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Physical, chemical and sensory properties of gluten-free kibbeh formulated with millet flour (Pennisetum glaucum (L.) R. Br.)

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Abstract
Pearl millet flour was utilized in kibbeh formulations instead of whole-wheat flour. Physicochemical properties, oxidation stability and sensorial characteristics of control kibbeh made with whole-wheat flour (CT) were compared with kibbehs prepared with millet flour (roasted or wet) and stored for 90 days (~18 °C). Kibbeh prepared with millet flour presented good oxidation stability (TBARS concentration). Baked kibbehs (with roasted millet flour) presented good acceptability and kibbeh samples did not differ significantly (p > 0.05) from the whole-wheat flour sample, when global appearance, texture and flavor were evaluated. Millet flour could be a suitable ingredient for kibbeh formulations, maintaining their nutritional value and sensorial quality in addition to being a gluten-free product.

Keywords: gluten-free products; alternative cereal; food stability; meat products; acceptability; roasting.

Practical Application: Kibbeh formulations with millet flour can be a good alternative for gluten-free diet.

1 Introduction
Millet (Pennisetum glaucum (L.) R. Br.) is a small cereal with seeds of 1.2-1.8 mm in diameter and a light brown-to-brick red colored seed coat with an undulated surface. Millet is a word derived from “mille”, which signifies a thousand grains. The major millet species in the world is pearl millet (Pennisetum glaucum (L.) R. Br.), followed by foxtail, proso and finger millet (Shahidi & Chandrasekara, 2013).

Pearl millet is a food that supplies a major proportion of calories and protein to large segments of populations in the semi-arid tropical regions of Africa and Asia (O’Kennedy et al., 2006). Recombinant DNA technology is a powerful tool that has been recently used to enhance the gene pools of sorghum and pearl millet crops, which are regarded as important crops, particularly in countries like Africa (O’Kennedy et al., 2006).

Millet is a gluten-free and low-cost cereal (approximately 40% lower than the price of corn), which is resistant to drought and nutrient-poor soils (Gomes et al., 2008). In 2011, the global millet production was about 27.5 million tones (Food and Agriculture Organization, 2015). Countries in Africa and Asia produced 56% and 41% of the total world production, respectively (Shahidi & Chandrasekara, 2013).

In years 2003/2004 millet cultivation area in Brazil corresponded to four million hectares, and the estimative to years 2008/2009 were about five million hectares (Brasil, 2008). There is no current data of millet production in Brazil because it is not commonly used for human feeding despite represents an important ingredient for feeding confined cattle, poultry and pork (Brasil, 2008). So, it is considered an underutilized cereal (Suma & Urooj, 2014), even in the agroecological systems where they grow (Shahidi & Chandrasekara, 2013).

Millet is a superior cereal with regard to nutritional quality and presents several health benefits (Krishnan et al., 2011). It is a rich source of dietary fiber, calcium, oleic acid (25%) and linoleic acid (46%) (Rooney, 1978) and of phytochemicals with nutraceutical potential (Malleshi & Hadimani, 1999). Moreover, millet is a potent source of antioxidants, due to its phenolic content (Dykes & Rooney, 2006; Shahidi & Chandrasekara, 2013) and is a staple food substitute for celiac patients who require gluten-free cereal (Shahidi & Chandrasekara, 2013).

Due to its nutritional characteristics and low cost, there is increased interest in millet due to its health benefits, hypoglycemic characteristics (Lakshmi Kumari & Sumathi, 2002) and due to the antimicrobial and antioxidant activities of its polyphenols (Chethan & Malleshi, 2007). Moreover, as millet does not contain gluten and is known for its low carbohydrate concentration and low glycemic index (Singh et al., 2010; Suma & Urooj, 2014), some authors has studied its viability in bakery products such as breads, biscuits and pasta (Rathi et al., 2004; Saha et al., 2011; Schoenlechner et al., 2013), aiming to replace whole-wheat flour with millet flour. The acceptability of the foods developed with millet flour, such as biscuit dough and breads, is reported to be very good (Saha et al., 2011; Schoenlechner et al., 2013).

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Unfortunately, no meat products that employ the use of millet or millet flour have been described in the literature. The evolution of nutritional and eating habits throughout human history has led to the modern custom of eating snacks (Danski, 2008). Despite originally being a Turkish cuisine, kibbeh is one of the most famous meat snack foods in Brazil. Kibbeh is an industrialized meat product, obtained from beef or lamb, which is minced, added to whole-wheat flour, spices and other ingredients (Brasil, 2000) and is usually commercialized raw (kibbeh nayeh), deep-fried or baked. However, in its original formulation, whole-wheat flour is an essential ingredient and this product should not be consumed by celiac patients.

Thus, the aim of this study was to develop different kibbeh formulations using millet flour and evaluate their nutritional, sensorial and technological quality following freeze storage (90 days at −18 °C), in order to replace whole-wheat flour and provide novel and alternative gluten-free products.

2 Material and methods

2.1 Material and reagents

Pearl millet (Pennisetum glaucum (L.) R. Br.) - ADR 7010 cultivar, a hybrid with the attributes of both straw and grain and great productive potential (50 bags ha⁻¹), was kindly provided from the “Sementes Adriana®” Company (Mato Grosso, Brazil). Other ingredients used to prepare kibbeh (ground beef, whole-wheat flour and spices) were purchased at a local market. All other chemical reagents used in this study were of analytical grade.

Millet flour

Seeds (Pearl millet) were ground using a mill (MCS 280-5 hp Vieira, Tatui, SP, Brazil) in sieves with a 3-mm particle size under continuous suction. The millet flour was stored (−22 °C) until analysis. Millet flour was roasted or hydrated to yield two types of flour; roasted millet flour (RM) and wet millet flour (WM). RM was prepared using 50 ± 0.1 g of millet flour, roasted on a domestic stove oven at 240 °C with stirring during five minutes. WM was prepared using 30 mL of boiling water added to 25 ± 0.1 g of millet flour during ten minutes. The excess of water was removed using a domestic sieve. The same hydration procedure was used to hydrate the wet whole-wheat flour, which was applied as a control (CT) for the kibbeh samples.

Kibbeh formulation and manufacturing

Kibbeh samples were formulated according to the Technical Regulation of Kibbeh Identification and Quality (Brasil, 2000, 2001). The formulations were prepared as described by Degáspari et al. (2002), with some modifications (Table 1). TM and WM were used to replace the whole-wheat flour in the original kibbeh formulations. All ingredients used in these processes (Table 1) were homogenized for ten minutes and the samples were then stored frozen for 90 days (Tₕ) (−18 °C). Chemical, color and texture profile analysis were performed before and after storage for 90 days. Before the tests, samples were previously defrosted and cooked (fried and baked). Fried kibbeh (FRM and FWM) were portioned (20 ± 0.1 g) and deep fried in soybean oil (120-130 °C) for three minutes until reaching a characteristic color (golden brown). The baked kibbeh samples (BRM and BWM) were placed in inox pans (30 × 20 cm) and baked in an electric oven (180 °C) for 35 minutes. As such, we obtained the following samples: Baked kibbeh with roasted millet flour (BRM); Fried kibbeh with roasted millet flour (FRM); Baked kibbeh with wet millet flour (BWM) and Fried kibbeh with wet millet flour (FWM). Water activity analysis, microbiology assay and TBARS analyses were performed in the defrosted samples following 90 days of storage (−18 °C).

Chemical analysis

Three samples were randomly separated from each kibbeh sample (FRM, FWM, BRM and BWM) for the nutritional composition analysis. Moisture, ashes and protein content were determined according to Association of Official Analytical Chemists (1990) methods. Protein content (total nitrogen content) was determined using the Micro-Kjeldahl method with a conversion factor of 6.25; the digestible carbohydrates content was determined by the difference and total lipids extracted in methanol: chloroform (Bligh & Dyer, 1959). Dietary fiber was determined according to the methodology described by Prosky et al. (1988). Water activity (aₕ) was measured during 90 days of storage using the Aqualab system (Decagon, model CX-2, Devices Inc., Pullman WA, USA) at room temperature (25 °C).

Microbiological analyses

The ingredients used in the kibbeh formulations, fried and baked kibbeh samples were submitted to microbiological analysis to control their sanitary quality, according to Resolution RDC number 12 of the National Health Surveillance Agency (Brasil, 2001). Analyses were performed in triplicate as described by the Compendium of Methods for the Microbiological Examination of Foods (Downes & Ito, 2001).

*Table 1. Kibbeh Formulation (g.100g⁻¹).*

| Ingredients       | CT    | RM    | WM  |
|-------------------|-------|-------|-----|
| Beef              | 85.00 | 85.00 | 85.00 |
| Whole-wheat flour | 8.50  | -     | -   |
| Wet millet flour  | -     | 8.50  | -   |
| Roasted millet flour | - | 8.50  | -   |
| Fresh garlic      | 0.34  | 0.34  | 0.34 |
| Fresh parsley     | 0.70  | 0.70  | 0.70 |
| Fresh mint        | 1.36  | 1.36  | 1.36 |
| Salt              | 1.40  | 1.40  | 1.40 |
| Soybean oil       | 2.70  | 2.70  | 2.70 |
| Total (g)         | 100.00| 100.00| 100.00|

*All samples were fried (F) or baked (B) for the analysis. CT: Control (whole-wheat flour). RM: Roast Millet Flour. WM: Wet Millet Flour.*
**Color and texture analyses**

The samples were defrosted, except T₀, and cooked (fried or baked) prior to starting color and the texture analysis. Color parameters were analyzed using a colorimeter (HunterLab, ColorQuest II, Reston, England) with Universal Software 4.10 on the CIELAB system. The color measurements were performed five times in different places for each sample. The texture parameters was evaluated using a texturometer (Stable MicroSystems, TA-XTplus, Surrey, UK) with SMS-P/5 aluminum probe under a uniaxial force up to 70% strain, using cylindrical specimens of size 2 × 2 cm, with 2 mm s⁻¹ pre-test speed, 1 mm s⁻¹ test speed, and 10 mm s⁻¹ post-test speed. Hardness, defined as the maximum peak force required to compress the sample, was evaluated and expressed in Newtons (N). Seven measurements were recorded for each sample.

**Lipid oxidation**

Lipid oxidation was assessed by measuring the thiobarbituric acid reactive substances (TBARS), according to the methodology described by Wyncke (1970) with some adaptations proposed by Capitani et al. (2009). TBARS concentration was measured after the storage time (90 days) after defrosting the samples. A calibration curve was constructed using TEP solutions of concentrations varying from 0 to 1.25 mg.mL⁻¹. The TBARS concentration was expressed as mg MDA.kg⁻¹ kibbeh.

**Sensory evaluation**

Sensory analysis was conducted in two steps. First, the samples were evaluated using the preference test based on a nine-point hedonic scale (1 = extremely dislike to 9 = extremely like) (Meilgaard et al., 2007). Global appearance characteristics were evaluated and the acceptability index (AI) of the kibbeh samples was calculated as AI (%) = (Average score ÷ highest score) x 100 (Teixeira et al., 1987). The sensorial test was applied in individual cabins illuminated by red lamps. Fresh kibbeh samples (fried and baked) were served on individual plates (20 ± 0.1 g), coded with a three-digit number, following a randomized block design, to 57 untrained volunteer panelists from the University staff. Panelists signed an approved informed consent form regarding the Ethics Protocols of Research involving Human subjects. The data were tested for normality (Shapiro-Wilk test), then submitted to analysis of variance (two-way ANOVA), followed by Bonferroni post-tests, to compare the replicate means by samples. The data for the oxidative stability of the kibbeh samples (Figure 2) reveal that the CT sample presented an increased TBARS activity values, microbiological analysis showed satisfactory sanitary conditions for all of the kibbeh samples and were below the limits established by the Brazilian sanitary legislation (<10 UFC.g⁻¹), representing accordance with the law of good manufacturing practices (Brasil, 2001).

After 90 days of storage (T₀ and T₉₀) significant differences (p<0.05) in the color parameters of the kibbeh samples were observed for the L*, a*, b* and Chroma (Table 2). Reductions in the lightness (L*) and redness (a*) parameters were recorded following 90 days of storage. FRM and CT fried samples showed a greater reduction in the L* parameter (ΔL* ~ 24.5 and 20.7, respectively), indicating increased opacity during storage.

The texture analysis showed significant differences (p<0.05) in hardness for almost samples following the storage time (90 days), except for the Control fried (Fried kibbeh with whole-wheat flour - CT fried) (Figure 1). Initially, samples prepared with roasted millet flour (FRM and BRM) were harder than other samples and this may be due to the lower water content in the RM. However freeze storage reduced the hardness (N) in almost samples (p<0.05), except for the Control fried (Fried kibbeh with whole-wheat flour - CT fried) and was more expressive for the FWM sample (T₀ = 132.7 ± 11.7; T₉₀ = 72.7 ± 4.6). The FRM sample did not differ significantly (p>0.05) from the Control fried after 90 days of storage.

The data for the oxidative stability of the kibbeh samples (Figure 2) reveal that the CT sample presented an increased TBARS concentration following 90 days of storage (–18 °C), where the TBARS concentration significantly increased (p<0.05) during freezing for 90 days (T₀ and T₉₀) at –18 °C. When ascorbic acid was added to the control sample (0.01 %), values of TBARS were not lowered when compared to those of the CT (without ascorbic acid).
Table 2. Color Parameters ($L^*$, $a^*$, $b^*$, and Chroma) of Fresh Kibbeh ($T_0$) and following 90 days of freeze storage ($T_{90}$), based on the CIELAB System. Results were expressed as medium ± SD (n = 5).

| Samples          | $T_0$    | $T_{90}$ | $T_0$    | $T_{90}$ | Chroma          |
|------------------|----------|----------|----------|----------|-----------------|
| CT fried         | 46.6 ± 0.1 | 25.9 ± 2.0 | 26.3 ± 0.3 | 11.3 ± 0.5 | 16.1 ± 1.8      |
| CT baked         | 50.4 ± 0.1 | 38.6 ± 2.4 | 24.9 ± 0.1 | 6.3 ± 0.4  | 7.1 ± 0.6       |
| FWM              | 36.1 ± 0.1 | 21.6 ± 3.0 | 36.0 ± 0.1 | 17.7 ± 1.1 | 44.1 ± 0.6      |
| FRM              | 47.7 ± 0.5 | 23.2 ± 2.4 | 30.9 ± 0.2 | 11.8 ± 0.8 | 31.2 ± 0.9      |
| BWM              | 44.8 ± 3.5 | 34.9 ± 2.7 | 24.2 ± 0.4 | 5.0 ± 0.9  | 14.0 ± 0.5      |
| BRM              | 45.8 ± 0.7 | 33.1 ± 1.5 | 23.1 ± 0.3 | 5.5 ± 0.6  | 21.3 ± 0.8      |

Different letters in the same columns represent significant difference (p < 0.05) between the samples. CT fried: Control fried (Fried kibbeh with whole wheat flour). CT baked: Control baked (Baked kibbeh with whole wheat flour), FWM: Fried kibbeh with wet millet flour, FRM: Fried kibbeh with roasted millet flour, BWM: Baked kibbeh with wet millet flour, BRM: Baked kibbeh with roasted millet flour.

Figure 1. Hardness (N) of the baked (B) or fried (F) kibbeh samples at the beginning ($T_0$) and after 90 days of freeze storage ($T_{90}$). Bars represent means (n = 7 ± SD). Bars labelled with different letters represents statistical difference (p < 0.05): Capital letter: comparison between samples at $T_0$ or $T_{90}$, of storage, for same kibbeh formulation; Small letter: comparison among all kibbeh formulations at same storage time. CT fried: Control, (Fried kibbeh with whole-wheat flour), CT baked: Control, (Baked kibbeh with whole-wheat flour), FWM: Fried kibbeh with wet millet flour, FRM: Fried kibbeh with roasted millet flour, BWM: Baked kibbeh with wet millet flour, BRM: Baked kibbeh with roasted millet flour.

Figure 2. Lipid oxidation of the samples at the beginning ($T_0$) and after 90 days of freeze storage ($T_{90}$), expressed as mg MDA.kg$^{-1}$ sample. Bars represent mean (n = 3) ± SD. Bars followed by different letters represents statistical difference (p < 0.05) between samples. CT AOX: kibbeh prepared with whole whole-wheat flour and with ascorbic acid (0.01% p/v); CT: kibbeh prepared with whole-wheat flour and without antioxidant; WM: kibbeh prepared with wet millet flour; RM: kibbeh prepared with roasted millet flour.

There were no statistical differences among the samples (p>0.05) when the acceptability indices (AI %) were analyzed. Untrained panelists preferred the baked CT sample (AI = 83%), followed by FRM (78.9%) and fried CT (75.4%). When potential consumers evaluated the baked kibbeh samples in the supermarket, no differences (p<0.05) were found between the CT sample and the other samples (BRM and BWM). All attributes received average grades of above six, indicating good acceptability compared with conventional preparations (CT).

4 Discussion

The nutritional composition of kibbehs prepared with millet flour were not different to the nutritional contents of the CT kibbeh samples. However, lipids content were slightly higher in the kibbeh samples prepared with millet flour and it could be explained mainly by the influence of the cooking procedure (Krishnan et al., 2011; Miyagusku, 2006), since all preparations used the same ingredients, with the exception of the millet. Despite these results, all kibbeh samples prepared with millet flour could be classified as a “source” and as a “food with high amount” of fiber and proteins (Brasil, 2012) due to their elevated amount of fiber and proteins (average 20.6 g.100g$^{-1}$).

In this study, we did not use sodium erythorbate to preserve or to fix the red color of the meat; the color of kibbehs is usually brown or dark, and we observed the reduction in the lightness ($L^*$) and redness ($a^*$) parameters during 90 days of storage. Usually, freeze storage (~18 °C) of beef samples reduced colorimetric parameters over storage time (Fernández et al., 2007; Scramlin et al., 2010). The excess of metamyoglobin, formed during freeze storage, could reduce the redness parameter ($a^*$) and, consequently, increase $b^*$ values (Martínez et al., 2006; Valencia et al., 2008). However, the $b^*$ parameter was significantly reduced in the FRM and BWM samples and these results did not corroborate with the literature. The changes in the lightness ($L^*$) and in the redness ($a^*$) values may not indicate loss in...
the quality parameters, unless the consumer disapproves this parameter when buying these products. However, there were no sensorial differences when color was analyzed.

Different cooking methods (Apple et al., 1999) influenced the texture of the food products; however, no differences between the hardness (N) of the baked or fried kibbeh were found in this study. According to the sensorial analysis, panelists related that the kibbeh samples prepared with RM flour were crisper and harder than the other preparations and the texture profiles confirm these findings. Following storage (90 days), the kibbeh samples demonstrated reduced hardness due to ice crystal formation by the meat products during freeze storage, which promotes the output of intracellular water by osmosis and causes modification in texture parameters after the defrosting process (Ordóñez, 2005). Considering formulations containing millet, Baked kibbeh (BWM and BRM) showed lower reduction of hardness than fried kibbeh (FWM and BRM) in relation to initial time and after storage under freezing. Despite of kibbeh containing whole-wheat flour (CT baked and CT fried) have showed lower hardness than kibbeh containing millet flour, consumers did not detected differences in the texture, because sensory acceptability showed no statistical differences among the samples (p>0.05) when the acceptability indices (AI %) were analyzed.

Ascorbic acid (applied at the 0.01% level) may have acted as a pro-oxidant in the CT samples, resulting in increased TBARS concentrations following storage. Moreover, antioxidant efficiency depends on the level of the antioxidant in the matrix composition (Capitani et al., 2013). In the other samples prepared with millet flour (RM and WM), TBARS concentration remained below the limits (<0.51 mg MDA.kg–1 kibbeh) during 90 days of storage, and this would suggest that there was no development of a rancid flavored (0.5 to 2.0 mg MDA.kg–1) sample (Gray & Pearson, 1984).

Some studies have reported antioxidant and potent radical scavenging activity in millet grains (Hegde et al., 2005; Dykes & Rooney, 2006; Amadou et al., 2013a; Shahidi & Chandrasekara, 2013; Pradeep & Sreerama, 2015) and the in vitro antioxidant activity of millet is usually attributed to its phenolic content, represented by vanillic and ferulic acids (Pradeep & Sreerama, 2015). More recently, Zhang & Liu (2015), observed that foxtail millet extracts inhibited the growth of human breast and liver cancer cells in culture. While the antioxidant activity and phenolic content of samples were not evaluated in this study, it is important to note that the presence of millet flour maintained TBARS levels lower during 90 days of storage. The antioxidant properties of millet grains could also provide health benefits to humans (Hegde et al., 2005; Amadou et al., 2013b) acting on management of cardiovascular, geriatric diseases and cancers (Zhang & Liu, 2015). The use of millet as a nutraceutical and specialty food is warranted (Shahidi & Chandrasekara, 2013) and should be studied in humans’ subjects to observe the impact of this food on health.

Our results corroborate with other studies reporting good acceptability of products formulated with millet flour (Krishnan et al., 2011; Saha et al., 2011; Schoenlechner et al., 2013). According to Singh et al. (2012), millets can replace wheat in the bread to some extent with good acceptability. However, the addition of millet flour to wheat bread caused a decline in bread quality due to the reduction in the final gluten content (Schoenlechner et al., 2013), which is essential to breads.

5 Conclusions
Roasted or wet millet flour could be used into kibbeh formulations to replace whole-wheat flour without affecting their nutritional and technological properties. Kibbeh formulations with roasted or wet millet flour presented good acceptability and showed oxidation stability measured by TBARS concentration during storage for 90 days at –18 °C. So, millet holds a huge potential for food production and, in particular, for replacing whole-wheat flour. As such, it can be used in several food products, such as kibbeh, making a valuable contribution to a healthy diet, particularly in individuals with celiac disease.

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