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In vitro IRON BIOACCESSIBILITY FROM TRADITIONAL LEGUME PREPARATIONS OF RURAL AND URBAN HOUSEHOLDS OF PUNJAB (INDIA)

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ABSTRACT

Five most consumed legumes by rural and urban households of Punjab (India) were evaluated for total iron, dialyzable iron and *in vitro* iron bioaccessibility. *In vitro* iron bioaccessibility varied from 3.07 to 9.72% in rural households and 2.78 to 6.39% in urban households. In rural households, iron bioaccessibility was maximum for white chickpeas (9.72%) followed by whole mungbean + mothbean (8.56%) and dehusked Bengal gram (8.41%). The least bioaccessibility was in Bengal gram (3.07%) followed by dehusked mungbean + dehusked lentil (3.51%). In urban households, iron bioaccessibility was maximum in dehusked Bengal gram (6.39%), followed by white chickpea (4.45%) and kidney bean (4.19%) Least bioaccessibility was in whole mungbean (2.67%) and dehusked mungbean + dehusked lentil (2.78%). The average iron bioaccessibility of legume preparations was higher in rural households than in urban households which may be attributed to the variation in food preparation practices.

Key words: Cooking, dialyzable iron, legumes, iron bioaccessibility, total iron, soaking

INTRODUCTION

Iron deficiency anemia continues to be a public health concern despite the implementation of several large scale nutritional intervention programs in most developing countries (Allen and Sabel, 2001). Iron deficiency costs India about 5% of its gross national product annually due to the loss of lives, resources and productivity (Sanghvi, 1996). In India, the prevalence of anemia is high because of poor dietary intake of iron as well as its poor bioavailability in phytate and fiber rich Indian diets. Iron is currently receiving global attention due to its nutritional value in vegetarian diets. With low intake of meat and increased intake of phytate containing cereals and legumes, absorption of iron is lower in vegetarians than in non-vegetarians (Hunt, 2003).

Legumes are fair source of iron for the population dependent on plant foods; however, the bioavailability of iron from legumes is poor (Sandberg, 2002). The form of dietary iron present in legumes is non-heme iron, the absorption of which is influenced by a variety of inhibitors and enhancers of iron absorption. In most parts of the world, including India, legumes are integral part of habitual diets contain iron in amount equivalent to amount in a non-vegetarian diet but the iron is likely to be substantially less available for absorption because of difference in the chemical form of iron and the accompanying constituent that may enhance or inhibit iron absorption (Hunt, 2003). Most of the vegetarian Indian population is exposed to the development of mild to severe degree of anemia attributed to the poor bioavailability of iron (Singh, 2013); hence determination of bioavailability of dietary iron is extremely important in evaluating the causes of iron deficiency anemia. Moreover, the cooking practices also influence the iron bioaccessibility which vary a lot in different communities. This work was aimed to study the legume consumption, its cooking practices and iron bioaccessibility from legumes by rural and urban households of Punjab, India where these are an essential part of daily diets.

MATERIALS AND METHODS

Selection of locale and collection of data

Abdullapur Chuhane, a small village in Sangrur district with a population of 1118, represented rural locale from where the data was collected while Ludhiana city (India) from centrally located district of Punjab represented the urban locale for the study. Thirty each rural and urban (50:50) households from the selected locales having monthly family income in between ₹ 25000 to 40000 were chosen to study the consumption frequency (days/week) of legumes as well as their legumes related cooking practices. A questionnaire was developed to assess consumption frequency and cooking practices of consumed by them. The legumes included whole and dehusked mungbean *(Vigna radiata)*, black gram (*Vigna mungo)*, lentil (*Lens culinaris)* and Bengal gram (*Cicer arietinum),* dehusked pigeon pea *(Cajanus cajan)*, moth bean *(Vigna aconitifolia)*, kidney bean *(Phaseolus vulgaris* and white chickpeas *(Cicer arietinum).* Bengal gram (locally called as *kala channa*, has small, darker seeds and a rough coat and is used to make *chana dal* which is a split Bengal gram with the skin removed. White chickpeas, also called *Kabuli channa*, is lighter-coloured, larger and has a smoother coat. The practice of inclusion of onion, garlic, tomato, spices and other ingredients in various legume preparations which may influence the iron bioaccessibility was also studied. The data was collected in August-September, 2014 by personally interviewing the home makers of the each household.

Collection of household samples for legume preparations

Six households each from rural and urban households were chosen for the collection of household samples for legume preparations in order to analyze total iron and dialyzable iron. The households were selected randomly. The samples of five most frequently consumed legumes in both rural and urban areas were collected. The home makers were made aware of the objectives of study. Each household was provided with 100 g of selected raw legumes and instructed to cook them in traditional manner in order to see inter-household variations, if any, in preparation method of selected legumes. The cooked preparations were packed in decontaminated polythene zip lock bags and immediately brought to Food Analysis Laboratory of the Department of Food and Nutrition, PAU, Ludhiana. The samples were stored at -18˚C till further chemical analysis.

In vitro iron bioaccessibility of collected samples

Total iron of samples was determined by atomic absorption spectrophotometer (Perkin Elmer 3100) after wet digestion. Bioaccessibility of iron in samples was determined using an *in vitro* method (Luten *et al.*, 1996). The samples were ground in a stainless steel grinder and subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) (16 g pepsin in 100 mL 0.1M HCl) at 37˚C for 2 h. Titratable acidity was measured in an aliquot of gastric digest by adjusting pH to 7.5 with 0.2 M NaOH in presence of pancreatin-bile extract mixture (4 g pancreatin + 25 g bile salt + 0.1 M Na_2CO_3). Titrable acidity was defined as the amount of 0.2M NaOH required to attain a pH of 7.5.

To simulate intestinal digestion, segments of dialysis tubing (molecular mass cut-off 10 kDa) containing 25 mL Na_2CO_3 solution being equimolar to the titrable acidity determined as above were placed in Erlenmeyer flasks containing gastric digest and incubated at 37˚C with shaking for 45 min. or longer until the pH of digest reached 5.0. Pancreatin-bile salt mixture (5 mL) was then added and incubation continued for 3 h or longer until the pH of digest reached 7.0. Iron and zinc present in dialysate at the end of simulated gastrointestinal digestion represented the bioaccessible fraction. Dialysates were mixed with 5 mL nitric acid and analyzed for dialyzable iron content using atomic absorption spectrophotometer (Perkin Elmer, AAnalyst 400) and dialysate iron expressed in percentage. The following equations were used to estimate dialyzable and total iron:

$$\text{Dialyzable iron (\%)} = \frac{\text{Iron } (\mu g\ mL^{-1}) \text{ dialysate} \times \text{dialyasate (mL)}}{\text{Iron } (\mu g\ g^{-1}) \text{ of legume sample} \times 20\ g} \times 100$$

Bioaccessible iron was calculated as under

$$\textit{In vitro} \text{ iron bioaccessibility (\%)} = \frac{\text{Dialyzable iron}}{\text{Total iron}} \times 100$$

All the estimations of iron bioaccessibility were made in 4 replicates and average values reported.

Statistical analysis

Mean and standard deviations were computed for the data as per standard procedure (Gnanadesikan 1997). Mean and standard deviation of the five most commonly consumed legumes by rural and urban households were computed.

RESULTS AND DISCUSSION

Consumption frequency of legumes

The suggested daily intakes of legumes for adult Indian man and woman are 75-120 and 60-90 g, respectively (ICMR, 2011). The mean weekly consumption frequency of some major legumes has been depicted in Table 3. The maximum consumption frequency score of rural households was observed for *sabut moth* (4.80) followed by dehusked Bengal gram (4.50), mung dal (3.97), Bengal gram (3.90), sabut mung (3.87) and dehusked mungbean (3.07). The highest consumption frequency score by urban households was for dehusked mungbean (3.90) followed by dehusked Bengal gram (3.70), kidney bean (3.70), Bengal gram (3.67), white chickpea (3.60) and dehusked

Table 1: Mean weekly consumption frequency score of major legumes consumed by rural and urban households (N = 60)

Legume diets	Weekly consumption frequency score		
	Rural (n = 30)	Urban (n = 30)	t-value
Whole mungbean	3.87±1.68	3.50 ± 1.41	ns
Dehusked mungbean	3.97 ± 1.59	3.17± 2.00	1.87**
Whole black gram	0.37 ± 0.96	3.13 ± 1.57	8.93***
Dehusked black gram	0.00 ± 0.00	1.17 ± 1.60	3.95***
Whole lentil	0.23 ± 0.73	1.83 ± 1.98	4.05***
Dehusked lentil	3.07 ± 1.80	3.90 ± 1.77	2.00**
Whole Bengal gram	3.90 ± 1.37	3.67 ± 1.49	ns
Dehusked Bengal gram	4.50 ± 1.63	3.70 ± 1.49	2.59***
Dehusked pigeon pea	0.17 ± 0.59	1.70 ± 2.23	3.60***
Moth bean	4.80 ± 1.95	2.03 ± 2.13	5.086***
Kidney bean	0.83± 1.56	3.70 ± 1.18	7.67***
White chickpea	2.97 ± 2.19	3.60 ± 0.81	1.38*

Frequency scores out of seven

mungbean (3.50). The consumption frequency score revealed that legume consumption was significantly higher for dehusked mungbean , mothbean and dehusked Bengal gram in the surveyed rural households as compared to the urban households; whereas the consumption frequency of whole black gram, dehusked black gram, whole mungbean, dehusked mungbean, dehusked pigeonpea, kidney beans and white chickpeas was significantly higher in urban households.

Soaking and cooking practices for legume preparations

Soaking is a common practice prior to cooking in case of whole legumes. The study revealed that out of the selected legume preparations of rural households, two were in combination i.e. whole mungbean + mothbean and dehusked mungbean + dehusked lentil. Similarly, dehusked mungbean + dehusked lentil was in combination while rest were individual legume preparations and only two preparations i.e. dehusked mungbean + dehusked lentil and dehusked Bengal gram were prepared from dehusked legumes in urban households. Soaking was followed by 41-100% of rural and urban households. Soaking has been reported beneficial to reduce phytic acid which is a major inhibitor of iron in legumes (Uppal 2011; Barakoti, 2004). For whole legume preparations, soaking time more than 20 min. was followed by 70-92% rural households while all the urban households soaked dehusked Bengal gram, kidney beans and white chickpeas for more than 20 min. The results revealed that more urban households followed soaking practice as compared to rural households which shows better awareness among urban households and could be attributed to their higher educational level. Use of soaking water was followed by 49-89 and 41-83% of rural and urban households, respectively. Soaking water needs to be discarded as it has anti-nutrients which hinder the absorption of minerals. The households appear unaware of this fact.

Open pan cooking was carried out for only dehusked mungbean + dehusked lentil in 60 and 15% of rural and urban households, respectively; while covered lid cooking was more prevalent in rural households for other legumes i.e. 50-69%. On the other hand 80 to 100% of urban households used to pressure cook for all the legume preparations. The longer cooking time [>25 min.] was reported by 60-83% of rural and 32-82% of urban households. The longer cooking time by larger percentage of rural families could be attributed to the method of cooking i.e. covered lid cooking

Table 2: Cooking practices for legume preparations by rural and urban households

Cooking practices		Rural (n = 30)					Urban (n = 30)				
		Mungbean whole + mothbean (n = 20)	Bengal gram dehusked (n = 29)	Mungbean dehusked + lentil dehusked (n = 20)	Bengal gram (n = 28)	White chickpeas (n = 20)	Mungbean dehusked + lentil dehusked (n = 20)	Bengal gram dehusked (n = 29)	Kidney beans (n = 28)	White chickpeas (n = 30)	Mungbean whole (n = 27)
Soaking		53	41	60	96	100	30	41	100	97	48
Time of soaking	5-10 min	0	24	9	0	0	66	0	0	0	39
	10-15 min	9	7	16	0	0	34	0	0	0	31
	15-20 min	21	24	25	8	10	0	0	0	0	0
	>20 min	70	45	50	92	90	0	100	100	100	30
Use of soaking water		49	68	42	89	80	83	68	43	41	54
Method of cooking	Open pan	0	0	60	0	0	15	0	0	0	0
	Covered lid	63	69	0	62	50	5	0	0	0	11
	Pressure cooking	37	31	40	38	50	80	100	100	100	89
Time of cooking	10-15 min	32	14	45	7	10	40	0	0	0	19
	15-20 min	5	21	15	3	5	50	0	7	7	30
	20-25 min	0	27	0	7	25	0	18	23	23	19
	25-30 min	63	7	0	0	0	10	54	37	37	22
	>30 min	0	31	45	3	60	0	28	33	33	10

method which requires more time for legumes for cooking as compared to pressure cooking. Deol (2007) observed higher reduction in iron content when cowpea was cooked by covered lid method (20.5%) as compared to pressure cooking (13.8%). Further, the enhancement of cooking time resulted in significant iron loss because of the leaching of iron from seeds to cooking water. Phytic acid and polyphenol reduction was maximum during pressure cooking as compared to covered lid or microwave cooking. Hemlatha *et al.* (2007) observed that dehusking and pressure cooking both result in significant reduction in phytin phosphorus and polyphenols. Barakoti (2004) observed that dehusking and pressure cooking of mungbean enhanced the iron bioavailability by 9-21%.

Use of onion, garlic, tomato and other ingredients in legume preparations

Onion was most commonly used ingredient among all the rural and urban households (Table 3). Use of garlic use was observed in 75-85% of rural households and 75-90% of urban households in different legume preparations. Similarly, ginger and tomato use was reported by 20-72% and 30-75% of rural households and 60-100 and 71-96% of urban households, respectively. The results revealed that onion and garlic used in legume preparations was comparable in rural and urban households but ginger and tomato use was much higher in urban households.

The use of coriander leaves as herb and green chilies in all the legume preparations was reported by majority of rural and urban households. Dietary components present in a preparation influences iron bioaccessibility. Gautam *et al.* (2010a) reported that dietary sulphur containing amino acids improves the mineral status of experimental animals. The beneficial effect of sulphur compounds rich *Allium* spices i.e. onion and garlic added to legumes has been highlighted by Luo *et al*. (2014). The presence of tomato in salads when consumed along with *dal* and *chapatti* enhanced the *in vitro* iron bioaccessibility by 5.2-28.9% (Singh, 2013).

Table 3: Ingredients used for legume preparations by rural and urban households (N = 60)

Ingredients		Rural (n=30)					Urban (n=30)				
		Mung-bean whole + moth bean (n = 20)	Bengal gram dehusked (n = 29)	Mung-bean dehusked + lentil dehusked (n = 20)	Bengal gram (n = 28*)*	White chick-peas (n = 20)	Mung-bean dehusked + lentil dehusked (n = 20)	Bengal gram dehusked (n = 28)	Kidney beans (n = 28)	White chick-peas (n = 30)	Mung-bean whole (n = 27)
Use of onion/ garlic/ tomato/ ginger	Onion	100	100	100	100	100	100	100	100	97	100
	Garlic	85	79	80	75	75	85	75	89	90	89
	Tomato	30	55	30	75	75	80	71	96	90	93
	Ginger	20	72	40	50	50	60	82	100	90	100
Procedure of addition	Sauted	100	97	100	100	100	100	96	89	90	100
	Direct	0	3	0	0	0	0	4	11	10	0
Other ingredients used*	Red chilli	85	69	82	100	100	85	71	89	67	93
	Garam masala	15	76	10	100	100	15	54	89	97	74
	Cumin seeds	72	90	65	65	65	90	100	100	97	100
	Coriander powder	20	0	10	0	0	20	29	18	17	15
	Coriander leaves	50	69	50	50	50	50	82	100	83	100
	Green chillies	85	55	80	100	100	85	71	79	87	93

Sauting was the preferred method for adding onion, garlic and tomato by majority of rural and urban households for all the legume preparations. Salt and turmeric powder was used by 100% of rural and urban households and the other ingredients namely red chilli, *garam masala* and cumin seeds were used by appreciable number of rural and urban households. The spices have been reported to impart positive effect on the iron bioavailability in food preparations. Curcumin, the

Table 4: Total iron, dialyzable iron and i*n vitro iron* bioaccessibility of legume preparations collected from rural households

Legumes	Total Iron (mg)	Dialyzable iron (mg)	*In vitro* iron bioaccessibility (%)
	Rural (n = 6)		
Whole mungbean + mothbean	5.74 ± 0.52	0.49 ± 0.03	8.56 ± 0.79
Dehusked Bengal gram	5.95 ± 0.84	0.39 ± 0.18	8.41± 4.04
Dehusked mungbean + dehusked lentil	5.35 ± 0.91	0.18 ± 0.03	3.51 ± 1.09
Bengal gram	7.24 ± 1.08	0.22 ± 0.03	3.07 ± 0.70
White chickpea	5.52 ± 0.53	0.53 ± 0.13	9.72 ± 2.27
	Urban (n = 6)		
Dehusked mungbean + dehusked lentil	5.60 ± 0.54	0.16 ± 0.08	2.78 ± 1.42
dehusked Bengal gram	6.10 ± 0.57	0.39 ± 0.14	6.39 ± 2.28
Kidney bean	6.33 ± 0.89	0.27 ± 0.16	4.19 ± 2.23
White chickpea	4.97 ± 0.52	0.22 ± 0.10	4.45 ± 2.02
Whole mungbean	7.34 ± 1.80	0.19 ± 0.15	2.67 ± 1.85

antioxidant phytochemical of turmeric, which has a structure partially similar to that of β-carotene has been reported to promote influence on mineral bioaccessibility as it significantly enhanced the bioaccessibility of iron from sorghum, green gram and chickpea (Gautam *et al*., 2010b).

Total iron, dialyzable iron and in vitro iron bioaccessibility of legume preparations

Five most frequently consumed legume preparations by surveyed rural and urban households were selected to access total iron, dialyzable iron and *in vitro* iron bioaccessibility. Total iron for whole mungbean + mothbean, dehusked Bengal gram, dehusked mungbean + dehusked lentil, Bengal gram and white chickpea ranged between 5.17 to 6.40, 4.84 to 7.35, 4.40 to 6.73, 5.26 to 8.36 and 4.56 to 6.13 mg 100 g^{-1} with mean values of 5.74 , 5.95, 5.35, 7.24 and 5.52 mg 100 g^{-1} dry matter, respectively (Tables 4 and 5).

The maximum dialyzable iron was found in bengal gram (0.53), followed by whole mungbean + mothbean (0.49), dehusked Bengal gram (0.39), white chickpea (0.22) and dehusked mungbean + dehusked lentil (0.18) in rural households. The *in vitro* iron bioaccessibility of whole mungbean + mothbean, dehusked Bengal gram , dehusked mungbean + dehusked lentil, Bengal gram and white chickpea ranged between 7.76 to 9.90, 3.80 to 14.66, 2.06 to 4.82, 2.19 to 3.81 and 6.32 to 12.73% with respective mean values of 8.56 , 8.41, 3.51, 3.07 and 9.72%. The maximum iron bioaccessibility was in white chickpea followed by dehusked Bengal gram and whole mungbean + mothbean. Hemlatha *et al.* (2007) found the dialyzability of iron in the range of 1.77 to 10.2% in commonly consumed pulses in India.

In urban households, maximum dialyzable iron was found in *channa dal* (0.39) followed by kidney beans (0.27), white chickpea (0.22), whole mungbean *(*0.19) and dehusked mungbean + dehusked lentil (0.16). The *in vitro* dialyzability of dehusked mungbean + dehusked lentil, dehusked Bengal gram , kidney bean, white chickpeas and whole mungbean ranged between 1.15 to 5.18, 2.61 to 9.15, 1.07 to 6.45, 2.20 to 7.91 and 0.65 to 4.75% with respective mean values of 2.78, 6.39, 4.19, 4.45 and 2.67 %. The maximum *in vitro* iron bioaccessibility was in dehusked Bengal gram followed by white chickpea, kidney bean, dehusked mungbean + dehusked lentil and whole mungbean . The average *in vitro* iron bioaccessibility for various legumes varied between 2.67 to 8.56%. Based on the classification of FAO/WHO (Zimmermann and Hurrell, 2007), the iron bioavailability of various legume preparations was found to be intermediate as it ranged between 5 to 10%. Though, there was no much variation in total iron for all the legumes prepared by different households but significant variation was observed in percent iron bioaccessibility. The varied bioaccessibility may be attributed to the variation in cooking methods and ingredients used by the selected households. Phytic acid content variation in different legumes had significant negative influence on iron dialyzability (Sandberg, 2002; Glahn *et al*., 2002; Gibson *et al*., 2010).

The study concluded that the weekly consumption frequency trend for legumes in rural households was mothbean > dehusked bengal gram > dehusked mungbean >white chickpea >whole mungbean , while in urban households it was dehusked lentil > dehusked Bengal gram >kidney bean >Bengal gram> white chickpea. Legume consumption frequency was more in rural households in comparison to urban households. Soaking prior to cooking was followed for most of the whole legumes by majority of households. Pressure cooking of legumes was a preferred method followed by majority of urban households while covered lid cooking was more practiced in rural households. Longer cooking time for legumes was reported by rural households as compared to urban households. In rural and urban households, iron bioaccessibility from different legume preparations varied between 3.07 to 9.72 and 2.67 to 6.39%, respectively. The average iron bioaccessibility of legume preparations from rural households was higher in comparison to urban households which may attribute to variation in food preparation practices.

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