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EFFECT OF INCORPORATION OF GERMINATED MILLET FLOUR ON IRON BIOACCESSIBILITY AND GLYCEMIC INDEX IN RTS FRUIT BEVERAGES

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ABSTRACT

The study was carried out to formulate and evaluate the suitability of different germinated millet flour incorporation in mango, pineapple and pomegranate ready-to-serve (RTS) beverages. Incorporation of germinated foxtail millet or little millet flour @ 1% in mango, pineapple and pomegranate RTS beverages showed high dialyzable iron in germinated foxtail millet incorporated (GFMI) mango (2.47 µg mL^{-1}), GFMI pineapple (2.68 µg mL^{-1}) and GMFI pomegranate (1.65 µg mL^{-1}) RTS beverages as compared to the germinated little millet incorporated (GLMI) mango (1.51 µg mL^{-1}), GLMI pineapple (1.11 µg mL^{-1}) and GLMI pomegranate (1.38 µg mL^{-1}) RTS beverages and respective control samples. Similarly, predicted glycemic index (GI) was 95.76 to 105.91, 93.86 to 103.90 and 98.62 to 110.60 in germinated foxtail millet flour, little millet flour incorporated beverages and their control samples, respectively; even though the GI of all the samples did not fall under low GI range. The findings suggest that germinated millet flours can be incorporated to increase dialyzable iron content and reduce GI of fruit beverages with further increasing the incorporation amount in fruit beverages and validation with adequate *in vivo* studies.

Keywords: Bioaccessibility, bioactive compounds, flour, fruit beverage, germinated millet, glycemic index, iron

INTRODUCTION

Health benefits of the consumption of fruits and their juices have been reviewed extensively (Henning *et al*., 2017; Angelino *et al*., 2019). The health benefits are attributed to the bioactive compounds such as phenolic acids, flavonoids, flavanols, terpenoids, dietary fibres, phytosterols, vitamins and minerals present in fruits and their juices (Zhan *et al*., 2017). Consumption of fruits and their juices have shown positive impact on human health. Many studies have shown that consumption of fruit juices helps in alleviating high blood pressure (Asgary *et al*., 2014; Kent *et al*., 2016), cardiovascular diseases (Scheffers *et al*., 2019) and micronutrient deficiency disease conditions (Widyaningsih *et al.,* 2017). In this context, consumers now prefer to consume fruit beverages with the ingredients which can provide additional benefits such as immunity enhancement, weight management, blood sugar management and other health advantages. The demand for fruit juice is increasing due to the availability of fortified juices with vitamins, minerals and bioactive rich ingredients. Many juice manufacturing industries have developed nutritionally rich packaged fruit beverages to meet the consumer's expectations *viz.*, mixed fruit juice with added vitamin D_2, orange juice with added dietary fibre and/or omega fatty acids, etc.

Functional foods with high consumer-preference include beverages because of the reasons like i) convenience and possibility to meet consumer's demand for container contents, size, shape, and appearance; ii) effortless distribution and better storage for refrigerated and shelf-stable products; and iii) the great opportunity to incorporate targeted nutrients and bioactive compounds (Sanguansri and Augustin, 2010; Wootton-Beard and Ryan 2011; Kausar *et al.,* 2012). Globally, functional beverage market size reached at US $ 206.41 billion in 2022 with a cumulative annual market growth of 6.64% which is projected to reach US $ 385.94 billion by 2032 (*https://www.precedenceresearch.com;* accessed on 18.4.2024). The addition of non-traditional ingredients such as minerals, probiotics, and various natural ingredients is in demand as functional beverages.

Owing to the nutritional quality and health benefits, Food and Agriculture Organization announced the year 2023 as 'International Year of Millets'. Millets are commonly referred to as "small seeded grasses" which are considered as high drought-tolerant crops. These includes pearl millet (*Pennisetum glaucum* L., finger millet (*Eleusine coracana* L.), foxtail millet (*Setaria italica* L.), proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum*), and little millet (*Panicum sumatrense*). Millets play a vital role in food and nutritional security of developing countries in Asia and Africa. However, the cultivation and consumption has declined due to the emergence of high yielding cereal crops. Therefore, more emphasis is given to increase its production and consumption. Many studies have reported that millets are very good source of protein, fat, minerals, vitamins and fiber, and many bioactive compounds having high antioxidant activity (Panwar *et al*., 2016; Sharma *et al*., 2018; Ofosu *et al*., 2020). Studies have shown that different millets have glycemic index (GI) ranging from 55-69, which is much lower than the polished rice and refined wheat flour (Anitha *et al*., 2021). Germination enhances the biochemical processes resulting in improved nutraceutical properties, especially in cereals and pulses.

Iron deficiency anaemia is a global public health concern. Besides crop biofortification, many processed foods have been used to fortify them by synthetic iron to increase iron intake (Siddique and Park, 2019; Blanco-Rojo *et al.,* 2019). Conventional iron supplements induce some adverse reactions in human body. Thus, there is need to explore and develop novel or existing products with high iron bio-availability/bioaccessibility. So, emphasis is laid on to identify natural fortificants to enhance iron content and its bioaccessibility to avoid side-effects like weakening of bone metabolism and infection due to high synthetic iron intake (Man *et al.,* 2022). Also, suitable fortifying vehicle is an important factor for iron fortification as iron compounds tend to interact with food (Shubham *et al.,* 2020). The dialyzability of iron compound, whether fortified with iron or present in food itself, is considered as an improved approach of solubility method where the levels of dialyzable iron are measured. Hence present study was aimed to explore the possibility of utilizing germinated millets in fruit beverages. However, development of any new product should possess acceptable organoleptic properties. Therefore, an attempt was made in this study to incorporate germinated millet flour into mango, pineapple and pomegranate RTS beverages to ascertain whether the addition of germinated millet flour could enhance the bioactive compounds, increase iron bioaccessibility and decrease GI of beverage. This study also provides information on the suitability of fruit beverages for incorporating germinated millet flours to attain organoleptically acceptable product with millet component for consumers.

MATERIALS AND METHODS

Materials and chemicals

Whole seeds of foxtail (*Setaria italica* L.) and little millets (*Eleucine coracana* L.) were procured from a local market in Bengaluru, India and stored under cool and dry conditions until use. Mango cv. 'Alphonso' was collected from the experimental field of ICAR-Indian Institute of Horticultural

Research, Bengaluru. Pineapple, pomegranate and refined cane sugar were procured from the local market. Sucralose was procured from ProFoods^{TM} online through Amazon India. The chemicals, standards and enzymes used in the study were of analytical grade procured from Himedia Laboratories Pvt. Ltd (Bengaluru, India), Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and Sigma Aldrich-Merck (St. Louis, Missouri, USA). Dialysis membrane (MWCO 6-8 kD, 20.4 mm diameter, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) used for *in vitro* study was procured from Serva Electrophoresis GmbH D-69115 Heidelberg, Germany. Double distilled water from Millipore was used for analysis of different parameters.

Preparation of germinated millet flour

Whole seeds of foxtail millet and little millet were cleaned and washed with potable water to remove any adhering dirt/debris. The surface contaminants were further reduced through a hot water dip treatment at 85°C for 40 sec., followed by immediate cooling using cold water (25±1°C) for 1 min. Chemical sanitation was subsequently carried out by soaking one part of whole millet seeds in three parts of sodium hypochlorite solution (2000 mg available chlorine L^{-1}) for 2 h. After decanting the chlorinated water, millets seeds were soaked in water (RO) [1:5] for 22 h (Bari *et al*., 2010). Millet seeds were removed from the soaked water and spread in a thin layer on a wet blotting paper on a perforated stainless-steel tray covered by another similar tray. Subsequently, trays were kept at a temperature of 28±1°C and 90±1% relative humidity for 48 h in a germination chamber. After germination, the millets were dried in hot air oven at 50±1°C for 5 h. Rootlets and shoots were removed by manual method followed by sieving through 1 mm^2 mesh sized sieve. Then germinated millets were ground into fine flour using blender (Usha Int Ltd, New Delhi, India).

Preparation of Ready-to-Serve (RTS) fruit beverage matrices

The fruit beverages were prepared using a previously standardised recipe developed in the laboratory as per the organoleptic acceptance by the panellists. The ingredients used were respective fruit pulp/ juice, cane sugar/sucralose, water, and citric acid to obtain the composition given in Table 1. Preliminary trials on replacing sucrose with sucralose resulted in non-acceptance of mango and pineapple beverages; however, pomegranate beverage was accepted by the panellists, with sucralose at a concentration of 17 ppm. Hence in pomegranate beverage sucralose was used as a sweetener in in millet flour incorporated beverages.

Table 1: RTS beverage formulation standardized for experiment by incorporating germinated millet flours

RTS beverage formulation	Pulp/ juice (%)	TSS adjusted with sucrose (˚Brix)	Acidity (% citric acid)	Germinated foxtail millet flour (%)	Germinated little millet flour (%)
MC	25	18	0.2	-	-
MFT	25	18	0.2	1	-
MLT	25	18	0.2	-	1
PC	20	18	0.2	-	-
PFT	20	18	0.2	1	-
PLT	20	18	0.2	-	1
PoC	30	15	0.3	-	-
PoFT	30	*	0.3	1	-
PoLT	30	*	0.3	-	1

*Sucralose 17 mg L^{-1}

MC: Mango RTS beverage control; MFT: Mango RTS beverage with germinated foxtail millet flour; MLT: Mango RTS beverage with germinated little millet flour. PC: Pineapple RTS beverage control; PFT: Pineapple RTS beverage with germinated foxtail millet flour; PLT: Pineapple RTS beverage with germinated little millet flour. PoC: Pomegranate RTS beverage; PoFT: Pomegranate RTS beverage with germinated foxtail millet flour incorporated; PoLT: Pomegranate RTS beverage with germinated little millet flour.

Incorporation of germinated millet flour in fruit beverage matrices

Germinated foxtail millet flour and germinated little millet flour was added at 1% concentration separately during preparation. Initially the concentration of millet flour addition was made up to 3% and subjected to sensory evaluation. However, above 1%, the addition was found unacceptable by the panellists due to the change in taste, flavour, colour and viscosity of beverages. Hence, addition of 1% germinated millet flours was selected for further studies. The prepared RTS mango, pineapple and pomegranate beverages incorporated with millet flour were pasteurized and stored at ambient temperature for analysis.

Biochemical parameters

TSS and titratable acidity: The total soluble solids (TSS) of beverage samples were measured by using digital refractometer (Digital refractometer PAL-3, ATAGO, Japan). The titratable acidity was estimated titrimetrically (Ranganna,1986).

Total polyphenols and antioxidant activity: Total polyphenols were determined as per Panwar *et al.* (2016) and the results expressed as gallic acid equivalent (GAE). Free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as per the method of Kang and Saltveit (2002) and the results expressed as ascorbic acid equivalent antioxidant capacity (AEAC). The reducing power assay was assessed as per the method of Benzie and Strain (1996) and results expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

Iron content: The iron concentration in digested samples were determined using atomic absorption spectrophotometer (Analyst 200 Perkin Elmer) as per the standard method (AOAC, 1995).

***In vitro* bioaccessibility of iron**: Bioaccessibility of iron was determined as per Haro-Vicente *et al.* (2006) in the sample *in vitro*.

Preparation of dialysate: For this, 10 g homogenous beverage sample was taken in 50 mL centrifuge tube. The pH of sample was adjusted to 2 with 0.05 N HCl and 1 mL pepsin digestion mixture (4 g porcine pepsin was suspended in 0.01 N HCl and diluted to 100 mL with 0.1 N HCl) added to it before incubating in shaking water bath for 2 h at 37°C. After incubation, the dialysis bag containing 20 mL of PIPES buffer [piperazine-NN-bis (2-ethane-sulfonic acid)] disodium salt dissolved in 100 mL water; 0.15 N, pH 8.5] was placed into each centrifuge tube and then incubated for 30 min. Then 5 mL pancreatin-bile mixture (0.5 g porcine pancreatin and 3.0 g bile extract dissolved in 100 mL of 0.01 N $NaHCO_3$, volume was made up to 250 mL with 0.1 N $NaHCO_3$) was added and incubated for 2 h in shaking water bath at 37°C. Then dialysis bag was removed and rinsed by dipping in DW.

Measurement of total iron in dialysate: Dialysate (2 mL) was taken in a test tube and 1 mL reducing protein precipitation solution (100 g trichloroacetic acid and 50 g hydroxylamine monohydrochloride dissolved in 500 mL distilled water (DW) to which 100 mL concentrated HCl was added. The final volume was made up to 1 L with DW. The test tubes were incubated at room temperature overnight; and centrifuged at 2575 rpm for 10 min at 17°C. Then 1 mL aliquot was taken into separate test tubes in duplicate, to which 0.25 mL ferrozine chromazen solution (125 mg ferrozine [3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonicacid)-1,2,4-triazine] disodium salt dissolved in 25 mL DW to reach a concentration of 5 mg mL^{-1}) and 2 mL HEPES buffer {[N-2-hydroethyl-piperazine-N-2-ethanesulfonic acid] sodium salt dissolved in 100 mL DW, 0.3 N, pH 8.5} was added and the mixture incubated for 1 h at room temperature before measuring absorbance at 562 nm.

Measurement of ferrous iron in dialysate: For this, the above procedure of total dialysate was followed but in place of reducing protein precipitation solution, non-reducing protein precipitation solution (100 g trichloroacetic acid dissolved in 500 mL DW to which 100 mL of concentrated HCl was added making the final volume to 1 L with DW) was added, incubated overnight and measured at 562 nm. The total dialyzable ferrous iron in the samples was calculated as follows:

$$\text{Dialyzable ferrous iron } (\mu g/mL) = \left(\frac{\text{Ferrous iron in dialysate } (\mu g/mL)}{\text{Total iron in the sample } (\mu g/mL)}\right) \times 100$$

$$\text{Bioaccessibility } (\%) = \left(\frac{\text{Dialysable ferrous iron}}{\text{Total iron in the sample}}\right) \times 100$$

Starch hydrolysis and in vitro glycemic index (GI)

Starch hydrolysis rate in millet flour incorporated beverages was estimated as per Goni *et al.* (1997) and Oboh *et al.* (2015). Beverage (50 mg) was taken in a 50 mL graduated test tube and 10 mL HCl-KCl (pH, 1.5) containing 1 mg pepsin was added to it before incubation in a shaking water bath for 1 h at 40°C. Then the volume was made up to 25 mL with 5 mL phosphate buffer (pH 6.9) containing 2.6 IU α-amylase and the solution was incubated in a shaking water bath for 3 h at 37°C. During this period, 0.1 mL sample was taken into a separate test tube at every 30 min interval starting from 0 to 180 min and the enzyme was inactivated by keeping the test tube in a boiling water bath for 15 min and transferred to refrigerator until the end of 180 min. Further, 1 mL sodium acetate buffer (pH 4.75) was added to test tubes, then 30 µL sodium acetate buffer containing 40 IU amylo-glucosidase was added and incubated for 45 min at 60°C. To these test tubes 200 µL dinitrosalicylic acid colour reagent was added and placed in boiling water bath for 5 min. The test tubes were cooled to room temperature and 5 mL DW added, centrifuged at 1200 rpm at 17°C and supernatant read at 540 nm. The rate of starch digested was expressed as percentage of starch hydrolysed per unit time. Glucose was used as a standard. Hydrolysis index (HI) was calculated as AUC (area under the curve) of test sample to AUC of white bread expressed in percentage (Goni *et al*., 1997). The estimated glycemic index (eGI) was calculated as per the equations given by Goni *et al*. (1997) and Granfeldt *et al.* (1992).

$$eGI_G = (0.549*HI) + 39.71$$ (Goni *et al*., 1997)

$$eGI_{Gr} = (0.862*HI) + 8.198$$ (Granfeldt *et al*., 1992)

Sensory evaluation

Sensory evaluation was performed by panellists comprising of scientists and research scholars of laboratory. The panellists included healthy male and female aged between 25 and 55 years. The samples were assigned codes and evaluated by participants 2-3 h after having morning breakfast. All the participants were previously exposed to different fruit beverages. Participants were asked to assess the beverages based on colour, flavour, mouthfeel, taste and overall acceptability using a 9-point hedonic scale comprisiing of: dislike extremely = 1, dislike very much = 2, dislike moderately = 3, dislike slightly = 4, neither like nor dislike = 5, like slightly = 6, like moderate = 7, like very much = 8 and like extremely = 9 (Larmond, 1977).

Statistical analysis

All the experiments were conducted in triplicate in a completely randomized design. Statistical analysis was performed by one-way analysis of variance (ANOVA) and the Fisher's LSD values were computed at $P_{0.05}$. Statistical analysis was performed by using SPSS statistical software (IBM SPSS Statistics 22.0. for windows, Armonk, NY: IBM Corp)**.**

RESULTS AND DISCUSSION

Biochemical and bioactive compounds in germinated millet flour incorporated fruit beverages

The biochemical and bioactive compounds in germinated millet flour incorporated RTS beverages is given in Table 2. Total soluble solids (TSS) and titratable acidity are important quality parameters that decide acceptability by the consumers. Appropriate blend of sugar and acidity enrich the taste of any fruit beverage. Acidity and TSS was adjusted in both control and treated samples. Total polyphenols ranged from 93.63 to 114.40 mg GAE 100^{-1}g in mango beverage and 61.85 to 68.67 mg GAE 100^{-1}g in pineapple beverage. Significant difference ($p<0.05$) was observed for polyphenol content in mango beverage but there was no significant change ($p<0.05$) in polyphenol content due to millet incorporation in pineapple beverage. The total antioxidant activity of MFT and MLT beverage was 12 and 15% high, respectively, as compared to MC which ranged from 0.41 to 0.47 mg AEAC g^{-1}. The antioxidant capacity of foods largely depends on many individual or combined antioxidants such as ascorbic acid and phenolic compounds. Similar to mango beverage, the millet incorporation in pineapple beverage also improved the antioxidant activities by 20% [from 0.50 mg AEAC g^{-1} in control to 0.60 mg AEAC g^{-1} in treated samples]. In case of pomegranate, treatments PoC, PoFT and PoLT showed significantly ($P < 0.05$) variable TSS due to the reason that PoC was prepared by adding cane sugar (sucrose) whereas sucralose was used in PoFT and PoLT treatments. Moldovan and David (2020)

Table 2: Biochemical and bioactive compounds composition in germinated foxtail and little millet flour incorporated mango, pineapple and pomegranate RTS beverages

Parameters	Mango			Pineapple			Pomegranate		
	MC	MFT	MLT	PC	PFT	PLT	PoC	PoFT	PoLT
TSS (°Brix)	18.10[c] ± 0.0	18.73[a] ± 0.05	18.53[b] ± 0.11	18.4[c] ± 0.0	19.4[a] ± 0.0	19.6[a] ± 0.0	16.16[a] ± 0.05	6.36[b] ± 0.05	6.20[c] ± 0.00
Titratable acidity (%)	0.22[a] ± 0.01	0.22[a] ± 0.00	0.18[b] ± 0.00	0.23[a] ± 0.01	0.18[b] ± 0.03	0.16[b] ± 0.02	0.32 ± 0.00	0.34 ± 0.03	0.32 ± 0.00
Total polyphenols (mg GAE 100 g^{-1})	114.40[a] ± 0.11	106.88[b] ± 0.11	93.63[c] ± 0.23	61.85[ns] ± 4.86	68.67[ns] ± 1.84	68.18[ns] ± 3.80	251.89[a] ± 0.57	206.39[b] ± 1.51	198.48[c] ± 2.28
DPPH (mg AEAC g^{-1})	0.41[c] ± 0.00	0.45[b] ± 0.00	0.47[a] ± 0.00	0.50[b] ± 0.0	0.60[a] ± 0.0	0.60[a] ± 0.0	1.05[a] ± 0.01	1.09[a] ± 0.02	0.94[b] ± 0.02
FRAP (mg AEAC g^{-1})	0.11[ns] ± 0.00	0.10[ns] ± 0.00	0.12[ns] ± 0.00	0.21[ns] ± 0.0	0.22[ns] ± 0.0	0.23[ns] ± 0.0	1.26[b] ± 0.0	1.31[a] ± 0.0	1.31[a] ± 0.0

Values are mean ± SD (n = 3). Values with different superscripts in a row under each parameter for same fruits are significantly different. MC: mango RTS beverage (control); MFT: germinated foxtail millet flour incorporated mango RTS beverage; MLT: germinated little millet flour incorporated mango RTS beverage. PC: pineapple control RTS beverage; PFT: germinated foxtail millet flour incorporated pineapple RTS beverage; PLT: germinated little millet flour incorporated pineapple RTS beverage. PoC: pomegranate control RTS beverage; PoFT: germinated foxtail millet flour incorporated pomegranate RTS beverage; PoLT: germinated little millet flour incorporated pomegranate RTS beverage

have reported reduced TSS in Cornelian cherry juice [from 15-25°Brix to 10°Brix] by replacing sucrose with artificial sweeteners (aspartame and acesulfame potassium) which are almost similar to our finding of replacing 100% sucrose with sucralose (17 mg L^{-1}) in PoFT and PoLT treatments. Total polyphenol content was significantly high in PoC (251.89 mg GAE 100^{-1} g) as compared to PoFT (251.89 mg GAE 100^{-1} g) and PoLT (251.89 mg GAE 100^{-1} g). Sucrose showed a protective effect on the degradation of bioactive compounds while artificial sweetener sucralose increased the degradation of bioactive compounds. Salar *et al.* (2022) found that sucralose addition caused 10% more loss in individual bioactive compound as compared to the sucrose addition, leading to only 5% loss in fruit beverage. Scanty work has been done on the impact of artificial sweeteners on individual compounds, yet the protective effect of sucrose may be due to the inhibition of oxidative enzymes polyphenol oxidase and peroxidises (Nicoli *et al.*, 1991). Hence, one should consider the usage of sucralose when providing bioactive rich beverage.

Effect of addition of germinated millet flour in beverage on bioaccessibility of iron

In present study, the total iron present in beverage and the dialyzable iron were measured in all fruit beverages using a membrane through which dialyzable iron passes and bioaccessibility was calculated (Table 3). The total iron content in beverages ranged from 2.35 to 5.87 µg mL^{-1} while total dialyzable iron was in the range of 1.11-2.68 µg mL^{-1}. A significant difference was observed in dialyzable iron

Table 3: Effect of incorporation of germinated millet flours on total iron, dialyzable total iron and bio accessibility in mango, pineapple and pomegranate RTS beverages

Treatments	Mango RTS beverage			Pineapple RTS beverage			Pomegranate RTS beverage		
	Total iron (µg mL^{-1})	Dialyzable iron (µg mL^{-1})	Bio accessibility (%)	Total iron (µg mL^{-1})	Dialyz-able iron (µg mL^{-1})	Bio-accessibility (%)	Total iron (µg mL^{-1})	Dialyz-able iron (µg mL^{-1})	Bio-accessibility (%)
Control (C)	4.96 ±0.48	1.13c ±0.05	22.99c ±2.79	5.87 ±0.15	1.25b ±0.17	21.40b ±2.72	2.47b± 0.22	1.43 ±0.13	58.73a ±10.1
Foxtail millet (FT)	4.64 ±0.16	2.47a ±0.08	53.28a ±1.66	5.40 ±0.32	2.68a ±0.09	49.75a ±1.55	2.35 b ±0.17	1.65 ±0.06	66.76a ±2.84
Little millet (LT)	4.99 ±0.38	1.51b ±0.07	30.52b ±3.24	5.39 ±0.15	1.11b ±0.05	20.66b ±0.69	2.61a ±0.30	1.38 ±0.13	52.87b ±1.10

Values are mean ± standard deviation (n=3). Values with different superscript letters in a column for each treatment for different fruits are significantly different.

due to the incorporation of millet flours in mango and pineapple beverages. However, pomegranate beverage did not show any significant difference among the treatments. The bioaccessibility was 22.99 to 53.28% in mango beverage, 20.66 to 49.75% in pineapple beverage, whereas pomegranate beverage had highest bioaccesibility (52.87-66.76%), even though the total dialyzable iron was less as compared to the other fruit beverages. The results elucidate that among all the treatments, foxtail millet flour incorporated treatments (MFT, PFT and PoFT) had high bioaccessibility. Addition of millet flour did not significantly increase the total iron content in beverages but significantly improved dialyzable iron. This may be due to the interaction of iron compounds with different constituents of fruits (Kapsokefalou *et al.,* 2005). Probably the formation of dialyzable iron was affected due to the interaction between iron compounds and the food in iron fortified milk product. The iron present in soybean hulls was expressed as highly bioavailable ferrous form (Latunde-Dada, 1991). Prabhavathi and Narasingha Rao (1979) have reported that germination enhances ionizable and soluble iron. Thus, dialyzability of iron compounds better explains iron bioavailability than its solubility.

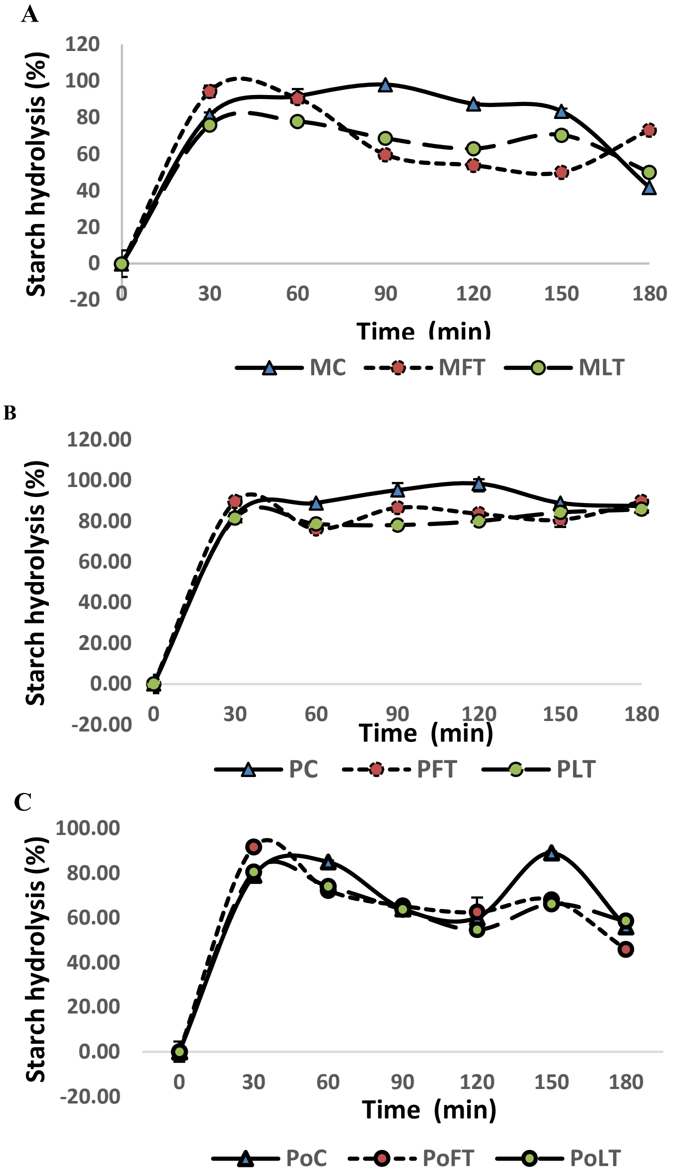

Fig. 1: *In vitro* starch hydrolysis of fruit beverages from 0 to 180 min incorporated with germinated millet flours (A: mango beverage; B: pineapple beverage; C: pomegranate beverage). MC: mango control RTS beverage; MFT: Germinated foxtail millet flour incorporated mango RTS beverage; MLT: Germinated little millet flour incorporated mango RTS beverage. PC: Pineapple control RTS beverage; PFT: Germinated foxtail millet flour incorporated pineapple RTS beverage; PLT: Germinated little millet flour incorporated pineapple RTS beverage. PoC: Pomegranate control RTS beverage; PoFT: Germinated foxtail millet flour incorporated pomegranate RTS beverage; PoLT: Germinated little millet flour incorporated pomegranate RTS beverage

Effect of addition of germinated millet flour in beverage on starch hydrolysis, HI and GI

At the beginning of digestion, the hydrolysis of starch was in the order of MFT > MLT > MC in mango beverage, PFT > PC > PLT in pineapple and PoFT > PoLT > PoC in pomegranate (Fig. 1). Since foxtail millet and little millets are relatively rich in starch than mango, pineapple and pomegranate, starch digestibility was high in millet flour incorporated beverages as compared to control fruit beverages due to the quick amylase activity on starch. Among the three fruit beverages, pineapple beverage showed high glucose release which ranged from 76.12 to 95.24% till 180 min

Table 4: Calculated hydrolysis index (HI) and estimated glycemic index (eGI) in mango, pineapple and pomegranate RTS beverages incorporated with germinated millet flours

Treatments	Mango RTS beverage			Pineapple RTS beverage			Pomegranate RTS beverage		
	HI	eGI_G	eGI_{Gr}	HI	eGI_G	eGI_{Gr}	HI	eGI_G	eGI_{Gr}
Control (C)	120.98 ±9.83	106.13 ±5.41	112.48 ±8.49	129.16^a ±0.27	110.61^a ±0.15	119.53^a ±0.23	107.30 ±8.68	98.62 ±4.76	100.69 ±7.48
Foxtail millet (FT)	102.55 ±20.52	96.01 ±11.27	96.59 ±17.70	120.59^b ±3.98	105.91^b ±2.19	112.15^b ±3.43	102.09 ±4.82	95.76 ±2.65	96.20 ±4.16
Little millet (LT)	101.53 ±1.54	95.45 ±0.85	95.72 ±1.33	116.93^b ±2.39	103.90^b ±1.31	108.99^b ±2.06	98.64 ±4.12	93.86 ±2.26	93.22 ±3.55

eGIG = (0.549*HI)+39.71; eGIGr = (0.862*HI)+8.198; Values are mean ± standard deviation (n = 3). Values with different superscript letters in a column for each parameter for different fruits are significantly different

digestion. In case of mango and pomegranate beverages, it ranged from 41.67 to 97.92 and 45.83 to 91.67%, respectively. Slow starch hydrolysis and glucose release in mango may be due to the presence of fibre in mango while less starch content in pomegranate beverage might have resulted in less glucose release. Germinated proso millet, buck wheat and pea flour showed rapid increase in starch digestibility *in vitro* in first 20 min. The study showed that increase in germination duration increased the starch digestibility due to the hydrolysis of starch by amylase fast (Yang *et al.*, 2021). However, as the digestion progresses further, control beverages from all the three fruits showed high glucose release as compared to the millet flour incorporated beverages. The GI of anyfood is defined as the incremental blood glucose area compared to test food which is expressed in percentage (Wolever *et al.*, 1991). The area under curve (AUC) is calculated according to the trapezoidal rule in geometry to calculate the GI. Lower GI values indicate the slower rate of carbohydrate absorption which means lower increase of blood glucose. Hence, estimating GI value of any food provides idea about how fast the rise in blood glucose level is. Therefore, as an alternative to *in vivo* studies, *in vitro* studies are also designed to simulate small intestinal digestion. The measurement of rate of starch digestion and thus hydrolysis index and estimated GI as formulated previously by Goni *et al.* (1997) and Granfeldt *et al.* (1992) are suitable *in vitro* models for understanding the pace of glucose release after consumption of foods. The HI ranged between 98.64 and 120.56 among the millet beverages while in control beverages it was ranged from 107.30 to 129.16. The predicted GI found in the range from 93.86 to 105.91 (eGI_G) and 93.22 to 112.15 (eGI_{Gr}) in all the three millet beverages (Table 4). Eventually mango, pineapple and pomegranate RTS beverages with added millet flours showed lower values for both HI and GI compared to control samples. Scientific report on low GI of millets infer that the protein from millets form a matrix around starch which prevents its hydrolysis by enzymes resulting in decreased glycaemic response over a time (Annor *et al.*, 2017). In case of pomegranate, the additional contributing factor must be the high polyphenol level which is a trait inherent to this fruit. Further, the sugar content was also low due to the replacement of sugar with the non-nutritive sucralose sweetener. Consumption of pomegranate juice and a polyphenol-rich pomegranate extract was studied to observe the glycaemic response after consuming high GI bread among 16 healthy volunteers by Kerimi *et al.* (2017). It was found that the polyphenols present in pomegranate beverage than the polyphenol rich extract reduced the postprandial glycaemic response of bread. It was also noted that polyphenols present in pomegranate beverage inhibited the activity of human α-amylase and maltase/sucrase activity in rat intestinal brush border in an *in vitro* experiment. These results support the results of this study. Further, the composition of macronutrients, fibre content and viscosity of food also affects the GI of food as reported by Nayak *et al.* (2014). On the account of this, millet flour incorporation into fruit beverages has a potential to reduce the GI of the fruit beverages which is advantageous for maintaining blood glucose level while relishing the RTS fruit beverage. However, more *in vitro* and *in vivo* studies are required to validate GI reduction in millet flour incorporated fruit beverage. Probably, this is the first research paper reporting on incorporation of germinated millet flour in to fruit beverages and its impact on various parameters.

Conclusion: This study explored the value addition of fruit juices of mango, pineapple and pomegranate through incorporation of germinated foxtail and little millet flour. Addition of millet flour significantly improved iron dialyzability and *in vitro* iron bioaccessibility of beverages. There was a reduction in glycemic index of beverages due to millet flour addition even though it did not fall under low GI foods. The increased iron bioaccessibility indicated that these beverages could be exploited for clinical evidences to enhance iron bioavailability when paired with certain foods. The study revealed the prospectus of improving the functional qualities of these popular beverages using millet flour as natural fortificant, opening new vistas for research and product development in this sector.

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