QUINOA AND AMARANTH: Multi-purpose agro-industrial crops

by

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ABSTRACT

Studies were conducted in 2018 with two multi-purpose agro-industrial crops [quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* sp.)] to characterize its chemical composition. Limited information is available on the nutritional value of quinoa grown in a tropical environment. The objective of this research was to developed an amino acids profile, determined crude protein, by two nitrogen procedures (Kjeldahl and Combustion), dietary fiber, total fat, starch and mineral (calcium, magnesium, phosphorous, potassium, iron and zinc) concentrations on 3-wk old leaves and mature seeds of three quinoa accessions [Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea)]. Compositional analysis was completed using AOAC, AACCI, and AOCS official methods. Leaves samples were dried in a forced air oven at 65° C for 72 hours, ground in a Wiley mill. Seeds at physiological maturity (15-wks) were harvested, dried and ground for analysis. Data was analyzed using SAS, and means were separated using Tukey's test, when significant differences were found. Lysine was higher than most of the staple grains. There was no significant ($P > 0.05$) difference between Pison, Copacabana and Kaslaea for crude protein of leaves and seeds in both N procedures. However, there was a significant difference ($P < 0.05$) between N procedures. Crude protein percentages were higher using the Combustion method with mean percentage of 33.3 and 16.6 %, leaves and seed respectively. Insoluble dietary fiber (IDF), total dietary fiber (TDF) percentage in the leaves differed significantly ($P > 0.05$) among quinoa accessions , whereas soluble dietary fiber (SDF) was similar. Seeds of quinoa did not differ significantly ($P > 0.05$) in percentage IDF, SDF and TDF, nor in total fat and total starch. Calcium (Ca) and phosphorous (P) concentrations were different ($P < 0.05$) among accessions, but not for magnesium (Mg), iron (Fe), potassium (K) and zinc (Zn), but among accessions seed there was no significant ($P > 0.05$) difference. This result show high nutritional properties (crude protein

and minerals) of quinoa accessions, with Kaslaea exhibiting higher total dietary fiber in their leaves. For the second study chemical components was determine on the leaves and seeds of four amaranth varieties. Information is lacking on the chemical composition of leaves and seed of *A. cruentus* (Juana, Aurelia, Elena) and *A viridis* (Callaloo) in Puerto Rico. The objective of this study was to develop an amino acids profile, and determine crude protein, by two nitrogen procedures (Kjeldahl and Combustion) dietary fiber, total fat, starch and mineral (calcium, magnesium, phosphorous, potassium, iron and zinc) concentrations on 3-wk old leaves and mature seeds of field grown *A. cruentus* (Juana, Aurelia, Elena) and *A viridis* (Callaloo). Compositional analysis was completed using AOAC, AACCI, and AOCS official methods. Harvested leaves were dried in a forced air oven at 65° C for 72 hours, and ground in a Wiley mill. Seeds at physiological maturity (15-wks) were harvested, dried and ground for analysis. Data was analyzed using SAS, and when means were significant were separated using Tukey's test. Lysine content of amaranth species was higher than common cereals. There were significant differences (P >0.05) in crude protein (CP) on leaves for both nitrogen procedures. But among amaranth seeds there was no significant difference (P >0.05). Combustion presented the higher CP percentage (22 %) and (19 %) leaves and seed respectively. Among amaranth leaves, there were significant differences (P >0.05) in IDF and TDF, while amaranth seeds differed (P >0.05) for IDF, SDF and TDF. While, total fat and starch in the seeds were not different (P >0.05). Calcium, Mg, and P concentrations differed in their leaves (P >0.05), but Fe, K and Zn, did not. Among amaranth seeds there were significant difference (P >0.05) for Ca, Mg, Fe, and P. This study demonstrates that amaranths are an excellent source of nutrients, with Elena and Aurelia having higher CP percentage in their leaves.

RESUMEN

Los estudios se llevaron a cabo en 2018 con dos cultivos multiusos agroindustriales [quinoa (*Chenopodium quinoa* Willd.) Y amaranto (*Amaranthus sp*.)] Para caracterizar su composición química. Se dispone de información limitada sobre el valor nutricional de la quinoa cultivada en ambiente tropical. El objetivo de esta investigación fue desarrollar un perfil de aminoácidos, determinar proteína cruda, mediante dos procedimientos de nitrógeno (Kjeldahl y Combustion), fibra dietética, grasa total, almidón y minerales (calcio, magnesio, fósforo, potasio, hierro y zinc) en hojas a la edad de 3 semanas y semillas maduras en tres accesiones de quinoa [Ames 13746 (Pison), Ames 13748 (Copacabana) y Ames 13745 (Kaslaea)]. El análisis de composición química se completó utilizando los métodos oficiales de AOAC, AACCI y AOCS. Las muestras de hojas se secaron en un horno de aire forzado a 65° C durante 72 horas, se molieron en un molino Wiley mill. Las semillas en la madurez fisiológica (15 semanas) fueron cosechadas, secadas y molidas para su análisis. Los datos se analizaron utilizando SAS, y cuando las medias fueron significativas, se separaron mediante la prueba de Tukey. La lisina fue más alta que la mayoría de los granos básicos. No hubo diferencia significativa (P >0.05) entre Pison, Copacabana y Kaslaea para la proteína cruda de hojas y semillas en ambos procedimientos de N. Sin embargo, hubo una diferencia significativa (P >0.05) entre los procedimientos de N. Los porcentajes de proteína cruda fueron más altos utilizando el método de Combustión con 33.3 y 16.6 %, hojas y semillas respectivamente. El porcentaje de fibra dietética insoluble (IDF), el total de fibra dietética (TDF) en las hojas difirió significativamente (P >0.05) entre las accesiones de quinoa, mientras que la fibra dietética soluble (SDF) fue similar. Las semillas de quinoa no difirieron significativamente (P >0.05) en porcentaje IDF, SDF y TDF, ni en grasa total y almidón total. Las concentraciones de calcio (Ca) y fósforo (P) fueron diferentes (P >0.05) entre las accesiones, pero no para magnesio

(Mg), hierro (Fe), potasio (K) y zinc (Zn), pero entre las semillas no hubo Diferencia significativa (P >0.05). Este resultado muestra altas propiedades nutricionales (proteínas y minerales) de las accesiones de quinoa, con Kaslaea quien exhibe un porcentaje mayor de fibra dietética total en sus hojas. Para el segundo estudio se determinaron los componentes químicos de las hojas y semillas de cuatro variedades de amaranto. No se cuenta con información sobre la composición química de las hojas y semillas de *A. cruentus* (Juana, Aurelia, Elena) y *A viridis* (Callaloo) en Puerto Rico. El objetivo de este estudio fue desarrollar un perfil de aminoácidos y determinar la proteína cruda, mediante dos concentraciones de nitrógeno dietético (Kjeldahl y Combustión), grasas totales, almidón y minerales (calcio, magnesio, fósforo, potasio, hierro y zinc). en hojas de 3 semanas de edad y semillas maduras de A. cruentus cultivada en el campo (Juana, Aurelia, Elena) y A viridis (Callaloo). El análisis de la composición se completó utilizando los métodos oficiales de AOAC, AACCI y AOCS. Las hojas cosechadas se secaron en un horno de aire forzado a 65° C durante 72 horas y se molieron en un molino Wiley mill. Las semillas en la madurez fisiológica (15 semanas) fueron cosechadas, secadas y molidas para su análisis. Los datos se analizaron utilizando SAS y cuando las medias fueron significativas, se separaron mediante la prueba de Tukey. El contenido de lisina en las variedades de amaranto fue más alto que en cereales comunes. Hubo diferencias significativas (P <0.05) en la proteína cruda (PC) en las hojas para ambos procedimientos de nitrógeno. Pero entre las semillas de amaranto no hubo diferencias significativas (P >0.05). El método de Combustión presentó el mayor porcentaje de PC (22 %) y (19 %) hojas y semillas respectivamente. Entre las hojas de amaranto, hubo diferencias significativas (P >0.05) en IDF y TDF, mientras que las semillas de amaranto difirieron (P >0.05) para IDF, SDF y TDF. Mientras, la grasa total y el almidón en las semillas no fueron diferentes (P >0.05). Las concentraciones de calcio, Mg y P difirieron en sus hojas (P <0.05), pero Fe, K y Zn no lo hicieron. Entre las semillas

de amaranto hubo una diferencia significativa (P <0.05) para Ca, Mg, Fe y P. Este estudio demuestra que el amaranto es una excelente fuente de nutrientes, y Elena y Aurelia tienen un mayor porcentaje de PC en sus hojas.

DEDICATION

My parents: *Julia Altagracia Torres y Francisco Vidal who always motivated me to strive to my best not only with their advices also with their example. Thanks for being the best parents over the world.*

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Thanks to God who protects me and give me the strength to wake up every day with a hope, knowing that who does the right things receive the best of blessings.

" When we want to achieve something in life, we must leave until our last breath on it , to see it done"

(Edil Vidal Torres)

TABLE OF CONTENTS

TABLE LISTS

TABLES **PAGES**

1 INTRODUCTION

Worldwide, the basic human diet includes carbohydrates (maize, rice and wheat), fats and proteins (meat and plants). There are over 50,000 edible plants species worldwide, but only a few hundred of them are partially or fully domesticated and contribute to our food supplies (Dogra & Awasthi, 2015). According to the Food Agriculture Organization (FAO), staple crops such as maize, rice, potatoes and wheat are our main source of nutrition and provide close to 80% of the dietary energy and nutrients and 60 % of the global requirements for calories and proteins (FAO, 2012). In the tropics, there are many traditional foods rich in carbohydrates (e.g., tubers), low in protein and high in starch (e.g., plantains), as well as cereal grains which, represent the majority of the food staples.

Nowadays food security, the increasing global food demand and mitigating climate change, are major concerns in food production. Therefore, increasing research towards the potential use of forgotten or underutilized crops in the tropics is needed. There is a need to explore nutritive, multi-purpose agro-industrial crops such as the pseudocereals [e.g., quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* sp.)]. Worldwide, both quinoa and amaranth are receiving significant interest because of their high nutritional values, positives health benefits (Jubete, Wijngaard, Arendt, & Gallagher, 2010), and their potential to thrive in extreme soil and climatic conditions (Shukla et al., 2006; Abugoch, 2009).

Amaranth (Family *Amaranthaceae*), a multipurpose crop (grains and leafy crop) has different centers of origin and domestication. Mesoamerica is considered the origin of the annual seed cultivated species that include *A. caudatus* (Peru), *A. hypochondriacus* (Mexico) and *A. cruentus* (Guatemala), (Kietlinski et al., 2014), and are used for human consumption. Quinoa (of

the goosefoot family *Chenopodaceae*) is a seeded annual crop native to the Andean region of South America (Goyat & Handa, 2018). Quinoa has multiples agro-industrial uses, its seeds can be processed and converted into flakes, bars, flour, tortillas, cookies, oils, nutritional drinks, and a wide range of bakery products, flower can be utilized as vegetable dyes and its green leaves can be consume fresh as a salad, incorporated in naturals drinks or for the elaboration of food supplements (Jancurova et al., 2009).

Today, there is a demand for healthy foods with high functional value. Functional foods are those that provide positive physiological effects beyond their nutritional function of providing nutrients (Rivera, García, & Monge, 2010). Proteins and amino acids of quinoa and amaranth are considered of high biological value and characterized of being gluten free (Bressani, 1989; Ruales & Nair, 1993; Carrasco et al., 2003). The edible parts involve leaves and grains, the latter being the most economically and scientifically explored. Both can be used for human or animal consumption, their leaves contain high concentrations of calcium, also rich in iron, potassium, magnesium, zinc, phosphorus and vitamins (Gálvez et al., 2010; Nascimento et al., 2014).

In Puerto Rico, there is a lack of information on quinoa and amaranth crop production and nutritional value. Studies are needed to characterize the nutritional value of quinoa accessions Ames 13745 (Kaslae), Ames 13746 (Pison) and Ames 13748 (Copacabana) and amaranth grain and leafy varieties types (Juana, Aurelia and Elena) and Callaloo (*Amaranthus viridis*) respectively. Is necessary to promote their cultivation, incentive their consumption and to be considered as alternatives and multipurpose crops of high nutritional value.

2 OBJECTIVES

i. Determine percentage of crude protein (by two methods) in leaves and seeds of three accessions of quinoa Ames 13745 (Kaslaea), Ames 13746 (Pison) and Ames 13748 (Copacabana); and in four amaranth varieties (Callaloo, Juana, Aurelia and Elena).

ii. Determine the content of dietary fiber in leaves and seeds of three accessions of quinoa Ames 13745 (Kaslaea), Ames 13746 (Pison) and Ames 13748 (Copacabana); and in four amaranth varieties (Callaloo, Juana, Aurelia and Elena).

iii. Evaluate percentage of amino acids, total fat, and total starch in seeds of three accessions of quinoa Ames 13745 (Kaslaea), Ames 13746 (Pison) and Ames 13748 (Copacabana) and; in four amaranth varieties (Callaloo, Juana, Aurelia and Elena).

iv. Assess minerals content (calcium, magnesium, iron, potassium, phosphorus and zinc) in the leaves and seeds of three accessions of quinoa Ames 13745 (Kaslaea), Ames 13746 (Pison) and Ames 13748 (Copacabana) and in four amaranth species (Callaloo, Juana, Aurelia and Elena).

3 LITERATURE RIVIEW

3.1 Quinoa and Amaranth as alternative crop for the tropics

Quinoa and Amaranth are dicotyledonous plants referred to as pseudocereals because their seeds have similar functions and composition of true cereals (e.g., wheat), however quinoa and amaranth are richer in fat and protein than traditional cereals (Jubete et al., 2010). In the past, both were important food crops of the Aztec, Mayan and Incas civilizations. Today, amaranth is still consumed in Central America and Mexico, and Callaloo variety in the Eastern Caribbean Islands as a leafy vegetable. Quinoa has a broader genetic diversity, and several species are adapted from the sea level to the Bolivian Altiplano Plateau. It grows on high salinity soils in southern Bolivia and northern Chile and it is mostly consumed as a breakfast cereal (Nowak, Du & Charrondière, 2016).

The main quinoa producers are Bolivia, Peru, Ecuador, and the United States of America. Bolivia and Peru are the major producer in the Andean region and in the world, planting over 75,000 and 45,000 hectares respectively during the year 2013 (Bazile, 2016). Currently the cultivation of quinoa is taking places in others countries (Tibet, Morocco, France, India, China, the United Kingdom, Sweden, Denmark, Netherlands, and Italy, among others) (Bhargava & Srivastava, 2013; Pulvento et al., 2010).Crop adaptation have been studied since the 1950s in the region of the Andes and around the world with the finality to understand better the crop and to obtain quinoa germplasm adapted to a wide range of environmental conditions. Quinoa can produce seeds in higher elevations in the tropics; it has also been successfully grown in the temperate and subtropical zones (Popenoe, King, Leon & Kalinowski, 1990).

In the case of amaranth, the production data is more limited but areas such as the tropical regions of South America, Africa (especially for leaves of the amaranth plant), Central, and Southeast Asia (especially India) are the higher producer, and North America region with minor production around the world. Also, amaranth is grown as a leafy vegetable in the Mediterranean region being Russia the greater cultivator with about 100,000 ha (Moudry, Pejcha, & Peterka, 1999).

3.2 Quinoa and Amaranth as a food crop

The nutritional value of both quinoa and amaranth and their multiple food uses are some of the most important aspects that generate interest to be consider as multifunctional food crops worldwide. According to Léder (2009), incorporating either quinoa or amaranth in food elaboration process is becoming popular, particularly among those that consciously eat healthy food. At the same time, they can improve the nutritional value of traditional cereals such as maize, rice, or wheat by adding quinoa and amaranth in the formulation.

Obesity, diabetes, high pressure, cardiovascular problems and food disorders are associated to malnutrition's habits. This has triggered a series of other unknown diseases and intolerances to certain food components, such as being gluten intolerance as one of the most recently discovered.

Gluten is defined for legislative purpose as "a protein fraction from barley, oats, rye , wheat, or their crossbred varieties and derivatives, to which some persons are intolerant and that is insoluble in water" (Codex Alimentarius, 1979 rev. 2018). The protein that form gluten are major storage proteins and represent between 70 % and 80 % of the total protein content on the grain. In

the bakery industry, whey gluten is widely used as a main ingredient for food production (Penella, Wronkowska, Smietana, & Haros, 2013).

The increased prevalence of celiac disease leads to an increased demand of gluten-free products. The rising need to alternative gluten free cereals (amaranth, maize, millet, quinoa, and sorghum) is a big challenge in food research and development. Gluten characteristic needs to be substituted by other means in order to achieve products with satisfying quality. Gluten free products available in the market today are based on starch and therefore, show poor nutritional quality and can be replaced with quinoa and amaranth (Schoenlechner et al., 2010).

A comprehensive review of quinoa and amaranth by Sousa and Farfan (2012); Porr (2012) focused on its effects in the human health (antitumor, antioxidant activity, blood glucose levels, celiac disease, hypertension, immune system and well liver function). Favorable effects on the elderly, athletes, children, and lactose or gluten intolerant among others have also been reported (Valcárcel & Caetano, 2012; Galvez et al., 2010).

3.3 Quinoa and amaranth in the food industry

Quinoa and amaranth are considered multipurpose agro-industrial crops. A review by Jacobsen (2003) reports the potential of quinoa as modified food products with diverse industrial applications for e.g. baking (good source of starch), cooking (boiled or popped), industrial uses (cosmetic and textile) among others. A wide range of products can be elaborated with flour of both quinoa and amaranth with excellent nutritional and sensory quality (Chamorro, 2003).

Interest in formulation of gluten-free products has increased worldwide. Caicedo and Torres (2015) reported that with the substitution of 13 % of quinoa flour in a wheat base formulation is

possible to produce bread with excellent sensorial characteristics and also increase the protein content by 6 %. Schoenlechner et al. (2010) assessed amaranth, quinoa and buckwheat to produce gluten-free pasta. This study results in a pasta with better agglutination effect when quinoa was added, while with amaranth the pasta presented least suitability (had low texture firmness, decreased cooking time and tolerance). However, by combining these three pseudocereals (one flour blend), dough matrix was improved resulting in high quality pasta.

Quinoa flour has been used in bread dough fermentation to improve palatability and general flavor appreciation, similar to those obtained with wheat flour; also, by Adding 10 to 20 % of amaranth flour to bread dough rheological properties were improved (Coda et al., 2010). Others authors compared quinoa and amaranth flour with rice flour, potato starch and cassava to elaborate bread with different sweeteners (stevia, sucralose, acesulfame-K), observing equal results (firmness and volume) in quinoa and amaranth compared to the control formulation (rice flour, potato starch and cassava) (Machado et al., 2015; Mlakar, Turinek, & Jakop, 2009). Incorporating quinoa and amaranth in food formulations the nutritional profile (greater amount of proteins and lipids) was improved, indicating that it is possible to produce gluten-free breads with pseudocereals maintaining at the same time excellent sensory and physicochemical properties as those produced with traditional cereals (Dziki et al., 2015).

Amaranth also has diversity of uses in food industry. Its flower has been utilized as a dye of biological origin in the elaboration of meat products. Amaranth red pigments (0.1 % to 0.3 %) were added in the formulation of pork sausage obtaining good sensory characteristics and satisfactory quality for the final product. Pigments obtained from amaranth flowers could be a

possible alternative substitution of nitrite an additive considered as a carcinogenic in the food industry (Zhou, Zhang, Wang & Chen, 2012).

Pseudocereals seed flour are also a potential raw material as meat extender (high protein and starch). Compared with other extenders, it is economical to use and therefore, reduces costs in the production of meat derivatives, facilitating the management and improving the nutritional content (Delgado & Albarracin, 2012).

Brewing, using gluten-free ingredients from quinoa or amaranth has gained interest, is possible to produce a gluten free beer with good acceptance by the consumers (Meo et al., 2011). However, quinoa and amaranth must undergo a malting process to elaborate this type of product (Mäkinen, Zannini & Arendt, 2013). The effectiveness of an amaranth-based beverage (CHO-P) on cycling performance and hydration status has also been evaluated. Total caloric content was higher in the amaranth beverage compared to the commercial sports beverage (CHO-P: 52.48 kcal per 100 mL vs. CHO: 24 kcal per 100 mL). These results suggest that an amaranth-based beverage may be equally effective as a commercial sports product for supporting optimal performance and hydration (González et al., 2018).

3.4 Nutritional Value of Quinoa and Amaranth

The nutritional content of pseudocereals can vary depending on species, agronomic practices, location and climatic conditions where crops are grown (Budin et al., 1996). Quinoa and amaranth are rich in macronutrients such as protein (exceptional balance of amino acids) lipid (essential fatty acids), carbohydrates (starch) as well as high level of micronutrient (minerals and vitamins) (Jubete, Arendt, & Gallagher, 2009; Filho, 2015). Several studies have been done to

determine the chemical composition and nutritional value of quinoa and amaranth seed , values of protein, total fat, total starch, dietary fiber and ash are presented in table 1.

Table 1. Chemical composition of quinoa and amaranth seeds.

Nutrient	Quinoa	Amaranth
	%	
Protein	15	14 – 17
Total fat	5-6	6-7
Total starch	64	61-65
Dietary fiber	14	21
Ash	3	3

Adapted from Jubete et al., (2009) and USDA (2018)

3.4.1 Protein and amino acids

The main nutritional values of pseudocereals are their amino acids and protein concentration, an important group of bio-macromolecules actively involved in physiological functions (Valcárcel & Caetano, 2012). Globulin and albumin are main protein compounds of quinoa and amaranth, contrary to common grains such as wheat (prolamin proteins). Prolamin is the toxic component in celiac disease, because of their low or lack of prolamin content, quinoa and amaranth are considered a gluten-free grain (Chamorro, 2003; Jubete, 2010; Grobelnik , 2009).

Protein percentage in quinoa and amaranth are generally higher than traditional cereals (maize, rice and wheat). Quinoa (14 %) and amaranth (13.6 %) have higher protein content than barley (10.8 %), maize (12 %), oats (11.6%), rice (8 %), and can be similar to wheat (15 %) (Carrasco, 2003; USDA, 2018).

Essential amino acids are those that cannot be synthesized by animals or humans and must be provided in the diet. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine,

tryptophan and valine are essential amino acids, and all of them are present in quinoa and amaranth seeds (Galvez et al., 2010; Bhargava , 2013).

Quinoa and amaranth proteins have higher or more adequate concentrations of essential amino acids than cereals (Ahamed, Singhai, Kulkarni, & Pal, 1998; Dini, Rastrelli, Saturnino, & Schettino, 1992; Gorinstein & Moshe, 1991). Highlighting lysine most limiting amino acid in traditional cereals (maize, rice and wheat) and vegetable proteins. For instance, lysine found in cereals like maize (3.8g /100g of protein, rice (3.8g /100g of protein) and wheat (2.6g /100g of protein) are lower than quinoa and amaranth 4.4 and 4.8g /100g of protein respectively, making them nutritionally superior (Kosiol, 1992; USDA, 2018). Ranhotra et al. (1993) studied the composition and nutritional quality of protein in quinoa and concluded that the protein found in quinoa matched to casein, which is the protein contained in the milk.

3.4.2 Carbohydrates

The carbohydrates content of quinoa (64.2g /100g) and amaranth (65.3g /100g) represent the major part of their composition (USDA, 2018). The carbohydrates present in these crops are considered as nutraceuticals because their positive effects in human nutrition and health, characterized for reducing bad cholesterol, hypoglycemia and decreases fatty acids (Qureshi, Lehman & Peterson, 1996).

Occurring in about 62 % of the total weight, starch represents the most important carbohydrate found in plants. It is the most abundant component of quinoa and amaranth seeds and has been studied since the early 1980s through different analytical methods (Valcárcel & Caetano, 2012). The physicochemical and rheological properties of starch play an important role in food

processing, however this properties undergo changes when summiting to thermal treatment or processing (Venskutonis & Kraujalis, 2013).

When comparing both crop's functionality, quinoa starch present lower levels of gelatinization temperatures, higher viscosity and solubility, high water-binding capacity, high swelling power, high enzyme susceptibility, and excellent stability under freezing and retrogradation processes (Atwell et al., 1983; Jancurová, Minarovičová & Dandár, 2009).

3.4.3 Total fat

Fats are an important source of calories, which promote the absorption of fat-soluble vitamins. Of the total fat content in quinoa and amaranth, more than 50 % comes from essential polyunsaturated fatty acids such as linoleic (omega 6) and linolenic (omega 3). Linoleic and linolenic acids are considered essential fatty acids, since the body is not able to produce them Carrasco et al. (2003); Ruales and Nair (1993) and Kosiol (1992). Their importance is related to a reduction of biological markers associated with many degenerative diseases such as cardiovascular problems, cancer, osteoporosis, inflammatory and autoimmune diseases (Chaun et al., 2007; Simopoulos, 2008).

The lipid content in quinoa and amaranth is between two and three times higher than other cereals such as maize and wheat (Jubete et al., 2010). Quinoa fat content is higher (5.2 to 9.7 %) when compared to others crops such as maize (4.7 %), but lower than soybean (18.9 %). Amaranth grain has higher lipid content (5.7 to 10.9 %) than quinoa. Lipids of both crops present a high degree of unsaturation (Schoenlechner et al., 2010). Even though quinoa and amaranth have high fat content and high degree of unsaturation, their lipids are reported to be generally stable against oxidation. Koziol (1992) demonstrated that the fatty acids of quinoa maintain its quality due to the

high natural value of vitamin E, acting as a natural antioxidant. The oil from quinoa's seed appears to be a high-quality edible vegetable oil, similar in fatty acid composition of soybean (Comai et al., 2007). In a conglomerate analysis of main fatty acids of 19 edible oils, including the isolated oil of amaranth (*A. cruentus*), amaranth was placed in level 1 of the 4 groups, close to wheat and barley. The profile of fatty acids in position 2 was also very similar of cereals and with some similarity with cottonseed and sesame seeds (Camacho et al., 2001).

3.4.4 Dietary fiber

According to Gordon (1999) dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.

The fiber content of amaranth and quinoa lies within the range of other cereals (Galvez et al., 2010). The total fiber content in both crops is somewhat similar. Quinoa and amaranth have 7 and 6.7 % of total fiber (USDA, 2018), and 14.2 and 20.6 % of dietary fiber, respectively (Jubete et al., 2009).

Dietary fiber of quinoa improves digestibility by facilitating the absorption process of other nutrients in the large intestine (Galvez et al., 2010). Amaranth seeds (depends on plant species and variety) are also a good source of dietary fiber. Usually, dietary fiber is presented as total (TDF), insoluble (IDF), and (SDF) Soluble dietary fiber. By the difference between total dietary fiber and insoluble dietary fiber, soluble dietary fiber can be determined (Carrasco et al., 2009). Punna and

Paruchuri (2004) studied green leafy vegetables, including amaranth, analyzing for TDF, IDF, and SDF by the gravimetric and enzymatic methods and observed significant variations in all the vegetables. The TDF and IDF contents of amaranth, hibiscus, basella, rumex, and spinach significantly increased during leaf maturation, whereas the SDF increased from tender to mature stage, but there was no further increase from mature to coarse stage, except in rumex. There was no significant effect on the TDF, IDF, and SDF contents in processing-cooking vegetables.

3.4.5 Mineral Concentrations

Mineral content is influenced by soil and environmental conditions present during plant growth and seed set. Iron, magnesium, calcium and zinc in quinoa and amaranth are two times higher than wheat, barley and most grains. High calcium content of amaranth and quinoa is associated with the treatment of osteoporosis in celiac patients (Siener et al., 2006; Jubete et al., 2010).

Table 2. Mineral composition in quinoa and amaranth seeds (mg/100g dry-weight basis).

Minerals	Quinoa	Amaranth
	mg/100g	
Iron (Fe)	10.28	29.35
Cupper (Cu)	1.55	1.25
Magnesium (Mn)	3.41	4.07
Sodium (Na)	7.31	4.14
Calcium (Ca)	108.41	283.14
Magnesium (Mg)	298.24	425.21
Potassium (K)	935.7	770.15
Phosphorous (P)	45.86	55.59

Adapted from Palombini et al., (2013).

Galan, (2013) conducted a study to evaluate the availability of Fe, Zn and Ca from extruded products from two amaranths mixed with maize using the dualizability method. The results of this

study reported Iron, Ca, and Zn concentrations of 64.0-84.0, 1977.5-2348.8, 30.0-32.1mg/kg, respectively. These values were much higher than control (6.2, 19.1, 9.7mg/kg, for Fe, Ca and Zn) respectively.

3.4.6 Vitamins

The vitamin content in quinoa is similar to cereals, presenting a significant amount of folate (0.18 %), riboflavin (0.32 %), thiamin (0.36 %), and vitamin B6 (0.49 %). Amaranth is also a good source of riboflavin (0.23 %) and ascorbic acid (4.50 %), (Abugoch, 2009). Furthermore, amaranth and quinoa are excellent sources of vitamin E, which contributes to the prolonged stability of oil and as a strong antioxidant. It has many essential physiological functions such as anticoagulation, regulation of the metabolic, inflammatory and anticancer processes in humans (Tang & Tsao, 2015).

Tocopherols represent the major vitamers of vitamin E, characterized for its fat-solubility and antioxidants capacity that act as scavengers of lipid peroxyl radicals (Ryan et al., 2007). In amaranth, α-tocopherol is the most abundant vitamin and was found in amounts of 248mg/kg in oil samples. Also, β-tocopherol was founded in a high concentration (546mg/kg oil). Quinoa seeds contain twice as much γ-tocopherols (5.3mg/100g) as α-tocopherols (2.6mg/100g), (Valcárcel & Caetano, 2012).

Both crops also contain ascorbic acid, one of the most important water-soluble vitamins. It is essential for collagen, carnitine and neurotransmitters biosynthesis, which cannot be synthesized by humans due to lack of an enzyme, (gulonolactone oxidase). The ascorbic acid content found in

amaranth samples range from 3.36 to 7.24mg/100g and in quinoa seeds from 4.0 to 16.4mg/100g (Akhilender, 2003; Koziol, 1992; Ruales and Nair, 1993).

3.5 Anti-nutritional factors

Some seeds contain enzymatic inhibitors, mainly anti-proteases and secondarily antiamylases. Anti-nutrients such as oxalic and phytic acid are also found. These tend to reduce the bioavailability of nutrients and the absorption of certain minerals (Akindahunsi & Salawu, 2005).

3.5.1 Phytates and oxalates

Phytic acid is present in most cereals and legumes in concentrations ranging from 1-3 % dry matter, but lower than in sesame seeds (toasted), soy protein concentrate, rice (unpolished and cooked), maize bread (unleavened) and peanuts (Chamorro, 2003; (Kumar, Sinha, Makkar, & Becker, 2010). Both amaranth and quinoa contain phytic acid; this component interferes with the absorption of Ca, Fe, Mg and Zn due to its ability to chelate divalent cationic minerals (Oboh, 2005).

Phytates can also interact with protein in a relatively broad pH range, decreasing protein solubility, enzymatic activity and proteolytic digestibility. Despite these potential negative impacts, research has indicated the anticancerogenic and antioxidant effects of phytates. The positive or negative impact of quinoa and amaranth originating from phytic acid on nutritional quality is yet to be systematically investigated (Tang & Tsao, 2015).

Siener et al. (2006) determined levels of oxalates in quinoa and amaranth plants using an HPLC-enzyme-reactor analysis and reported greater accumulation in the plant tissues (leaves,

stems and root), but higher percentage in the leaves and stems. Gélinas and Seguin (2007) evaluated the oxalate content in amaranth grain and reported amaranth as a high source of oxalate (insoluble form). They concluded that because of its high calcium and magnesium concentrations, oxalate absorbability is low.

Processing techniques on nutrient and anti-nutrient content of grain amaranth showed that boiling, soaking or heat processing amaranth seed significantly decreased anti-nutrients components (tannins, oxalates, and phytates), while increasing protein digestibility (Njoki, Sila, & Onyango, 2014). The oxalic acid maximum safe intake is 5g, which would be contained in about 2kg of fresh amaranth leave or seed; this amount exceeds the average amaranth consumed in a day. Under other conditions, levels of phytates in amaranth species were reported, resulting in 2mg/100g which is below the toxic level established and would not cause any significant effect in bioavailability of minerals and proteins (Muriuki, Sila, & Onyango, 2014).

4 MATERIALS AND METHODS

4.1 Quinoa and amaranth leaves and seed sample production

Two plantings were conducted to obtain quinoa and amaranth leaves and seeds for nutritional quality evaluation. At the University of Puerto Rico, Mayagüez Campus, three quinoa accessions were seeded in pots filled with topsoil (vertisol), seedlings were unfertilized, irrigation were provided with irrigation drip tape, weeding and pest control when necessary. The leaves samples were taken in a period when the leaves and petiole completed the right time to be consume fresh, then it was dried in a forced air oven and grounded for laboratory analysis. The seeds were harvested when they reached physiological maturity, dried and ground following the procedures described before.

In the second experiment, four commercial amaranths were planted in the field on a well-tilled seedbed (Oxisol) at the Agricultural Experiment Substation of Isabela, University of Puerto Rico. Plots were irrigated, weeded and pests were controlled. Young and succulent leaves were harvested then dried and ground as described for quinoa. Seeds were harvested in the optimum maturity stage, and dried and ground for laboratory analysis.

Crude protein (Kjeldahl method) and mineral concentrations were determined at the Tropical Agricultural Research Station (TARS), USDA, Puerto Rico, and crude protein (Combustion method), total fat, total starch and dietary fiber was conducted at the North Dakota State University (NDSU), Cereal Laboratory. Amino acids profile was determined at the University of Missouri, Agricultural Experiment Station, and Chemical Laboratory.

4.2 Crude protein by two methods

4.2.1 Kjeldahl method

The Kjeldahl method by block digestion and steam distillation was used for determination of nitrogen (6.25 factor was used to convert nitrogen to crude protein (Hoganas, 2002).Two grams of sample previously dried at 70° C were weighed and then transferred to a Kjeldahl digestion flask. It was then digested with 1.5g of $K_2S.O4$, 0.15g of $CuSO_4.5H_2O$, 5ml of concentrated H_2SO_4 and 3ml of 30 % H_2O_2. The samples were mixed in a vortex and then placed in the digestion block. The digestion was determined to be complete when the samples were clear. Samples were then removed from the digestion block to cool down. Within the distillers, 40ml of 40 % NaOH were added in the digestion tube, (an excess amount to neutralize the acid and create strong alkaline pH) and distill approximately 150ml in a 250ml Erlenmeyer flask for 5 minutes. The distillate is a solution of boric acid at 4 % in addition to bromocresol and red methyl indicator. Distillate was titrated with standardized 0.2 N HCL to determine the total nitrogen. A blank was also run with the sample.

$$\%N = mL\ HCLsample - mL\ HCLblank\ x \frac{\text{N Factor}}{\text{g sample x } 10}$$

% Crude protein = % N x 6.25

4.2.2 Crude protein (Combustion method)

Protein was determined using a LECO FP-528 (Leco Corp. St. Joseph MI). The protein was analyzed according to AACCI approved method 46-30.01 (AACCI, 2009).

The combustion analysis technique was used; this method prescribes a generic combustion method for the determination of crude protein. Combustion at high temperature in pure oxygen sets nitrogen free, which is measured by thermal conductivity detection. The total nitrogen content of the flour sample is determined and converted to equivalent protein by multiplication with a factor of 6.25 to obtain the protein content.

4.3 Amino Acids Profile

The amino acids profile was determined (essential and nonessential amino acids) following the procedures of AOAC Official Method 982.30 E (a, b, c) (Horwitz & Latimer, 2006). Nitrogen was determined using LECO FP-528 (Leco Corp. St. Joseph MI). The samples underwent acid hydrolysis with 6N HCl under nitrogen at 110° C. For determination of methionine and cysteine, the samples underwent performic acid oxidation followed by acid hydrolysis. Alkaline hydrolysis was also conducted on the samples prior to analysis to result in a complete amino acid profile. An anion exchange chromatography amino acid analyzer with post column derivatization analyzed the three hydrolysates. Standard amino acid solutions were used to calibrate the analyzer at least every 24 hrs. Each amino acid peak should have $\geq$85 % resolution. When alkaline hydrolysate is analyzed, tryptophan must be separated from lysinoalanine. It was then computed for each of the following amino acids, ASP, THR, SER, GLU, PRO, GLY, ALA, VAL, MET, ILE, LEU, TRY, PHE, LYS, HIS, AMM, ARG, CYS, and TRP.

4.4 Total fat

The total fat content of the samples was determined with a Soxhlet apparatus and the lipid was extracted with hexane according to AOCS method Ba 3-38 (AOCS, 1998).

Thimbles were prepared from Whatman No. 1 filter paper sheet. 5g of moisture free samples were transferred to the thimble and plugged with cotton. The thimble was placed in the Soxhlet assembly and hexane was added in the flask. Water circulation was open and let it run for 18 hours. All the fatty constituents were dissolved in the hexane. The hexane with the fat resulting was evaporated in the crucibles on water bath and then fatty constituents left in the flask were put into the oven and weighed.

$$\text{Crude fat \%} = \frac{\text{weight of fat (g)}}{\text{weight of sample (g)}} \times 100$$

4.5 Dietary fiber

Soluble (SDF) and Insoluble (IDF) dietary fiber (AOAC 991.43, AACC 32-07.01, NMKL 129, 2003) using the ANKOM ^{TDF} Dietary Fiber Analyzer.

Total dietary fiber (TDF) was determined with 0.5 ± 0.05g of dried food samples (duplicate) and then subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase. Samples were cooked at ~ 100°C with heat stable α-amylase to give gelatinization, hydrolysis and depolymerization of starch; incubated at 60°C with protease (to solubilize and depolymerize proteins) and amyloglucosidase (to hydrolyze starch fragments to glucose); and treated with four volumes of ethanol to precipitate soluble fiber and remove

depolymerized protein and glucose (from starch). The residue was filtered; washed with 78 % ethanol, 95 % ethanol, and acetone, dried and weighed.

4.6 Total starch

The total starch content of samples was determined using a Megazyme (Wicklow, Ireland) test kit following the approved method (AACC-I 76-13.01, 2009). The samples were hydrolyzed with α-amylase and amyloglucosidase and the glucose released was measured with glucose oxidase/peroxidase (GOPOD) solution, incubated at 50^{o} C for 20 mins and absorbance at 492 nm was read to determine total starch content.

4.7 Mineral content

The dry ash method for plant tissues (Pekin-Elmer, ICP-AES) was used. In a crucible 0.5g approximately of sample previously dried to 70^{o} C were weighed. Samples were placed in an oven at 500^{o} C for 4 hours, and then left at room temperature for 1 day. When removing the crucible from the oven-demineralized, water was added to avoid sample loss. 20ml of 33 % HCL were also added to be digested on a hot plate until it reached approximately 10ml. The remaining solution was transferred into a 100ml volumetric flask with demineralized water by boiling, through a filter paper # 541. The samples were kept to at room temperature and then completed the volumetric flask with distillated water to the mark. A combined inductible plasma (ICP) read the mineral concentration.

4.8 Statistical analysis

The experiment was a completely randomized design (DCA) with three accessions of quinoa (Pison, Copacabana and Kaslaea,) for two growth stages (leaves and seeds) with four repetitions. four varieties of amaranth (Juana, Elena, Aurelia, and Callaloo) by two harvest stages (leaves and seeds) with four repetitions was used. The results were submitted to variance analysis (ANOVA) and means were compared using the Tukey test if differences were found, through the Statistical program, SAS version 9.4. The significance level used for rejection of the null hypothesis was 5 % ($P < 0.05$).

5 EXPERIMENT I: EVALUATION OF THE CHEMICAL COMPOSITION OF QUINOA LEAVES AND SEEDS

5.1 INTRODUCTION

Worldwide, there are over 50,000 edible plant species, but only a few hundred are either partially or fully domesticated and contribute to our food supplies (Dogra and Awasthi, 2015). Staples crops such as maize, rice and wheat are our main source of nutrition (FAO, 2012). Nowadays, food security and mitigating climate change are major concerns. Therefore, increasing research towards the potential use of forgotten or underutilized crops such as quinoa (*Chenopodium quinoa* Willd) in the tropics is needed.

Quinoa is native to the Andean regions of Chile, Peru, Ecuador and Bolivia, and its cultivation dates back thousands of years. It is an annual plant of the Amaranthaceae family, subfamily Chenopodiaceae, genus Chenopodium; an herbaceous dicot that reaches a height of 0.2 to 3.0 m. It is not a true grain, like a typical cereal (monocot), and most often called a pseudo-cereal or even a pseudo-oilseed. Its leaves and seeds are known for its exceptional nutritional value and well-balanced protein, carbohydrate and fat (FAO, 2012; Filho, 2017).

Its high nutritional value and potential use as a multipurpose agro-industrial crop is receiving significant interest (Jubete et al., 2010). Also, it is a crop with the potential to thrive in extreme soil and climatic conditions (Shukla et al., 2006; Abugoch, 2009).

Quinoa (leaves and seeds) has multiples agro-industrial uses; its seeds can be processed and converted into flakes, bars, flour, tortillas, cookies, oils, nutritional drinks, and a wide range of bakery products and leaves still treated as a worthless waste product, are edible and may be

consumed in salad, and also used as a valuable food supplement (Świeca, Sęczyk, Gawlik-Dziki, & Dziki, 2014; Jancurova et al., 2009).

Today, there is a demand for healthy foods with high functional value. Functional foods are those that provide positive physiological effects beyond their nutritional function of providing nutrients (Rivera et al., 2010). Proteins and amino acids of are consider of high biological value and characterized of being gluten free (Bressani, 1989; Ruales & Nair, 1992; Carrasco, et al., 2003). Quinoa seeds and leaves can be used for human or animal consumption, their leaves contain high concentrations of calcium, phosphorus and vitamins (Gálvez et al., 2010) and rich in iron, potassium, magnesium and zinc (Nascimento et al., 2014).

Background data on chemical characteristics of quinoa accessions are limited and addressing its nutritive value for potential use as multi-purpose agro-industrial crop in Puerto Rico is important. Is necessary to promote their cultivation, incentive their consumption and to be considered as alternatives and multipurpose crops with high nutritional value.

The objective of this research was to compare crude protein percentage (Kjeldahl and Combustion) and amino acid; evaluate percentage of dietary fiber, total fat and total starch content (seeds only), and minerals (calcium, magnesium, iron, potassium, phosphorus and zinc) concentrations from the leaves and seeds of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea).

5.2 METHODOLOGY

The first phase of the study consisted of seeding three quinoa accessions Ames 13745 (Kaslaea), Ames 13746 (Pison) and Ames 13748 (Copacabana) from the USDA seed germplasm bank to obtain leaves and seeds for its chemical characterization. At the Alzamora greenhouse of University of Puerto Rico, Mayagüez campus, three accessions (four replicates) were seeded in 3.75 liters pot filled with topsoil (vertisol) obtained from the Agricultural Experimental Substation, Lajas on December 2018.

Pots were irrigated to achieve a uniform germination and watered as needed during the study. Plants were not fertilized; weeds and insects were controlled when necessary utilizing an insecticide powder (Sevin-5). Four weeks after sowing, representative leaves and petiole samples were taken from each replication by accession. A composite sample were prepared then samples were dried in a forced air oven at 65° C for 72 hours and ground in a Willey mill to pass a 1mm screen for laboratory analysis. The seeds were harvested when they reached physiological maturity (13 weeks after planting) then property cleaned, dried and ground following the procedures described above.

Table 3. Summary of chemical composition procedures used for quinoa leaves and seeds.

Determination	Method
Protein	Kjeldahl: block digestion and steam distillation. Combustion (LECO EP-528) AACCI 46-30.01.
Amino acids	A.O.A.C 982.30, chromatography ion exchanges high performance, HPIC.
Total starch	Megazyme test kit method 76-13.01.
Total fat	Soxhlet Extraction: hexane as solvent. A.O.A.C Ba 338.
Dietary fiber	AACC 32-07.01 ANKOM analyzer.
Macro and microminerals	Dry ash method for plant tissues, Perkin-Elmer, (1994).

5.3 RESULTS AND DISCUSSION

5.3.1 Protein content in quinoa leaves and seeds by two nitrogen analytical methods (Kjeldahl and Combustion)

There was no significant interaction (P >0.05) between N procedures by quinoa accessions, also there were no differences for crude protein on leaves and seeds among accessions. However, N procedure (P <0.05) presented differences in both leaves and seeds between quinoa accessions (Table 4). The protein content in leaves using Combustion (33.32 %) was higher than Kjeldahl (29.37 %), (approximately four percentage units higher). Similar tendency occurred with the seeds, 16.62 and 12.43 %, for Kjeldahl and Combustion, respectively.

Table 4. Summary of analysis of variance for crude protein (CP) on leaves and seeds of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana), and Ames 13745 (Kaslaea) comparing two N procedures (NP).

Source of variation	Leaves	Seeds
Quinoa accessions	0.1097	0.4418
N procedures	<0.001*	0.0001*
NP*Quinoa accessions	0.5167	0.4022

*Significantly different (P <0.05)
Quinoa accessions= Ames 13746 (Pison) Ames 13748 (Copacabana), and Ames 13745 (Kaslaea)
N procedures=Kjeldahl and Combustion

Daun and DeClercq (1994) compared Combustion (LECO) and Kjeldahl procedures to determine nitrogen in oilseeds. Higher values were found on samples analyzed with Combustion and lower nitrogen content for Kjeldahl samples. Combustion method is considered faster than Kjeldahl, and capable of handling relatively large number of samples with a semi-automatic operation with less environmental impact. The recent development of combustion-type nitrogen analyzers offers a potential replacement for Kjeldahl for direct determination of nitrogen.

Caballero et al. (2015) evaluated protein concentration of quinoa at five phenological stages of growth and found higher percentage of crude protein in the leaves (20 %) using the Kjeldahl method. However, crude protein in quinoa leaves averaged 29.37 and 33.32 % for Kjeldahl and Combustion, respectively. The results in this study was nine percentage higher using Kjeldahl, and much higher using Combustion (Table 5). The protein content in quinoa leaves was significantly higher than in seeds using the two analytical methods (Table 5).

Table 5. Effect of N procedure (Kjeldahl and Combustion) on crude protein on leaves and seeds of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana), and Ames 13745 (Kaslaea).

	Leaves		Seeds	
Accessions	Kjeldahl	Combustion	Kjeldahl	Combustion
	%			
Pison	30.31 b	34.09 a	12.34 b	18.01 a
Copacabana	29.69 b	32.87 a	12.52 b	16.69 a
Kaslae	28.12 b	33.00 a	12.43 b	15.17 a

Means followed by different letters in the same row are significantly different by Tukey's test.

As can be observed in Table 5, seed protein analyzed by Combustion method is higher (16.62 %) than the corresponding values reported by Jancurova (2009) for common cereals, like barley (10.8 %), maize (10.22 %), oat (11.6 %), rice (7.6 %), rye (13.4 %) and wheat (14.3 %). Studies by Jubete et al. (2009); Kosiol (1992) and Schoenlecner (2010) reported 12.9 to 16.5 % crude protein in quinoa seed using Combustion. In this study similar result was observed (16.62 %). Nascimento et al. (2014) and Dini et al. (1992) reported 12.10 to 12.5 % of protein, similar value was observed on this study (12.43 %), the average of three quinoa accessions (Table 5) using Kjeldahl method. However, Gonzales et al. (1989) studied the chemical composition of quinoa and reported a lower value (11.2 %).

5.3.2 Amino acids profile on quinoa seeds

Pooled samples (four replications) were used to develop an amino acids profile of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea) and are presented in Table 6 and 7.

Table 6. Essential amino acids in seed of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea).

	Quinoa seed			
Amino acids	Pison	Copacabana	Kaslae	Mean of accessions
	g/100g dry basis			
Threonine	0.55	0.53	0.48	0.52
Valine	0.69	0.68	0.62	0.66
Methionine	0.31	0.31	0.28	0.30
Isoleucine	0.61	0.58	0.54	0.58
Leucine	0.98	0.94	0.86	0.93
Phenylalanine	0.65	0.63	0.56	0.61
Lysine	0.92	0.89	0.81	0.87
Histidine	0.46	0.46	0.41	0.44
Tryptophan	0.20	0.17	0.16	0.18
Total	5.37	5.19	4.72	5.09

The protein quality (biological value) is determined for the amount of essential amino acids present in a source. According to Galvez et al. (2010) and Bhargava (2013) quinoa seeds contain essential amino acids that cannot be synthesized by animals or humans and must be provided in the diet, highlighting lysine as the most limiting amino acid in vegetable proteins (Table 6).

The essentials amino acids expressed in g/100g seed; leucine (0.93), lysine (0.87) ,phenylalanine (0.61) and valine (0.66) presented higher concentrations (Table 6). These results are slightly higher compared to those reported by Palombini et al. (2013) and USDA (2018). They reported leucine (0.84), lysine (0.70) phenylalanine (0.50) and valine (0.57) grams in 100g of seed.

Kosiol (1992) reported leucine (1.09), lysine (0.99), phenylalanine (6.9) and valine (4.5) g/100g seed, much higher than our values quinoa proteins have higher or adequate concentrations of the essential amino acids sufficient for adults' diets (Filho, 2017).

Table 7 presents a list of the nonessential amino acid for each accession and their mean average values in g/100g on a dry basis.

Table 7. Nonessential amino acids in seeds of three quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea).

	Quinoa seeds			
Amino acid	Pison	Copacabana	Kaslae	Mean of Accessions
	g/100g dry basis			
Taurine	0.02	0.02	0.01	0.02
Hydroxyproline	0.04	0.05	0.04	0.04
Aspartic Acid	1.26	1.22	1.09	1.19
Serine	0.6	0.57	0.52	0.56
Glutamic Acid	2.24	2.27	2.00	2.17
Proline	0.64	0.61	0.56	0.60
Lanthionine	0.00	0.00	0.00	0.00
Glycine	0.89	0.89	0.82	0.87
Alanine	0.67	0.64	0.58	0.63
Cysteine	0.29	0.30	0.26	0.28
Tyrosine	0.48	0.40	0.36	0.41
Hydroxylysine	0.04	0.03	0.02	0.03
Arginine	1.37	1.35	1.18	1.30
Ornithine	0.01	0.01	0.01	0.01
Total	8.55	8.36	7.45	8.12

5.3.3 Dietary fiber content in quinoa leaves and seeds

Table 8, presents the analysis of variance (ANOVA) for both leaves and seeds of the quinoa accessions. There were significant differences (P >0.05) in insoluble dietary fiber (IDF), total dietary fiber (TDF), but no difference was found for soluble dietary fiber (SDF). Regarding the

seeds of the quinoa accessions, there was not significant difference (P >0.05) for IDF, SDF and TDF.

Table 8. Summary of analysis of variance showing the sources of variation in leaves and seeds of quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana), and Ames 13745 (Kaslaea) on percentage insoluble dietary fiber (IDF) soluble dietary fiber (SDF) and total dietary fiber (TDF).

Source of variation	IDF	SDF	TDF
	p-value		
Quinoa accessions (leaves)	<0.0001*	0.7869	<0.0001*
Quinoa accessions (seeds)	0.0908	0.1904	0.5545

*Significantly different (P >0.05)
An ANOVA was done for each factor (leaves and seeds) individually, the table above summary the results. Quinoa Accessions= Ames 13746 (Pison) Ames 13748 (Copacabana), and Ames 13745 (Kaslaea).

Kaslae IDF was > 2.5 percentage units higher than those of Copacabana or Pison, as well as TDF. The mean average for SDF in the three quinoa accessions was 6.4 % (Table 9). Among quinoa accession seeds dietary fiber did not differ averaging 6.20, 5.23 and 11.43 %, IDF, SDF and TDF, respectively.

Table 9. Percentage insoluble (IDF), soluble (SDF) and total (TDF) dietary fiber in leaves of quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea).

Quinoa Accessions	Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber
		%	
Pison	11.90 c	6.45 a	18.35 c
Copacabana	13.60 b	6.27 a	19.87 b
Kaslaea	16.20 a	6.35 a	22.55 a

Ruales and Nair (1994) determined dietary fiber in raw and processed quinoa seeds. They reported a higher value (13.4 %) of total dietary fiber compared. In this study, TDF was much higher for all three accessions. Several authors (Jubete et al., 2009; Gonzales et al., 1989; Miranda, Gálvez, Fuentes & Rodríguez, 2013: Nowak, 2016) reported dietary fiber content range from 7.8 to 14.2 % attributing this range of variations to the varietal differences. However, lower contents

of dietary fiber were reported by Kurek, Karp, Wyrwisz, and Niu (2018); Santis, D'Ambrosio, Rinaldi & Rascio (2016) in IDF (5.66 %), SDF (3.88 %) and TDF (9.54 %) compared to the values obtained in this study 6.20, 5.23 and 11.43 %, for IDF, SDF and TDF, respectively. Therefore, the reported high content of quinoa fibers in the literature as well as this study can improve digestibility by facilitating the absorption process of the other nutrients present in quinoa in the large intestine (Filho, 2017).

5.3.4 Total fat and total starch content in quinoa seeds

There was no significant difference (P >0.05) in total fat and total starch among the quinoa accessions studied (Table 10).

Table 10. Analysis of variance for total fat and total starch among quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana), and Ames 13745 (Kaslaea).

	Total fat	Total starch
Source of variation	p-value	
Quinoa accessions (seeds)	0.3678	0.7700

*Significantly different (P >0.05)

The mean average percentage total fat among quinoa accessions seeds was 5.9 %. Carrasco and Serna (2009) reported an average of 5.71 % in four quinoa varieties, while Kosiol (1992) reported a range of 5.2 to 7.2 %. On the other hand, Ruales and Nair (1993) reported higher percentage of total fat (9.7 %) in quinoa seeds. Other authors like Gonzales et al. (1989) reported much lower value (4.0 % of total fat). Navruz and Sanlier (2016) compared fat content in quinoa seed (6.07 %) with traditional cereals such as rice (3.2 %), barley (1.3 %), wheat (2.47 %), maize (4.74 %) and rye (1.63 %). The percentage fat in this study agrees with those presented by the above-mentioned authors.

Total fat as well other chemical compound is highly influenced by quinoa varieties. The fat content of quinoa seed is lower when compared to soy beans (19 %) but with similar fatty acid composition (linoleic and linolenic), essential fatty acid that our body can produce (Filho 2015; Abugoch, 2009).

Starch plays a crucial role in functional properties of quinoa and related food products. The mean average of total starch between accessions was 49.21 %. Miravalles and Mahony (2018) investigated the nutritional composition of quinoa and reported 60 $\pm$ 2.58 % of total starch. This value is much higher than those found in this study. Nascimento et al., (2014); Ruales and Nair (1994); Carrasco et al., (2003) and Albugoch (2009) reported values ranging from 51.6 to 64.2 % of total starch, being this value closer to the results from this study.

5.3.5 Mineral concentration on quinoa leaves and seeds

Table 11 presents the analysis of variance for leaves and seeds of the quinoa accessions. Among accessions leaves, there was significant difference (P >0.05) in calcium (Ca) and phosphorous (P). However, there was no significant difference (P >0.05) for magnesium (Mg), iron (Fe), potassium (K) and zinc (Zn). Also, there was no significant difference (P >0.05) for Ca, Mg, Fe, K, Zn and P among seeds of quinoa accessions.

Table 11. Analysis of variance for calcium (Ca), magnesium (Mg), iron (Fe), potassium (K), zinc (Zn) and phosphorous (P) of quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana), and Ames 13745 (Kaslaea).

	Ca	Mg	Fe	K	Zn	P
Source of variation			p-values			
Quinoa accessions (leaves)	0.0342*	0.8052	0.7691	0.5153	0.0896	0.0461*
Quinoa accessions (seeds)	0.7701	0.9680	0.5879	0.9860	0.6899	0.8237

*Significantly different (P >0.05). An ANOVA was done for each factor (leaves and seeds) individually, the table above summary the results.

Pison calcium concentration was higher than Copacabana and similar to Kaslae, whereas Mg average 1.52 % among accessions (Table 12). Percentage of phosphorous (P) presented differences between accessions, Kaslae was higher than Copacabana, but Kaslae and Pison did not differ averaging 0.74 %.

Table 12. Macro and microminerals concentration in leaves of quinoa accessions Ames 13746 (Pison), Ames 13748 (Copacabana) and Ames 13745 (Kaslaea).

	Ca	Mg	P	K	Fe	Zn
Quinoa Accessions			%		mg/100g	
Pison	0.97 a	1.57 a	0.70 a b	14.32 a	15.48 a	7.83 a
Copacabana	0.76 b	1.49 a	0.59 b	14.35 a	14.96 a	7.01 a
Kaslae	0.91 a b	1.50 a	0.78 a	15.61 a	13.64 a	9.48 a

Mineral concentration on seeds did not differ among quinoa accessions averaging 0.04 0.15, 0.30 and 0.79 % for Ca, Mg, P, and K, respectively, whereas Fe and Zn averaged 14.69 and 8.10 mg/100g, respectively. Jubete et al. (2009), Dini, Tenore, & Dini (2005), Ranhotra et al. (1993) and Kozioł, (1992) reported a wide variation in minerals concentration for quinoa seeds. Difference in the values obtained by the various authors may be related to the fact that the samples were of different genotypes and regions with varying soil types and/or fertilizer use (Gálvez et al., 2010).

Quinoa seed is a good source of iron, magnesium and zinc when compared to the daily mineral recommendations. Also, have higher mineral contents compared to other cereals especially Fe (14.69 mg/100g) in maize (2.9 mg/100g), rice (0.6 mg/100g) and wheat (3.8 mg /100g) and Zn (8.10 mg/100g), maize (2.1 mg/100g), rice (0.6 mg/100g) and wheat (4.7 mg/100g), (Kosiol, 1992). Lower values of Fe in quinoa seeds (10.28 Mg/100g) has been reported (Palombini et al., 2013). In this study, higher Fe concentration (14.69 mg/100g) was observed. According Jubete et al. (2009) gluten-free products are deficient of main mineral like calcium, magnesium, and iron. The pseudocereal quinoa is a good source of Ca (0.04 %) and Mg (0.15 %) and other important minerals for celiac disease patients.

6 CONCLUSION AND RECOMMENDATIONS

This thesis research evaluated three quinoa (Pison, Copacabana and Kaslaea) accessions grown in Puerto Rico. Overall, this study provided significant information on the nutritional value of quinoa leaves and seeds. Pison, Copacabana and Kaslaea portrayed an excellent amino acid profile, especially the essential amino acids leucine, lysine, phenylalanine and valine showing a more balanced profile than any cereal grains. Crude protein concentration in three-week old leaves of three quinoas was 33 % suggesting that it can be an excellent source of protein if freshly consumed. While, CP in the seeds averaged 17 %, exceeding most cereals grains. Total dietary was different among quinoas leaves with Kaslaea having three percentage units higher than either Copacabana or Pison. This study also demonstrated that all three quinoas are an excellent source of the key nutrients (fat and starch) and also a good source of minerals.

Future research studies should address seed production in the different environmental conditions, particularly the mountainous and cooler areas of Puerto Rico. Seed processing is also a key component for quinoa needs to be addressed as this may affect its nutritive value. Research should also assess sensory attributes of fresh leaves and also determine if its sensory quality can be affected by environmental and stage of maturity. In addition, culinary attributes to involve consumer acceptance is needed.

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7 EXPERIMENT II. EVALUATION OF THE CHEMICAL COMPOSITION OF AMARANTH LEAVES AND SEEDS

7.1 INTRODUCTION

Climate changes is one of the factors which affects food security, therefore a strategy recommended to improve food demand is the use of drought tolerant crops. Amaranth is adapted to drought tolerant conditions and their leaves and seeds are edible (Filho et al., 2015). Amaranth (grains and leafy vegetables) has different centers of origin and domestication. Mesoamerica is considered the origin of the annual seed cultivated species that include *A. caudatus* (Peru), *A. hypochondriacus* (Mexico) and *A. cruentus* (Guatemala) (Filho et al., 2015; Venskutonis & Kraujalis, 2013). Amaranths are either annual or short-lived perennial consisting of approximately 60 species (Mlakar et al., 2009). Its leaves are of high nutritive value (Abbott & Campbell, 1982) and comparable to spinach (*Spinacia oleracea* L.) widely eaten in the humid tropics.

Grain amaranths are annual herbaceous plant (C_4 dicot), with an erect stem and enormous inflorescence of various colors. Some anatomical characteristics of amaranth and its C_4 photosynthesis pathway result in increased efficiency of CO_2 use under a wide range of temperature (from 25 to 40º C). These plants also are adapted to high light intensity and moisture stress environments. This contributes to the crop's wide geographic adaptability to diverse environmental conditions (Kigel, 1994). The species can be distinguished by inflorescence type (pistillate flower structures) and the seed color varies from white, gold, brown, pink to black (Sauer, 1967; Kigel, 1994).

Worldwide amaranth is receiving significant interest because of its high nutritional values and its positive health benefits (Jubete et al., 2010). The nutritional characteristics of amaranth seeds and leaves have previously been analyzed, and it has been established that both have a unique protein composition, highlighting essential amino acids, and good source of fat and dietary fiber (Becker et al., 1981; Schoenlechner et al., 2009). According to Chamorro (2003) amaranth can be also incorporated in a wide range of human consumption products (bread, crackers, bars, drinks, dyes among others).

In Puerto Rico, information is non-existent on the uses of leaves or seeds of Amaranth for human consumption. For these reason, the objective of this study was to compare crude protein percentage (Kjeldahl and Combustion) amino acids; evaluate percentage dietary fiber, fat and starch content (seeds only), and minerals (calcium, magnesium, iron, potassium, phosphorus and zinc) from the leaves and seeds of *A. cruentus* (Juana, Aurelia, Elena) and *A viridis* (Callaloo) .

7.2 METHODOLOGY

The study consisted of seeding four amaranths varieties (Callaloo, Juana, Aurelia and Elena) to obtain leaves and seed for its chemical characterization. In a greenhouse of the Alzamora Laboratory Farm, University of Puerto Rico, Mayagüez campus, commercial varieties originating from Guatemala were seeded in 3.75 liters pot filled with topsoil obtained from the Agricultural Experimental Substation of Lajas on March 2018. The experimental design was completely randomized with four replications.

Pots were irrigated manually to ensure germination and seedling emergence. Thereafter, water and weeding were conducted as needed. Three weeks after planting, young and succulent leaves were harvested, samples were dried in a forced air oven at 65° C for 72 hours and ground in a Willey mill to pass a 1mm screen and placed on whirl packs for laboratory analysis.

To obtain seeds, Callaloo, Aurelia, Elena and Juana were field planted on a well-tilled seedbed at the Agricultural Experiment Substation of Isabela, University of Puerto Rico. The experiment was established on 2 x 4-m plots on February 2018 in a completely randomized design with four replicates. Plots were irrigated to obtain uniform germination and maintained weed and pest free. Seeds were harvested when the plant reached the optimum stage of maturity (15-weeks after planting), and dried and ground in Wiley mill for subsequent analysis (Table 6).

7.3 RESULTS AND DISCUSSION

7.3.1 Protein content in amaranth leaves and seeds by two nitrogen analytical method (Kjeldahl and Combustion)

There was no interaction (P >0.05) between amaranth varieties by N procedure for crude protein in leaves and seeds. There was, however, significant difference (P >0.05) between N procedure (Kjeldahl versus Combustion) and among amaranth varieties (leaves only, Table 13).

Table 13. Summary of analyses of variance for crude protein on leaves and seeds of four varieties of amaranth (Juana, Elena, Callaloo and Aurelia) using two N procedures.

Source of variation	Leaves	Seeds
Amaranth Variety (AV)	0.0003*	0.0715
N procedure	0.0076*	0.0039*
AV x N procedure	0.9515	0.0455

*Significantly different (P >0.05)
Variety = Juana, Elena, Callaloo and Aurelia
N procedure (Kjeldahl and Combustion)

Table 14 presents crude protein mean values by N procedures, showing higher CP for both leaves and seeds by the Combustion method. According to Akubugwo et al. (2007) and Mnkeni, Masika, & Maphaha, (2007) the CP content in amaranth leaves ranges from 17.5 to 22.8 %. The results in this study are comparable, 19.76, 21.30 % of crude protein, for Kjeldahl and Combustion respectively. Another study by Andini et al. (2013) evaluating the protein variation in leaves of grain, vegetable and weedy types of amaranths (*A. viridis, A. blitum L. and A. dubius*) reported 12 to 29 %. This study was in the range with Elena (22.70 %) and Aurelia (20.84 %) presenting higher CP values compared to 19.51 % and 19.05 %, for Callaloo and Juana, respectively.

The nutritional value of leafy amaranth is similar or higher than common vegetables (e.g., spinach). Kavitha and Ramadas (2013) evaluated the nutritional composition of spinach and reported 11.10 % of protein, a lower percentage than the amaranths evaluated in this study (Table 14).

Table 1. Effects of N procedures (Kjeldahl and Combustion) on crude protein percentage in leaves and seeds of four amaranth varieties (Juana, Elena, Callaloo and Aurelia).

	Leaves		Seeds	
Variety	Kjeldahl	Combustion	Kjeldahl	Combustion
	% CP			
Juana	18.06 b	20.11 a	15.87 b	21.64 a
Elena	21.99 b	23.41 a	17.62 b	20.02 a
Callaloo	18.91 b	20.11 a	15.67 b	16.93 a
Aurelia	20.09 b	21.58 a	17.92 b	17.72 a

Results expressed as mean for analysis in four replications

Studies by Mlakar et al., 2009, Valcárcel and Caetano (2012) and USDA (2018) on the nutritional value of amaranth seeds reported a range of 13.1 to 21.0 % of CP. Results from this study are within the protein presented above (Table 14). Becker et al. (1981) and Bressani (1989) reported lower values (12.5 % to 15.0 %).Traditional cereals such as maize (10 %), sorghum (12 %) and wheat (13 %) presents lower values of crude protein (Fulho et al., 2015; Nieto, Barba de la Rosa & Lopez, 1994).

7.3.2 Amino acids profile in amaranth seeds

Pooled samples (four replications) were used to develop an amino acids profile of amaranths varieties (Juana, Elena, Callaloo and Aurelia). Table 15 and 16 presents essential and non-essential amino acids expressed in g/100g of grain (dry basic).

Table 2. Essential amino acids content for amaranth seed in the four varieties.

Amino acid	Amaranth seeds				
	Juana	Elena	Callaloo	Aurelia	Mean of varieties
	g/100g dry basic				
Threonine	0.69	0.62	0.56	0.56	0.61
Valine	0.84	0.77	0.66	0.67	0.74
Methionine	0.42	0.36	0.32	0.33	0.36
Isoleucine	0.75	0.71	0.62	0.61	0.67
Leucine	1.11	1.04	0.9	0.9	0.99
Phenylalanine	0.84	0.78	0.67	0.68	0.74
Lysine	1.14	1.02	0.92	0.93	1.00
Histidine	0.51	0.49	0.43	0.43	0.47
Tryptophan	0.28	0.28	0.23	0.22	0.25
Total	6.58	6.07	5.31	5.33	5.82

Valcárcel & Caetano (2012) found that amaranth seed contain a well-balanced amino acid composition for pseudocereals, with a higher concentrations in essential amino acids. The amino acids profile obtained in this study agrees with those of Grobelnik et al. (2009), Koziol (1992) and Becker (1981). Amaranth presented higher value of lysine (1.00 g/100g), the more limiting amino acid in traditional cereals such as maize (0.55 g/100g), rice (0.73 g/100g) and wheat (0.56 g/100g) also in vegetables protein.

The essentials amino acids are expressed in g/100g of amaranth seeds. Leucine (0.99), lysine (1.00), phenylalanine (0.74) and valine (0.74) presented the higher concentrations. This results are slightly higher to the reported by Palomini et al. (2013) and USDA (2018), with values for leucine, lysine, phenylalanine and valine, ranging from 0.86-0.88, 0.75-0.83, 0.56-0.61, 0.57-0.67 respectively.

Table 3. Nonessential amino acids content for amaranth seeds in the four varieties.

	Amaranth Seeds				
Amino acid	Juana	Elena	Callaloo	Aurelia	Mean of varieties
	g/100g dry basic				
Taurine	0.01	0.03	0.02	0.02	0.02
Hydroxyproline	0.11	0.12	0.09	0.09	0.10
Aspartic Acid	1.58	1.48	1.29	1.3	1.41
Serine	1.00	0.94	0.85	0.85	0.91
Glutamic Acid	3.24	2.87	2.62	2.64	2.84
Proline	0.79	0.73	0.67	0.65	0.71
Lanthionine	0.00	0.00	0.00	0.00	0.00
Glycine	1.52	1.36	1.25	1.28	1.35
Alanine	0.74	0.68	0.59	0.6	0.65
Cysteine	0.49	0.4	0.38	0.37	0.41
Tyrosine	0.66	0.58	0.51	0.52	0.57
Hydroxylysine	0.02	0.02	0.02	0.02	0.02
Arginine	1.78	1.60	1.42	1.45	1.56
Ornithine	0.01	0.02	0.01	0.01	0.01
Total	11.95	10.83	9.72	9.80	10.58

7.3.3 Dietary fiber content in amaranth leaves and seeds

Table 17 presents the analysis of variance for both leaves and seeds of the four amaranth varieties. There were significant differences (P >0.05) in insoluble dietary fiber (IDF), total dietary fiber (TDF), but no difference for soluble dietary fiber (SDF) among varieties. Among amaranth seed varieties there was significant difference (P >0.05) for IDF, SDF and TDF.

Table 17. Summary of analysis of variance showing the sources of variation in amaranth leaves and seeds of Juana, Elena, Callaloo and Aurelia for insoluble dietary fiber (IDF) soluble dietary fiber (SDF) and total dietary fiber (TDF).

Source of variation	IDF	SDF	TDF
Amaranth varieties (Leaves)	<0.0001*	0.0802	<0.0001*
Amaranth varieties (Seeds)	<0.0001*	0.0002*	<0.0001*

*Significantly different (P >0.05)
An ANOVA was done for each factor (leaves and seeds) individually, the table above summary the results.

Percentage IDF of Aurelia was nine units higher than Callaloo, and five units higher than those of Juana and Elena. This differences were also reflected on percentage TDF, but among amaranth varieties, SDF were similar (5.80 %), Table 18. The TDF for amaranth leaves was higher compared to amaranth seeds among varieties, highlighting Aurelia (leaves) as the variety who presented better dietary fiber value in both IDF (32.08 %) and TDF (38.18 %).

Table 4. Percentage insoluble (IDF), soluble (SDF) and total (TDF) dietary fiber in leaves of amaranth varieties Juana, Elena, Callaloo and Aurelia.

Amaranth Varieties	Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber
		%	
Juana	26.85 b	5.78 a	32.63 b
Elena	27.18 b	5.85 a	33.03 b
Callaloo	22.95 c	5.48 a	28.43 c
Aurelia	32.08 a	6.10 a	38.18 a

Means in the same column followed by same letter are not significantly different by Tukey's test.

Table 19 shows the percentage of insoluble, soluble and total dietary fiber in seeds of the four amaranth varieties. IDF in Juana and aurelia was three units higher than Callaloo, and one unit higher than Elena. This difference was also reflected on percentage TDF, but in this case Juana and Aurelia presented four and two units higher than Callaloo and Elena, respectively. Among Juana, Elena and Callaloo, SDF were similar (4.95 %) and higher for Aurelia (6.25 %).

Table 5. Percentage insoluble (IDF), soluble (SDF) and total (TDF) dietary fiber in seeds of amaranth varieties Juana, Aurelia, Elena and Callaloo.

Variety	Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber
		%	
Juana	11.20 a	5.35 b	16.55 a
Aurelia	10.73 a	6.25 a	16.98 a
Elena	9.93 b	4.75 b	14.68 b
Callaloo	7.95 c	4.75 b	12.70 c

Means in the same column followed by same letter are not significantly different (P >0.05).

Mlakar et al., 2009; Carrasco and Serna (2009) reported percentage of dietary fiber on amaranth seeds from 12.0 to 20.6 %. The values found in this study (12.70 to 16.78 %) are within the range. Lower dietary fiber percentages (8.8 %) have been reported by Fulho et al. (2015). Kurek et al. (2018). Reported IDF, SDF and TDF of 6.56, 4.56 and 11.15 %, respectively. These values are much lower than those found in this study. Amaranth grains are considered a good source of insoluble fiber but should be noted that the percentage of dietary fiber depends on the variety evaluated.

7.3.4 Total fat and total starch in four amaranth seeds varieties

There was no significant difference (P >0.05) for fat and total starch among the amaranth varieties Juana, Elena, Callaloo and Aurelia (Table 20).

Table 20. Summary of analysis of variance for fat and total starch for amaranth varieties Juana, Elena, Aurelia and Callaloo.

	Total fat	Total starch
Source of variation	p- value	
Amaranth varieties (seeds)	0.6369	0.0943

*Significantly different (P >0.05)
Varieties=Juana, Elena, Callaloo and Aurelia

The percentage of total fat on seeds of four amaranth varieties (Juana, Elena, Callaloo and Aurelia) averaged 6.74. Studies by Palombini et al. (2013) reported 6.43 % of total fat, Bressani (2009) a range of 5.9 to 6.70 %, while Mendonça et al. (2013) and Buddin et al. (1996) reported a range 5.2 to 7.7 % of total fat, similar range to found in this study. Other studies by Nascimento et al. (2014) reported higher value (10.9 %) in amaranth seeds. Amaranth contain more fat per 100g of dry weight compared to beans (1.1g), maize (4.7g), rice (2.2g) and wheat (2.3g) (FAO,2013).

Total fat is also dependent on species or genotype and can vary from 1.9 to 9.7 % (Berger, 2003, Becker, 1984 and Valcárcel & Caetano, 2012).

Total starch averaged of 53 % among amaranth varieties. Miravalles and Mahony, (2018) reported 52.8 % of total starch in Amaranth seeds, while Venskutonis and Kraujalis (2013) reported values ranging from 40 to 52 %, similar percentages to those reported in this study. However, Nascimento et al. (2014), Kosiol (1992) and USDA (2018) reported higher values of total starch ranging from 55.30 to 57.27 %. A study by Pedersen, Hallgren, Hansen and Eggum (1987), shows that amaranth (62.7 %) are comparable with maize (72.8 %), wheat (65.7 %) and sorghum (70.1 %).

7.3.5 Mineral concentration on amaranth leaves and seeds

There was significant difference (P >0.05) in amaranth leaves for Ca, Mg and P, but no significant difference (P >0.05) was found for K, Fe, and Zn. In seeds, similar difference (P >0.05) for Ca, Mg, P, K, Fe was observed, except for Zn.

Table 21. Summary of analysis of variance for calcium (Ca), magnesium (Mg), iron (Fe), potassium (K), zinc (Zn) and phosphorous (P) of amaranth varieties Juana, Elena, Aurelia and Callaloo.

Source of variation	Ca	Mg	Fe	K	Zn	P
			P-value			
Amaranth varieties (leaves)	<0.0001*	<0.001*	0.0511	0.0023	0.0002	<0.0001*
Amaranth varieties (seeds)	0.0207*	0.0247*	0.0044*	0.0011*	0.7340	0.0154*

*Significantly different (P >0.05)
An ANOVA was done for each factor (leaves and seeds) individually, the table above summary the results.

Juana Ca concentration was higher than Elena, but Callaloo and Aurelia were similar. Among Juana and Elena, Mg (1.41), P (0.95) and K (4.4) were higher than for Callaloo and Aurelia. Among amaranth varieties, Fe (14.72 mg /100g) and Zn (21.65 mg/100g) was higher in Juana and Elena compared to Callaloo and Aurelia (6.11 mg/100g) (Table 22).

Amaranth leaves present good source of minerals. According to FAO,WHO (2001) the daily iron intake for children (7-15mg/day) and women in reproductive age (15.18 mg/day) is satisfied consuming amaranth leaves and it is more affordable than common vegetables. Srivastara (2001) reported values of Iron from 12.23 to 14.55mg/100g similar to those in this study.

Table 22. Mineral concentration in leaves of Juana, Elena, Callaloo and Aurelia.

	Ca	Mg	P	K	Fe	Zn
Amaranth varieties	%				mg/100 g	
Juana	2.67 a	1.43 a	0.99 a	4.37 a	10.95 a	9.13 a
Elena	2.35 b	1.40 a	0.91 a	4.54 a	12.56 a	9.40 a
Callaloo	1.57 c	1.13 b	0.51 b	3.90 ab	16.96 a	6.23 b
Aurelia	1.41 c	1.00 c	0.62 b	3.20 b	18.42 a	5.99 b

For seeds, Callaloo, Aurelia and Juana did not differ in the percentage of Ca (0.20 %) but Aurelia, Juana and Elena had similar percentage. Aurelia, Juana and Callaloo presented the higher percentage of Mg (1.42), but Callaloo was similar to Elena (0.23 %). Juana, Callaloo and Aurelia had similar P percentage, but Elena was lower (0.35). Potassium was higher for Callaloo and Juana (52 %), than Elena and Aurelia. Iron concentration was higher for Callaloo (98.39 mg/100g), with Aurelia, Elena and Juana averaging (28.23 mg/100g). Zinc concentration averaged 6.18 mg/100g, among amaranth varieties (Table 23).

Table 23. Mineral concentration in seeds of Juana, Elena, Callaloo and Aurelia.

	Ca	Mg	P	K	Fe	Zn
Amaranth varieties	%				mg/100g	
Juana	0.18 a b	0.26 a	0.49 a	0.51 b a	38.24 b	6.86 a
Elena	0.16 b	0.20 b	0.35 b	0.37 c	20.34 b	5.83 a
Callaloo	0.21 a	0.25 b a	0.45 b a	0.54 a	98.39 a	5.91 a
Aurelia	0.20 ab	0.26 a	0.45 b a	0.41 b c	26.12 b	6.10 a

A study by Akubugwo et al. (2007) reported Fe concentration of 13.58 mg/100g in *A. hybrids*, while Chege (2014) reported 5.75 mg/100g in *A. cruentus* leaves. Iron concentration in both leaves and seed in this study are much higher those reported by other researchers.

Amaranth is characterized as a good source of iron, magnesium and zinc when compared to the daily mineral recommendations. Also, have higher mineral contents compared to other cereals especially Fe (28.23 mg/100g) in maize (2.9 mg/100g), rice (0.6 mg/100g) and wheat (3.8 mg/100g) and Zn (6.18 mg/100g), maize (2.1 mg/100g), rice (0.6 mg/100g) and wheat (4.7 mg/100g), (Kosiol, 1992. According Jubete et al. (2009) gluten-free products are deficient of main mineral like calcium, magnesium, and iron. The pseudo-cereal amaranth is a good source of Ca (0.20 %) and Mg (1.42 %) and other important minerals for celiac disease patients.

This study presents a wide variation in mineral concentrations. Differences obtained by various authors and in this study may be related to genotypes and regions with varying soil types and fertilizer used in the research studies (Gálvez et al., 2010).

8 CONCLUSION AND RECOMMENDATIONS

This study provides significant information and a basic characterization about the chemical composition of grain amaranths Juana, Elena, Aurelia and the leafy-type Callaloo. Research's results demonstrate that amaranths are an excellent source of nutrients. Amaranth contain a balanced set of amino acids in both their leaves ad seeds. Lysine, an amino acid that is most limited in cereals is significantly higher. Additionally, CP percentages in the leaves of Elena and Aurelia were much higher (3 percentage units) than the leafy Callaloo. The seeds averaged 19 % CP, which are much higher than any cereal grain. Total dietary fiber in the leaves and seeds of Juana, Elena and Aurelia, were much higher than Callaloo, indicating an excellent source of fiber. Total fat and starch in the seeds of amaranth were similar, while Ca, Mg, and P mineral concentrations differed among amaranth leaves and seeds and are comparable with most other studies.

Future research studies should address seed production in different environmental conditions of Puerto Rico (drought or saline soil) as well as processing as this may affect the nutritive value of Amaranths. In addition, sensory evaluation of these amaranth's varieties at different stages of growth (leaves) and seeds produced by year or location as can influence sensory attributes. Finally, address culinary attributes to involve consumer acceptance as research is limited in this area.

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