Antioxidant activity and Total Phenolic Content of Millet flour

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Abstract

This study explored the antioxidant activity and total phenolic content of individual millet flour and the composite millet flour using the selected millets like sorghum, pearl and foxtail. The millet grains were procured cleaned, roasted at 80 – 90°C and made into flour using hammer mill and sieved. Composite millet flour was prepared by mixing equal quantities of the millet flour. The individual millet flour and the composite millet flour were assessed for its Total Phenolic Content (TPC) and antioxidant activity - DPPH Free Radical Scavenging method. The results of the study revealed that the antioxidant activity of the millet flour ranged from 3.68 to 4.23 % whereas Total Phenolic content ranged from 1.2 to 1.28 µmol FAE/g. Foxtail millet exhibited highest antioxidant activity and total phenolic content among selected the flour. The study also proved that there exist a direct proportional relationalship between TPC and antioxidant activity of the flour.

Keywords: *antioxidant activity, millet, composite flour, DPPH, TPC*

1. Introduction

Millets are small seeded cereals that are cultivated in semi-arid and tropical regions of Asia and Africa. Millet belongs to the grass family, *Graminae*. The different varieties of millets are pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), proso millet (*Penicum miliaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*) and barnyard millet (*Echinochloa utilis*). They are known as coarse cereals beside maize (*Zea mays*), sorghum (*Sorghum bicolor*), oats (*Avena sativa*) and barley (*Hordeum vulgare*) (Saleh *et al*, 2013).

Millet grains are receiving specific attention in the developing countries like India, China and some countries from African Continent in terms of utilization as food. But still, millets are underutilized and neglected due to unawareness on the methods of processing and issues like lower cooking quality, taste and low bioavailability of millets. These problems can be solved and make them valuable as food for poor families to combat malnutrition (Sarita and Singh, 2016).

Millets are being recognized as potential future crops owing to its nutrient content similar to other major cereals and non – nutrient compounds having proven health benefits. Studies have shown that millet grains are rich sources of non - nutrients, especially phenolic compounds. There are evidences to show that phenolic compounds can act as antioxidants within the human body to protect against oxidative stress and to reduce the risk of Non Communicable Diseases. Millets are rich in phenolics, tannins and phytate which act as ‘antioxidants’ (Kumari *et al,* 2017).

An antioxidant can be defined as “any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate” In foods, antioxidants prevent undesirable changes in flavour and nutritional quality of a product (Zielinski and Kozłowska, 2015).

Free radicals cause autoxidation of unsaturated lipids in foods. Hence, antioxidants may break the free radical chain resulting from oxidation and donate hydrogen to form a stable product which does not promote further oxidation (Suma and Urooj, 2012). Anti-oxidants being free radical scavengers may inhibit or slow down the oxidation reactions that lead to degenerative diseases such as cancer, inflammation, anemia, neuro-degeneration, cardiovascular diseases and ageing (Abioye *et al,* 2018*).*

Considering the antioxidant benefits provided by the millets, a study was designed to formulate composite millet flour using sorghum, pearl and foxtail millet and to study the antioxidant properties of the formulated composite millet flour.

2. Materials and methods

2.1 Selection of millets

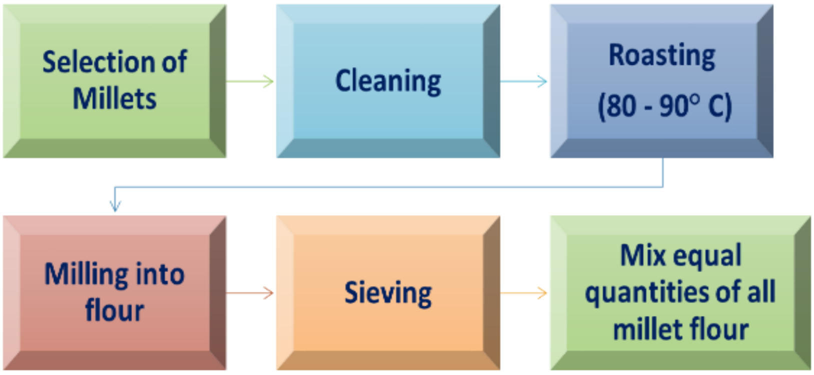
Millets varieties such as sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*) were selected for the preparation of composite millet flour. Sorghum and pearl millet has nutrient content better than or similar to rice and wheat since there is a considerable variation in sorghum for levels of proteins, lysine, lipids, carbohydrates, fiber, calcium, phosphorus, iron, thiamine, and niacin (Kumar *et al,* 2016). It also contain high fiber, starch and non-starchy polysaccharides with some unique characteristics (Unhale, 2012).

Pearl millet was found to have high levels of calcium, iron, zinc, lipids and good quality proteins with high concentration of threonine and tryptophan with adequate leucine, than other cereals (Balasubramaniam *et al*, 2012). Foxtail millet also known as Italian millet (Verma *et al*, 2014) is a good source of protein (12.3 g/100g) and dietary fiber (14 g/100g) whereas its carbohydrate content is low (60.9 g/100g). Besides, it is rich in minerals (3 g/100g) and phytochemicals. Foxtail millet is a good source of β - carotene (126-191 µg/100g, Goudar *et al*, 2011).

2.2 Preparation of composite millet flour

The selected millets procured from local shops were carefully cleaned and roasted separately at 80 – 90°C in a roaster and cooled to room temperature and ground into flour using Hammer mill. The individual millet flour was cooled, sieved and packed separately. Equal quantities of each millet flour were mixed and made into Composite Millet Flour (CMF). The flow chart for the preparation of composite millet flour in given in *Figure 1.*

Figure 1 – Preparation of Composite Millet Flour



2.3 Determination of antioxidant properties

Total Phenolic Content (TPC) and antioxidant activity using DPPH (2, 2 diphenyl-1-1 picrylhydrazyl) method (IS 1797:1985) were evaluated for the prepared individual millet flour as well as composite millet flour.

DPPH Radical Scavenging Activity

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of foods. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of foods. One gram of the sample was extracted with 10 ml methanol for two hours and centrifuged at 3000 rpm for 10 minutes. 100 µl of the supernatant was reacted with 3.9 ml of DPPH solution and the absorbance was read at 0 and 30 minutes against a methanol blank (Prakash, 2000). .

Total Phenolic Content (TPC)

50 µl of the sample was made upto 500µl using distilled water and added 250 µl of folin – ciocalteau reagent and 1.25 ml of 20 per cent sodium carbonate solution. The tubes were vortexed and then incubated in dark for 40 minutes. The colour developed was read in a spectrophotometer at 725 nm (Kumari *et al*, 2017).

3. Results and Discussion

The results obtained from the determination of free radical scavenging activity and total phenolic content of the individual millet flour and composite millet flour prepared using sorghum, pearl millet and foxtail millet is given in Table 1 and *Figure 1* respectively.

Table 1 - Free Radical Scavenging Activity of Millet Flour

Flour	DPPH Activity (g/100 g)
Sorghum	3.68
Pearl Millet	3.75
Foxtail Millet	4.23
Composite Millet	3.89

DPPH activity: DPPH is a stable free radical and accepts an electron and becomes a stable diamagnetic molecule. The reduction capacity of DPPH radicals was estimated by the decrease in its absorbance at 517 nm prompted by antioxidants in the sample. Lower absorbance indicates high free radical scavenging activity of the sample (Itagi and Singh, 2012). It is clear from *Table 1* that the DPPH free radical scavenging activity of foxtail millet was found to be higher (4.23 g/100 g) than the composite millet flour (3.89 g/100 g) whereas sorghum flour exhibited least DPPH activity (3.68 g/100 g). Suma and Urooj (2012) confirmed that the methanolic extract of foxtail millet flour (whole) showed considerably higher free radical scavenging activity compared to ethanolic and aqueous extracts.

The DPPH activity of dehulled pearl millet flour was found to be 13.8 µmol FAE/g whereas foxtail millet flour showed 6.77 µmol FAE/g in a study conducted by Chandrasekara *et al*, 2012.

Figure 2 – Total Phenolic Content of Millet Flour (µmol FAE/g)

It is evident from the above *Figure 2* that the foxtail millet has appreciable quantities (1.28 µmol FAE/g) of Total Phenolics followed by composite millet flour (1.24 µmol FAE/g).

Sorghum flour has exhibited a lower total phenolic content (1.13 µmol FAE/g) than other millet flour but found to be within the range of total phenolic content expressed by different varieties of sorghum which ranged from 0.976 – 2.128 µmol GAE/g (Pasha *et al*, 2015).

The total phenolic content of the foxtail millet (1.28 µmol FAE/g) and pearl millet (1.2 µmol FAE/g) of the present study were found to be lower than that of the study conducted by Chandrasekara *et al* (2012) in which the dehulled grains of foxtail millet and pearl millet has a total phenolic content of 3.80 µmol FAE/g and 8.50 µmol FAE/g respectively.

The composite millet flour prepared with sorghum, pearl millet and foxtail millet exhibited a total phenolic content of 3.89 per cent and DPPH activity of 1.24 per cent. The DPPH activity of the composite millet flour was found to be lesser than the composite multigrain mix formulated with a mixture of cereals, millets, legumes, nuts and oilseeds (Itagi *et al*, 2012).

Therefore, it is apparent from the present study that there exist a positive correlation ($R^2 = 0.933$) between the DPPH free radical scavenging activity and the Total Phenolic Content of the flour.

4. Conclusion

High antioxidant activity was observed in foxtail millet flour. The current study also clearly revealed that the total phenolic content is directly proportional to the DPPH radical scavenging activity of the millet flour. The results of this study explore the possible potential utilization of millet grains in food formulations and product development especially functional/health foods which may improve the consumption and health status. Further research has to be carried out to explain and validate its therapeutic uses to the human mankind.

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