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EFFECTS OF SOAKING AND GERMINATION OF TOTAL AND INDIVIDUAL POLYPHENOLS CONTENT IN THE COMMONLY CONSUMED MILLETS AND LEGUMES IN INDIA

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ABSTRACT

Millets and legumes are rich sources of bioactive phytochemicals which are powerful antioxidants. Phenolic compounds are bioactive components which are act as a part of the defense mechanisms of plants and they have antimicrobial, anti oxidative and anti- carcinogenic properties. This may help to promote optimal health and to reduce the risk of chronic diseases. The aim of the present study was to investigate the effect of total and individual polyphenolic content of germinated and non germinated (0, 12, 24, 48 and 72 hrs) commonly consumed millets and legumes in India. Millets and legumes were germinated in metabolic kitchen under room condition (25 ºC). Samples were analyzed through Folin – Ciocalteu (F-C) assay and RS- HPLC. Total polyphenolic content (TPC) was analyzed in the commonly consumed food samples. Among the food samples, the red gram (whole) contained higher total polyphenolic content (479.45 mg/100g) where as ragi contained lesser total polyphenolic content (186.99 mg/100g) during 0 hr on dry weight basis. Further these food samples were soaked for 12 hrs then germinated for various time periods (24, 48 and 72 hrs). The total polyphenolic content in these foods ranged from 40 -79% (12 h), 32-62 % (24h), 17 -50 % (48 h) to 9-35% (72h). Total polyphenols were further separated and quantitated into individual polyphenols by using RP-HPLC. We found phenolic acids such as protocatechuic acid, p-coumaric acid, gallic acid, syringic acid, ferulic acid, sinnapic acid and flavonols such as kaempferol, quercetin and its glycosides. It is evident that the soaking and germination processes significantly ($p<0.001$) reduced the total and individual polyphenolic contents in the selected commonly consumed millets and legumes. From the study it is recommended that soaking and 24hrs germination could be helpful for minimizing various polyphenols losses through germination.

Key words: Soaking, Germination, Total and Individual Polyphenols, HPLC.

INTRODUCTION

Polyphenols are the abundant antioxidants in our diet since the average daily intake is about 1 g, which is almost 10 - fold the intake of vitamin C, 100 – fold the intake of vitamin E, and 500 fold the intake of carotenoids (Scalbert and Williamson, 2000). In addition to their antioxidant properties, Polyphenols may have other biological activities including anti-mutagenic, anti-oestrogenic, anticarcinogenic and anti-inflammatory effects that might potentially beneficial in human health, such as in the treatment and prevention of cancer, cardiovascular diseases, and other pathologies (Bravo L., 1998). There is clear evidence that they have the potential to act in the three general areas specified (i.e., transition metal ion complexation, as antioxidants in cellular pro-oxidant states, and by association with proteins and peptides) (Haslam E., 1996).Experimental data indicate that most of these biological actions can be attributed to their intrinsic antioxidant capabilities. Dietary polyphenols may offer an indirect protection by activating endogenous defense systems and by modulating cellular signaling processes such as NF-κB activation, AP-1 DNA binding, glutathione biosynthesis, PI3-kinase/Akt pathway, MAPK proteins (ERK, JNK and P38) activation, and the translocation into the nucleus of Nrf2 (Shen et al., 2007 ; Molina et al., 2003; Chen et al., 2000).Their main dietary sources are fruits and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Vegetables, cereals, millets, chocolate, and dry legumes also contribute to the total polyphenol intake (Scalbert et al., 2005). Despite their wide distribution in plants, the health effects of dietary polyphenols have come to the attention of nutritionists only rather recently.

Millets and legumes form staple food for the population of low income groups in India and also worldwide (Pradeep et al., 2011). Millets and Legumes are rich in phenolic acids, tannins and phytate which act as an 'antinutrient' (Thompson, 1993). However, it is now well established that these antinutrients are known to reduce the risk of various degenerative diseases. Also the food

industries are more and more opting for development of functional foods specially designed for the vulnerable population. So it is necessary to understand the polyphenolic content changes while germination. The term 'germination' is often used to describe the soaking or steeping process of cereal in water (Roohinejad et al., 2010). Therefore, the objective of the present work was to evaluate the total and individual phenolic content in few millets and legumes during soaking and germination, so as to identify their suitability for polyphenols enriched functional food formulations.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Methanol (HPLC grade), Acetone (GR), Sodium dihydrogen phosphate dihydrate ($Na_2H_2Po_4.2H_2O$), Sodium Carbonate (Na_2Co_3), Folin – Ciocalteu (F-C), Dimethyl sulfoxide (DMSO). Reagents were highest analytical grade and purchased from Merck. Reference compounds for HPLC were from Extrasynthase and Sigma Aldrich. Gallic acid was chosen as standards for measurement of the Total Polyphenols content. The stock solution of Gallic acid (1mg/10 ml) was prepared in 70 % Acetone (GR). The stock solution was protected from light with aluminium foil and kept in refrigerator and stable for at least one month. The working standard was prepared freshly from the stock solution for each new measurement.

FOOD SAMPLES

Very commonly consumed food samples such as Green Gram whole (phaseolus aureus Roxb.), Bajra (Pennisetum tyhoideum), Red Gram whole (Cajanus cajan), Rajmah (Phaseolus vulgaris), Ragi (Eleusine coracana), Black Gram whole (Phaseolus mungo Roxb.), Bengal Gram whole (Cicer aritinum) and Horse Gram whole (Dolichos biflorus) were selected. The millets and legumes were cleaned manually to remove any stones and dirt. Then they were finely grounded by using Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado, USA).

GERMINATION PROCESS OF MILLETS AND LEGUMES

Germination of millets and legumes was conducted and slightly modified according to the method described by Donkor et al., (2012). Food samples were washed and cleaned with tap water and dried before soaking for 3 hr in room temperature (28°C). After 3 hr, samples were soaked in tap water for 12 hr and the excess water was removed, millets and legumes were placed under wet filter paper kept in a sterilized Petri dish and they were wrapped with sterile cheesecloth to maintain the moisture. The total germination length included the time for steeping and the time for sprouting. The millets and legumes were sprinkled with tap water for 5 min at 12 h intervals and aerated by hand once every 24 h in order to prevent rootlets matting, ensure that on average all samples were treated as nearly evenly as possible, break up hot spots and reduce its bulk density and ensure that the samples bed was saturated with water vapour and left germinated for 24, 48 and 72 hr in room temperature (28°C) without direct contact with sun light. After soaking and germination periods, samples were harvested, carefully and analyzed for moisture. Subsequently, the samples were ground using mortar and pestle into fine powder. The millets and legumes which were non germinated, soaked and germinated were analyzed for total and individual polyphenolic compounds.

EXTRACTION AND DETERMINATION OF TOTAL PHENOLIC CONTENT

Total phenol content (TPC) of the millets and legumes extracts was determined according to George et al., (2005) using solid phase extraction method. Samples were weighed and ground into a fine powder with 70% acetone and stirred further for 30 min in a magnetic stirrer (10 set, IKAFLON, IKA Pvt. Ltd.,) and supernatant was collected after centrifugation (Sigma, 3-18 K, made in Germany) at 5000×g for 7 min. This is called as raw extract. Raw extracts (3ml) were passed into the SPE - HLB (Solid Phase Extraction, Hydrophilic – Lipophilic Balance) cartridge (Model No. WAT094225, Oasis HLB 1 cc Vac Cartridge, 30 mg Sorbent per Cartridge, 30 µm Particle Size, Waters, UK).The recovered volume is called as washing extract (WE).This technique was used to remove some of the interfering components (sulfur, non protein nitrogenous compounds, reducing sugars and ascorbic acid) which are highly reactive towards the Folin Ciocalteu Reagent. Thus, This SPE cartridge method assist to reduce the interfering compounds and help to prevent the over or under estimation of phenolic compounds in the food samples.

Gallic acid was used as a standard. The calibration curve of absorbance vs. concentration of standard was used to quantify total polyphenols content (r^2 =0.999). Results were expressed as mg Gallic acid equivalents per 100 g of food sample (mg GAE/ 100g). 3.5ml of water diluted Folin – Ciocalteu reagent (1/10) was added to the different extracts (RE and WE). The mixture was incubated for 2 min at room temperature, and 2.5 ml of sodium carbonate (75g/L) was added. The mixture was incubated for 15 min at 50ºC. The specific absorbance at 760 nm was immediately measured (George, et al., 2005).

EXTRACTION AND QUANTIFICATION OF INDIVIDUAL PHENOLIC COMPOUNDS

Raw extracts were evaporated using rotary evaporator (B'U'CHI, R-215, made in Switzerland) and the samples were reconstituted with 1.5 and 3ml of DMSO. The extracts were centrifuged at 4200 rpm for 10 min and filtered through a Whatman 0.2µm syringe filter (PTFE) before the HPLC analysis.

HPLC analysis for characterization of the phenolics in food samples were detected by using the following individual standards namely Gallic acid, Gallocatechin, Protocatechuic acid, 3,4-Dihydroxy benzoic acid, Epigallocatechin, Chlorogenic acid, Catechin,

Syringic acid, Vanillic Acid, 3-OH Benzoic acid, Epicatechin, Caffeic acid, Sinapic acid, (-)Catechin gallate, Procyanidin B2, Epigallocatechin gallate, Ferulic acid, Isoferulic aicd,4-Coumaric aicd, Hesperdien, Gallocatechin gallate, Epicatechin galate,2-Coumaric acid, Rosmaric acid, Isovitexin,Luteolin-7-O-Glucoside, Hespertin, Quercetin-3-Beta-Galactoside, Hyperoside, Ellagic acid, Rutin, Physcion, Daidzein, Galangin, Salicyclic acid, Rhoifolin, Naringenin, Flavonone(internal standard), Quercetin, Luteolin, Chrysophanol, Chalcone, Rhein, Isorhamnetin, Myricetin, Emodin, Kaempferol, Cinnamic aicd and Curcumin. Calibration curves were made by diluting stock solutions with DMSO to give concentration of the standard in the range 1mg/1ml for standards. The calibration curves were constructed from chromatograms as peak area vs. concentration of standard. All phenolic standards gave linear calibration curves within the concentration range studied (r = 0.9992–0.9999). The limits of detections (LOD) were calculated from the parameters obtained from calibration curves, using the formula LOD = 3.3 Sa/b, where Sa is the standard deviation of the y-intercept of the regression line and b is the slope of the calibration curve (Ribani, Collins, & Bottoli, 2007). The calculated LOD was 0.027 mg/ml for gallic acid.

The HPLC system employed was a Dionex HPLC series Ultimate 3000 (Germany) equipped with Dionex model Chromeleon software, autosampler U-3000, and 3000 RS diode array detection system to monitor at all wavelengths from 200 to 600nm. For the column, Dionox PA_2 RSLC 120 A, Column C_{18} Acclaim RSLC, (100mm x 2.1dm) i.e., 2.1µ Thermo Scientific Ltd was used at 35 ºC. Gradient elution was performed with solution A, composed of 50mM Sodium phosphate (pH 3.3) and 10% methanol, and solution B, comprising of 70% methanol, delivered at a flow rate of 0.47 ml/min as follows: initially 100% of Solution A; for the next 0.03 min, 70% A; for another 2.65 min, 65 % A; for another 7.9 min, 60% A; for another 11.5 min 50% A and finally 0% A for 13.1 min , again 17 min 100 % A and 20.25 min 100% A. The injection volume of the extract was 5µl (Sakakibara, et al., 2003).

STATISTICAL ANALYSIS

Values are expressed as mean values and standard deviation of results obtained from 5 separate experiments (quintuplicate). One- way Analysis of variance (ANOVA) followed by the Least significant Difference (LSD) test for multiple comparisons, was used to assess differences among the millets and legumes. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

ANALYSIS OF TOTAL PHENOLIC CONTENT

The total phenolic content of two millets and six legumes assessed in this study is shown in Table 1. The 0 hr total polyphenolic content of commonly consumed millets and legumes. Among these red gram whole contains high phenolic content (440.07±4.42) followed by Green gram whole (397.72±2.06), Black gram whole (370.86±4.09), Rajmah (306.61±2.06), Horse gram whole (294.21±4.52), Bengal gram whole (251.31±4.83), Bajra (241.47±2.18) and the lesser content was observed in Ragi (161.64±1.54).Overnight soaking (0-hr germination) of Legumes and millets in water brought about a significant reduction in total polyphenols content. In addition increasing the germination period to 24, 48, and 72 hrs had further decreasing effect on the total polyphenols content (Fig.1).It can be observed from the table that the soaking (12 hrs) process can reduce the total Polyphenolic content from 20 - 50% among the millets and legumes were studied. The maximum reduction (50%) of total polyphenols was observed in green gram whole which is 397.72 ± 2.06 to 205.4± 3.57. The minimum reduction of polyphenolic contents were observed in bajra (22%) and ragi (20%) as 241.47±2.18 to 184.43±3.44, 161.64±1.54 to 128.97±1.73 respectively.

Germination of the lotus seeds caused the total phenolics of the seeds to decrease significantly at 95% confidence level. The same significant decrease was observed in tannins and catechins (Purintraphiban.S and Yan–bin.X., 2012).Our results agree with those of Mubarak (2005) who found that tannin content of mung bean seeds (Phaseolus aureus L.) was reduced after water soaking for 12 h. The reduction of tannins after soaking is mainly due to the fact that those compounds, in addition to their predominance in seed coats (Reddy & Pierson, 1994), are water soluble (Kumar, Reedy, & Rao, 1979) and consequently leach into the liquid medium. Reduction of total phenolics and tannins after germinating might have been caused by the activity of polyphenol oxidase or fermentation microflora (Reddy & Pierson, 1994).Similar tends were reported by Paramjyothi and Anjali (2005) for chickpea, Ramakrishna et al., (2006) for mung bean and Khandelwal et al., (2010) in Indian pulses. Xu and Chang (2008 a and b) suggested that the phenomena of the difference of reduction on total phenolic content by soaking might be due to the differences on distribution and content of phenolic compounds in the seed coat and cotyledon between the tested seeds.

In the 24 h germination process, red gram whole was decreased with its total polyphenolic content from its initial 0 hr total phenolic content (440.07±4.42) to (166.73±2.11). And further decreasing was observed in 48 hrs and 72 hrs germination as (132.64±3.05), (43.58±4.21) respectively. In the case of 24, 48 and 72hr germination process in the green gram whole, the total phenolic content was decreased to (170.14±2.80), 48 h (121.86±4.38), 72 h (72.67±2.99) from the 0 hr non germinated phenolic content (397.72 ± 2.06). The observed reduction in the polyphenols particularly after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. The reason for loss of tannins during germination process is binding of polyphenols with other organic substances such as carbohydrate or protein. Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be

Table 1 - Total phenolic content in soaked, germinated and non-germinated grains

Sample	0 hr (GAE mg/100g)	Soaking (12 hrs) (GAE mg/100g)	Germination (24 hrs) (GAE mg/100g)	Germination (48hrs) (GAE mg/100g)	Germination (72 hrs) (GAE mg/100g)
Bajra (Pennisetum *tyhoideum*)	241.47±2.18 (260.78)[9.83]	184.43±3.44[a] (252.19)[26.87]	122.25±1.92[b] (193.46)[36.81]	41.23±4.19 (75.84)[45.64]	38.65±2.44[e] (69.66)[44.52]
Ragi (Eleusine *coracana*)	161.64±1.54 (186.99)[13.45]	128.97±1.73 (177.20)[27.22]	101.36±2.54 (157.70)[35.73]	81.92±1.51[c] (131.33)[37.78]	57.8±4.59 (98.60)[41.38]
Bengal Gram whole (Cicer *aritinum*)	251.31±4.83 (273.65)[8.09]	107.61±3.42 (216.21)[50.23]	90.68±3.01 (200.48)[54.77]	54.43±3.62[c] (128.88)[57.57]	31.68±4.12[e] (73.64)[56.98]
Black Gram whole (Phaseolus *mungo Roxb*.)	370.86±4.09 (419.35)[11.29]	152.93±3.38 (274.41)[44.27]	121.95±1.36b (247.51)[50.73]	97.64±1.24 (207.56)[52.96]	66.69±4.72e (154.69)[56.89]
Horse Gram whole (Dolichos *biflorus*)	294.21±4.52 (328.58)[9.38]	223.35±2.09 (315.95)[29.31]	158.01±1.76 (290.45)[45.60]	81.49±2.79[c] (196.78)[58.59]	40.02±2.91[e] (99.47)[59.77]
Green Gram whole (Phaseolus *aureus Roxb*.)	397.72±2.06 (447.99)[11.66]	205.4±3.57 (435.72)[52.86]	170.14±2.80[b] (410.17)[58.52]	121.86±4.38 (332.76)[63.38]	72.67±2.99[e] (197.84)[63.27]
Rajmah (Phaseolus *vulgaris*)	306.61±2.06 (349.92)[12.59]	184.06±2.26[a] (329.91)[44.21]	126.92±2.03[b] (296.05)[57.13]	53.65±1.87[c] (120.77)[55.58]	31.57±3.81[e] (66.12)[53.77]
Red Gram whole (Cajanus *cajan*)	440.07±4.42 (479.45)[10.04]	258.21±2.09 (445.49)[42.04]	166.73±2.11[b] (253.85)[34.32]	132.64±3.05 (234.67)[43.48]	44.58±4.21[e] (106.05)[58.91]

Values are mean ± SD of five determinations on fresh weight basis. Values in parenthesis ( ) are in dry weight basis. Values in parenthesis [ ] are moisture content of the sample. [a-e] Significantly different.

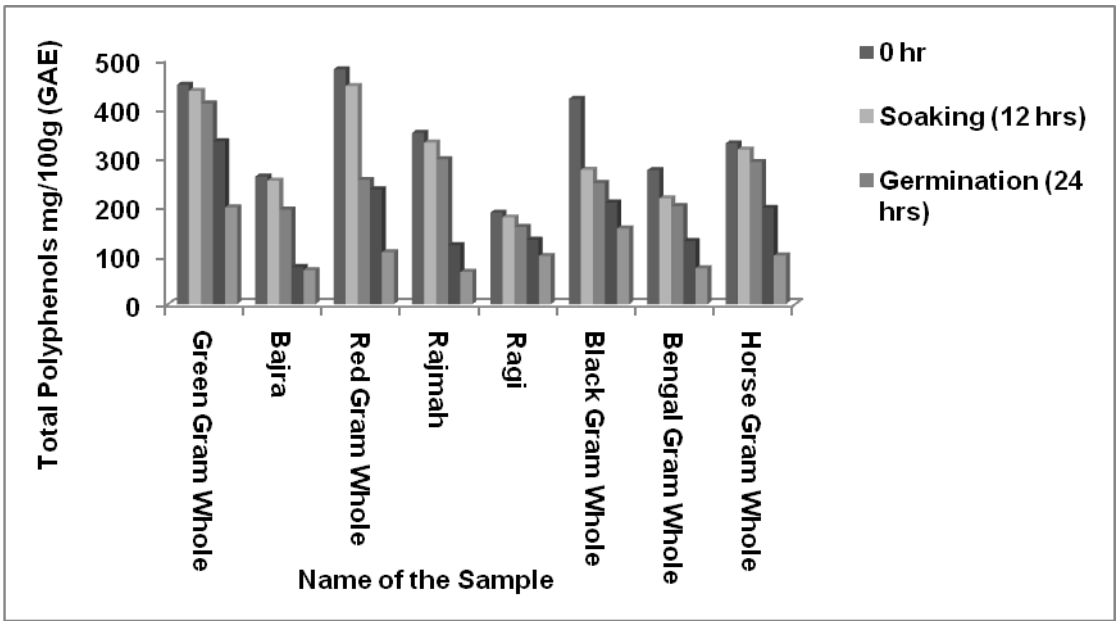


Fig.1.Total phenolic content changes during Soaking & Germination of millets and Legumes (GAE mg/100g) (DW)

activated, resulting in degradation and consequent losses of polyphenols. Germination process causes a reduction percent in total phenolic compounds ranged from 32.8 % after 24 hr of germination to 60.8 % after 120 hr of germination in kidney bean. Longer period of germination caused significant greater losses in total phenolic

compounds in kidney bean .For the soybean, the reduction percent in total phenol content was 27.0 % after 24 h. germination and reached 45.0 % after 96 h. of germination.

On the other hand, the mung bean displayed high losses of phenolic compounds which reached 66.8% after 48 h.of germination (Moahamad et al., 2011). A decrease in polyphenol contents was observed by Giami et al., (2001) for germinated cowpea (41.5 to 51.7%) and for Indian pulses by Khandelwal et al.,(2010). The reduction of total phenolic compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis (Rao and Deosthale, 1982).

However an increase in phenolic compounds was observed in soybean and mung bean during the progressive germination and peaked after 120hr of germination. These means that the highest reduction in phenolic compounds was achieved in after 48, 120 and 72 h. of germination for mung bean 66.8 %, kidney bean 60.8 % and soybean bean 45.0 %, respectively. However, the lowest reduction in phenolic compounds for the tested germinated legumes was observed for soybean after 48 h. of germination. Also, an increase of total phenols after germination was reported by Khattak et al. (2007) for chickpea, Duenas et al., (2009) for lupines and Tain et al., (2010) for oat.

Duenas et al., (2009) reported that germination caused significant changes in the phenolic composition (increasing) mainly due to endogenous enzymes activation and the complex biochemical metabolism of seeds during this process. Fig. 1 shows the TPC changes during soaking and germination on dry weight basis.

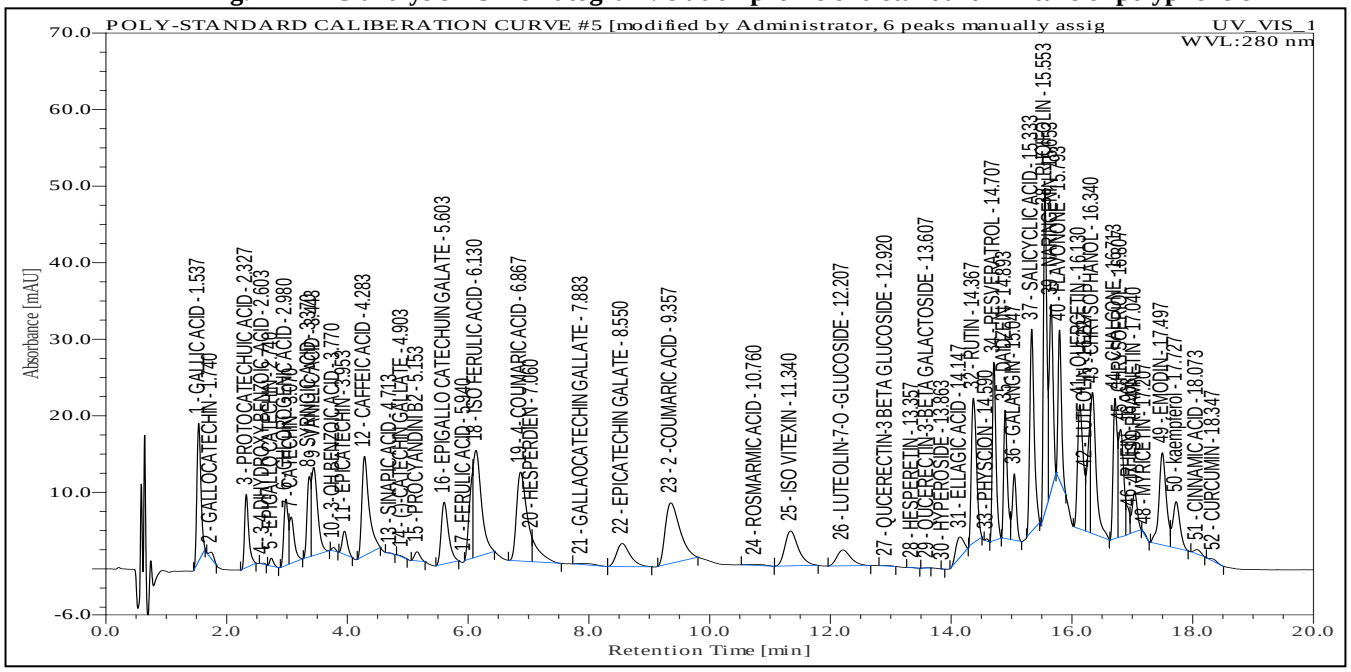
Table 2 - HPLC quantification of phenolic compounds in soaked, germinated and non germinated millets and legumes (mg/100g)

Sample	Individual Polyphenols	0 hr	12 hr	24 hr	48 hr	72 hr
Bajra	Caffeic acid	0.815 ±0.09	0.204±0.01	0.176±0.02	0.143±0.03	0.136±0.02
	Ferulic acid	0.928±0.10	0.233±0.01	0.244±0.04	0.163±0.04	0.155±.02
	P-coumaric acid	0.358±0.04	0.09±0.01	0.094±0.02	0.063±0.01	0.06±0.01
	4-OH-Benzoic acid	1.449±0.16	0.364±0.02	0.381±0.07	0.254±0.06	0.242±0.03
Ragi	Syringic acid	5.562±0.60	3.764±0.04	3.499±0.15	2.555±0.10	2.41±0.07
	Ferulic acid	0.349±0.04	0.236±0.01	0.219±0.01	0.16±0.01	0.151±0.01
	Gallic acid	1.241±0.13	0.84±0.01	0.781±.03	0.57±0.02	0.538±0.02
	Protocatechuic acid	0.847±0.09	0.573±0.01	0.533±0.02	0.389±0.02	0.367±0.01
	Vanillic acid	0.683±0.07	0.462±0.02	0.43±0.02	0.314±0.01	0.296±0.01
	4-OH-Benzoic acid	0.544±0.06	0.368±0.04	0.342±0.01	0.25±0.01	0.236±0.01
Bengal gram whole	Epigallo catechin	20.862±0.64	14.268±0.74	14.858±1.73	9.482±1.59	8.119±1.74
	Catechin	0.383±0.01	0.262±0.01	0.272±0.03	0.174±0.03	0.149±0.03
	Epicatechin	2.811±0.09	1.923±0.1	2.002±.23	1.278±0.21	1.094±0.23
	Syringic acid	5.686±0.17	3.889±0.2	4.05±0.47	2.584±0.43	2.213±0.47
	Epigallo catechin gallate	3.744±0.11	2.561±0.13	2.667±0.31	1.702±0.29	1.457±0.31
	Ferulic acid	5.386±0.16	3.684±0.19	3.836±.45	2.448±0.41	2.096±0.45
	Epigallo catechin	0.356±0.01	0.244±0.01	0.254±0.03	0.162±0.03	0.139±0.03
Black gram whole	Chlorogenic acid	1.146±0.08	1.236±0.18	1.35±0.22	0.699±0.02	0.827±0.19
	Ferulic acid	0.239±0.02	0.257±0.04	0.281±0.05	0.146±0.02	0.172±0.04
	Sinapic acid	0.07±0.01	0.075±0.01	0.082±0.01	0.042±0.01	0.05±0.01
	Kaempferol	0.417±0.03	0.45±0.07	0.492±0.08	0.255±0.01	0.301±0.07
	Daidzein	0.352±0.03	0.38±.06	0.415±.07	0.215±0.02	0.254±.06
Horse gram whole	Epigallo catechin	13.344±0.26	14.81±0.71	14.638±1.48	8.964±0.43	8.315±0.24
	Syringic acid	3.65±0.09	4.037±0.19	3.99±0.40	2.443±0.12	2.266±0.06
	Catechin	0.295±0.08	0.272±0.01	0.268±0.03	0.164±0.01	0.152±.01
	Epicatechin	1.834±0.09	1.996±0.10	1.973±0.20	1.208±0.06	1.121±0.03
	Chlorogenic acid	1.113±0.05	1.215±0.06	1.201±0.12	0.735±0.04	0.682±0.02
	Caffeic acid	0.224±0.04	0.222±0.01	0.22±0.02	0.134±0.01	0.125±0.01
	P-coumaric acid	0.101±.02	0.097±0.01	0.096±0.01	0.059±0.01	0.055±0.01
	Ferulic acid	0.239±0.02	0.253±0.01	0.25±0.03	0.153±0.01	0.142±0.01
	Gallic acid	0.83±0.04	0.901±0.04	0.891±0.09	0.545±0.03	0.506±.01
	Protocatechuic acid	0.565±0.03	0.615±0.03	0.608±0.06	0.372±0.02	0.345±0.01
	Vanillic acid	0.461±0.03	0.496±0.02	0.49±0.05	0.3±0.01	0.278±0.01
Green gram whole	P-coumaric acid	0.081±0.02	0.086±0.01	0.098±0.01	0.0540±0.02	0.075±0.01
	Luteolin	0.737±0.16	0.783±0.02	0.892±0.04	0.498±0.15	0.688±0.11
	Myricetin	1.872±0.41	1.988±0.05	2.266±0.09	1.264±0.37	1.749±0.27
	Quercetin	0.056±0.01	0.06±0.01	0.068±0.01	0.038±0.01	0.052±0.01
	Kaempferol	0.366±0.08	0.389±0.01	0.443±0.02	0.247±0.07	0.342±0.05
	Gallic acid	0.745±0.17	0.792±0.02	0.902±0.04	0.503±0.15	0.696±0.11
	Protocatechuic acid	0.508±.11	0.54±0.01	0.616±0.02	0.343±0.10	0.475±.07

Rajmah	Epigallo catechin	16.213±0.29	15.485±3.73	15.487±1.71	10.101±0.46	9.036±1.79
	Catechin	0.326±0.05	0.284±0.07	0.284±0.03	0.185±0.01	0.166±0.03
	Epicatechin	2.308±0.21	2.087±0.50	2.087±0.23	1.361±0.06	1.218±0.24
	Epigallo catechin galalte	2.91±0.05	2.779±0.67	2.78±0.31	1.813±.08	1.622±0.32
	Ferulic acid	0.294±0.03	0.265±0.06	0.265±0.03	0.173±0.01	0.154±0.03
	P-coumaric acid	0.132±0.04	0.102±0.02	0.102±0.01	0.066±0.01	0.059±0.01
	Quercetin	0.088±0.02	0.071±0.02	0.071±.01	0.046±.01	0.041±0.01
	Gallic acid	0.998±0.03	0.942±0.23	0.942±0.10	0.615±0.03	0.55±0.11
	Protocatechuic acid	0.677±0.02	0.643±0.15	0.643±0.07	0.419±0.02	0.375±0.07
Red gram whole	Epigallo catechin	15.83±0.76	13.827±0.69	16.485±2.81	7.301±0.67	8.744±1.49
	Catechin	0.29±0.01	0.254±0.01	0.302±0.05	0.134±0.01	0.16±0.03
	Epicatechin	2.133±0.10	1.863±0.09	2.222±0.38	0.984±0.09	1.178±0.20
	Apigenin	1.472±0.07	1.286±0.06	1.533±0.26	0.679±0.06	0.813±0.14
	Rutin	5.333±0.26	4.658±0.23	5.554±0.95	2.46±0.23	2.946±0.50
	Gallic acid	0.963±0.05	0.841±0.04	1.003±0.17	0.444±0.04	0.532±0.09
	Protocatechuic acid	0.657±0.03	0.574±0.03	0.684±0.12	0.303±0.03	0.363±.06
	Vanillic acid	0.53±0.03	0.463±0.02	0.552±0.09	0.244±0.02	0.293±0.05
	4-OH - Benzoic acid	0.422±0.02	0.369±0.02	0.44±0.07	0.195±0.02	0.233±0.04

Values are expressed as mean ± SD of five determinations on fresh weight basis.

Fig.2- HPLC analysis - Chromatogram: elution profile of a standard mixture of polyphenols



ANALYSIS OF INDIVIDUAL POLYPHENOLS

Polyphenol identification was determined and quantified using RP-HPLC analysis comparing the retention time of reference standards (Table 2). The reference standard mixture is shown in [Fig.2.Chromatograph]. Comparing the retention times of the peaks obtained from the selected soaked, germinated, non germinated 70% acetone extracts with the peaks obtained from the standard mixture, it was possible to identify 22 components: Benzoic acids such as protocatechiuc acid , vanillic acid, gallic acid, 4-OH benzoic acid and Cinnamic acids such as p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid and Flavones such as apigenin , luteolin and Flavonols such as kaempferol, quercetin, rutin, myricetin and Isoflavones daidzein, and catechins such as (+)-catechin, (-) gallo catechin, (-) epicatechin, (-) epigallocatechin, (-) epicatechin gallate and epigallo catechin gallate (Table 2).

The differences observed between the elution times related to the extract and the standard mixture could be due to the matrix effect. The effect depends upon variations of ionization efficiency in the presence of coeluting substances and causes analytes to elute at different retention times, considering the same polyphenolic compound dissolved either in a specific buffer or in a whole raw extract. The amounts of each identified polyphenol were determined by RP –HPLC analysis using standard peak area values obtained by serial dilutions of the polyphenols standard mixture (Donkor et al., 2012). Data reported in Table 2 shows the presence of phenolic components concentrations in the selected

soaked, germinated and non germinated samples. Phenolic compounds such as ferulic acid, protocatechuic acid, p- coumaric acid were commonly present in the selected millets and legumes.Millets such as bajra and ragi were rich in gallic acid and protocatechic acid. The individual polyphenolic contents are decreasing from non germinated to 72 hr germinated grains. Epigallocatechin is in high in bengal gram and the phenolic compounds concentrations also increasing from 12 hr to 24 hr. Later in 48 and 72 hr phenolic compounds reduction has been observed. Similar trends were observed in the horse gram whole and rajmah. Other than this, whole legumes such as black gram, green gram and red gram whole individual phenolic content also ranges from 0 hr > 12 hr < 24hr >48 hrs< 72 hr .

Some studies quoted that germination also a convenient process to enhance polyphenolic contents and related antioxidant activity (Cevallos-Casals & Cisneros-Zevallos 2009; Duenas and others 2009; Shi et al., 2010).Overall, different legume seeds have different responses towards germination. Although some legume seeds belong to the same species, they still have different responses towards germination (Wu et al., 2011).According to Hat and Sridhar (2008), different methods of processing such as dry heating, cooking, roasting, germination, fermentation need not necessarily reduce or completely eliminate polyphenols and phytic acid. The increase in the tannins to catechins ratio could be attributed to the imbibing of seed during the beginning of the germination process (Ayet et al., 1997).

CONCLUSION

To our knowledge this is the first study in India showing the individual polyphenolic values of commonly consumed millets and legumes. From this study it has been observed that the individual phenolic concentrations may vary with different time intervals from 0 hr to 72 hrs of non germinated, soaked and germinated millets and legumes. Also vary between the inter species and individual phenolic concentration within the species. So it is necessary to determine the individual phenolic values during soaking and germination process before developing a functional food. It is also possible that these functional foods contain significantly higher concentration of phytochemicals (such as flavonoids, phenolic acids) that may lead to many health enhancing benefits (e.g. antioxidant, anti-inflammation, anti-cancer, anti-obesity, cholesterol-lowering and anti-aging). However, we should establish a relatively comprehensive metabolite profiling of the sprouts and analyze the safety of these food in next way to develop this novel food and validate our proposition.

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