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Fermented pearl millet (*Pennisetum glaucum*) with *in vitro* DNA damage protection activity, bioactive compounds and antioxidant potential

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ABSTRACT

In the present study, pearl millet cultivar PUSA-415 was fermented by solid state fermentation (SSF) process using *Aspergillus sojae* (MTCC-8779) as starter culture. The fermentation was carried out for the period of ten days. The effect of SSF on phenolic content, condensed tannin content, antioxidant potential and DNA damage protection of pearl millet during different fermentation period was determined. Results showed that SSF and thermal processing significantly affect the bioactive profile and antioxidant potential of bio-transformed pearl millet. Extracts prepared from 6th days fermented pearl millet flour exhibited the highest TPC, antioxidant potential and DNA damage protection activity. Qualitative and quantitative analysis of bioactive compounds were done by HPLC. During SSF, production of enzymes (α-amylase, β-glucosidase and xylanase) as well as specific bioactive compounds (ascorbic acid, gallic acid and p-Coumaric acid) was significantly increased. Thus, bio-transformed *Aspergillus sojae* fermented pearl millet could be used in preparation of functional foods and novel nutraceuticals in health promotions. Chapatti was formulated from unfermented as well as fermented flour and the effect of thermal processing on bioactive compounds and antioxidant potential was studied. Thermal processing resulted in decrease in TPC of both, AFM and UFM by 4.75–16.27% and increase in CTC by 38.52–67.41%.

1. Introduction

The development of cost effective technology for the enhancement of bioactive compounds from natural resources especially cereal grains is of utmost importance. SSF process has emerged as a rapidly growing process technology used by many researchers/food scientists and industrialists to improve the nutritional quality of food and food products. Biological methods are quite safe, eco-friendly and rely on the use of specific and suitable microorganisms (Salar, Certik, & Brezova, 2012; Salar & Purewal, 2016). Although, a variety of microorganisms are available that can modulate the bioactive compounds of cereal grains, microorganisms that belong to *Aspergillus* group are gaining more interest from the researchers. Earlier published reports on SSF technology supports the use and benefits of starter cultures *Aspergillus awamori* on pearl millet (Salar, Purewal, & Bhatti, 2016); *Aspergillus awamori* on wheat cultivars (Bhanja, Kumari, & Banerjee, 2009; Sandhu, Punia, & Kaur, 2016) and *Aspergillus candidus* on rice (Yen, Chi, & Su, 2003). Pandey (2003) reported that selection of suitable substrate and microorganism is a sole factor that results in maximal recovery of bioactive compounds from natural resources.

Other factors that contribute to enhancement of bioactive compounds after SSF is completed are solvent types; solvent concentration, extraction temperature and extraction time (Salar et al., 2016). Depending on the type of substrate chosen, the extraction phase plays an important role. Some researcher strongly suggests the use of absolute solvent whereas other recommends the addition of aquatic phase with organic solvents (Bhanja et al., 2009; Espinosa-Pardo, Nakajima, Macedo, Macedo, & Martinez, 2017; Liyana-Pathirana & Shahidi, 2005; Salar et al., 2016; Salar, Sharma, & Purewal, 2015; Sandhu et al., 2016). In our earlier published work twelve important pearl millet cultivars of Indian origin were studied for their bioactive properties. Among these cultivars studied the best results in terms of higher phenolic compounds, antioxidant potential and DNA damage protection activity was observed in PUSA-415 (Salar & Purewal, 2017). Therefore, we chose PUSA-415 for further enhancement and evaluation of its bioactive properties by adopting SSF technique. After the fermentation, the flour from PUSA-415 was studied for phenolic compounds, condensed tannin content, antioxidant potential and DNA damage protection activity. Pearl millet grains after fermentation were milled to flour: the chapatti was formulated and the effect of baking on bioactive compounds and antioxidant potential was also studied.

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2. Materials and methods

2.1. Pearl millet, starter organism, and chemicals

Pearl millet cultivar PUSA-415 was procured from Indian Agricultural Research Institute, PUSA, New Delhi, India. The grains were washed thoroughly with tap water, dried in shade, stored at room temperature in airtight containers till further use. Fungal strain *Aspergillus sojae* (MTCC 8779) was purchased from Microbial Type Culture Collection, IMTECH, Chandigarh, India and used as starter culture for SSF process. Fungal strain was maintained on malt extract agar and malt extract broth at 25 ± 2 °C. Spore suspension was prepared by washing the mycelium grown on malt broth for 5 days at 25 ± 2 °C, with an aqueous solution of 0.1% (w/v) Tween 80. Spore suspension (1 ml) containing approximately 1×10^5 spores was used for inoculating the autoclaved pearl millet grains. All chemicals and solvents used in the present study were of analytical grade.

2.2. SSF of pearl millet

SSF process was used to prepare pearl millet koji in Erlenmeyer flask (1:5 w/v). Grains were soaked in equal quantity of malt extract broth at room temperature for 12 h. Excess media was decanted before sterilization process. Pearl millet grains were steam-cooked in an autoclave at 121 °C for 20 min. After cooling, the sterilized substrate was inoculated with 1 ml of spore suspension of *Aspergillus sojae*. After thoroughly mixing, the flasks containing grains (with or without spore suspension) were incubated for a period of 10 days at 25 ± 2 °C. The substrate was removed from the flasks after regular intervals of incubation period (2 days), dried in an oven (NSW, India) at 45 °C for 24–48 h and finally converted in to powdered form using mixer grinder (Sujata, India).

2.3. Extraction of enzymes

Unfermented pearl millet grains (UFMG) and fermented pearl millet grains (AFMG) were suspended in adequate amount of double distilled Millipore water (1:10 w/v) and agitated at 120 rpm on Orbitek shaker (Scigenics Biotech, India) for a period of 20 min and filtered through Whatman no. 1 filter paper. The filtrate was then centrifuged at 8000*g* for 10 min at 4 °C. The clear supernatant was assessed for the presence of enzymatic activity (α-amylase, β-glucosidase and xylanase).

2.3.1. Enzymatic activity

α-Amylase, β-glucosidase and xylanase activity were determined by following the method described by Bhanja et al. (2009).

2.4. Extraction of bioactive compounds

UFMG and AFMG was taken out of the Erlenmeyer flasks at every 48 h intervals and dried in an oven at 45 °C for 24 h. Flour from dried AFMG and UFMG substrates was obtained by grinding in mixer grinder (Sujata, India). Defatting of UFMF (unfermented millet flour) and AFMF (*Aspergillus sojae* fermented millet flour) was carried out with hexane fraction from petroleum according to method described by Bhanja et al. (2009). Extraction was carried out with aqueous ethanol (50%) (1:20 w/v) at 44.5 °C for 23.8 min. Extracts were prepared according to the method described by Salar et al. (2016).

2.5. Analytical assays

2.5.1. Total phenolic content (TPC)

TPC in the extracts prepared from UFMF and AFMF was determined using Folin-Ciocalteu reagent method described by Salar et al. (2012). TPC was calculated as mg gallic acid equivalents/g dry weight basis (mg GAE/g dwb) from the equation generated from standard calibration curve of gallic acid. All determinations were carried out in triplicates.

2.5.2. 2,2-Diphenyl–1′ picrylhydrazyl radical scavenging assay (DPPH)

The radical scavenging capacity of different extracts was measured by the DPPH scavenging method as described by Yen and Chen (1995).

Percent (%) DPPH scavenging activity was calculated using formula:

$$\text{DPPH Scavenging Activity (\%)} = (A_C - A_E/A_C) \times 100$$

where A_C and A_E are the absorbance of control and extracts, respectively.

2.5.3. 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$)

The radicals scavenging activity in all extracts against radical cation ($ABTS^+$) was estimated according to the method described by Re, Pellegrini, Proteggente, Pannala, and Evans (1999).

Percent (%) scavenging activity was calculated using formula:

$$\text{Decolorization Activity (\%)} = (A_C - A_E/A_C) \times 100$$

where A_C and A_E are the absorbance of control and extracts, respectively.

2.5.4. Hydroxyl free radical scavenging activity (HFRSA)

The antioxidant potential of extracts against hydroxyl radicals was analyzed by following the method described by Smirnoff and Cumbes (1989). The percentage of scavenged OH^- by extracts was calculated using the following formula:

$$\text{Scavenged } OH^- \% = [(Ac - A_E)/Ac \times 100]$$

where, Ac is absorbance of control and A_E is absorbance of extract.

2.5.5. Cupric reducing antioxidant capacity (CUPRAC)

Cupric ion reducing capacity was measured by following method as described by Apak, Guclu, Ozyurek, and Karademir (2004). The absorbance was recorded at 450 nm. Ascorbic acid was used as a positive control. CUPRAC value expressed as mg AAE/g dwb.

2.5.6. Total antioxidant capacity (TAC)

TAC of extracts was determined by following the method described by Prieto, Pineda, and Aguilar (1999). Absorbance was recorded at 695 nm. Ascorbic acid was used as standard to compare antioxidant activity of extracts.

2.5.7. Reducing power assay (RPA)

The reducing power was measured using the method described by Oyaizu (1986). Absorbance was recorded at 700 nm. Quercetin was used as standard to compare the reducing power potential of extracts.

2.5.8. Metal chelating assay

Extracts (100 µl) was mixed with 50 µl of ferrous chloride (2 mM) followed by addition of 1.5 ml ethanol (50%). After an interval of 5 min reaction was initiated by addition of ferrozine (5 mM/l). The mixture was incubated for a period of 10 min at room temperature. Absorbance was recorded at 560 nm.

2.5.9. Condensed tannins content (CTC)

CTC of extracts was estimated by using vanillin-HCl method according to the method described by Julkunen-Titto (1985). The absorbance against blank was read at 500 nm. Catechin was used to make the standard curve (mg/ml). Each extract was analyzed in triplicate and the results were expressed as milligrams Catechin equivalent per 100 g dried weight basis of pearl millet (mg CE/100 g dwb).

2.6. DNA damage protection activity

DNA damage protection activity of all the extracts prepared from