FUNCTIONAL AND ANTIOXIDANT PROPERTIES OF RAW AND POPPED AMARANTH (*Amaranthus cruentus*) SEEDS FLOUR

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Abstract
This study was carried out on raw and popped amaranth (Amaranthus cruentus (PI538326)) seed flour in order to provide scientific data on its functionality. Raw amaranth seeds were milled into flour. The same raw seeds were also popped at 180ºC – 200ºC for 20 seconds and milled into fine flour as well. The resulting flour from the processing were analyzed for the proximate composition (protein, fat, crude fiber, ash, moisture and carbohydrate by difference) and functional properties (water and oil absorption capacity, bulk density, gelation properties, emulsion capacity, swelling capacity and foaming capacity). The antioxidant properties were also determined. The result of the proximate composition showed that Raw Amaranth Flour (RAF) and Popped Amaranth Flour (PAF) had 15.39% and 16.78% for the protein and 9.41% and 3.70% for the moisture, respectively. Water Absorption Capacity (WAC) values of 16.50% and 58.17%, Oil Absorption Capacity (OAC) of 19.30% and 24.40%, Emulsion Capacity (EMC) of 0.51% and 0.66%, Foaming Capacity (FC) of 3.67% and 0.63% were observed for RAF and PAF respectively. The RAF shows a better ability in scavenging the free radical compared to the PAF. The results from this scientific study suggest the potential use of the flour in food formulations.

Keywords: functionality, proximate composition, antioxidant properties, scavenging, free radicals.

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I. Introduction

Amaranth is one of the underutilized multi-purpose crop which can supply both grains and tasty leafy vegetables of high nutritional quality as a food and animal feed. Additionally, because of its attractive inflorescence colouration, amaranth can be cultivated as an ornamental plant. Although the crop was one of the staple foods in the pre-colonized South American civilizations, the cultivation and knowledge fell into oblivion and thus nowadays it could be classified as a new, forgotten, neglected and alternative crop of great nutritional value. The poor nutritional values of the few, but most produced, crop species in the world today and consecutively occurred genetic erosion of cultivated land are some of the reasons for renewed interest in alternative crops. The use of alternative crops would result in product competitiveness, rich nutritional value, tradition, locality and special quality (Bavec and Bavec, 2006).

Amaranth grains belongs to the order *Caryophyllales*, the amaranth family *Amaranthaceous*, sub-family *Amaranthoideae*, to the genus *Amaranthus*, and according to Sauer (1967), into the section *Amaranthus*. The genus *Amaranthus* includes approximately 60 species, most of which are available as weeds growing worldwide, associated with difficulties in cultivation practices after soil disturbance and seed exposure to light and cultivated amaranth species which can be used as food grain, leafy vegetables, forage and ornamental. Based on utilization pattern, amaranth species has been divided into grain and vegetable amaranths. The amaranth grain belongs to a group of cereal-like grain crops or pseudocereals. According to the proposed definition of Shewry, (2002), pseudocereals are dicotyledonous plants which are not closely related to each other or to the mono-cotyledonous true cereals. The name is derived from the production of small grain-like seeds and the group comprises three crops, amaranth

(*Amaranthus spp., family Amaranthaceae*), quinoa (*Chenopodium quinoa, family Chenopodiaceous*) and buckwheat (*Fagopyrum esculentum, family Polygonaceae*). All contain major groups of globulin storage proteins with smaller amounts of albumins. The cultivated grain amaranths differ from their wild and weedy relatives by bearing pale, rather than black seeds, which are superior for many culinary uses (Steckel, 2007). According to Sauer's taxonomic key, (1967) as reported by Steckel (2007), three principal species of genus Amaranthus, originating from South America, are considered for grain production:

• *Aamaranthus hypochondriacus* - prince's feather

• *Amaranthus cruentus* - bush greens, red amaranth and

• *Amaranthus caudatus*. - love bleeding

Among each grain species there are several grain types or races defined by their common branching pattern, height, inflorescence size and form, days to maturity, seed size and colour, and other morphological characteristics (Breene, 1991).

Functional properties are those characteristics properties, which aid in the processing and the utilization of agricultural produce industrially. They predict uses in food system. These properties include, water and oil absorption capacity, foaming capacity, emulsification capacity, gelation, protein (Nitrogen) solubility properties. Protein component are largely responsible for most of the functional properties of most food material. However the insoluble carbohydrate may also have contributed few functional properties (Enwere, 1998).

Grain amaranth are not typically true grain but are typically associated with the grain family due to their similar composition (Joanne, 2004) numerous phytochemical, some common in many plant food (phytate and phenol compound and some unique to grain products (avenanthramides, avenalumic acid), are responsible for the high antioxidant activity of whole grain food (Miller *et al.*, 2000a). The primary protective function of antioxidant in the body is the reaction with free radicals. These free radicals are known to cause attack on DNA, lipid and protein in human body. Antioxidant also protects the DNA from oxidation changes and mutation, leading to cancer. It has been reported that if the dietary antioxidant reduce free radical activity, in the body then disease potential is likely to be reduced (Joanne, 2004). It is evident that a lot of studies has been carried out in the cereals such as: processed breads indicate that they are a rich source of antioxidant (Miller *et al.*, 2002b); processing effect have been found to open up the food matrix, thereby allowing the release of tightly bound phytochemicals from the grain structure (Fulcher and Duke, 2002). Likewise, studies with rye found that many of the bioactive compounds are stable during food processing and their level may even be increased with suitable processing (Liukkonen *et al.*, 2003). However, there is death information on the functional and antioxidant properties of amaranth seed flour, a new forgotten crop. Investigation into the functional and antioxidant properties of raw and popped amaranth seed flour will increase its utilization and prevent it from going into extinction.

Therefore, there is need to explore this forgotten seed, whose leaf is only important to us in this part of the world, for industrial utilization and in the treatment of secondary related heart diseases. Therefore the objectives of this research work were to:

- determine the proximate components and functional properties of raw and popped amaranth seed flours
- evaluate the antioxidant properties of raw and popped amaranth seed flours.

2. MATERIALS AND METHODS

Raw Materials

The Amaranth seeds *(Amaranthus hybridus, PI538326*) used in this study was obtained from National Horticulture Research Institute (NIHORT), Ibadan.

Methods

Preparation of Raw Amaranth Seed to Flour: The raw amaranth seed was sorted and cleaned (as shown in Figure 1). It was oven dried 50 ^{0}C - 60 ^{0}C for 48 hours. Seeds were milled into fine powder using Perten Lab Milling Machine and sieved to an average particle size of 0.5 mm.

Preparation of Popped Amaranth Seed to Flour (PAF): The amaranth seed were popped at temperature of about 190 ^{0}C – 210 ^{0}C for 21 seconds and milled into fine flour. As illustrated in Figure 1 below.

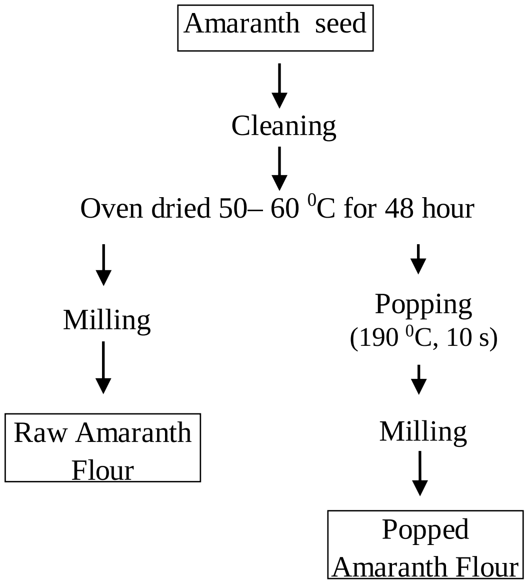

Figure 1: Flow chart for the production of raw and popped amaranth seed flour

Proximate Composition: The methods described by AOAC, (2005) were used to determine protein, moisture, crude fibre, ash content and the carbohydrate content were determined by difference on the raw and popped amaranth seed flour.

Functional properties of raw and popped amaranth seeds flour: Water and Oil absorption capacity were determined according to Tomotake *et al.*, (2002) with minor modifications. Emulsifying activity and stability were evaluated using the method described by Aluko *et al.*, (2001). Foaming capacity and stability were assayed according to the method of Gamel *et al.*, (2006). Likewise, the gelation properties (gelling properties) were measured by the method described by Coffman and Garcia (1977).

Antioxidant properties assays: The Ferric reducing property was measured according to the method described by Pulido *et al.*, (2000). The radical scavenging ability of the extract against DPPH was evaluated using the method described by Gyamfi *et al.*, (1999). Measurement of Superoxide anion scavenging activity assay was based on the method described by Selvakumar *et al.*, (2001). The hydroxyl radical scavenging activity of the extract was assayed based on a previous method reported by Bao *et al.*, (2005). The metal chelating activity by the fractions and standards were estimated by the method of Puntel *et al.*, (2005).

Statistical Analyses: Analyses were done in triplicate and the data were analysed using one way Analysis of Variance (ANOVA) (Steel and Torrie, 1980).

3. Results and discussion

Chemical Composition of Raw and Popped Amaranth Flour: The proximate composition of raw amaranth flour (RAF) and popped amaranth flour (PAF) is as shown in Table 1. Processing amaranth grain had a significant effect ($p \leq 0.05$) on its proximate composition; protein, ash, fiber and fat, except for moisture reduction during drying, thus raising the nitrogen free extract. These grains have been considered to have a special composition of protein, carbohydrates and lipids; thus it can provide a balanced nutrient uptake with lower amounts of consumption compared with other cereals (Lehman, 1989). The proximate compositions obtained were in agreement with those reported by other researchers for *A. cruentus* species. Muchová *et al.,* (2000) reported value ranges of crude protein 12-19%, crude fat, 5-8% crude fiber, 4-5% and ash, 2-4% on dry matter basis. This study recorded that grain amaranth of *Amaranthus cruentus* species has a higher protein content (12 -18%) than cereal grains. These could be as a result of its significantly higher lysine content, which makes it particularly attractive for use as a blending food to increase the biological value of processed foods as earlier reported by Mburu *et al.,* (2012).

Processing of amaranth seed into popped form has a significant effect on the protein content of popped amaranth flour (PAF) having 16.78%

of the protein content and the raw amaranth flour (RAF) having 15.39%. Likewise processing also causes a slight difference in the crude fat which is higher in the PAF 7.49% compared to the RAF 6.68%. The popped amaranth seed flour has a moisture content of 3.70% lower than that of the raw amaranth seed flour 9.41%, this is as a result of the high temperature it was subjected to during popping and this has help reduce spoilage of the flour and also prolong its shelf life. Both raw and popped amaranth seed flour contain the same percentage of ash content of about 2.78% (raw) and 2.58% (popped) which is determining factor to see whether the seed is of good quality. The ash content is the remaining organic residue after the organic matter has been burnt away. It is not necessarily of exactly the same composition as the mineral matter present in the original flour as there may be losses due to vapourization or some interactions between constituents. This grain has nutritional properties that are superior to almost all common cereals. Among the outstanding nutritional attributes of amaranth grain is its high protein content. From the study it can be concluded that there were significant effect on the proximate analysis of the raw and popped amaranth seed flour. The protein content of the popped amaranth seed flour (16.78%) was higher than that of the raw amaranth seed flour (15.39%) which makes the product rich in nutrient and also for consumption purposes.

Table 1: Chemical composition of raw and popped amaranth flour

Proximate Composition	RAF	PAF
Protein	15.39 ± 0.22^{b}	16.78 ± 0.14^{c}
Fat	6.68 ± 0.09^{a}	7.49 ± 0.06^{b}
Moisture	9.41 ± 0.11^{a}	3.70 ± 0.81^{a}
Ash	2.78 ± 0.15^{d}	2.58 ± 0.06^{e}
Crude fiber	3.74 ± 0.05^{a}	2.51 ± 0.03^{a}
Carbohydrate*	62.00±0.00	66.94±0.00

*Values are means of triplicate determinations ± standard deviation. Values in columns with different superscript letter are significantly different (p≤0.05). *=Calculated by difference. RAF=Raw Amaranth Flour, PAF=Popped Amaranth Flour.*

Functional Properties of Raw and Popped Amaranth Flour

The functionality of flour plays an important role in the manufacturing of food product. Table 2 shows the functionality of raw and popped amaranth seed flour. Water absorption capacity represents the ability of a product to associate with water under conditions where water is limiting (Giami and Alu, 1994). In addition, Osundahunsi *et al.*, (2003) described water absorption capacity as the ability of flour to absorb water and swell for improved consistency in food. It is of optimum importance in food processing plants to improve yield and consistency and give body to the food. The water absorption capacity (WAC) of RAF was found to be significantly different from that of PAF (Table 2) ($p<0.05$). From the result, it shows that popped amaranth seed flour has good ability to bind water than that of raw amaranth seed flour. WAC is an important characteristic of flours because physicochemical properties such as viscosity and gelation are dependent on them and give valuable information on the behaviour. WAC are attributed to the protein and starch granules present in the samples as well as their arrangements, and the degree of packing of the granules which determine the intermolecular spaces available at the surfaces of the products. The WAC as an index of the amount of water retained within the protein matrix shows the functional capacity of the product protein in thickening and food formulations (Mburu *et al.*, 2011). In addition heat treatment of raw materials has been reported by Philip *et al.*, (1988) to affect their hydration properties of flour materials.

The oil absorption capacity (OAC) of popped amaranth seed flour (24%) was significantly higher than that of raw amaranth seed flour (19%) ($p<0.05$). These could be due to the presence of high lipid content of the amaranth grain that is made more available as a result of processing effect. The mechanism of oil absorption capacity has been attributed mostly to the physical entrapment of oil, but as well may be influenced by its hydrophobicity power. The oil absorption capacity (OAC) of flour is equally important as it improves the mouth feel and retains the flavour of food product (Kinsella, 1976) and could be used to develop new food product with beneficial components.

Table 2: Functional properties of raw and popped amaranth seed flour

FUCNTIONAL PROPERTIES	RAF (%)	PAF (%)
Water absorption capacity	16.50^{b}	58.17^{a}
Oil absorption capacity	19.30^{b}	24.40^{a}
Emulsifying capacity	0.51^{b}	0.66^{a}
Foaming capacity	7.67^{a}	3.67^{b}
Foaming stability	3.67^{a}	0.67^{b}

Values are means of triplicate determinations. Values in row with different superscript letter are significantly different ($p \leq 0.05$).

From the Table 2, there were significant difference in the emulsifying capacity between the RAF and PAF. The relatively low emulsion capacity of the raw flour could be due to the nature and type of protein. Sathe and Salunkhe, (1981) reported that emulsion capacity and stability is higher in protein with globular nature. Protein can emulsify and stabilize the emulsion by decreasing surface tension of the oil droplet and providing electrostatic repulsion on the surface of the oil droplet, while some types of polysaccharides can help stabilize the emulsion by increasing the viscosity of the system (Dickinson, 1994).
The result in Table 2 shows that the foaming capacity and stability (FC&S) for the RAF is higher than the PAF which could be as result of temperature effect on the grains causing denaturation of globular protein. Because foaming capacity is assumed to be dependent on the configuration of protein molecules. Flexible proteins have good foaming capacity but highly ordered globular molecule gives low foam ability (Graham and Philips, 1976). The foam expansion and foam stability have been correlated with water-dispersible nitrogen (Yasumatsu *et al.*, 1972). Food ingredients with good foaming capacity and stability can be used in numerous leavening bakery products such as cakes, biscuits e.t.c. (Akubor *et al.*, 2000). The foaming stability of the raw amaranth seed flour was 3.67% while the popped amaranth seed flour was 0.67% lower than that of the raw amaranth seed flour. The result shows an inverse relationship between Foaming Capacity and Foam Stability. This work as also supported the previous research reported by Jitngarmkusol *et al*., (2008).
The results (Table 3) of the gelation properties show minimum gelation concentration at 14% for RAF. This could be because the higher the concentration, the stronger the gel formation. But this could not hold for PAF as gel was formed at lower concentration of 6%. This could be as a result of effect of hydration on PAF. This has been reported by Philip *et al.*, (1988) that heat treatment has been observed to affect the hydration properties of flour materials. Least gelation concentration (LGC) is considered as the gelling ability of flour which provide structural matrix for holding water and other water soluble materials like sugars and flavours. The LGC of different flours may vary depending on the relative ratios of different constituent like proteins, carbohydrates and lipids (Sathe *et al.*, 1982). It could also serve as a good binder or provides consistency in food preparations especially the semi-solid products as reported by Ayo *et al.*, (2010). The increasing concentration of proteins in the flour facilitates the gelation properties which may be due to the enhanced interaction among the binding forces (Lawal, 2004).

The Antioxidant Properties of Raw and Popped Amaranth Flour

The antioxidant compounds found in our food or farm produce plays an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (Esan and Fasasi, 2013). Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Table 3: Gelation properties of raw and popped amaranth seeds flour

SAMPLE	FLOUR (%w/v)									
	2	4	6	8	10	12	14	16	18	20
RAF	NG	NG	NG	NG	NG	NG	G	G	G	G
PAF	NG	NG	G	G	G	G	G	G	G	G

NG: No gel formed; the sample slipped from the inverted test tube
G: Gel formed; the sample did not fall from the inverted test tube

Plant food contains antioxidant sources such as Vitamin C, Vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Miller *et al.*, 2000b).

Ferric Reducing Antioxidant Power: The assay evaluate the ability of the antioxidants in the plant sample to reduce the ferric-tri-piridyltrazine (Fe^{3+} -TPTD) in the sample ferrous plant to form (Fe^{2+}) with absorbance at 593nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive ability was measured in terms of Fe^{3+} to Fe^{2+} transformation in the presence of different concentrations of the extract. Figure 2 shows that the raw amaranth flour reduced the ferric-ion better than the popped amaranth flour, simply put. The lower the percentage reducing power the stronger the ability to donate electrons that kill or destroy the free radicals present in human body. The reducing ability of a compound generally depend on the presence of the reductant which have been exhibited by antioxidative potential by breaking the free radical chain (Huda *et al.*, 2009.)

DPPH Radical Scavenging Activities: DPPH (2, 2-diphenyl-1-picryhydrazyl) is not a biologically relevant radical. It is widely used to evaluate the antioxidant activity of the natural compound (Udenigwe *et al.*, 2009). The relatively stable DPPH radical in methanol has been widely used to test the ability of some compound to act as free radical scavenger or hydrogen donors and thus to evaluate the antioxidant activity (Shimada *et al.*,1992). The scavenging of the effect of the plant extract using 10mg/ml concentration, which has little or no effect on popped amaranth flour compared to the raw amaranth flour (65%) which is more potent. These could be as a result of the popping temperature (Figure 3).

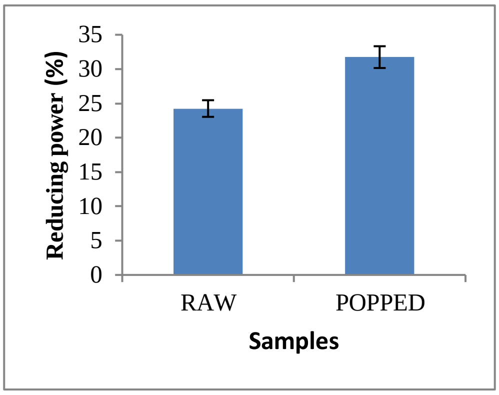

Figure 2: Ferric reducing antioxidant power of raw and popped amaranth flour

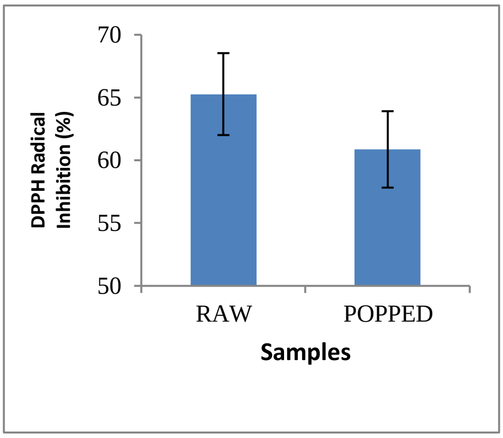

Figure 3: DPPH radical scavenging activities of raw and popped amaranth seed flour

Super Oxide Anion Scavenging Activities: Superoxide radicals scavenging activities of raw and popped amaranth seed flour are generated in PMS–NADH systems by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture was incubated at 25^{0}C for 5 minutes; the absorbance was read at 560 nm by spectrophotometer (Schimadzu UV- Vis 1700) against blank samples using l- ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity. Superoxide anion radicals are produced endogenously by flavor enzymes like xanthine oxidase, which converts hypoxanthine to xanthine and subsequently to uric acid in ischemia-reperfusion (Zheng and Wang, 2001). Exogenous chemical and endogenous metabolic process in the human body or in the food system might produce highly reactive free radical, especially oxygen derived radical, which are capable of oxidising biomolecule resulting in the death of cell and tissue. As shown in Figure 4 the PAF demonstrated good superoxide scavenging activity compared to the RAF which exhibited a poor superoxide scavenging activity. According to Pownall *et al.*, (2010), those fractions exhibiting superoxide scavenging activity could contain proline which plays an important vital role in superoxide scavenging activity (Figure 4).

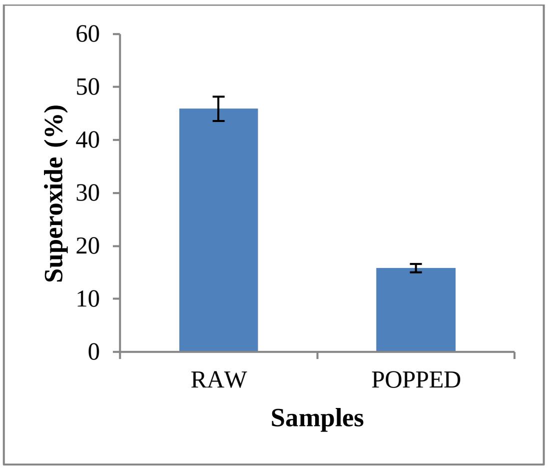

Figure 4: Super oxide anion scavenging activities of raw and popped amaranth flour

Hydroxyl Radical Scavenging Activity: The hydroxyl radical scavenging activity of raw amaranth seed flour was found to be lower in value than that of the popped amaranth seed but in contrast very potent than the PAF in scavenging the free radicals at 10mg/ml of the extract concentration (Figure 5). Conversely studies have shown that thermal processing of fruit, vegetable and cereal can have a detrimental effect on the flavonoids compound (Alvarez–Jubete *et al.*, 2010c). The extent of flavonoid loss was due to the processing has shown to be highly dependent on factors such as the type of the substrate and the processing condition, mainly the length and the temperature of the process. As already mentioned antioxidants, such as hydroxyl radical scavenging/ polyphenol, have significant potential health benefit; they protect cell constituent against oxidative damage and therefore limit the risk of various degenerative disease associated with oxidative stress such as cancer cardiovascular disease and osteoporosis.

Metal Chelating Scavenging Activity: The chelating by the fractions and standards were estimated in raw and popped amaranth seed flour. The absorbance of the solution was measured spectrophotometrically at 562 nm by using a UV-Visible Spectrophotometer (Schimadzu UV-Vis 1700). From the result the raw amaranth seed had stronger metal chelating power than that of the popped amaranth seed. One of the mechanisms of antioxidative action is chelation of transition metals, thus preventing catalysis of hydroperoxide decomposition and fenton type reactions (Gordon, 1990).

Estimation of the chelating activity of the coexisting chelator

The transition metal ion, fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Figure 6).

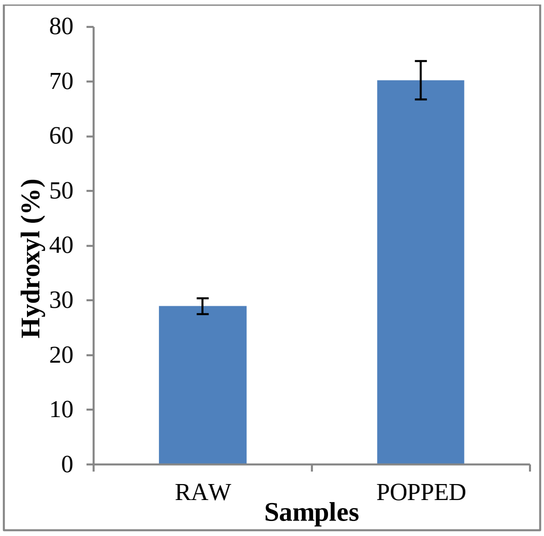

Figure 5: Hydroxyl radical scavenging activity of Raw and Popped Amaranth seed

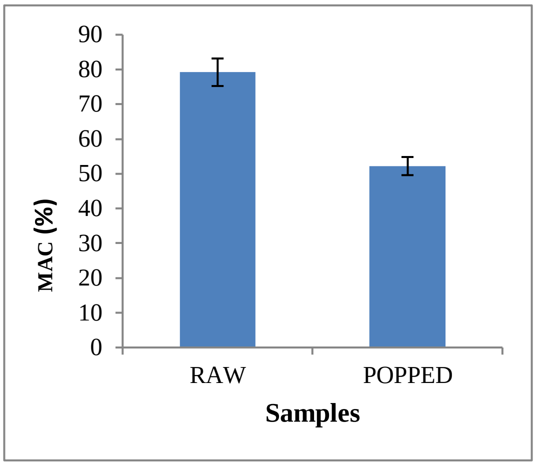

Figure 6: Metal chelating scavenging activities of raw and popped amaranth seed flour

4. CONCLUSION

Popping has significant effect on the proximate composition of the amaranth seed flour. The popped amaranth seed will make a good snack because of its protein content and also suitable for the celiac patient. PAF gave a good gelation ability, high water absorption capacity, oil absorption capacity and these will encourage its usage in the industry as a good binding agent. In addition, RAF has a low bulk density, of which it is a desirable factor for infant food processing. The raw amaranth seed flour was also high in its foaming capacity which has a positive effect in the brewing industry and ice cream production. The oil absorption capacities therefore would be potentially useful in flavour retention, improvement of palatability and extension of product(s) shelf life. These seeds flour could serve as an alternative protein source with great potential to alleviate protein malnutrition in developing countries and to improve the overall nutritional status of functional foods in both the developed and developing countries. An assay of total antioxidant capacity helps to understand the functionality properties of foods. From the result it was concluded that raw amaranth flour has higher antioxidant properties owing to the fact that scientific evidence suggests that antioxidants reduce the risk of chronic diseases including cancer and heart disease. Based on the chemical composition of this study, it is recommended that amaranth seeds should be cultivated on a large scale to encourage its usage on household and industrial uses as a good alternative to other cereals.

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