degree of coverage of the daily necessary bio-accessible micro-elements reached the following values: 64.89% in Cu in men and women; 19.5% in Fe in men and 8.67% in Fe in women; 8.52% in Mn in men and 10.89% in Mn in women; 4.65% in Zn in men and 6.40% in Zn in women.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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