Processing of Pearl millet Grains to Develop Nutri-Smart Food

Overview

The world is facing agrarian as well as nutritional challenges, especially in developing countries. Today's world is at the edge of nutritional transition and its associated chaos: which has created the triple burden of under, over, and imbalanced nutrition. The day to day changes in climate have been alarming the resilience in the global food system. Although food grains such as wheat, rice and corn are globally the most popular staple food crops globally to feed more than 7 billion people, but little change in our routine diet including diversity in cereals and other food commodities can help us to achieve both the food as well as the nutritional security. India being a food secure country, is facing the major challenge of nutritional insecurity. The major reason that contribute to the multiple burdens of malnutrition are intake of high levels of under-nutrient foods, lack of availability, accessibility, stability, and utilization of the foods with vital nutrient profiles.

Millets are being nutritionally superior to rice, wheat, and maize in terms of protein, bioactive compounds, micronutrients, and dietary fibre can act as a potential alternative to alleviate malnutrition. Recognizing the potential of millets, the Government of India tagged millets as "nutricereals" and included them in the government flagship 'Poshan Abhiyan' to fight malnutrition and hidden hunger. Due to inherent capacity of superior nutrition, UN General Assembly adopts India-Led Resolution and declared year 2023 as the "International year of millets". With the need to increase pearl millet landraces production and to expand studies on potential genetic diversity impacts on nutritional components, this bulletin is mainly focusing on nutritional constituents (protein, starch, lipid, iron, zinc) as well as nutritional quality (protein digestibility and "sweetness" profiles) in pearl millet genotypes including Indian landraces. Besides highest production and productivity in the country, among millets, pearl millet play an important role in addressing hidden hunger because of its excellent nutritional quality which is higher than commonly consumed cereals, makes it more desiring for the tote of "smart-food."

Pearl millet – Gold mine of human nutrition

- Pearl millet has 56–65 % carbohydrates and has higher amount of amylose (20–22 %). The resistant starch content in the range of 2.8–5.1 % makes it suitable to   manage blood glucose levels   and alter gastrointestinal tract function in a manner that improves human health.

- Pearl millet dietary fibre (11.9–13.3%) serve as an important modulators of starch digestibility, and helpful in controlling   cholesterol level, coronary heart disease, stroke, hypertension and bowel health.

- Dietary fibres can slow the absorption of sugar and fat from food, and therefore helps to prevent spikes in blood sugar and blood fat after eating, possibly reducing the inflammatory response to food. Dietary fibres in pearl millet causes slow release of glucose, hence helps to attain lower glycemic index.

- Approximately 11 to 15% of protein in pearl millet has higher level of essential amino acids especially sulfur-containing amino acids such as methionine and cysteine range from 2.38 to 9.71 g/100g   which is usually deficient in cereal grains   owing to create smart food for complete quality protein.

- Considerably higher lipid content ranging from 5 - 7%, of which ~70% is PUFA especially 39–45 % linoleic acid (C18:2) and 21–27 % oleic acid (C18:1) which positively modulate human health and metabolism.

- Phenolics (~1478 µg/g) are the major bioactive compounds in pearl millet that are readily absorbed through intestinal tract walls. Due to their potential antioxidant property, they promote anti-inflammation capacity of human beings.

- Pearl millet is also a rich source of essential micronutrients such as iron (40 to 85 ppm), zinc (40 to 55 ppm) including copper, potassium, calcium, sodium, magnesium and phosphorus. These minerals are essential for human growth, development, maintenance of physical activity, work capacity, resistance to infection and improves immune system.

Nutritional Profiling of Pearl millet germplasm based on NIR Spectroscopy Prediction Model

- Near-infrared reflectance spectroscopy (NIRS) uses diffusely reflected near-infrared radiation of the electromagnetic spectrum (from 400 nm to 2500 nm), helps in speedy and precise determination of biochemical parameters for large germplasm.

- Modified Partial Least Squares regression based NIRS prediction models were used to assess various biochemical and nutritional parameters such as starch, resistant starch, amylose, protein, oil, total dietary fibre, phenolics, total soluble sugars, phytic acid for high throughput screening of pearl millet germplasm. Overall illustration of the experimental procedure is given in figure1.

- All these biochemical and nutritional parameters for 87 diverse pearl millet germplasm were assessed through NIRS prediction model and represented as range, mean value, and the standard deviation as presented in the table 1. The accuracy and predictive capacity of the models were evaluated using global statistical values like RSQ, slope, bias, RPD and SEP.

Table 1: Model statistics for the validation set

TRAIT	N	% RANGE (Calibration)	% RANGE (Predicted)	Math Treatment	$RSQ_{external}$	SLOPE	BIAS	SD	SEP (C)	RPD
Protein	34	8.91–18.15	7.96-17.00	4,5,4,1	0.929	1.011	0.039	1.928	0.538	3.58
Oil	34	5.24–9.99	6.12-9.23	4,5,4,1	0.909	1.076	-0.049	0.618	0.216	2.86
Total dietary fibre	34	7.68–16.18	8.46-17.27	4,5,4,1	0.922	0.966	-0.017	1.843	0.52	3.54
Starch	34	52.49–63.25	54.80-61.43	2,8,4,1	0.915	1.13	0.126	2.132	0.784	2.71
Amylose	34	19.88–26.50	20.24-26.36	2,8,4,1	0.903	1.118	-0.001	1.635	0.629	2.59
Resistant Starch	34	1.49–3.52	1.63-2.82	4,5,4,1	0.860	1.076	-0.037	0.331	0.146	2.26
Total Soluble Sugars	34	1.62–3.22	1.60-2.82	4,7,4,1	0.879	1.109	-0.037	0.253	0.108	2.34
Phenols	34	0.04–0.21	0.07-0.18	3,4,4,1	0.821	1.001	0.001	0.023	0.011	2.09
Phytic Acid	34	0.54–1.43	0.65-1.19	2,4,4,1	0.859	0.972	-0.005	0.157	0.062	2.53

RSQexternal = coefficient of determination for valiation; SD = standard deviation; SEP(C) = standard error of performance; RPD = residual prediction deviation; N=Number of samples; the values of traits are expressed as g/100 g.

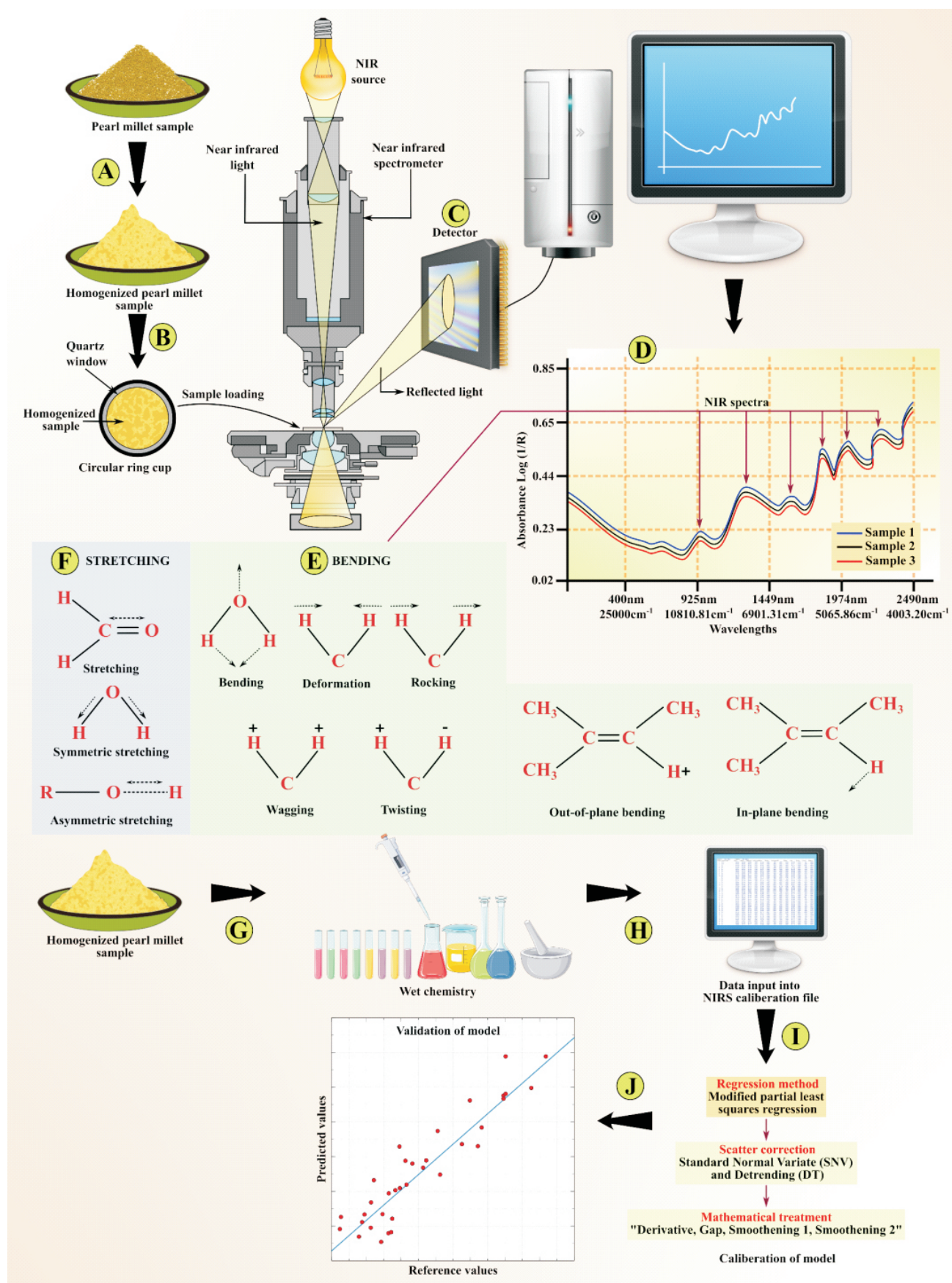The results were reproduced from Tomar et al. (2021)

Pearl millet sample

A

Homogenized pearl millet sample

B

Quartz window

Homogenized sample

Circular ring cup

Sample loading

NIR source

Near infrared light

Near infrared spectrometer

C

Detector

Reflected light

D

NIR spectra

Absorbance Log (1/R)

0.85

0.65

0.44

0.23

0.02

Sample 1
Sample 2
Sample 3

400nm
25000cm⁻¹

925nm
10810.81cm⁻¹

1449nm
6901.31cm⁻¹

1974nm
5065.86cm⁻¹

2490nm
4003.20cm⁻¹

Wavelengths

F STRETCHING

Stretching

Symmetric stretching

Asymmetric stretching

E BENDING

Bending

Deformation

Rocking

Wagging

Twisting

Out-of-plane bending

In-plane bending

Homogenized pearl millet sample

G

Wet chemistry

H

Data input into NIRS caliberation file

I

Regression method
Modified partial least squares regression

Scatter correction
Standard Normal Variate (SNV) and Detrending (DT)

Mathematical treatment
"Derivative, Gap, Smoothening 1, Smoothening 2"

Caliberation of model

J

Validation of model

Predicted values

Reference values

Figure 1: Overall illustration of the experimental procedure. (A) Pearl millet samples were ground, homogenized and sieved through 1 mm sieve; (B) The spectra were obtained by loading the homogenized sample in a circular ring cup with a quartz window (3.8 cm in diameter and 1 cm in thickness); (C) Detection of the reflected NIR light by the detector; (D) A typical NIRS spectrum indicating the peaks; (E) A molecule absorbs the incoming IR radiation which causes a change in bond length (stretching) and/or (F) bond angle (bending); (G) Wet chemistry analysis of homogenized pearl millet flour for nutritionally relevant biochemical parameters like starch, resistant starch, amylose, protein, oil, total dietary fibre, phenolics, total soluble sugars, and phytic acid; (H) Input of wet chemistry data into the NIR calibration file; (I) Calibration of model using modified partial least squares regression. This was followed by the application of various mathematical algorithms scatter correction and preprocessing the spectral data including the Standard Normal Variate (SNV) and Detrending (DT); (J) Representation of the prediction accuracy by scatter plot between the reference and predicted values.

Pearl millet – Protein Quality

- The protein quality depends on the content of amino acids especially the dietary indispensable amino acids, the physiological utilization of specific amino acids after digestion (or protein digestibility) as well as on the bioavailability of the amino acids.

- The nutritional quality of the dietary proteins can be assessed using a variety of different markers and approaches such as amino acid score (AAS), nitrogen balance(NB), protein efficiency ratio (PER), net protein ratio,(or retention) (NPR), net protein utilization (NPU), protein digestibility, biological value (BV) and Protein digestibility corrected amino acid score (PDCAAS).

The results were reproduced from Tomar et al. (2021)

- PDCAAS method was approved and recommended by FAO in 1991 for use in estimating protein quality and is the most widely used method and considers the availability of essential (indispensable) amino acids and protein digestibility. These two factors are the most important in protein evaluation.

- In this method of PDCAAS, at first, amino acid profile of a food protein is compared to a reference value and an amino acid score is determined by the ratio of the limiting amino acid content in the test protein to that of the reference protein. The amino acid score is then corrected by multiplying with digestibility (true digestibility, fecal digestibility or in vitro digestibility) of the protein to generate a PDCAAS value.

Pearl millet – Protein Quality

- Pearl millet genotypes were analysed for crude protein content and found protein levels in the range of 8.84 to 15.71 g/100g.

- The amino acid profiles of the pearl millet genotypes showed higher content of glutamic acid ranging from 17.58 to 22.614 g/100g, followed by leucine (7.714 to 11.865 g/100g), aspartic acid (6.265 to 10.774 g/100g).

- The most limiting amino acid was found to be lysine in all the varieties with the content ranging from 0.119 to 2.845 g/100g.

- Essential amino acid score (EAAS) was generated for pearl millet genotypes, wherein lysine has lowest EAAS of 2.39 out of allotted score i.e. 57 indicating extremely low availability of Lysine to full fill the basic requirement of the body.

- The daily value (DV) of EAA of pearl millet genotypes showed sufficient amounts of all the essential amino acids for adults as recommended by FAO 1991, except for lysine.
- The in vitro protein digestibility (IVPD) and PDCAAS of pearl millet genotypes was determined. The product of the IVPD and the lowest amino acid score were calculated to provide the PDCAAS values. PDCAAS values ranged from the 39.4% to 60.18%.

Table 2: Percentage Daily Value (% DV) of essential amino acids from pearl millet that an adult weighing 60 kg can obtain based on per capita consumption of 100 g of pearl millet.

	His %DV	Lys %DV	Leu %DV	Ile %DV	Thr %DV	Met + Cys % DV	Phen + Tyr % DV	Val %DV
HHB-67(IMP)	70.24	31.11	169.57	93.68	88.29	50.89	86.15	78.89
PC-443	64.16	21.22	149.74	92.08	86.91	43.89	79.68	68.02
MPMH-17	54.94	12.15	144.02	156.25	104.95	51.827	87.37	92.46
86M86	55.94	22.22	132.26	104.37	80.71	51.84	81.14	72.02
Dhanshakthi	67.47	13.22	117.96	106.54	82.14	48.44	86.25	80.44
HHB-299	54.94	12.15	144.02	156.25	104.95	51.87	87.37	92.46
AHB-1200	70.77	20.74	150.85	103.27	82.16	34.58	82.11	89.99
GHB-558	56.32	1.81	127.11	101.16	78.27	45.56	87.37	76.71
Chadi Bajri	57.28	13.76	139.58	138.79	104.85	40.66	70.19	76.51
Dhodhsar Local	58.66	10.55	132.03	109.88	82.19	42.18	75.47	81.81
PC-701	54.94	12.15	144.42	156.35	104.85	51.27	87.67	92.56
PC-612	63.49	11.93	158.52	103.17	82.46	61.85	84.34	87.26
Damodar Bajra	45.34	22.56	139.71	85.23	88.33	33.56	78.48	82.73
Chanana Bajra	61.88	13.87	166.18	141.89	81.26	44.56	82.01	89.89
Gadhwal ki Dhani	70.67	13.76	110.22	176.99	96.92	44.47	126.64	96.86

Pearl millet – Starch quality

- Pearl millet genotypes were analysed for starch quality as well as their inherent glycemic potential.

- Optimized in vitro assay mimicking gastro-intestinal (GI) simulation developed, was used as a tool to analyse nutritional starch fractions – rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS).

- Higher proportion of SDS and RS was found as compared to white rice.

- Starch digestograms revealed 43-68% starch bio-accessibility (Figure 2).

- To unravel the observed low digestibility, molecular structure of starch contributed by amylose and amylopectin in terms of crystallinity was characterized.

- X-ray diffraction (XRD) analysis revealed millet starch with distinct 2θ angles at 14.7°, 17.5°,20.7°, 24.3°, 26.5°, and 29.9°. The observed A type crystallinity was well correlated with the digestibility profile.

- The observed low inherent glycemic potential owing due to millet phenolics were further validated by using hepatocyte model which validated the role of phenolics in intracellular uptake and glucose transporter (GLUT) expression.
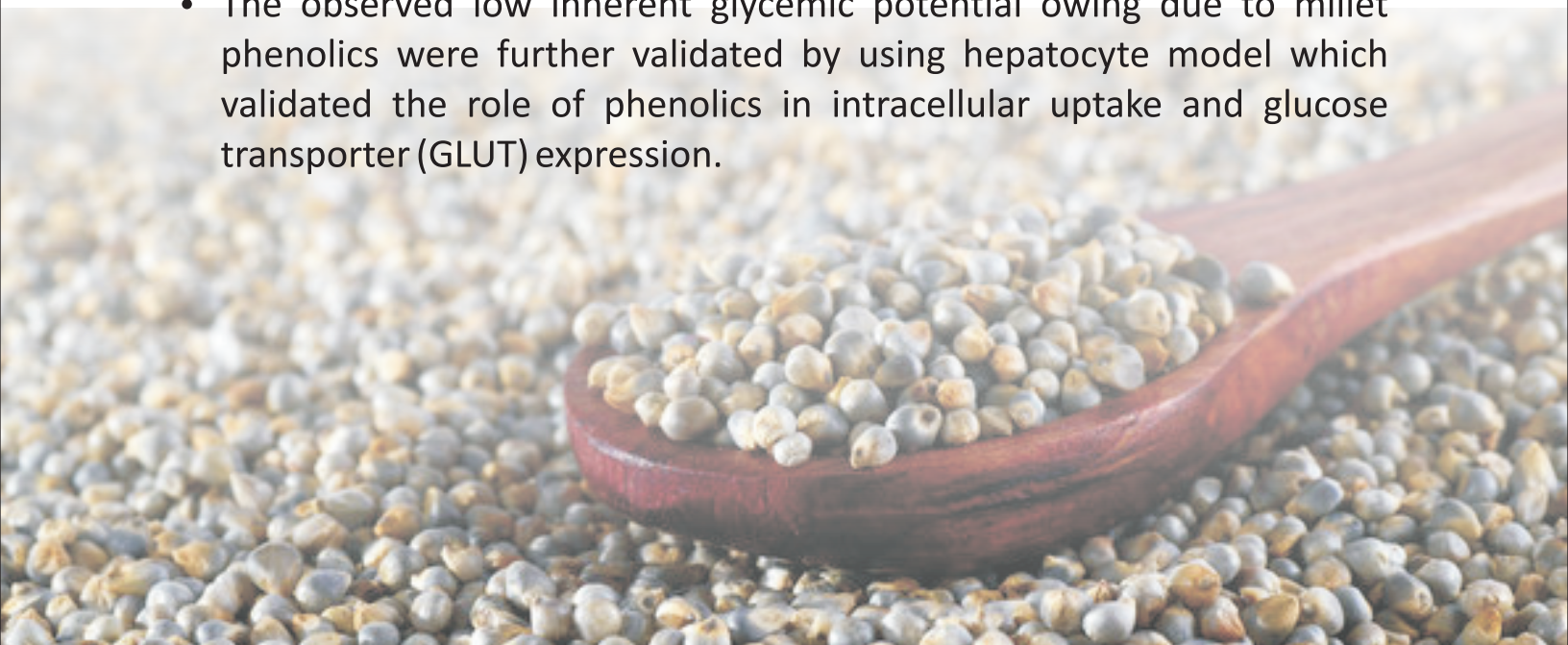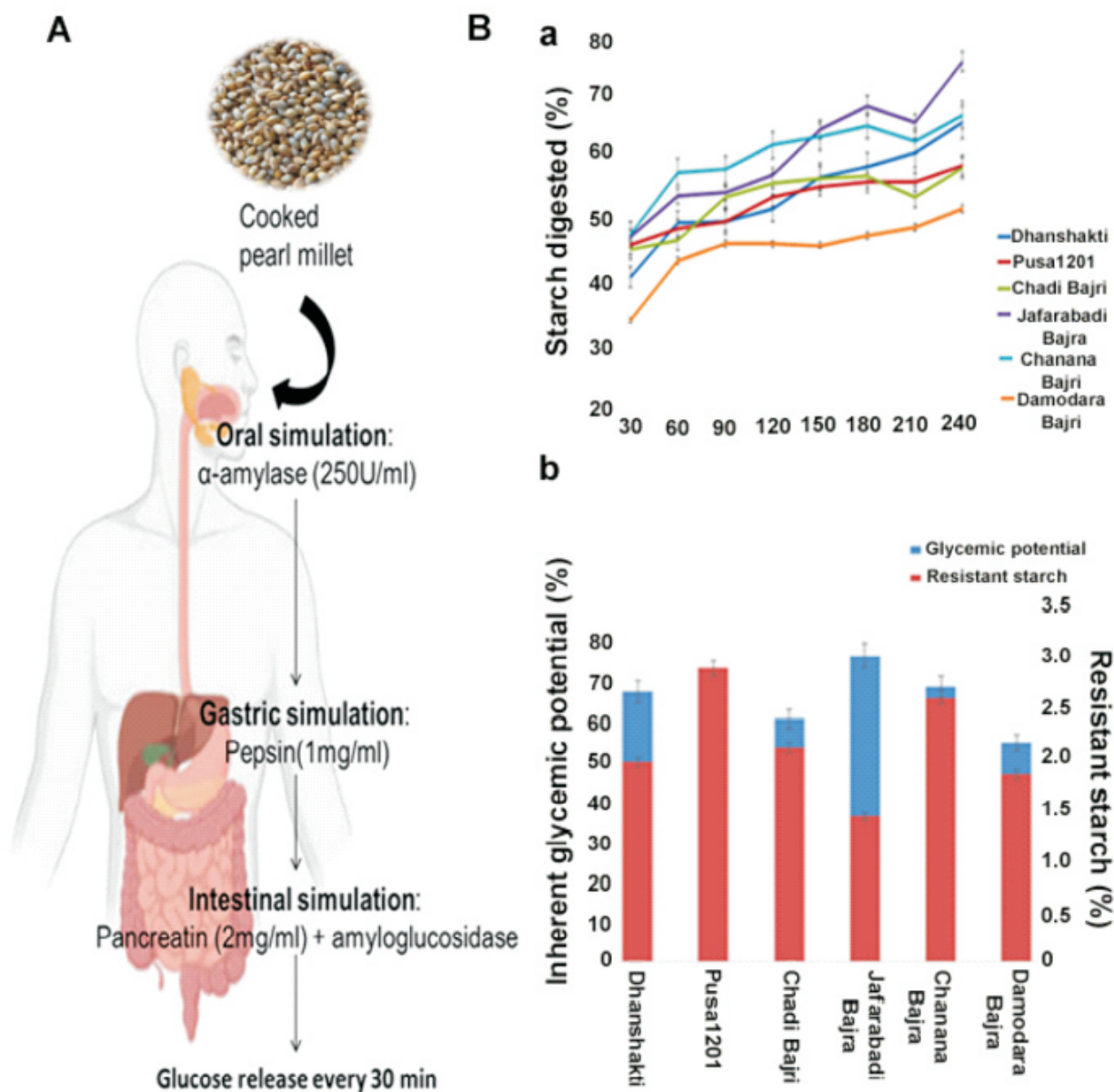
Figure 2: In vitro starch digestibility endorsing inherent glycemic potential (IGP) and resistant starch (RS) content in pearl millet (PM) genotypes. A. Flow chart of in vitro starch hydrolyzation kinetics (SHK) simulating human oro-gastro-intestinal digestion. B. (a) Starch digestogram depicting the time course millet starch digestion from 30 to 240 min using in vitro SHK based on glucometry. (b) Comprehensive histogram depicts the variations in in vitro glycemic potential calculated based on the proportion of rapidly digestible starch (RDS) normalized with total starch content (TSC) along with RS content. Results are expressed in percentage and are mean ± SE of three independent experiments.

The results were reproduced from Veda et al. (2021)

Pearl millet lipid quality

- Lipid content and composition was analysed in pearl millet genotypes and it ranged from 3-7.5 %.

- The fatty acid profiling revealed 65 to 70% unsaturated fatty acids (USFA) of total lipid present in pearl millet genotypes.

- Unsaturated fatty acid composition showed highest content of linoleic acid (C18:2) followed by oleic acid (C18:1). Saturated fatty acid composition revealed highest amount of palmitic acid (C16:0) followed by negligible amount of stearic acid (C18:0) and arachidic acid (C20:0).

- Among the available FAs in PM, linoleic acid majorly contribute in developing oxidative rancidity in the flour.

Landraces posses superior nutritional characteristics

- With the need to increase pearl millet landraces production and to expand studies on potential genetic diversity impacts on nutritional components, this bulletin is mainly focusing on nutritional constituents (protein, starch, lipid, iron, zinc) as well as nutritional quality (protein digestibility and "sweetness" profiles) in the Indian landraces of pearl millet, especially "Jafrabadi" from Gujrat.

- All the nutritional profiles/quality resides in "Jafrabadi" was compared with the other landraces from Rajasthan such as Chanana Bajri, Chadi Bajri and Damodar Bajri.

- Jafrabadi landrace was distinctive for significantly higher levels of starch (4 - 8 %), amylose (2 – 4%) and reducing sugars (8-12 %), as compared to landraces from Rajasthan (Table 3).

- Thus, these results depicting an unique characteristic of "sweetness", due to higher levels of reducing sugars and it stimulates the taste sensation as salivary amylase depolymerize it into glucose (Figure 3).

- Although, total protein content (~12 %) was on par with the other landraces, In vitro protein digestibility (IVPD) was found to be lower (4 – 9 %) but retained higher total essential amino acid content upto 3 % (Table 1).

- The essential amino acid composition analysis revealed significant levels of sulfur-containing amino acids including methionine and cysteine, ranging from 2.38 to 9.71 g/100g of flour, with an essential amino acid score ranging from 95 % to 388.40 % in all the landraces.
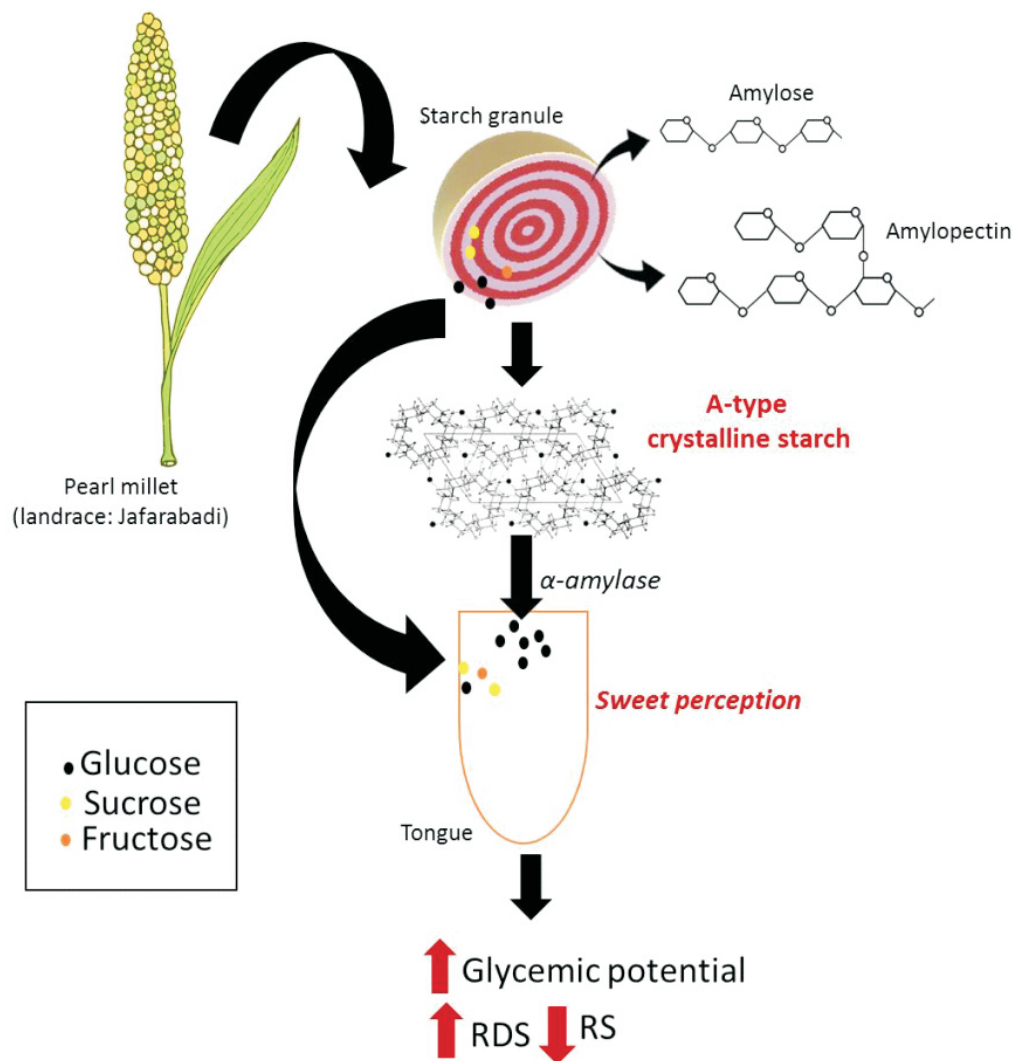
Figure 3: Starch components in the matrix of grains/flour that affect the "sweetness" profile. Fast releasing carbohydrates like reducing sugars and starch stimulates the taste sensation as salivary amylase depolymerize it into glucose. Based on the structural integrity of starch which results in its digestibility, starch fractions have been classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The time course dependent digestion of these starch fractions resulted in ultimate increased sweetness and glycemic amplitude which majorly depends on its molecular crystallite structure.

The results were reproduced from Veda et al. (2021)

Table 3: Nutritional Characteristics of pearl millet genotypes

Pearl millet Landraces	Carbohydrate					Protein			Minerals	
	Starch (%)	Starch Quality***				Protein (%)	Protein Quality**		Fe (ppm)	Zn (ppm)
		Reducing Sugars (%)	Amylose (%)	Resistant starch (%)	In vitro glycaemic potential		Protein Digestibility (%) (PDCAAS)	Essential amino acid content (%)		
Jafrabadi (Gujrat)	67.80	2.75	27.77	1.42	77.05	12.25	47.04	36.94	56.66	50.0
Chanana Bajri (Rajasthan)	61.78	0.72	25.87	2.58	69.03	12.54	51.14	38.56	37.00	33.0
Chadi-Bajri (Rajasthan)	58.97	0.70	24.08	2.09	61.11	12.25	55.15	34.75	39.00	30.0
Damodar Bajri (Rajasthan)	59.75	0.67	23.56	1.83	55.10	10.50	58.82	34.00	35.00	34.0
Pusa 1201 (Pusa Hybrid)	59.96	2.30	-	2.87	61.5	15.45	45.81	63.27	56.00	45.0
Dhanshakti (ICRISAT-Composite)	57.51	2.76	24.50	1.95	67.8	11.66	47.62	33.97	83.00	44.0

*Rancidity: Measured as Comprehensive Peroxide Value (CPV) after 10th day of milling. Based on CPV10, all the landraces are in Green category (CPV10; Green: up to 60; Orange: 61-90; Red 90 and above). Lower the value, better is the keeping quality.

**Protein Quality: Measured by keeping milk protein as reference. Protein digestibility analysed using PDCAAS [(Protein Digestibility Corrected Amino Acid Score) and total EAA (Essential Amino Acid) content denotes the sum of all EAA (Arginine, Histidine, Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine). With reference to milk protein (protein digestibility – 100 % and total EAA content - 100%).

*** Starch Quality: High reducing sugar and high glycaemic potential makes Jafrabadi sweeter.
 Note: Results represented here are the mean values of two biological and three technical replicates of single experimental study, done in pearl millet flour.

Genetic potential of Pearl millet landraces to develop low rancid varieties

- Lipids, its composition and lipid hydrolysing enzymes such as lipase and lipoxygenase are the key determinants of off flavour development in pearl millet flour.

- Four pearl millet landraces (Jafrabadi, Chanana Bajra-2, Chadi Bajri, and Damodara Bajri) were analysed for off flavour development and nutritional quality, and their results were compared to a bio fortified pearl millet PM variety (Dhanshakti).

- All four landraces showed longer shelf life and have better shelf life.

- Linoleic acid, the main substrate for the lipoxygenase (LOX) enzyme, was found to be lower in the landraces than Dhanshakti.

- Due to the limited availability of the substrate, the activity of LOX in landraces was shown to be substantially lower.

- Comparatively low levels of key micronutrients (iron and zinc) were found in landraces than biofortified varieties, with an exception of the landrace- Jafrabadi.

- Pearl millet landraces are potential genetic material to develop a low-rancid with nutritionally superior variety of PM.
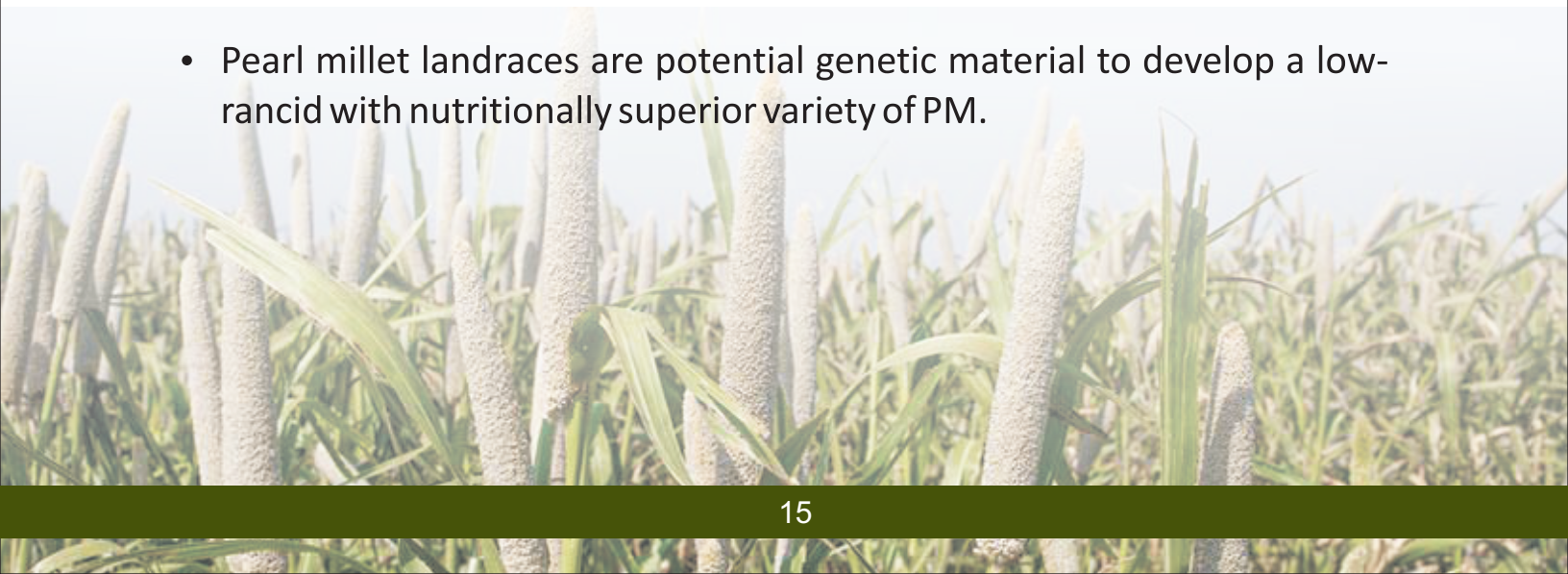
Pearl millet processing to improve shelf life

- Pearl millet is a nutrient-dense cereal, however its flour is underutilised due to off flavour develops during storage.

- Pearl millet grains were subjected to a preliminary hydro-treatment (HT). Subsequently, the hydrated grain-wet flour have undergone individual and combined thermal treatments viz., hydrothermal (HTh) and thermal near infrared rays (thNIR) (Figure 4).

- Effects of these thermal treatments on the biochemical process of hydrolytic and oxidative rancidity were analyzed in stored flour.

- A significant ($p < 0.05$) decrease in the enzyme activities of lipase (47.8 %), lipoxygenase (84.8 %), peroxidase (98.1 %) and polyphenol oxidase (100 %) in HT-HTh-thNIR treated flour was found compared to the individual treatments was documented.

- Upon storage (90 days), decline of 67.84 % and 66.4 % of free fatty acid and peroxide contents were observed in flour under HT-HTh-thNIR treatment without altering starch and protein digestibility properties.

- A significant increase in the starch digestibility was observed in HT-HTh 486 (67.17 %) and HT-thNIR (65.18 %) treated flours compared to HT-HTh-thNIR (57.28 487 %) and native flours (55.91 %).

- No significant difference in RDS was observed 488 between HT-HTh-thNIR treated and native flour, where as increased content of RDS 489 was found in HT-HTh (52.61 %) and HT-thNIR (51.13 %).
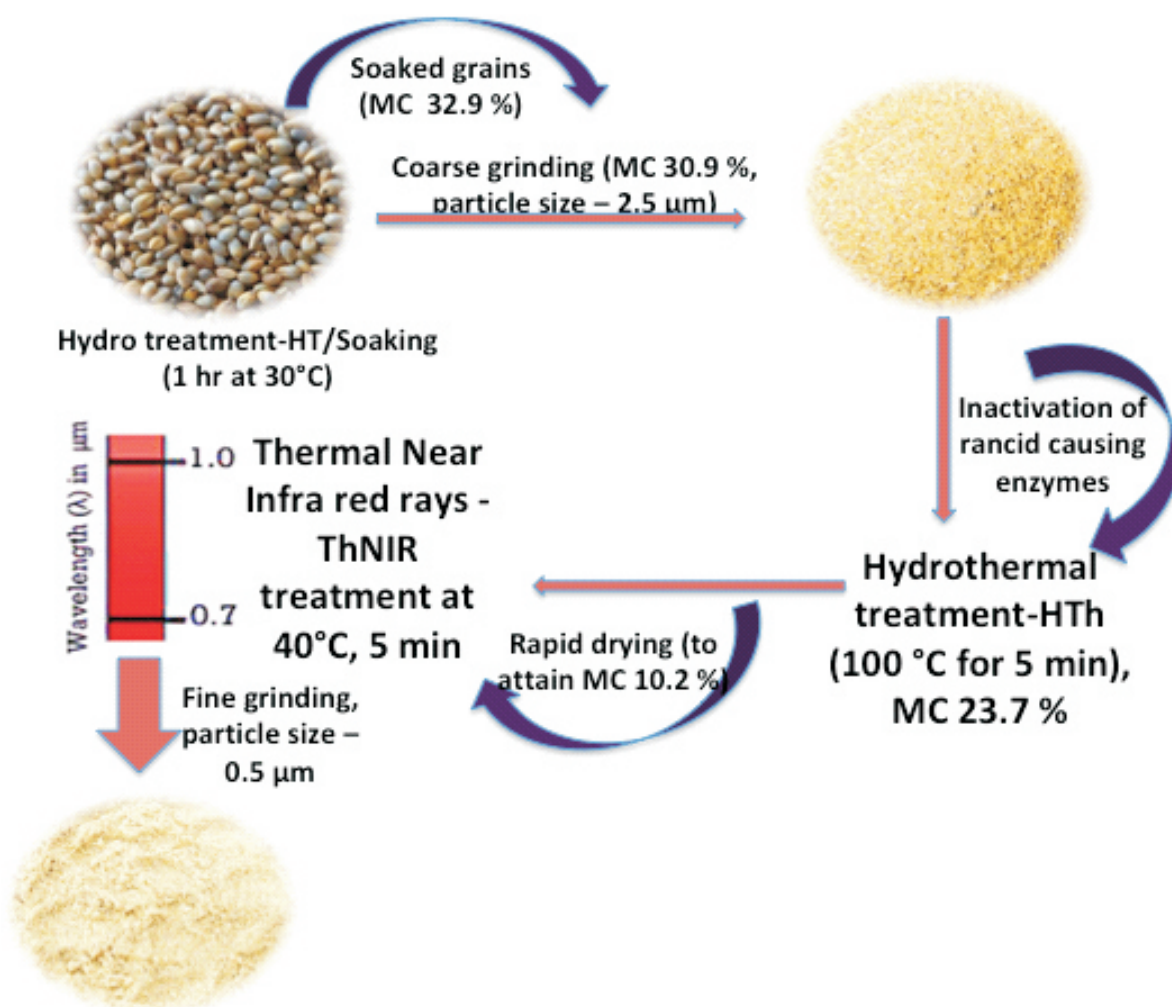
Results reproduced from Vinutha et al. (2022)

Figure 4: Three step treatment of soaking, hydrothermal (HTh) and near infrared (NIR) rays. Initially, grains were soaked (MC -32.9 %) for one hour and wet milled to a coarse particle size of 2.5 µm (MC – 30.9 %) and subjected to HTh treatment of 5 min at 100°C (MC -23.7 %) followed by thNIR treatment for 5 min to bring down the MC < 14 %. These treated samples were finely ground to a particle size 0.5 µm for all the biochemical and physico-chemical and structural analysis.

Results reproduced from Vinutha et al. (2022)

Fortification of Pearl millet : a new alternatives to healthier bakery products

Vital wheat gluten fortified Soft Bajra Atta

- Pearl millet is nutri-rich cereal, due to lack of good dough making quality. its not popular among consumers, which limits its use in bakery products and in chapatti making.

- Soft Bajra Atta was developed to solve this issue by reconstituting vital wheat gluten (VWG) in bajra flour to enhance viscoelastic properties for improved dough and chapatti making quality as good as wheat.

- The regeneration was optimized with different percentage of VWG in bajra flour and tested for dough quality parameters.

- Soft Bajra Atta developed by the effort of the research team at Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi has the potential to move as a sustainable alternative to wheat in many baked and cooked preparations; as a new staple food owing to its easy availability throughout local markets, supermarkets and e-commerce sites.

- This product not only can positively impact these markets but also has the potential to address nutritional challenges, as the hidden hunger and malnutrition is associated with the huge investment from the Indian government with the estimated economic cost of 0.8 to 2.5 per cent of the total GDP which is equivalent to $15–46 billion.

HEALTHY INDIA

Towards
Mission
Healthy India