IJFANS

International Journal of Food And Nutritional Sciences

Volume 3, Issue 1, Jan-Mar-2014, www.ijfans.com e-ISSN: 2320-7876

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.3, Iss.1, Jan-Mar 2014

Research Paper *Open Access*

EFFECT OF GERMINATION AND FERMENTATION ON POLYPHENOLS IN FINGER MILLET (*Eleusine coracana*)

Sudha Rani .R and Usha Antony*
Centre for Food Technology, Department of Biotechnology,
Anna University, Chennai, Tamilnadu, India.

*Corresponding author: usha.antony@gmail.com

ABSTRACT

Finger millet is nutritionally significant in terms of its notably high calcium content, iron, zinc and dietary fibre. It also contains polyphenols which are phytochemicals with antioxidant activity. The common processes in the preparation of indigenous foods from finger millet are germination and spontaneous fermentation. This study focuses on the variation in the polyphenol content of four finger millet varieties of different seed coat color subjected to germination followed by natural fermentation. The polyphenols content analysed at 0, 6, 12, 18, 24, 36 and 48 h showed a significant decrease from the Folin-Ciocalteu assay. High Performance Liquid Chromatography analysis showed significant changes in catechin, through the period of fermentation lasting 48 h.

Keywords: Finger millet, Germination, Fermentation, Polyphenols.

INTRODUCTION

The challenge of meeting the increasing need for food, feed and fuel demands increased agricultural production or a change in current crop consumption patterns (Licker *et.al.*, 2010). Diversification of food grain production to include millets would ensure ecological and economical sustainability contributing to food and nutrition security. Providing traditional whole-grain and multigrain substitutes for refined carbohydrates can be one important dietary modification that promotes health while promoting utilization of minor-grain foods (Singh, 2012). Finger millet is one of the minor grains produced and consumed in India and Africa. It is nutritionally superior to other major cereals such as wheat, rice and maize and being an excellent source of calcium, iron and zinc in addition to dietary fibre (Verma, 2013). In spite of the nutritional characteristics, utilization of finger millet as food is still mainly limited to populations in rural areas at the household level. This is due to lack of novel processing and preparation methods to provide safe and improved foods at a commercial scale that can be used to feed large populations in urban areas (Ushakumari et.al., 2004). Controlled processing conditions as opposed to traditional processing are needed to provide consistent production in industry. Finger millet is unique in having a colored seed coat rich in polyphenols that is strongly attached to the endosperm (Emmambux, 2013). Polyphenols are reducing agents that protect the body's tissues against oxidative stress. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others. They are the most abundant antioxidants in our diets (Scalbert). It has been reported that populations consuming sorghum and millet have lower incidences of esophageal cancer than those consuming wheat or maize (Van, 1981). Furthermore, a recent study has demonstrated that millet phenolics may be effective in the prevention of cancer initiation and progression in vitro (Chandrasekara, 2011). Some of the phenolic acids present in finger millet grain from HPLC analysis are gallic acid, protocatechuic acid, p-hydroxybenzaldehyde, p-coumaric acid, trans-ferulic acid, catechin, taxifolin-o-hexoside, gallocatechin, procyanidin dimer B1, epicatechin, taxifolin, vitexin, isovitexin, myricetin hexoside, myricetin, quercetin-o-pentoside, procyanidin dimer B2 (Chandrasekara, 2011). But the effects of germination and spontaneous fermentation on the polyphenols have not been studied. The aim of this study is to analyse the effect of traditional processing methods on polyphenols in finger millet with different seed coat colours and varying polyphenol content.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

The four finger millet varieties used in this study were CO 9, CO 13 and CO 14 from Coimbatore and OUAT 2 variety from Orissa. Catechin, and gallic acid were obtained from Sigma-Aldrich Chemicals Co., St. Louis, Missouri, USA. Folin-Ciocalteu reagent, sodium carbonate and ethanol (HPLC-grade) were from Merck, Mumbai, India.

The test samples were cleaned manually to remove husks, damaged grains, stones, dust, light

materials, glumes, stalks, undersized and immature grains and other extraneous materials. Cleaning was done by winnowing and hand sorting. The cleaned grains were divided into two sub-portions. The first sub-portion not subjected to any treatment, served as a control.

PROCESSING METHODS AND THEIR COMBINATION

The processing methods used were soaking, germination, fermentation and their combinations. After each step the samples were ground to obtain fine powder. Following each processing method polyphenols were analyzed using standard procedure. The finger millet grains were washed three times using tap water. Then, the cleaned and washed grains were soaked in a volume of water 3 times the weight of seeds (3:1) for 24h in a stainless steel vessel at room temperature (25±1°C). The grains were germinated for 48h at 30°C followed by spontaneous fermentation. The samples were drawn at 6h intervals for upto 48h and dried in a hot-air oven at 60°C. All samples were then packed in airtight polyethylene bags and stored at 4°C until further analysis. The processing was done in 3 batches.

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

To an aliquot of the extract, 1% HCl in methanol was added in the ratio 1:10. The mixture was kept in water bath maintained at 70°C for 20 min followed by centrifugation for 10 min at 3500 rpm. The extracts were analysed using Folin-Ciocalteu (FC) reagent following a modified method by Singleton and Rossi (1965) (Singleton *et.al.,* 1965). The extract (0.5 mL) was mixed with 2.5 mL FC reagent and 7.5 mL of 20% (w/v) sodium carbonate within 8 min and incubated at 30°C for 2 h. The absorbance was read at 760 nm. Gallic acid (GA) was used as standard and the amount of total phenolics was expressed as gallic acid equivalents (GAE). The analyses were performed in duplicates.

PREPARATION OF EXTRACT FOR HPLC ANALYSIS

Samples were mixed with 40% ethanol in the ratio 1:30 (w/v). The mixture was refluxed on a water bath at 70°C for 20 min. The extraction was repeated three times and the combined extracts were filtered and evaporated to dryness on a rotary-evaporator (70°C). The residue was dissolved in 70% ethanol, left 14-16 h at -12°C and filtered. The clear solution was evaporated to dryness and the residue was stored under nitrogen at -18°C until used (Kovatcheva *et.al.,* 2008).

HPLC ANALYSIS OF PHENOLIC COMPOUNDS IN EXTRACTS

A modification of the method of Rødtjer et al., 2006 was used. Extracts were dissolved in 70% ethanol, filtered and 10 µL aliquots of the solutions were injected into the HPLC. Analysis was carried out with a gradient-forming Hewlett Packard series 1050 HPLC system (Palo Alto, CA, USA) that was coupled to an Agilent Technologies series 1100 autosampler (Palo Alto, CA, USA). The HPLC system was equipped with a Hichrom Ltd. H1000DS-10C Guard column (Reading, UK) and the separations were performed on a 25 cm x 4.6 mm (i.d.) Macherey-Nagel ET Nucleosil® reversed phase 10 µm C_{18} stainless-steel column (Düren, Germany). The mobile phase consisted of 2.5% acetic acid in water (solvent A) and a mixture of water, methanol and acetic acid (2.5:95:2.5) (solvent B). The gradient applied at a flow rate of 1.0 ml/min was; 0–10 min, 100% A; and 10–110 min, 100% A to 50% A. A diode array detector (DAD) was used for detection. Identification and quantification was achieved by comparison with retention times of pure phenolic compound.

RESULTS AND DISCUSSION

PHENOLIC COMPOUNDS IN THE EXTRACTS

The extract from the raw flour natural fermented samples (RFNF) contained higher amounts of total phenolics than the extract of germinated flour natural fermented samples (GFNF). CO13 and CO14 (brown varieties) had higher total polyphenols when compared to CO9 and OUAT2 (white varieties) (Table 1 and Fig. 1). The profile of phenolic is species-specific and typical for most plant material (Maillard, 1995). In finger millet also the profiles of catechin in the extract differs significantly between different varieties but is commonly present in all the extracts. HPLC analysis with diode array detector of the extracts revealed that catechin was predominant at 18h in both RFNF and GFNF of CO13. The increase in the levels of catechin upto 18h followed by a decrease may be due to the growth of the microorganisms during fermentation which can be related to the growth curve of the natural flora present in the finger millet. An increase in the catechin levels was observed at the end of 48h which may be due to the conversion of complex molecules to simple forms during the process of fermentation. As in the case of total polyphenols, the catechin level was also high in RFNF (Fig. 2). The antioxidant activity of any of the compounds is normally dose-dependent (Adom, 2002). Since appreciable amount of polyphenols are found in finger millet, especially in the brown varieties, they can be considered as a rich source of antioxidants among cereals and this information may be useful in understanding the health benefits of the millet.

Table 1. Total polyphenols (µg/mL GAE) in extracts of raw, germinated and fermented finger millet varieties

Treatment	Time (h)	CO13	CO14	Co9	OUAT2
Raw	0	10.376	10.753	6.615	3.869
Germinated	0	9.261	9.038	4.707	3.623
Raw fermented	24	6.961	4.000	3.423	2.000
	48	3.269	1.730	0.392	1.015
Germinated fermented	24	6.061	3.353	1.330	1.630
	48	1.900	1.561	0.276	0.246

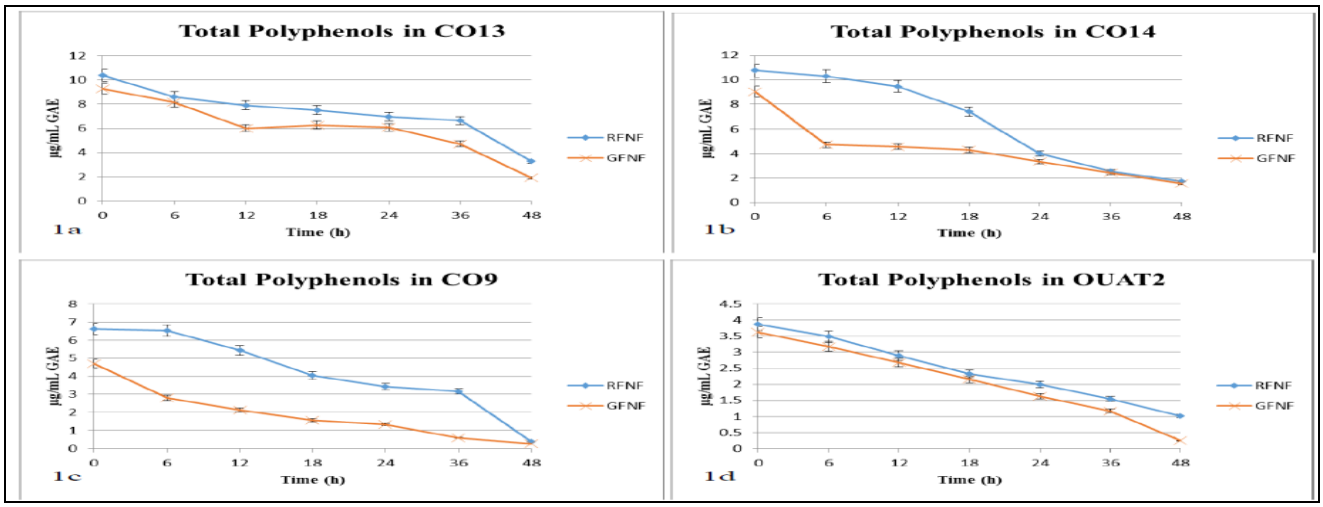

Figure 1a. Total polyphenols in extracts of raw, germinated and fermented finger millet CO13, Figure 1b. Total polyphenols in extracts of raw, germinated and fermented finger millet CO14, Figure 1c. Total polyphenols in extracts of raw, germinated and fermented finger millet CO9, Figure 1d. Total polyphenols in extracts of raw, germinated and fermented finger millet OUAT2

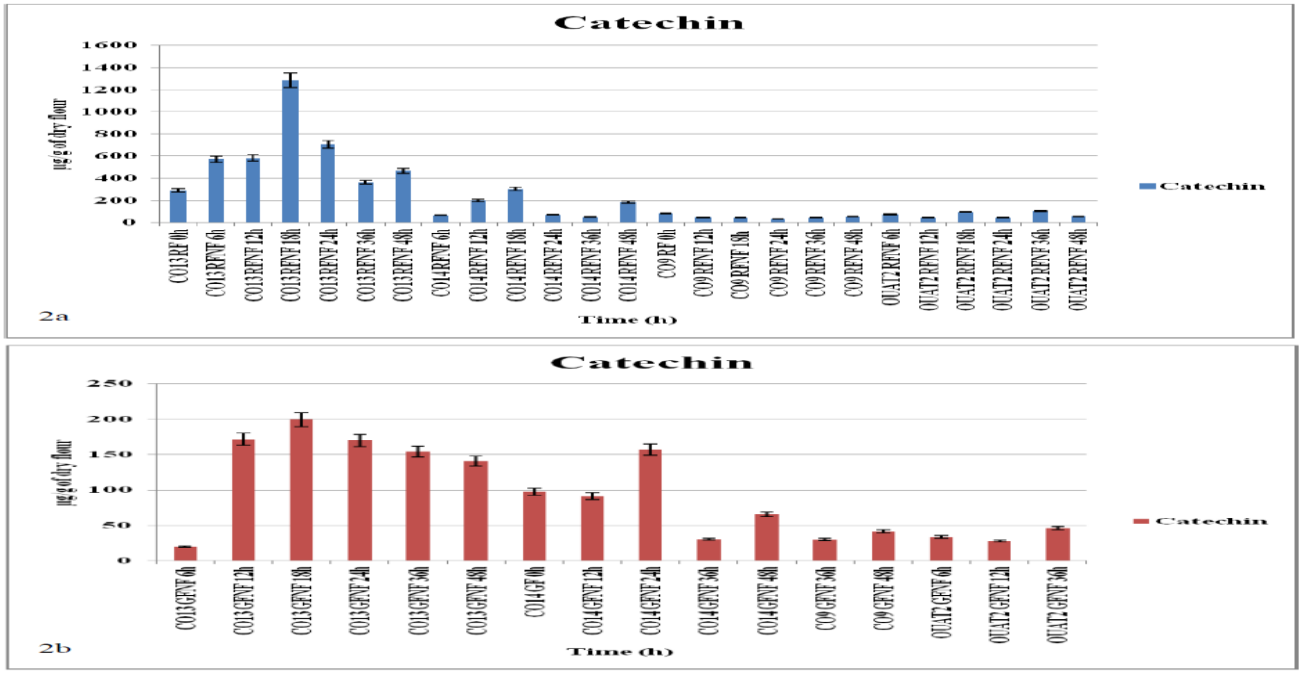

Figure 2a. Catechin in extracts of raw and fermented finger millet varieties (CO13, CO14, CO9, OUAT2), Figure 2b. Catechin in extracts of germinated and fermented finger millet varieties (CO13, CO14, CO9, OUAT2).

CONCLUSIONS

The effect of processing methods like germination and fermentation on the varieties of finger millet with different seed coat color has a high impact on the polyphenols content. The essential content of polyphenols and specifically catechin will promote the use of finger millet as a functional food among other cereals like rice and wheat. The changes in polyphenols during traditional processing methods in the preparation of indigenous foods will meet the needs of the development of novel dietary products. The exploration of phytochemicals with antioxidant properties is essential for human health and nutrition research.

REFERENCES

- Licker R, Johnston M, Foley JA, Barford C, Kucharik CJ, Monfreda C, Ramankutty N. Mind the gap: how do climate and agricultural management explain the 'yield gap' of croplands around the world? Global Ecology and Biogeography. 2010; 19 (6): 769-782.
- Singh P, Raghuvanshi RS. Finger millet for food and nutritional security. African J Food Sc. 2012; 6 (4): 77-84.
- Verma V, Patel S. Value added products from nutri-cereals: finger millet (*Eleusine coracana*). Emirates J Food Agri. 2013; 25 (3): 169-176.

- Ushakumari SR, Latha S, Malleshi NG. The functional properties of popped, flaked, extruded and roller-dried foxtail millet (*Setaria italica*). Int. J Food Sc Tech. 2004; 39 (9): 907-915.
- Emmambux MN, Taylor J. Morphology, physical, chemical, and functional properties of starches from cereals, legumes, and tubers cultivated in Africa: A review. Starch-Stärke. 2013.
- Scalbert A, Williamson G. Chocolate: Modern science investigates an ancient medicine
- van Rensburg SJ. Epidemiologic and dietary evidence for a specific nutritional predisposition to esophageal cancer. J Nat. Cancer Inst. 1981; 67 (2): 243-251.
- Chandrasekara A, Shahidi F. Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. J Func. Foods. 2011; 3 (3): 159-170.
- Chandrasekara A, Shahidi F. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS^n. J Func. Foods. 2011; 3 (3): 144-158.
- Singleton, VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American J Enol. Viti. 1965; 16 (3): 144-158.
- Kovatcheva-Apostolova EG, Georgiev MI, Ilieva MP, Skibsted LH, Rødtjer A, Andersen ML. Extracts of plant cell cultures of *Lavandula vera* and *Rosa damascena* as sources of phenolic antioxidants for use in foods. Eur. Food Res. Tech. 2008; 227 (4): 1243-1249.
- Rødtjer A, Skibsted LH, Andersen ML. Antioxidative and prooxidative effects of extracts made from cherry liqueur pomace. Food chem. 2006; 99 (1): 6-14.
- Maillard MN, Berset C. Evolution of antioxidant activity during kilning: role of insoluble bound phenolic acids of barley and malt. J Agri. Food Chem. 1995; 43 (7): 1789-1793.
- Adom KK, Liu RH. Antioxidant activity of grains. J Agri. Food Chem. 2002; 50 (21): 6182-6187.