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Process optimization for anti-nutrient minimization of millets

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ABSTRACT

The millets were processed by soaking, germination, microwave treatment and fermentation (open & closed). Application of the process parameters for pearl millet, finger millet and sorghum showed significant differences on nutrients and anti-nutrients components. The protein and fiber value increased significantly whereas anti-nutrients *viz.* tannin and phytic acid decreased maximally to ~90% and 80% respectively by fermentation. Reduction in tannin follows the order Untreated >Microwave Treated > Soaking >Germination >Open Controlled Fermentation>Closed Controlled Fermentation. Germination favors maximum reduction of polyphenolics to a level of 75% and least with both open and close controlled fermentation.

Key words: Anti-nutrients, Antioxidant, Millets, Polyphenol, Pre-processing, Proximate composition.

INTRODUCTION

The millets are small-seeded cereals with excellent nutritional attributes that are even superior to staple cereals i.e. wheat and rice (Ragaee *et al*., 2006). They rank as sixth most important cereal that feeds one third of the total world population (Saleh *et al*., 2013). Being drought resistant and ability to grow in too hot or cold climate, millets serve to work for malnourished inhabitants of underdeveloped countries. (Adekunle, 2012).

Millets are non-glutinous, non-acid forming and easy to digest (Thilagavathi *et al.,* 2015), loaded with high phytochemicals and antioxidant levels (Banumathi *et al.,* 2015). These facts recognize them as grain of future being drought resistant and pest tolerant features, helpful in term of climate change and global warming issues.

Pearl millet (*Pennisetum glaucum*), commonly known as Bajra, is one of the most important cereal grown in tropical and semi-arid regions of the world primarily in Africa and Asia. It has low glycemic index and is gluten free. Similarly Finger millet (*Eleusine corucana*) also known as '*ragi'* is the third most important millet of India, next to sorghum and pearl millet, and an important staple food for people belonging to the low socio-economic group. It is a major source of dietary carbohydrates for a large section of society (Patel & Verma 2015). It is also recognized for their beneficial health effects, anti diabetic, anti tumerogenic, antioxidants and anti microbial properties.

Sorghum is the fifth important cereal crop after wheat, rice, corn and barley. It is a traditional belief that they promote the health of unborn babies and act as therapeutic against various digestive disorders (Awika *et al*., 2004) but affect the minerals (divalent cations), proteins and other nutrients inhibiting their absorption in human gut (Mohamed *et al*., 2011).

The wildly and widely grown millets can be exploited as a major part of human consumption rather than to use them as animal fodder. Keeping in mind this fact, an attempt has been made to evaluate the effect of some pre-processing methods on to the proximate characteristics, polyphenolics and anti-nutrient properties of millets so that they would attain the status of staple cereals in human diet.

MATERIALS AND METHODS

Millet grains were purchased from local market of Mohali, Punjab. Pearl millet, finger millet and sorghum were treated against a series of pre-processing parameters for anti-nutrient minimization and proximate analysis.

Sample preparation: Cleaning of pearl millet, finger millet and sorghum seeds were initially done manually by removing sticks, stones, other crop seed, weed seed and foreign matter. Grain seeds were washed with clean potable water. Cleaned grains followed by sun drying were stored in polyethylene laminates. This cleaning step is common and applicable to all the treatments. These were then utilized as sequential steps for further processing as follow:

Soaking: The cleaned raw grains were soaked in aerated fresh water for 24 h at 28°C with 0.1% formaldehyde added to it to arrest mould growth. A portion of soaked seeds were then dried in hot air oven, set at 55°C overnight and milled

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in home scale cabinet flour mill (Natraj & Co, New Delhi) and sieved through 16 mesh sieve size to obtain flour.

Germination: As per the suggested method of Ajay *et al.,* 2014, the remaining soaked millet seeds were allowed to germinate at 25°C for 48 to 72 h till the growth of 3 to 4 mm long rootlets placed inside the incubator (Metrex, New Delhi). On germination, these were dried in hot air oven at 55°C, milled and sieved through 16 mesh sieve size to get germinated millet flour.

Microwave treatment: Millets were adjusted to 18% moisture content by adding distilled water (Yadav *et al*., 2008). The tempered samples were placed in a glass beaker and treated (900 W, 2,450 MHz) in a microwave oven for 40-100 sec. The samples were then removed from microwave oven and cooled by spreading in trays at room temperature. Cooled treated samples were ground and sieved through 16 mesh size to form millet flour which was then packed in airtight HDPE (High Density Polyethylene) laminates of 80micron thickness (Yadav *et al*., 2012).

FERMENTATION

Open controlled fermentation: Previously cleaned, dried raw millet seeds were pulverized to form flour and sieved through 16 mesh size. It was then suspended in distilled water at 300 g/ L concentration which was found to be the optimal concentration for fermentation according to Doblado *et al.* 2002. The suspension was allowed to ferment naturally with microorganisms present in the seeds and in the surrounding atmosphere for 48 h. Completion of fermentation and inactivation of microbes was achieved by drying slurry mass at 55°C in hot air oven for 24 h (Fadahunsi, 2009). Dried flakes were ground to make fine flour and packed in air-tight bags at low temperature (4°C) for further analysis.

Closed controlled fermentation: About 250 g of millet flour was weighed into 500 mL flat bottom flask and autoclaved at 121°C for 15 min. Moisture content of flour samples was adjusted to 25% before aseptic inoculation with spore suspension of *Aspergillus niger*, containing 1.064×10^7spores/ 25 g of flour (Bhat *et al*., 1997), and incubated at room temperature (29±3°C) for 48 h. After fermentation, fungal growth was terminated by drying at 55°C in oven for 24 h (Fadahunsi, 2009) and re-ground to form flour and stored in air-tight bags at low temperature (4°C) for further use.

The AOAC methods, hot air oven for moisture content (AOAC 2010), muffle furnace for ash content (AOAC 2010), fibraplus for crude fiber (AOAC 2000), soxhlet for fat content (AOAC 2000) and protein estimation by lowry method were used. Carbohydrate and energy value was calculated by difference as:

% Carbohydrate = 100- (% protein + % fat + % fiber + % ash + % moisture)

Energy Value = % protein × 4 + % Carbohydrate × 4 + % fat × 9.

Anti-nutrient analysis: Phytate and tannins are the major problems associated with millet consumption. They were minimized by applying various minimal home scale pre-processing applications.

Tannin content of raw and processed flour was quantified by using butanol-HCl assay as described by Porter *et al.,* (1986). Phytate content of raw and treated flour was quantified by using Megazyme phytic acid kit.

Antioxidant study: Antioxidant capacity was evaluated with DPPH (2, 2-diphenyl-1-pikrahydrazyl) solution. Aliquot of the sample extract was added to 0.1mM methanolic solution of DPPH. Each mixture was vortexed vigorously and incubated for 30 min. in dark at room temperature.The samples were analyzed spectrophotometrically at 517 nm (Bembem and Sadana, 2013) and calculation was done by using following formula:

[1-(absorbance of sample/absorbance of blank)]×100

Polyphenol content: Polyphenol content of processed flour samples was estimated by using Folin's Ciocalteu Reagent and Gallic acid was used for standard curve formation. The sample extract was mixed with 10% Folin's Ciocalteu Reagent and incubated in dark at room temperature for 8 min. Then 7.5% Na_2CO_3 was added to the mixture and incubated for 2h in dark at room temperature. Samples were analyzed spectrophotometrically at 765 nm using distilled water as blank (Chumyama *et al*., 2013).

Statistical analysis: Statistical study in triplicates was conducted by means using ANOVA (Analysis of Variance) and depicted in significant form ($p<0.05$).

RESULTS AND DISCUSSION

Proximate composition of raw and processed millets: The proximate composition of raw and differently processed millets is given in Table 1. Protein content ranged from 9.67±0.42-11.69±0.18, 10.27±0.44-14.44±0.31 and 6.16±0.28-15.56±0.09 in pearl millet, finger millet and sorghum respectively and revealed non-significant difference ($p>0.05$). There was little reduction in fiber content due to enzymatic degradation of fiber in fermented samples compared with untreated sample in a similar manner as reported by Gunashree and associated researchers in 2014. Energy and moisture content of pearl millet was also minimized after processing. Low value of energy for pearl millet gives significant results ($p<0.05$). On the other hand, moisture content, protein, ash and carbohydrate showed non-significant differences ($p>0.05$). Lower value of fat for sorghum and fiber for pearl millet seems to be highly significant ($p<0.05$) after all treatments. This reduction in fat level of all the processed samples lessens the chances of rancidity thus contribute in increasing shelf life (Vadivoo *et al*., 1998).

Table 1: Proximate analysis of millet grain flour after different processing

Parameters	Grains	Untreated	Soaking	Germination	MT	OCF	CCF
Moisture Content (%)	1. PM	10.36±0.09	9.30±0.15	6.68±0.38	4.28±0.48	4.59±0.48	15.67±0.98
	2. FM	12.08±0.56	10.31±0.58	5.46±0.97	6.20±0.52	3.34±0.25	14.87±0.50
	3. S	10.87±0.42	8.13±0.42	9.63±0.5	6.78±0.48	2.84±0.57	14.45±1.07
Protein (%)	1. PM	9.67±0.42	10.91±0.35	11.56±0.18	10.93±0.08	11.69±0.18	11.39±0.36
	2. FM	10.07±0.44	12.48±0.26	11.25±0.22	14.48±0.37	14.44±0.31	8.97±0.16
	3. S	6.16±0.28	8.59±0.27	12.68±0.17	15.56±0.09	14.14±0.26	13.33±0.32
Fat (%)	1. PM	4.36±0.45	2.96±0.25	2.59±0.14	2.24±0.11	1.8±0.45	1.6±0.51
	2. FM	1.8±0.2	1.36±0.25	1.26±0.30	1.4±0.3	1.4±0.26	1.33±0.11
	3. S	4.6±0.2	3.26±0.25	3.33±0.51	3.63±0.25	2.86±0.25	2.9±0.3
Fiber (%)	1. PM	2.56±0.30	2.13±0.12	2.48±0.15	2.22±0.25	2.09±0.06	2.18±0.13
	2. FM	3.53±0.30	3.36±0.32	3.26±0.20	3.16±0.20	3.3±0.36	3.03±0.20
	3. S	2.33±0.30	1.8±0.2	2±0.2	1.73±0.30	1.93±0.40	1.93±0.56
Ash (%)	1. PM	1.74±0.08	1.80±0.06	3.07±0.13	1.39±0.16	1.03±0.18	1.49±0.14
	2. FM	1.89±0.07	2.12±0.11	2.07±0.06	3.09±0.02	1.92±0.09	2.39±0.06
	3. S	1.64±0.10	1.66±0.04	1.92±0.02	1.37±0.09	0.97±0.09	1.52±0.21
Carbohydrate (%)	1. PM	82.87±0.68	83.35±0.64	80.45±0.20	83.70±0.25	83.50±0.19	83.58±0.12
	2. FM	82.86±0.06	80.15±0.26	81.49±0.49	79.42±0.94	80.16±1.04	84.82±0.41
	3. S	85.83±0.15	85.75±0.28	82.54±1.21	78.14±0.16	80.50±0.15	81.35±0.17
Energy (kcal)	1. PM	410.2±1.8	402.7±1.6	394.6±3.3	400.6±2.4	401.7±2.7	398.5±1.7
	2. FM	392.5±5.4	386.2±3.5	389.1±1.9	384.6±4.3	388.8±2.9	387.5±2.5
	3. S	409.9±1.0	408.5±1.7	405.4±1.9	408.8±1.4	406.5±1.9	406.7±2.7

MT = Microwave Treatment, **OCF** = Open Controlled Fermentation, **PM** = Pearl Millet, **CCF** = Closed Controlled Fermentation, **FM** =Finger Millet, **S** = Sorghum

Anti-nutrient analysis: Tannin and phytate are prominent in millets. All the pretreatments like soaking, germination, fermentation (controlled closed and controlled open) and microwave treatment reduce their level.

Tannin and Phytate: Table-2 showed that anti-nutrient level in pearl millet, finger millet and sorghum flour was significantly reduced after processing. Eltayeb *et al*., (2007) observed the same effect of anti-nutrients minimization using pre processing applications in case of millet varieties. Sorghum is the main source of tannin (0.601±0.05mg/100g) among pearl millet (0.459±0.02mg/100g) and finger millet (0.301±0.04mg/100g). Fermentation treatment suits more appropriately among all treatments stated ahead because of its effect in tannin reduction to more than 10 times compared to untreated samples.

Germination and fermentation give beneficial results significant results ($p<0.05$) by lowering the antinutrients factors due to leaching of polyphenols during soaking in water (Jood *et al*., 1987) and increased enzymatic activity while germination (Bishnoi *et al*., 1994). Microwave treatment has the capacity to reduce tannin to approximately half value to initial one.

Table 2: Tannin and Phytate content in PM, FM and Sorghum flour after pre-processing:

Treatment	Tannin (mg/100g)			Phytic acid (g/100g)		
	PM	FM	SORGHUM	PM	FM	SORGHUM
Untreated	0.459±0.023	0.301±0.043	0.601±0.051	0.050±0.003	0.086±0.009	0.034±0.008
Soaking	0.222±0.019	0.185±0.018	0.020±0.001	0.037±0.005	0.019±0.003	0.022±0.007
Germination	0.032±0.007	0.094±0.025	0.014±0.002	0.020±0.003	0.018±0.003	0.021±0.005
MT	0.250±0.012	0.165±0.007	0.135±0.010	0.015±0.005	0.036±0.007	0.030±0.007
OCF	0.035±0.011	0.054±0.008	0.010±0.002	0.025±0.006	0.030±0.004	0.018±0.004
CCF	0.016±0.002	0.014±0.003	0.014±0.002	0.012±0.005	0.012±0.003	0.023±0.005

PM = Pearl Millet, **FM** = Finger Millet, **MT** = Microwave Treatment, **OCF** = Open Controlled Fermentation, **CCF** = Closed Controlled Fermentation

Table 3. Antioxidant and total polyphenol of millet flour after different processing:

Treatment	Antioxidant Activity (Trolox eq.ìg/g)			Polyphenol Conent (GAEmg/g)		
	PM	**FM**	**SORGHUM**	**PM**	**FM**	**SORGHUM**
Untreated	77.14±0.95	86.81±0.69	82.91±0.52	25.03±0.69	26.06±0.86	27.49±1.18
Soaking	59.62±0.41	65.90±0.65	45.60±0.71	20.74±0.48	14.96±0.87	13.22±0.35
Germination	44.02±0.60	62.80±0.65	42.23±0.75	10.00±0.28	5.68±0.40	6.74±0.934
MT	31.73±0.84	43.66±0.50	40.70±0.84	11.51±0.96	10.06±0.22	8.66±0.90
OCF	15.88±0.30	34.81±0.95	23.58±0.53	11.57±1.20	20.56±0.79	13.80±0.89
CCF	23.53±0.80	25.77±0.51	48.77±0.29	14.78±1.00	20.65±0.58	17.43±1.16

PM = Pearl Millet, **FM** = Finger Millet, **MT** = Microwave Treatment, **OCF** = Open Controlled Fermentation, **CCF** = Closed Controlled Fermentation

The treatments for reducing tannins, follows the given order UT < MT < Soaking < Germination < Fermentation. Similar trend was suggested by the findings of Fasasi *et al* (2009).

Untreated samples have 0.050±0.003g /100g, 0.086±0.009g/100g and 0.034±0.008g /100g phytic acid in PM, FM and sorghum respectively. There was much reduction in phytic acid by fermentation compare to other treatment and thus corroborate the findings of Tizazu *et al.,* (2011). Soaking also minimized phytic acid as they thus leach or wash away in soaking stage (Alonos *et al*. 2000). Germination works similarly in reduction of phytic acid significantly as per the findings of Gunashree *et al*., 2014, where phytate reduced to 0.020±0.003g/100g in PM, 0.018±0.003g/100g in FM and 0.022±0.005g/100g in sorghum after germination. This reduction in phytic acid is due to activation of endozymes i.e. phytases that feeds on phytate while germination (Badau *et al*., 2005).

Antioxidant and Polyphenol analysis: Antioxidant power (µg/g) evaluated with DPPH in raw samples of finger millet (86.81±0.69), sorghum (82.91±0.52) and pearl millet (77.14±0.95) follows the decline behavior with varied treatments as depicted in Table-3. Previously discussed treatments give non-significant findings ($p>0.05$) and lowers antioxidant power with the sequence as follows:

Untreated > Soaking > Germination > Microwave Treatment > Fermentation

Jemima and Mohan (2012) evaluated that DPPH radical scavenging activity of soaked and germinated millets samples was higher compared to other methods due to lower heat treatment provided to soaked and germinated samples.

Soaking significantly ($p<0.05$) reduced polyphenolics. Soaking and germination contributed more pronounced effect in its reduction than all the treatments taken into consideration (Table-3). This reduction in polyphenols during germination and soaking is attributed to activation of phenolic Oxidase, amylase and also to their leaching to some extent as corroborated by Naveena *et al* (2013).

Polyphenolics reduction after fermentation follows the sequence suggested by Elyas *et al*., (2002). Polyphenolics reduction after close controlled fermentation 14.78±1.00, 20.65±0.58 and 17.43±1.16 for PM, FM and Sorghum respectively effected not to that extent as for previous treatments of soaking and germination.

CONCLUSION

It is concluded that different processing treatments i.e. soaking, germination, microwave treatment and fermentation (closed & open) contribute significantly in rise of crude protein and fibre content of millet flour but effect crude fat negatively. They don't increase shelf life of grains and its products but also improve sensory properties. Approximately 90% tannin was minimized by fermentation and 80% reduction was reported in phytic acid by fermentation compared to other treatments. Efficacy of treatments effected antioxidant power and polyphenol negatively that follows the given pattern: Untreated > CCF > OCF > Soaking > Microwave Treatment > Germination. Soaking has more pronounced effect in its reduction than all the treatments taken into consideration in present study. A total of 70% polyphenols reduction were observed compare to untreated samples. So fermentation is the best among discussed minimal home scale pre processing that minimizes anti-nutrients and favors them nutritionally.

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