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Iron Content of Liver Spreads

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Abstract: Analysis of the iron content of three locally manufactured liver spreads by flame atomic absorption showed calculated concentrations ranging from 6.50-21.15 ppm Fe. Two brands are comparable, while one brand is lower than the similarly determined 18.42 ppm iron content of raw chicken liver. All samples are significantly lower than 62.89 ppm iron in raw pork liver. ANOVA showed that there are significant differences among the samples. Tukey-Kramer test verified that iron in processed liver spreads have significant variation with raw pork liver but not in raw chicken liver. Liver spread Fe content varies among available brands and may reflect degree of extender use. The method used was validated and found to have LOD of 0.3429 ppm and LOQ of 1.143 ppm solutions; recovery of 99.09 ± 9.977; precision at %RSD < 11%; and linearity to 5.0 ppm at a correlation coefficient of 0.9952 and 0.9993.

Key Words: Fe, liver spread, AAS

1. INTRODUCTION

According to the Philippine Nutrition Profile surveyed by the Food and Agriculture Organization of United the Nations, iron deficiency anemia (IDA) is the most alarming micronutrient deficiency in the Philippines. This greatly affects infants, pregnant and lactating women and older persons. Four out of the 16 regions of the Philippines show a high number of IDA in most of the infants and preschoolers while 12 out of 16 regions for women who are pregnant and lactating. Causes of IDA include the lack of iron intake, decrease in the absorption of iron, iron loss and high amounts of iron requirement. Most cases of IDA are prescribed of oral iron therapy either by eating iron rich foods or by taking iron supplements.

Dietary iron also contributes in the treatment of IDA. It improves and maintains iron status through promoting both iron-containing and iron-rich foods as well as foods that enhance absorption of iron. Foods rich in iron can be mostly found in meat, organs of fish, cattle, and poultry and even in green leafy vegetables.

Iron stores in liver due to the degradation of hemoglobin, making liver as one of the most important sources of iron. Liver, however is an acquired taste for many, particularly children. And although a common ingredient used in the Filipino cuisine like kaldereta, menudo, dinuguan, sisig, and lechon, it is not readily eaten on its own, reducing its effectivity as a source of Fe. Processed liver products present alternatives with modified and potentially

more palatable flavors. These include liver spreads, pate and liverwurst. In the Philippines, liver spreads are the most commonly used among the three due to practicality and accessibility. In this paper, the iron content of three local liver spread brands was determined by Flame Atomic Absorption Spectroscopy (FAAS) and compared against raw liver samples of chicken and pork. Figures of merit were determined to validate the procedure used but were limited to limits of detection and quantitation, accuracy and recovery, precision and linearity.

2. METHODOLOGY

2.1 Detection and Quantitation Limits

The Limit of Detection, LOD and Limit of Quantitation, LOQ were verified by calculating the standard deviations of the blank samples. Eight replicates of the blank were prepared and the absorbance of each was obtained by FAAS.

LOD was determined using the formula (3SD)/m while LOQ was calculated with the expression (10SD)/m where SD is the standard deviation of the absorbance of the blank samples prepared and m is the slope of the calibration curve.

2.2 Accuracy and Recovery

Accuracy and Recovery of the method were confirmed by spiking blank samples with known concentrations of Fe. The concentrations added into the blanks must cover the range of concern and those concentrations that are close to the Limit of Detection. Seven replicates of the blank samples were each spiked with the following concentrations of Fe: 0.10, 0.30, 0.50, 0.75, 1.00, 2.50 and 5.00 ppm. Each blank sample contained 8mL of nitric acid and 4mL of hydrogen peroxide with a known amount of iron then diluted with distilled water. Recovery was calculated using the expression (Cs/Cm)×100 where Cs is the concentration of the sample and Cm is the concentration of the metal spiked to the blank sample.

2.3 Precision

The degree of the precision of the method performed was expressed in terms of percentage relative standard deviation, %RSD. Blank samples were prepared and were spiked with 1 ppm of Fe. Three trials were run through the flame atomic absorption spectrophotometer and the % RSD of each run was identified by the instrument. This validation was performed for three consecutive days.

2.4 Linearity

Calibration curve was plotted from the values obtained from the preparation of standard solutions mentioned in the proposed method and was presented in absorbance vs concentration. Linearity of the curve was identified using the correlation coefficient, r.

2.5 Analysis of Iron Content

One gram of each of the liver spread samples was placed in a 30-mL beaker. A mixture of 8 mL HNO_3 and 4 mL H_2O_2 was added in the sample and heated up to 85°C for about 5-10 mins in the fume hood until the mixture homogenizes. The solution was cooled to room temperature. Digested samples were then subjected filtration with Whatman filter papers followed by dilution in 25-mL volumetric flasks with distilled water. Blanks were carried out in the same way. The method was repeated for the wet digestion process of chicken and pork liver. Each sample was analyzed in triplicates. Absorbance of iron in liver spreads and chicken and pork liver were measured on a Shimadzu AA-6300 Spectro-photometer. The instrument was set at 248.3 nm with a slit width of 0.2 nm. The lamp current was fixed at 5.0 mA and Air/Acetylene flame was utilized.

3. RESULTS AND DISCUSSION

The LOD and LOQ calculated for iron were 0.34 ppm and 1.14 ppm, respectively. Concentrations below the LOD will be undetectable by the instrument. The accuracy which can be measured by the % recovery was calculated as 99.09±9.9773 which falls within the acceptable range of 80-110% for a concentration range of 1 to 10 ppm. For days 1, 2, and 3, %RSDs were 6.65%, 0.61% and 0.85%, respectively. AOAC expected precision at 1 to 10 ppm is 11-7.3% indicating that the method had good reproducibility. The accuracy and precision test for iron analysis demonstrated that the method was applicable and free from interferences. In the case of the linearity, results from the correlation coefficients are within the required values of $r > 0.995$, having $r=0.9952$ and $r=0.9993$, thus proves the linearity of the curve. Results of the method validation showed the reliability and applicability of the method.

At the manufacturer's recommended serving size of 42.5 g liver spread, 0.8987±0.214, 0.7929±0.133 and 0.2764±0.159 mg of Fe were determined for Brands X, Y and Z respectively. The same portion of raw chicken liver had 0.7829±0.197 mg iron while raw pork liver had 2.6729±0.249 mg.

None of these are sufficient to supply the recommended nutrient intake for iron which ranges from 8 mg for one-year-olds to 38 mg for pregnant women in the third trimester. Assuming, the samples analyzed was the sole source of iron, larger servings are required to gain the needed amount of iron of one's body. Iron deficiency anemia patients require greater amount of iron compared to the usual and thus more servings must be consumed by the patient to attain the amount of iron necessary in a day. Fortunately, many foods are rich in iron which can be found in meat, organs of fish, cattle, and poultry and even in green leafy vegetables. The concentrations converted to ppm are presented in Table 1.

Table 1. Determined Fe concentrations

Sample	Concentration, ppm
Brand X	21.15
Brand Y	18.66
Brand Z	6.504
Raw chicken liver	18.42
Raw pork liver	62.89

The results showed that pork liver had the most iron among the dietary sources of iron and Brand Z liver spread had the least. In addition, the data confirmed that the amount of iron in raw samples is greater than the liver spread samples. It was also observed that the iron content of raw samples is generally greater than the content found in the three liver spread samples. Processed foods seldom have greater amount of nutrients than the raw ingredients. The production of liver spread involves several processing techniques such as chopping, mixing, homogenizing, cooking, pasteurization and emulsification. Among these, cooking and mixing greatly affect the amount of Fe in the processing of liver spread. Cooking is known to degrade and convert heme iron to nonheme iron when cooked at high temperature at long periods. The differences in the amount of iron among liver spread brands could be due to the alternatives in ingredients, like soy protein extenders or cereals, and variations in processing.

4. CONCLUSIONS

The iron content of all the samples studied cannot supply the RENI amount of iron to all population groups except infants. Iron deficiency anemia patients require greater amount of iron compared to the usual and thus more servings must be consumed by the patient to attain the amount of iron necessary in a day. However, when added to supplements such as dietary iron tablets, drops and syrups, these samples could help increase the amount of iron that can be absorbed by the body.

5. REFERENCES

Aljaff, P., O.Rasheed, B., & M. Salh, D. (2014) Assessment of heavy metals in livers of cattle and chicken by spectroscopic method. IOSR Journal of Applied Physics, 6(1), 23-26.

Croghan, C. and Egeghy, P. (2003). Methods of dealing with values below the limit of detection using SAS. [online] US Environmental Protection Agency. Retrieved from http://analytics.ncsu.edu/sesug/2003/SD08-Croghan.pdf&ved=0ahUKEwjnrNOzp-nQjCNEfbFhC7XlmtAnF-NIDzewUnMKRCw&sig2=G8BKRVMwyNnD8SchCpIBQg

Demirel, S., Tuzen, M., Saracoglu, S., & Soylak, M. (2008) Evaluation of various digestion procedures for trace element contents of some food materials. Journal of Hazardous Materials, 152(3), 1020-1026.

Hibbert, D. Quality Assurance in the Analytical Chemistry Laboratory (2010). Oxford: Oxford University Press, USA.

Food and Agriculture Organization of the United Nations. (2010) Nutrition country profiles: Philippines summary. Retrieved 11 November 2016 from http://www.fao.org/ag/agn/nutrition/phl_en.stm

McLean, E., Cogswell, M., Egli, I., Wojdyla, D., & de Benoist, B. (2008) Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Public Health Nutrition, 12(04), 444.

Official Methods of Analysis of AOAC International. Appendix F: Guidelines for Standard Method Performance Requirements. [online] Retrieved 7 Dec. 2016 from http://www.eoma.aoac.org/app_f.pdf.