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Determination of Zinc and Copper micronutrients and Lead and Cadmium contaminants in non-alcoholic malt beverages by anodic stripping voltammetry

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ABSTRACT

Non-alcoholic malt beverages are nutritional soft drinks and suitable to replace with alcoholic drinks due to increased public concern about harms associated with synthetic sugars and alcohol. Malt beverages are rich sources for minerals such as zinc and copper, two essential minerals for normal human body functions. However, malt beverages may contain high levels of heavy metals, including lead and cadmium. Anodic Stripping Voltammetry was used to determine concentrations of these elements in non-alcoholic malt. Non-alcoholic malt beverages of 119 bottles representing 13 different brands in 11 different flavors were purchased from the market in Tehran. The mean concentration of zinc, copper, lead and cadmium were 1.34±1.00, 0.51±0.05, 0.04±0.02 and 0.05±0.03 mg/100 ml respectively. The results indicated that zinc and copper concentrations found in the malt beverages were too low to promote the beverages as sources of zinc and copper. Levels of lead and cadmium were below acceptable daily intake (ADI) established by the US Food and Drug Administration, which may alleviate concerns regarding heavy metal intake and malt beverages. The result of this study showed that malt beverages might be enriched with zinc and copper to be considered important sources for the minerals. Additionally, it can also be concluded that despite the differences between flavors and brands were not meaningful, but the bigger sample size may definite that brand 3 has the most micronutrients and the classic or plain flavored beverage has the least amount of Cd and Pb.

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1. Introduction

Non-alcoholic malt beverages are similar to common beer, but lack detectable amounts of ethanol. These beverages are produced in two different ways. In the first method, ethanol is removed from the beverage post-fermentation. In the second method, measures are taken to prevent the generation of ethanol during processing. Removing alcohol is favored by beer producing companies because they do not have to change their production line, but only add an alcohol removing step. This method, however, cannot remove 100% of the ethanol and traces of ethanol are usually present (0.05% to 0.5%). As consumption of ethanol is prohibited in I. R. Iran, malt beverage producers favor the second method which prevents ethanol production during processing (1).

Non-alcoholic malt beverages may have positive effects on human health such as prevention of osteoporosis (2), cardiovascular diseases (3), and kidney stones formation prevention (4), and reduction

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of the risk of Alzheimer's disease (5), while being a good source of key vitamins (6).

Malt beverages also are believed to have rich mineral content, including zinc and copper, which are focal points of this study. Zinc is the second trace element in highest concentration in the human body after the iron and has many key-roles in body functions such as immune-system modulation (7), learning and cognitive processes (8, 9), wound healing (10), age-related macular degeneration; AMD prevention (11) and fertility (12 , 13).

Copper, another essential trace element, is present in smaller concentrations of 1.4 to 2.1 mg per Kg human body. Copper acts as an important co-enzyme in iron metabolism, ATP production and many other vital functions like radical scavenging (14-22). Copper is also necessary for proper growth (23), bone formation (24), CNS function (25), and cardiovascular system function (26).

Some foods can serve as sources for heavy metal exposure, either due to pollution or the processing method (27). Lead and cadmium are believed to be most significant pollutants of malt beverages and are believed to enter the product at various points in production including, soil, seeds and even packaging. Lead is the heaviest stable metal and can affect the hematopoietic system (28), renal functions (29) and can cause infertility and CNS mal-function (30, 31). Cadmium, with no known function in high organisms, mostly harms lung (32) and kidney (33) function and bone development (34). Thus, lead and cadmium levels in foods and beverages should be closely monitored to ensure product safety. The purpose of this study was to determine the levels of zinc and copper micronutrients and lead and cadmium contaminants in non-alcoholic malt beverages by anodic stripping voltammetry.

2. Materials and methods

Non-alcoholic malt beverages (119 bottles, can and pet representing 13 different brands in 11 different flavors) were purchased from retail markets chosen randomly from Tehran, Iran. To evaluate element amounts, a 746 VA Trace Analyzer was used (Metrohm AG Ltd., Switzerland).

The cell was a three-electrode system with an Ag/AgCl electrode as a reference electrode, a hanging mercury drop electrode (HMDE) as the working electrode and a platinum electrode as auxiliary electrode (35).

Reagents and solutions were the tartaric acid, CH_3COOH, CH_3COONa, Pb $(NO_3)_2$, Cd (NO_3), Cu $(NO_3)_2$, Zn $(NO_3)_2$ and HNO_3 used were from Merck (pro- analysis grade). The metal stock solutions (1 g/L) were prepared in 0.005 M HNO_3. The acetate buffer was 0.2 M and pH 4.7 containing 0.2 M tartaric acid, as a supporting electrolyte.

To prepare a sample solution, 2ml of each bottle was weighed, and then heated until dried. After the remaining powder cooled to room temperature, 10ml of 65% concentrated nitric acid was added to make a solution. The solution was again heated until dried. The remaining powder was placed in a 450°C muffle furnace for 24 hours to give ash. After returning of previous section remnants to room temperature, the powder was washed with 10% concentration nitric acid and filtered until 25ml solution was acquired. This solution was the sample (35).

To prepare the blank solution, the same pattern of sample preparation was followed, except the first step, that de-ionized water was used instead of the malt beverage.

The buffer solution is needed to maintain pH during analysis despite water electrolysis. To prepare buffer solution, 34g of Sodium Acetate ($C2H_3O_2Na_2H_2O$) and 1.5gr Tartaric acid ($C_4H_6O_6$) was poured in a least possible amount of water and was inserted in the ultrasonic device until totally dissolved. More water added until the pH is set between 4.6-4.8. The solution was then diluted to achieve the volume of 200ml.

Standard solutions were prepared separately for each element. Goal concentration for each element in solution was 500 mg/L. To achieve this concentration, the proper amount of each element was calculated and the closest amount possible was weighed, then the exact concentration of the prepared solution was calculated to be used in further calculations.

The amounts of 0.227g of zinc nitrate (Zn $(NO_3)2.6H_2O$), 0.183g of Cu (II) nitrate (Cu $(NO_3)2.3H_2O$), 0.079g of lead nitrate (Pb $(NO_3)_2$) and 0.137g of cadmium nitrate (Cd $(NO_3)2.4H_2O$) were weighed and their related concentration was calculated 500mg/L respectively. The standard solution of the four metals was then mixed together.

The analyzing procedure started with calibrating the device by blank solution to ensure preparation methods have not contaminated the samples. Then 0.5ml of prepared sample and 10ml of buffer solution were added to sample flask of the device and the concentration was recorded. In 3 different steps, then 0.1ml of the standard solution obtained from the mixture of four standard metal solution which added to sample flask and related concentrations were recorded. This process was done two times for each sample and final concentration reported for each sample was the average of duplicate tests.

3. Results

Table 1 shows the amount of each element in all of the malt beverage:

Table 1. Amount of elements in all of the malt beverage

Element	Mean±SD (mg/100ml)
Zinc	1.342 ± 1.003
Copper	0.507 ± 0.035
Lead	0.038 ± 0.025
Cadmium	0.038 ± 0.005

Tables 2 to 6 show the amount of each element according to beverage flavor:

Table 2. Average amount of Zinc, Copper, lead and Cadmium in Peach flavor malt beverages (mg/100ml)

B	Zinc	Copper	Lead	Cadmium
1	0.937±0.391	0.121±0.011	0.058±0.004	0.053±0.007
3	2.797±0.161	0.448±0.373	0.034±0.025	0.035±0.012
5	3.278±0.697	1.032±0.107	0.023±0.016	0.025±0.014
7	2.377±1.168	0.833±0.390	0.028±0.000	0.011±0.009
8	0.431±0.248	0.123±0.094	0.033±0.003	0.006±0.003
9	1.651±0.585	0.301±0.168	0.068±0.023	0.098±0.081
10	0.549±0.417	0.176±0.056	0.061±0.025	0.096±0.058
12	0.740 ±0.549	0.387±0.238	0.089±0.005	0.055±0.030

B: Brand

Table 3. Average amount of Zinc, Copper, lead and Cadmium in flavor malt beverages (mg/100ml)

B	Zinc	Copper	Lead	Cadmium
3	0.905±0.308	0.062±0.007	0.077±0.032	0.099±0.044
4	1.456±0.352	0.110±0.097	0.078±0.001	0.091±0.049
8	2.958±0.634	1.119±0.009	0.012±0.010	0.021±0.003
9	0.605±0.316	0.143±0.039	0.023±0.010	0.025±0.019
12	0.472±0.390	0.310±0.248	0.0165±0.015	0.026±0.014

B: Brand

Table 4. Average amount of each element in Lemon flavor malt beverage (mg /100ml)

B	Zinc	Copper	Lead	Cadmium
4	0.830±0.409	0.300±0.052	0.069±0.035	0.070±0.039
5	1.481±1.060	0.825±0.053	0.060±0.001	0.080±0.069
8	0.869±0.709	0.534±0.029	0.018±0.013	0.016±0.012
10	2.535±1.659	1.012±0.009	0.037±0.025	0.067±0.001

B: Brand

Table5. Amount of Zinc, Copper, lead and Cadmium in Apple flavor malt beverages (mg /100ml)

B	Zinc	Copper	Lead	Cadmium
1	1.083±0.941	0.605±0.519	0.104±0.066	0.044±0.037
3	3.315±0.193	1.183±0.072	0.015±0.008	0.010±0.000
4	0.404±0.066	0.201±0.085	0.035±0.009	0.038±0.029
9	0.879±0.109	0.256±0.141	0.079±0.031	0.039±0.025
10	0.841±0.474	0.399±0.452	0.054±0.032	0.067±0.057

Table 6. Amount of Zinc, Copper, lead and Cadmium in malt flavor beverages (mg /100ml)

B	Zinc	Copper	Lead	Cadmium
1	0.438±0.145	0.049±0.011	0.030±0.012	0.018±0.012
3	4.648±0.913	0.565±0.469	0.041±0.003	0.048±0.013
2	1.276±0.446	1.1069±0.032	0.041±0.031	0.074±±0.033
5	0.376±0.285	0.142±0.006	0.031±0.003	0.039±0.003
6	1.481±0.003	0.728±0.051	0.027±0.011	0.068±0.049
7	1.452±1.000	0.514±0.450	0.003±0.001	0.019±0.002
8	2.706±0.808	1.041±0.000	0.010±0.005	0.010±0.004
9	0.480±0.208	0.211±0.113	0.013±0.007	0.014±0.005
10	0.234±0.146	0.105±0.071	0.020±0.007	0.019±0.012
11	0.096±0.027	0.189±0.117	0.012±0.009	0.022±0.016
12	1.007±0.275	0.093±0.036	0.064±0.030	0.089±0.005
13	1.983±0.019	2.712±1.079	0.045±0.001	0.108±0.081

B: Brand

The standard amount for zinc, copper, lead and cadmium in malt beverages is 5mg, 5mg, 0.5mg and 0.5mg per 100ml respectively.

4. Discussion

Average contents of Zinc, Copper, Lead and Cadmium in the samples of this study were significantly lower than suggested standard amount ($p<0.05$).

Brand 11 had the lowest zinc content and Brand 3 had the highest, even though the effect of the brand name in zinc content was not significant ($p>0.05$). For copper, maximum and minimum amount was measured in Brand 13 and Brand 11 respectively. Brand name effect on Copper content was insignificant ($p>0.05$). The highest and lowest concentration of lead was found in Brand 1 and Brand 11 respectively. Brand name was not a significant factor on Lead content ($p>0.05$). Most and least polluted to cadmium samples were Brand 13 and Brand 7. Cadmium pollution amount was not related to brand names in a significant way ($p>0.05$). Maximum average of zinc, copper, lead and cadmium was found in Lemon flavor, Tropical fruits flavor, Apple flavor and Tropical fruits respectively.

Minimum average in the same order was measured in Peach flavor, Malt flavor and Apple flavor. But statistics showed that effect of flavor on heavy metal content was insignificant ($p>0.05$).

This elements level could be attributed to the different manufacturing practices, variations in the quality of raw materials, finished products and packaging containers used by malt drinks manufacturers. Some analysis was performed on lead, copper and cadmium in wine and beer using potentiometric stripping method. The studies concluded this method was even better than the old ways (36, 37). Technical errors could also affect the accuracy and precision of results such as inevitable deterioration in the performance of the equipment as well as the aging of reagents.

Spanish researchers in 2005 conducted a study to determine copper, lead and cadmium in aniseed spirits and reported Cu concentration in a range of 6 - 473 µg/L and Pb and Cd less than 6 and 1.4µg/L respectively (38), which in comparison to our study results are lower significantly. This may be due to the kind of product difference or water used preparation, even the concentration of the elements in the soil and environment of the farming space.

Another study carried out in Tehran University of Medical Sciences in 2013 in order to measure the amount of tyramine present in the market of Tehran by spectrophotometry and its interaction with MAOI drugs compared to imported products with internal production, there was a significant difference in tyramine level (39).

Our study showed that non-alcoholic malt beverages available in I.R. Iran market can be considered as safe in lead and cadmium average levels which were significantly lower than maximum allowed level.

Zinc and copper are two micronutrients necessary for human body, but lower average of these metals than recommended value, shows malt-beverages can't be considered as a proper source for zinc and copper.

Because brand names and flavors, do not affect the zinc, copper, cadmium and lead content level significantly, none of the brand names or flavors can be suggested or refrained.

5. Conclusion

Non-alcoholic malt beverages studied here, are "safe" based on lead and cadmium content. Also likewise, they are not rich in copper and zinc in this research, but could prepare some part of daily need to these nutrients.

Conflict of interest

The authors have no conflict of interest.

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