

# Study of the Potassium Content in Amaranth Protein Using the Gamma Spectrometry Technique

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**Abstract:** *Amaranthus* is a dicotyledonous pseudocereal and one of the new world's oldest crops, having originated from Meso-America and was a major food crop of the Aztecs. Popularity in the cultivation and consumption of *Amaranthus* seed in the modern era began in the mid-1970s with the rediscovery and promotion of amaranth due to its superior nutritional attributes as compared to cereal grains. Amaranth plant has a high-quality protein, carbohydrates, unsaturated oil, squalene, dietary fiber, tocopherols, phenolic compounds, flavonoids, vitamins and minerals. The amaranth's grain was collected at San Martin Pahuacan, Estado de Mexico, Mexico. The used methodology is gamma spectrometry with Hyperpure Germanium detector (HPGe) and Multichannel Analyzer (MCA) with Maestro<sup>®</sup> software. The result shows a unique concentration of radioactive potassium content in the amaranth protein isolated from amaranth grains is 424.1 mg/100 g of amaranth protein. There is an excellent agreement between the amount of potassium determined by atomic absorption spectroscopy in our laboratory and the value obtained in this work using the Gamma Spectrometry Technique.

Key words: Potassium, amaranth (Amaranthus hypochondriacus), gamma spectrometry, K-40, HPGe.

# 1. Introduction

Amaranth was a staple food of pre-Hispanic Mexicans and was important as a source of high quality nutrients. The family Amaranthaceae was generally considered as the Amaranth family. The word Amaranthus is basically derived from the Greek word "Anthos" (Flower) which means everlasting or unwilting. At the present time it is also called a third millennium crop plant [1]. Amaranth was a part of the daily diet, in addition to corn and beans. At the present time it is also called a third millennium crop plant [1]. Currently it is widely cultivated and consumed throughout India, Nepal, China, Indonesia, Malaysia, Philippines; whole of Central America, Mexico; Southern and Eastern Africa [2]. Recently, an increased interest in amaranth appeared in the 1980s, when the U.S. National Academy of Sciences entitled Underexploited Tropical Plants with Promising Economic Value, performed in 1975, amaranth was elected from among 36 of the world's most promising crops and since then, extensive research has been carried out [3]. Amaranth is considered as a "super food" because it counts with high nutraceutical values such as: a high-quality protein, unsaturated oil, squalene, dietary fiber, tocopherols, tocotrienols, phenolic compounds, flavonoids, vitamins and minerals. Compared to other grains, amaranth has higher amounts of protein, dietary fiber, calcium, iron and magnesium therefore although it is an ancient crop nowadays it is considered as a millennium crop or super food with relevant nutraceutical values. The research was performed on the grain and described its high nutritional value and agronomic potential [4, 5].

The aim of this work is to present the study of gamma spectroscopy technique for quantification of elemental potassium in Amaranth protein isolated from *Amaranthus hypochondriacus* grain. The sample protein was analyzed by means of the detection and quantification of potassium-40 (<sup>40</sup>K), radioactive

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isotope content of natural and innate in any product containing potassium [6]. The determination is based on measuring the gamma radiation emitted naturally by the protein immersed in potassium, this radiation is caused by the radioactive decay of potassium nuclide ( $^{40}$ K) (0.0118% of potassium) per unit time. Thus, when considering the balance between secular nuclides daughters and parents of this radioisotope, the rate of emission of gamma radiation is proportional to the amount of  $^{40}$ K in the study sample from the ratio in the sample (specific activity of the sample) and the specific activity of potassium theoretically established. One can determine the total elemental concentration of potassium in the sample.

As we can see in this work, Gamma spectrometry allows the identification and/or quantification of radionuclides by analysis of the gamma ray energy spectrum produced in a gamma ray spectrometer and is an alternative and powerful analysis method with great advantages, which includes a non-destructive technique, and does not require complex sample preparation and allows obtaining results with high reliability and accuracy in a relative short time.

Potassium is a chemical element with symbol K and atomic number 19. It was first isolated from potash, the ashes of plants, from which its name derives. In the periodic table, potassium is one of the alkali metals. Potassium is a mineral and an electrolyte. It helps your muscles work, including the muscles that control your heartbeat and breathing. Potassium comes in many foods you eat, for example, bananas, cantaloupe, grapefruit, prunes, raisins, potatoes, mushrooms peas amount many others. Your body uses the potassium it needs. The extra potassium that your body does not need is removed from your blood by your kidneys. It constitutes 5% of the total mineral content of the human body [7]. Natural potassium comprises the isotopes <sup>39</sup>K, <sup>40</sup>K and <sup>41</sup>K, where <sup>39</sup>K and <sup>41</sup>K are stable elements and <sup>40</sup>K is a radioactive isotope (isotropic abundance 0.0118%) with a half-life of  $1.28 \times 10^9$  years, and is the most abundant radioactive substance in the human body. <sup>40</sup>K decays to <sup>40</sup>Ca by  $\beta$  decay (89%) and to <sup>40</sup>Ar by  $\beta$ + decay (11%), the latter associated with the emission of a gamma ray of 1,462 keV which is used for the determination of <sup>40</sup>K concentration [8].

HPGe gamma spectrometry is a nondestructive analytical method and used here for the analysis of  ${}^{40}$ K in amaranth protein. The secular equilibrium between daughter nuclides and the naturally occurring parents implies the gamma ray emission rate for the decay to  ${}^{40}$ Ar is proportional to the amount of  ${}^{40}$ K in amaranth protein [9]. The aim of this work is to assess the potassium concentration in amaranth protein.

# 2. Methodology

## 2.1 Specific Activity of Potassium

The specific activity of potassium (number of disintegration per second per gram) is given by Eq. (1), where  $N_A$  = Avogadro's constant (6.02 × 10<sup>23</sup> mol<sup>-1</sup>), a = isotropic abundance of <sup>40</sup>K (0.0118%),  $M_w$  = molecular weight of potassium (39.1 g/mol) and  $T_{1/2}$  = half-life of <sup>40</sup>K (1.27 × 10<sup>9</sup> years) [10, 11].

$$Ac = \frac{N_{AG} \ln 2}{100 M_{W} T_{1/2}} = 31.20 B_{q/g} K$$
(1)

#### 2.2 Detection Efficiency

The detection efficiency  $E_{ff}$  is given by the number of counts per second per gram from the KCl standard divided by the specific activity of potassium  $A_c$  in Eq. (2), where  $C_{st}$  = total counts (1,461 keV peak) from the standard (including background);  $A\gamma$  = percentage gamma radiation emission from <sup>40</sup>Ar;  $C_B$  = total counts from the background radiation,  $M_{KSt}$  = mass of K in the KCl standard (g) and T = analysis time (s).

$$E_{ff} = \frac{C_{st} - C_B}{A_{\gamma} A_C M_{KSt} T} = \frac{162057 - 9570}{31.20 \times 0.11 \times 192.27 \times 86400}$$
(2)

The specific activity of a particular sample  $A_S$  is given by Eq. (3), where  $C_S$  = total count (1,461 keV peak) from the sample;  $C_B$  = total counts from the

background radiation;  $M_{KSt}$  = mass of the sample (g); T = analysis time (s) and  $E_{ff}$  = detection efficiency [12].

$$As = \frac{(C_{st} - C_B) / T}{W_S E_{ff} A_{\gamma}}$$
(3)

Finally, dividing the specific activity of a sample  $A_S$  by the specific activity of potassium Ac yields the concentration of potassium of the sample (Eq. (4)). Here the authors express the results as a percentage, or, equivalently, the number of grams of potassium per 100 g (or mL) of sample.

$$\% K = \frac{As}{Ac} \times 100$$
 (4)

#### 2.3 Sample Description

Amaranth is becoming a super food as its

composition includes high quality of carbohydrates, dietary fiber, lipids as omega-3 and omega-6, essential amino acids and other important constituents, such as squalene, tocopherols, phenolic compounds, flavonoids, vitamins and minerals.

Fig. 1 shows the *Amaranthus hypochondriacus* plant and it can be easily recognized among other crops as each plant has an "immortal colorful flower" that based on our own experience, contains more than 1 kg of grains in each big flower. The seed morphology, it is smooth, with slightly yellow color, shiny and slightly flattened with a lens-shaped form. Its size varies between 1.1 and 1.4 mm of length and 1.0 and 1.3 mm of width while its weight is usually between 0.6 and 1.0 mg.



Fig. 1 Amaranthus hypochondriacus plant.

An important nutritional fact is that besides containing a high amount of protein [13], Amaranth's grain helps to have a balanced protein diet because they provide several amino acids including those considered as essential that needs to be taken from the diet.

The amount of minerals per 100 g of Amaranth's seed [14] and 100 g of Amaranth protein determined in our laboratory using the atomic absorption spectroscopy technique is given in Table 1.

#### 2.4 Sample Selection

The selection of amaranth grains (Amaranthus hypochondriacus) of the protein sample analyzed collected in the town of Panhuacan, Estado de México near the volcano's Popocatépetl and Iztaccíhuatl. "Popocatépetl and Iztaccíhuatl" refers to the volcanoes Popocatépetl ("the Smoking Mountain") and Iztaccíhuatl ("white woman" in Nahuatl, sometimes called the Mujer Dormida "sleeping woman" in Spanish) which overlook the Valley of Mexico. Popocatépetl is one of the most active volcanoes in Mexico and the most famous, having had more than 15 major eruptions since the arrival of the Spanish in 1519. It towers to 5,426 m, 70 km SE of Mexico City to form North America's 2nd-highest volcano. The amaranth grains were collected in the town of Panhuacan, located in the Municipality of Ayapango (in the State of México). Pahuacán is 2,536 meters above sea level and the geographic coordinate's lat. and lon. are 19° 06' N and -98°48' W, respectively.

#### 3. Instrumentation

#### 3.1 Sample Preparation

Amaranth's seed meal was obtained by grinding whole grain at 6 °C in a mill (Tekmar, Germany) attaining an average particle size of ~60 mesh. The seed sample was defatted by extraction with *n*-hexane suspension (1:10, w/v) under continuous stirring for 24 h. The defatted meal was separated by centrifugation at 800 g for 20 min at 4 °C. The flour was dried at room temperature and stored in a hermetic bowl at 4 °C until it was used. A 36 kDa amaranth globulin was extracted by sequential precipitation using ammonium sulfate and purified by gel filtration and cationic exchange columns [13].

The equipment used included an EG & G Ortec® Hyper Pure Germanium (HPGe) detector, an Ortec439® bias supply, an Ortec 570® amplifier, an OrtecACE 4K® multichannel card and a PC. The gamma ray spectra were obtained using the Maestro 2®program and analyzed using the Gamma Vision program. A  $36.4 \times 33.6 \times 35$  cm old lead box of wall thickness 7.5 cm was used to reduce background radiation [7]. Old lead was used to ensure better and low background

## 3.2 Determination of the Background

In order to determine the analysis system background, five spectra of 24 h were taken with an empty Marinelli. The environmental <sup>40</sup>K photopeak of the background spectrum appears as usual.

Minerals	Amaranth's grain (g per100g)	Amaranth's protein (g per 100 g)
Calcium	159 mg	298 mg
Iron	7.61 mg	0.76 mg
Manganese	3.33 mg	4 mg
Phosphorus	557 mg	862 mg
Potassium	508 mg	424 mg
Sodium	4 mg	22 mg

 Table 1
 Mineral's content in g per 100 g of amaranth's seed and 100 g of amaranth's protein.

### 3.3 Energy Calibration

The HPGe analysis system was calibrated in energy using the Gamma Vision program with the radioactive sources of  $^{241}$ Am (60 keV),  $^{137}$ Cs (662 keV) and  $^{60}$ Co (1,173 keV and 1,332 keV) and natural potassium  $^{40}$ K (1,460 keV).

## 3.4 KCl Standard Calibration

The potassium standard used was 366.6 g of potassium chloride crystal assayed by the supplier Backer, JT. The KCl was placed inside the 500 mL Marinelli beaker and analyzed with the spectrometer system over 24 h. The energy of the photopeak was 1,460 keV and the energy resolution (FWHM) was 2.5 keV.

# 3.5 Measurement Conditions

The measurements of the different samples were carried out under identical conditions. The amaranth

globulin protein was distributed homogenously inside the Marinelli beaker and covered the top of the detector. The relevant detection efficiency of 0.27% was calculated above. Triplicate assays that were made for the amaranth globulin protein were made to determine their potassium concentrations.

## 4. Results and Discussion

Fig. 2 shows the gamma spectra of potassium ( $^{40}$ K) of the amaranth globulin protein sample, and can observe a relatively high peak that corresponds to potassium. The photopeak analysis was done using ORTEC-MAESTRO® software, and the proposed methodology and calculation on the previous section. The experimental values obtained for a sample weight, net area, specific activity of potassium, detection efficiency, specific activity of a amaranth protein sample and the number of miligrams of potassium per 100 g of protein sample are: 500 g, 31.20, 0.26%, 0.1323, 424.1, respectively.



Fig. 2 Gamma spectra of amaranth protein sample in the <sup>40</sup>K region (1,460 keV).

# 5. Conclusions

Existing evidence suggests that nutrition, especially staple based foods such as amaranth, when part of a balanced pattern, contribute important protein, polyunsaturated fatty acids, minerals, appropriate dietary fiber, vitamins, and antioxidants, all of these nutrients could help mitigate or reduce the risk of several diseases. The amounts of minerals, mg per 100 g in the amaranth grains are: phosphorous 557 mg, potassium 508 mg, calcium 159 mg, iron 7.61, and sodium 4.0 mg [14]. Potassium is one of the major minerals in amaranth grains, and as opposed to sodium, potassium mineral is crucial for muscle contraction, nerve conduction as well as providing acid-alkaline balance in the body. It is involved in a health blood pressure and heart beat. There is an excellent agreement between the amount of potassium determined by atomic absorption spectroscopy in our laboratory and value obtained in this work using the gamma spectrometry technique [15].

From scientific knowledge and experience it is obvious that *Amaranth* seeds surpass traditional cereals in a number of nutritional values, and in food they can provide a good supplement to them. It is reasonably well balanced food with functional properties that have been shown to provide medicinal benefits. Thanks to its properties, amaranth gives us a wide range of possibilities for using it in human nutrition, including active health support. It can be applied when we need more easily digestible quality proteins, e.g. in children, sportsmen and elderly people.

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