Received: 20 March 2019 | Revised: 22 July 2019 | Accepted: 17 September 2019
DOI: 10.1111/jfpp.14230

ORIGINAL ARTICLE

Journal of Food Processing and Preservation
WILEY

Characterization of two new rice varieties, *Amankwatia* and *AGRA*, grown in Ghana

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Abstract

The proximate and mineral contents of two Ghanaian rice varieties (*Amankwatia and AGRA*) were assessed by standard methods. Results showed significant difference as for proteins solubilized by media with various dissociating abilities and revealed the presence of several disulfide-rich proteins. The two varieties showed similar contents as for ash, protein, crude fiber, and carbohydrate, whereas significant difference ($p < .05$) in the fat content was observed. Protein overall structure was addressed by solubility and SDS-PAGE electrophoresis, while water binding capacity, swelling power, and bulk density accounted for the functional properties. No significant difference ($p > .05$) existed in the bulk density, except for swelling power and water binding capacity of the rice varieties. Potassium was the predominant mineral in both rice flours and significant differences existed among the contents of manganese, zinc, iron, magnesium, sodium, and potassium. Thus, the rice varieties have very useful constituents for developing new rice-based food products.

Practical applications

The study showed the presence of hydrophobic interactions and disulfide bonds stabilizing proteins in the *Amankwatia* and *AGRA* rice flours. Structural features of the protein network in the two aromatic rice flours developed in Ghana was also revealed through SDS-PAGE electrophoresis. This is essential towards understanding the chemistry of the rice flours and to appreciate their subsequent utilization in food product development. The higher water absorption capacity of *AGRA* rice flour makes it useful as thickener in food formulations. The excellent nutrient profile of *Amankwatia* and *AGRA* rice flours, compared with others implies these two rice varieties, can compete with other imported rice varieties which are generally perceived to be of higher quality in Ghana. The need to encourage the consumption of these two locally produced rice varieties is justified.

1 | INTRODUCTION

Rice is an important staple food in Sub-Saharan Africa including Ghana with consumption projected to increase by 32% in the year 2026 (USDA, 2017). Increased urbanization and population growth coupled with low rice production are the predominant factors in Sub-Saharan Africa that have triggered the increased rice demand. Consequently, rice demand in Sub-Saharan Africa is satisfied through importation, and the quantity of imported rice is predicted to increase from 12.3 million tons in 2017 to 15.4 million by 2026 (USDA, 2017).

Efforts to improve rice production in Africa have compelled stakeholders to implement several initiatives. The Coalition for

Africa Rice Development (CARD), jointly proposed by the Alliance with a Green Revolution in Africa (AGRA) and the Japan International Cooperation Agency (JICA), is one such initiative with the overarching goal of responding to the increasing demand for rice (Bado, Djaman, & Mel, 2018). Ghana, a member of CARD, launched its National Rice Development Strategy (NRDS) for the decade 2009–2018 in May 2008 with the objective of doubling domestic production by 2018. This was geared towards promoting a 10% of annual production growth rate and enhancing rice quality to stimulate demand for domestically produced rice (FAO, 2016).

Despite the implementation of several strategies to improve rice production in Ghana, locally grown rice is confronted with the challenge of poor acceptance by consumers. This is due to the perception that imported rice is of higher quality than locally grown rice varieties (Demont & Ndour, 2015; Diako, Sakyi-Dawson, Bediako-Amoa, Saalia, & Manful, 2010). However, it has been reported that rice consumers have higher preference for locally produced rice with improved qualities including increased nutritional value, appealing appearance, and aroma (Ehiakpor, Apumbora, Danso-Abbeam, & Adzawla, 2017). Demont and Ndour (2015) reported that the importation of rice can be drastically reduced if stakeholders in the rice sector increased the production of good quality rice. Improvement in the nutritional quality of locally produced rice cultivars is essential to make them competitive to their imported counterparts (Demont & Ndour, 2015). In this context, the CSIR-Crops Research Institute (CRI), which is part of CSIR-(Council for Scientific and Industrial Research) of Ghana, continues to breed new varieties of local rice to address food security challenges and thus leading to the production of the two local aromatic rice varieties (*Amankwatia* and *AGRA* rice).

Several studies have evaluated the functional and nutritional composition including proximate and mineral content of rice cultivars grown in Nigeria (Otemuyiwa, Falade, & Adewusi, 2018), India (Verma & Srivastav, 2017), and Thailand (Kraithong, Lee, & Rawdkuen, 2018). Currently, there is a paucity of data on the properties and nutritional profile of these two local aromatic rice varieties (*Amankwatia* and *AGRA* rice). This study aimed at investigating the overall quality parameters of locally grown rice varieties (*Amankwatia* and *AGRA*) and understanding some of the molecular determinants of their properties, that may help in defining practical applications for these varieties, and eventually add value to local African raw materials. Therefore, addressing the biochemical, functional, and nutritional properties of flour from different rice cultivars can offer guidelines for the use of rice flours in specific production processes and in the formulation of peculiar products that would meet the consumers' expectations (Falade & Christopher, 2015; Kraithong et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Raw materials

Two local rice varieties namely *Amankwatia* and *AGRA* rice, developed by the CSIR-CRI at Fumesua in the Ashanti Region of Ghana, were used in the study. The paddy rice obtained was milled to obtain polished rice using SB30 rice miller (China). The polished rice was subsequently milled into flour using a Hammer mill at the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology (KNUST). A 500 µm of pore-sized sieve was used to sieve the milled rice sample to obtain fine rice flour. The rice flour samples were sealed in Ziploc double zipper high-density packages (Ziploc Brand Products, WI, USA) and kept in a cold room at 4°C for analyses.

2.2 | Proximate composition

The moisture, ash, crude fat, fiber, and protein contents of the two local rice flours were determined according to the procedure by AOAC (2000). The carbohydrate content was determined by difference.

2.3 | Determination of functional properties

Bulk density was determined according to the method of Suresh (2013). The swelling power determination was carried out, based on modifications of the method by Leach, McCowen, and Scoch (1959). A 1.0 g of powdered sample was weighed into a previously weighed 40 ml capacity centrifuge tube and distiled water added to the 40 ml of mark. The samples were then heated in the water bath at 85°C for 30 min with constant shaking. The tubes were allowed to dry and cool to room temperature before centrifuging at 2,200 rpm for 15 min using a centrifuge. The sediment paste obtained after centrifugation was weighed and used to calculate the swelling power.

The water absorption capacity (WAC) procedure used by Falade and Christopher (2015) was followed. One gram (1.0 g) of the sample was weighed into 10 ml capacity centrifuge tube. A 10 ml of distiled water was added. This was followed by mid-speed vortexing for 1 min and centrifuged at 3,500 rpm for 30 min. The supernatant was decanted, and the tubes were air-dried. The bound water was calculated from the increase in the weight of the samples. WAC was expressed as percentage of water adsorbed by 100 g of sample. All samples were analyzed in triplicate.

2.4 | Determination of mineral content

A 3.50 g sample of the *AGRA* and *Amankwatia* flour was weighed using an analytical balance and placed in a crucible. The crucible and content were placed in the muffle furnace for 3 hr at 550°C. The samples were then allowed to cool in an oven (Binder drying heating oven, 78,532, Tuttingen, Germany) to 100°C for 30 min, cooled to ambient temperature (28°C) in a desiccator and weighed. The ash was dissolved in 25 ml of 10% HNO_3 and filtered through an acid-washed filter paper. The filtrate was made up to 100 ml mark using deionized water in a standard flask and shaken prior to analysis. Standard solutions were prepared to determine the various elements using a stock solution of 1,000 mg/L. A simple dilution formula of $C_1V_1 = C_2V_2$, where C_1 and C_2 and V_1 and V_2 represents (initial and final) concentration and (initial and final) volume, respectively, was used. The diluent used was 10% HNO_3 (v/v). A 10 ml of stock solution was pipetted into a 100 ml volumetric flask and diluted with 10% HNO_3 to obtain 100 mg/L. The same step was repeated to prepare other standard solutions for the calibration of the spectrophotometer, prior to the potassium (K), iron(Fe), sodium (Na), calcium(Ca), magnesium(Mg), manganese (Mn) and zinc (Zn) content determination using Atomic Absorption Spectrometer (Varian AA 240FS) and standard curves were plotted, out of which the various concentrations were calculated based on the absorbance readings K, Fe, Na, Ca, Mg, Mn and Zn were analyzed at wavelengths of 766.5, 248.3, 589, 422.7, 285.2, 279.5 and 213.9 nm respectively (AOAC, 2002). All samples were analyzed at least in triplicate.

2.5 | Protein solubility

The nature of interactions stabilizing protein aggregates in the rice flours was addressed by measuring soluble proteins in various buffers as described in Marengo et al. (2018). In particular, 0.12 g of finely ground samples (<250 μm) were dispersed in 5 ml of 0.05 M sodium phosphate buffer, pH 7.0, containing: (a) 0.1 M NaCl; (b) 0.1 M NaCl, 6 M urea; and (c) 0.1 M NaCl, 6 M urea, and 0.01 M dithiothreitol (DTT). After stirring at room temperature for 60 min, suspensions were centrifuged at 10,000 ×g for 20 min at 20°C, and the protein content in the supernatant was assessed by a dye-binding method (Bradford, 1976), using bovine serum albumin as a standard protein. Results were expressed as mg proteins/g sample.

2.6 | Electrophoretic pattern of the extracted proteins

SDS-PAGE was performed according to Iametti et al. (2013). A fixed volume of the proteins solutions resulting from the solubilization in the various buffers was treated with an equal volume of denaturing buffer (0.125 M Tris–HCl, pH 6.8, 50% glycerol, 1.7% SDS; 1% 2-mercaptoethanol; 0.01% Bromophenol Blue). Samples were boiled for 10 min, and volumes corresponding to 0.015 mg protein were loaded onto a fixed porosity gel (12% monomer). SDS-PAGE was carried out in a Mini-PROTEAN apparatus (Bio-Rad, Richmond, VA, USA), and gels were stained with Coomassie Brilliant Blue. Molecular mass markers covered the range between 14 and 97 kDa.

2.7 | Statistical analyses

Independent sample *t* test was used to establish significant differences among means at 95% confidence interval using the Tukey's test. All the analyses were done with IBM SPSS version 24.0 software.

3 | RESULTS AND DISCUSSION

3.1 | Proximate composition of *Amankwatia* and *AGRA* rice flours

The proximate composition of the flours from the two rice varieties is shown below (Table 1). Moisture contents of 9.11% and 9.65% were recorded for the *Amankwatia* and *AGRA* rice flours, respectively. These values compare well with moisture content ranging from 8.8% to 14.1% for rice varieties grown in Nigeria (Otemuyiwa et al., 2018), but higher than what was reported for organically grown white Thai rice (5.47%; Kraithong et al., 2018). Rice with moisture contents below 11% is reported safe against deterioration (Gani et al., 2017; Padmavathi, Babu, & Waghray, 2015). *Amankwatia* and *AGRA* rice flours had ash content of 0.55% and 0.54%, respectively. These values were consistent with ash content of 0.38%–0.73% in aromatic rice varieties (Verma & Srivastav, 2017) but lower than in Nigerian grown types (0.88% to 1.67%; (Otemuyiwa et al., 2018). In terms of protein content, the *Amankwatia* and *AGRA* rice flours recorded 8.00% and 8.13%, respectively. This was comparable to protein content of 7.23%–9.51% in aromatic rice varieties (Verma & Srivastav, 2017), but higher than Nigerian cultivars (6.1%–6.6%; (Otemuyiwa et al., 2018). *Amankwatia* rice flour contained about twice the amount of crude fat compared to the *AGRA* rice flour. The fat content in *Amankwatia* and *AGRA* rice varieties were 1.06% and 0.47%, respectively. These were generally lower than the fat content of 2.90% to 5.90% in Nigerian rice cultivars (Otemuyiwa et al., 2018) but comparable to aromatic varieties grown in India (Verma & Srivastav, 2017). Crude fibre content of 0.59% and 0.60% were obtained for the *Amankwatia* and *AGRA* rice varieties, respectively. The values were comparable to 0.48%–0.85% aromatic varieties grown in India (Verma & Srivastav, 2017). *Amankwatia* and *AGRA* rice flours contained almost the same content of carbohydrate (80.58% and 80.59%, respectively), closely comparable to the Kalanamak aromatic rice variety (80.94%; Verma & Srivastav, 2017). However, the carbohydrate concentration values for the two varieties investigated here were higher than those reported for the rice varieties grown in Nigeria (Otemuyiwa et al., 2018). Variations in the nutritional composition of rice cultivars are dependent on factors including differences in geographical location and genetic variation (Du et al., 2018; Oh, Park, Yeo, Park, & Kim, 2015). Apart from the crude fat composition,

TABLE 1 Proximate composition of *Amankwatia* and *AGRA* rice flour

Constituent (%)	*Amankwatia*	*AGRA*
Moisture	9.11 ± 0.28^a	9.65 ± 0.29^a
Ash	0.55 ± 0.04^a	0.54 ± 0.03^a
Protein	8.00 ± 0.00^a	8.13 ± 0.00^a
Crude fat	1.06 ± 0.02^a	0.47 ± 0.01^b
Crude fibre	0.59 ± 0.03^a	0.60 ± 0.02^a
Carbohydrate	80.58 ± 0.23^a	80.59 ± 0.20^a

Note: Mean values with different superscript within a row are significantly different ($p < .05$).

no significant difference was observed for the *Amankwatia* and *AGRA* rice varieties. Rice largely contributes calories mostly from its carbohydrate components (Table 1).

3.2 | Functional properties of rice flours

Bulk density is a determinant of flour expansion and an indicator of the porosity of food products (Kraithong et al., 2018). The bulk densities of the two rice varieties were 0.77 g/ml (Table 2) and were not significantly different ($p > .05$). These values compared well with that reported for flour from white Thai organic grown rice that recorded bulk density of 0.70 g/ml (Kraithong et al., 2018) but were higher than un-germinated rice flour which recorded bulk densities that ranged from 0.53 to 0.64 g/ml (Moongngarm, Moontree, Deedpinrum, & Padtong, 2014).

WAC was significantly different between the two rice flours with the *Amankwatia* variety recording 206.6% and *AGRA* rice recording 310%. Factors including differences in the structure of starch which consequently results in variations in the internal forces of association that maintains the structure of granule, and the extent to which starch chains interact to form hydrogen and covalent bonds, and the presence of water binding sites accounts for the variations in the WAC of rice flours from different varieties (Falade & Christopher, 2015). The higher WAC of the two rice varieties in this present study makes them promising ingredients that could be used as thickeners in liquid and semi-liquid foods owing to their ability to absorb water and swell for improved consistency in food (Fasasi, Adeyemi, & Fagbenro, 2007).

Swelling power indicates the water holding capacity of flours, which has generally been used to demonstrate differences between various types of starches (Crosbie, 1991). Swelling power values of 6.76% and 7.75% were recorded for *Amankwatia* and *AGRA* rice varieties with a significant difference ($p < .05$) existing between them. This was a bit higher than swelling power of 6.25 and 6.41 reported for *Digang* and *Nerica-1* rice varieties grown in Ghana (Eshun, 2009). The high swelling power values recorded for the *Amankwatia* and *AGRA* could be attributed to the higher water absorption capacities of the rice flours as indicated by Falade and Christopher (2015). The high swelling power suggests that the rice flours could be useful in food systems where swelling is required.

3.3 | Mineral composition of rice flours

Evaluation of the mineral composition of the rice is essential to provide a baseline idea of the micronutrient content to allow for the fortification of food products when the rice flour is used in food product development. This is essential to address micronutrient deficiencies. The mineral content of the *Amankwatia* and *AGRA* rice flour are presented (Table 3). Calcium, for instance, is essential for nerve impulse transmission, vascular dilation, and contraction (Beto, 2015). The calcium contents of the *Amankwatia* and *AGRA* rice varieties were 2.28 and 2.45 mg/100 g, respectively, with no significant difference ($p > .05$) observed between them. This compares well with calcium concentrations of 3.07–7.46 mg/100 g, reported for rice cultivars grown in India (Prasad, Hymavathi, Babu, & Longvah, 2018). The values were, however, lower than calcium values of 7.275–9.875 mg/100 g reported for aromatic varieties of rice (Verma & Srivastav, 2017). Iron content of 1.60 and 0.89 mg/100mg were obtained for the *Amankwatia* and *AGRA* rice varieties with significant difference ($p < .05$) existing between them. These values were consistent with the iron content of 0.37–1.21 mg/100 g reported for rice varieties in India (Prasad et al., 2018). The values were generally lower compared to rice varieties grown in Nigeria (4.2–10.6 mg/100 g; Otemuyiwa et al., 2018) and to those reported in previous studies for three different rice varieties (4.35, 7.254 and 11.97 mg/100 g; David et al., 2019). Zinc is an essential micronutrient, which supports immune function and impairs inflammation in the body (Wong, Rinaldi, & Ho, 2015). The *Amankwatia* and *AGRA* rice flours recorded zinc content of 1.95 and 2.14 mg/100 g, respectively. These values compare well with that recorded by Anjum, Pasha, Bugti, and Butt (2007) who reported zinc content levels of 2.97, 1.44, 2.33, and 1.97 mg/100 g for Irri 6, Irri 9, Sarshar, and Dr- 83 Pakistani rice cultivars. There was a significant difference ($p < .05$) in the zinc content of the *Amankwatia* and *AGRA* rice flours. Manganese is also involved in the metabolism of macronutrients including fats, carbohydrate and proteins (Lewicka et al.,2017). The amount of manganese recorded for the *Amankwatia* and *AGRA* rice varieties were 2.26 and 1.16 mg/100 g, respectively.

TABLE 3 Mineral composition of *Amankwatia* and *AGRA* flour

Mineral (mg/100 g)	*Amankwatia*	*AGRA*
Calcium	2.28 ± 0.01^a	2.45 ± 0.11^a
Iron	1.60 ± 0.03^b	0.89 ± 0.07^a
Zinc	1.95 ± 0.00^a	2.14 ± 0.01^b
Manganese	2.26 ± 0.02^b	1.16 ± 0.03^a
Potassium	114.70 ± 0.06^a	139.24 ± 5.68^b
Magnesium	38.04 ± 0.07^a	44.11 ± 0.02^b
Sodium	6.89 ± 0.03^a	14.20 ± 0.21^b

Note: Mean values with different superscript within a row are significantly different ($p < .05$).

TABLE 2 Functional properties of *Amankwatia* and *AGRA* flour

Properties	*Amankwatia*	*AGRA*
Bulk density (g/ml)	0.77 ± 0.00^a	0.77 ± 0.00^a
Water binding capacity (%)	206.6 ± 11.55^a	310.0 ± 10.00^b
Swelling power (%)	6.76 ± 0.00^a	7.75 ± 0.00^b

Note: Mean values with different superscript within a row are significantly different ($p < .05$).

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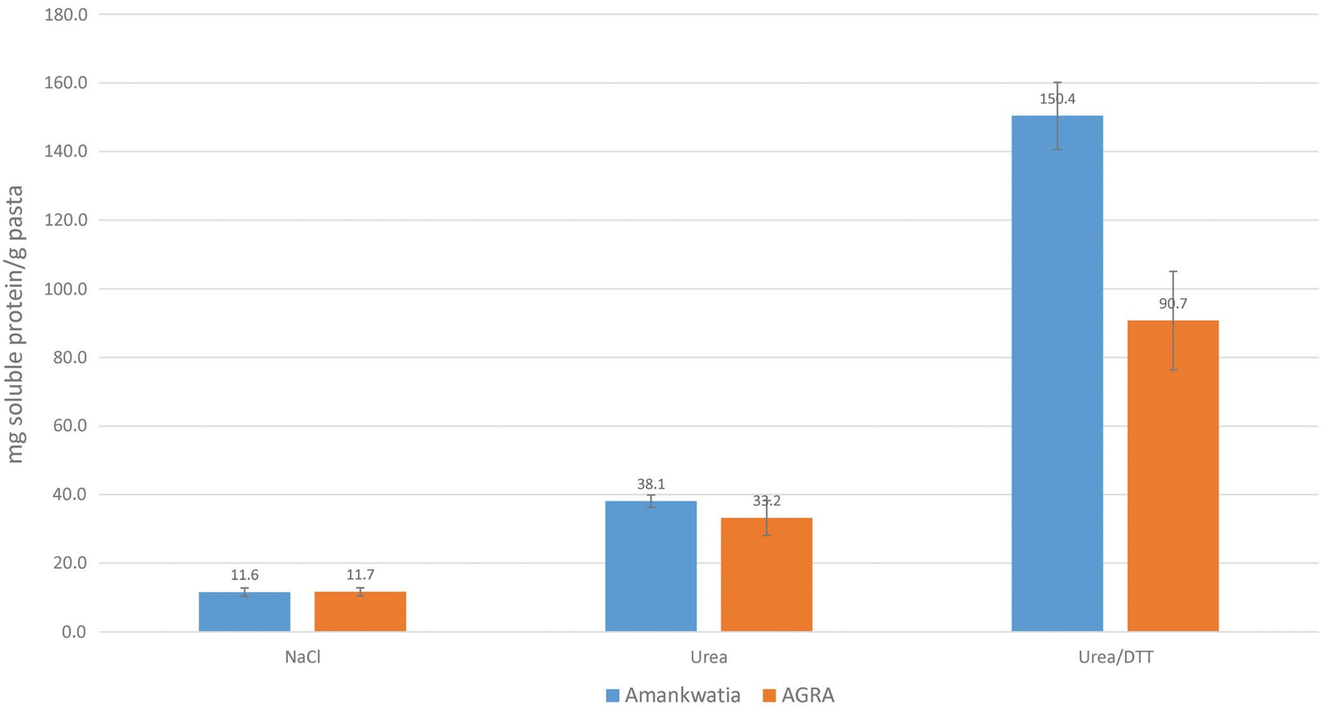

FIGURE 1 Effect of different buffer treatments on the soluble protein content of Amankwatia and AGRA rice flour samples

Comparable values ranging from 0.59 to 1.35 mg/100 g has been reported for rice varieties cultivated in India (Prasad et al., 2018). However, the values of manganese recorded for the *Amankwatia* and *AGRA* rice varieties, in this study, were higher than manganese value of 0.4 mg/100 g recorded for white rice in Brazil (da Silva, Paim, & Silva, 2018). A significant difference ($p < .05$) was observed between the manganese content of *Amankwatia* and *AGRA* flours. The consumption of foods rich in potassium has the potential of promoting vascular and endothelial functioning, consequently improving cardiovascular function (Blanch, Clifton, & Keogh, 2015). Potassium was the most abundant mineral in the two rice varieties used in this study. Potassium content of 114.70 and 139.24 mg/100 g were recorded for the *Amankwatia* and *AGRA* rice varieties, respectively. This compares well with potassium content ranging from 105 to 156 mg/100 g recorded for Indian rice cultivars grown in India (Prasad et al., 2018). There was a significant difference ($p < .05$) in the potassium content of the rice flours. Magnesium is involved in blood pressure and insulin regulation (Houston, 2010). The amount of magnesium recorded for the *Amankwatia* and *AGRA* rice flour in this present study were 38.04 and 44.11mg/100 g with a significant difference ($p < .05$) existing between them. These values were higher than magnesium content of 24.3 mg/100 g in white rice variety cultivated in Brazil (da Silva et al., 2018). However, the values were a bit lower than the magnesium levels of 67 and 82 mg/100 g for raw Ofada and Aroso varieties (Albert T. Ebuehi & Christiana Oyewole, 2008). The role of sodium in maintaining homeostasis in the cells of the body and fluid balance is well known (Farquhar, Edwards, Jurkovitz, & Weintraub, 2015).

Sodium content for the *Amankwatia* and *AGRA* rice flours were 6.89 and 14.20 mg/100 g, respectively, with significant difference ($p < .05$) between them. The value for the *Amankwatia* compared well with sodium concentrations, reported for aromatic rice varieties (4.14–6.89 mg/100 g) (Verma & Srivastav, 2017) but not the *AGRA* rice flours. Several factors including the genetic composition, geographical location, and type of soil reportedly impact on the mineral composition of rice (Du et al., 2018; Oh et al., 2015).

3.4 | Characterization of proteins from rice flours

Structural features of proteins in flours from *Amankwatia* and *AGRA* rice flour were assessed by extracting proteins in buffers with different dissociating ability toward inter-protein bonds. This approach provides insights into the types of bonds that stabilize aggregation and/or association in protein-based aggregates. As presented in Figure 1, the amount of proteins from the rice sample soluble in the saline buffer is very low and comparable for the two rice varieties. The presence of a denaturing agent (urea) in the extraction buffer leads to dissociation of aggregates stabilized by hydrophobic interactions (Moroni, Iametti, Bonomi, Arendt, & Dal Bello, 2010), therefore resulting in a significant increase of the amount of soluble proteins. A further increase in soluble proteins was detected when both urea and the disulfide-reducing agent DTT were present, due to the destabilization of protein aggregates where disulfide linkages have a main structural role, as previously reported for other cereals and cereal-based foods (Marengo et al., 2018). Findings from the present study is consistent with that reported by Marti et al. (2014), who showed that the amount of proteins in rice pasta enriched with proteins from different sources, was very low and increased when a denaturing and/or reducing agents were added. All together, these results suggest the presence in both rice varieties of a comparable amount of protein aggregates stabilized by hydrophobic interactions.

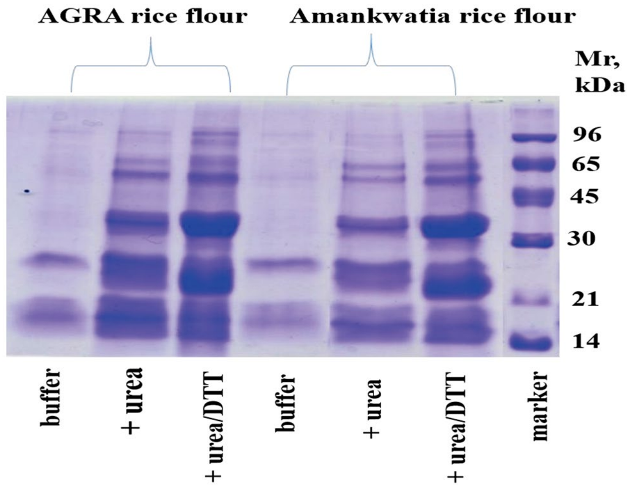

FIGURE 2 SDS-PAGE profile of proteins solubilized from the various samples in different buffer systems. Aliquots of the various rice samples were suspended under stirring in 0.05 M sodium phosphate, 0.1 M NaCl, pH 7.0, in the presence of 6 M urea and 10 mM DTT, as indicated. Separations were run on protein samples denatured in the presence of 2-mercaptoethanol

However, a significantly higher amount of proteins was extracted in both varieties when both urea and DTT were present indicating that disulfide interaction were involved in the stabilization of proteins aggregates. In this contest, it can be noted that a higher amount of protein was detected in *Amankwatia* rice, suggesting that in this variety disulfide bounds have a major role in stabilizing protein network.

Proteins solubilized from the rice varieties in the various buffer systems used in the studies presented in Figure 1 were separated by SDS-PAGE, which allowed a comparison among samples in terms of protein families and size. The results of SDS-PAGE carried out in the absence/presence of 2-mercaptoethanol—a disulfide-reducing agent—are presented in Figure 2. As expected from the conditional solubility results, the number and the intensity of bands increase when urea or urea/DTT are used for protein extraction, confirming the presence of protein aggregates stabilized by hydrophobic interactions and disulfide bonds. The tracings do not offer evidence of dramatic differences as for the soluble proteins between the rice varieties considered in this study, although they most likely have a different structural overall organization, as indicated by results presented in Figure 1.

4 | CONCLUSION

This study has revealed that the two new rice varieties had varied degrees of solubility in three-buffer treatments: saline, urea, and DTT. The rice varieties also had several disulfide-rich proteins. Both had very good mineral contents with potassium being the predominant. This shows the two local rice varieties have a high nutritional value. Even though the functional properties of the two rice flours were comparable to those found in other rice varieties, the WAC of AGRA rice flour was found to be much higher than that reported for *Amankwatia*. The very high WAC of AGRA rice flour makes it useful as thickeners in food formulations.

ACKNOWLEDGMENT

The authors express their gratitude to the staff from the Department of Food and Environmental Sciences of University of Milan, Italy for assistance with the biochemical characterization of the rice flours.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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How to cite this article: Amoah I, Lutterodt HE, Adubofuor J, et al. Characterization of two new rice varieties, *Amankwatia* and *AGRA*, grown in Ghana. *J Food Process Preserv*. 2019;43:e14230. https://doi.org/10.1111/jfpp.14230