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Effect of Processed Beverages on Dialysable Iron From Rice Based Meals in Comparison with Fresh Fruits

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ABSTRACT

The investigation is aimed at analysing the effect of fruits and processed beverages on the dialysable iron content of a rice based meal with four different green leafy vegetables (GLV). Rice (150g), red gram (30g) and a GLV (150g) forming the meal components were pressure-cooked and homogenised. To each of the meal, one of the following was added at 400 g or ml/meal level - fresh fruit pulp or juice (four varieties), reconstituted juice powders (RJP), fruit squashes, ready to drink fruit beverage (RTD) and carbonated beverages (CB). Two brands of each processed beverages were used. Dialysable iron content of diets increased by 16-104% on addition of fruit pulp and by 45-263% on addition of fruit juices over the respective control diets. Among the processed juices, RJP enhanced the dialysable iron by 30-81%, fruit squashes by 20-138%, RTD by 33-96% and carbonated beverages by 1-131% with different brands. Differences observed in the two brands of processed beverages could be attributed to their ascorbic acid and citric acid contents. Hence, it can be concluded that addition of fruit/fruit juice, either in natural or synthetic forms containing organic acids i.e., citric, ascorbic or maleic acids will definitely increase the iron dialysability of cereal meal indicating enhanced iron bioavailability.

INTRODUCTION

Iron is one of the highly essential trace elements on which a wide range of body functions are dependent. Deficiency of iron is known to result in anaemia. Iron deficiency anemia is a nutritional problem of multiple etiologies of which poor bioavailability of the mineral is identified to be one of the important factors. "Bioavailability (or biological availability) is key to nutrient effectiveness," which is defined as the proportion of the nutrient in food that can be utilised and absorbed (Ballot *et al.,* 1987; Gilloly *et al.,* 1983). The absorption of iron depends on the form of iron in the food i.e., heme iron from animal foods, that is better absorbed than that of non-heme iron from plant foods. The absorption of non-heme iron is governed by the meal components and other factors operating in the stomach and small intestine (Benito & Miller, 1998).

The meal components that influence the bioavailability of iron include enhancers or chelating ligands that solubilise iron as ascorbic acid, citric acid, maleic acid and tartaric acid and inhibitors that form insoluble complexes as tannins, phytates, dietary fiber and oxalates that would hinder absorption at the brush border (Reddy & Cook, 1991; Hallberg & Rossander, 1984; Hazell & Johnson, 1987; Gillooly *et al.,* 1983).

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Fruits and fruit juices that are rich in organic acids have been shown to enhance iron availability in cereal meals and cereal products. Fruits such as grapes, apples, peaches, oranges etc. given at a level of 100g per 200g meal fed to human volunteers showed a marginal increase, while those given in the form of juices at the same level showed higher increase in the hematological parameters which indicated iron absorption. The extent of increase was observed to correlate well with the ascorbic acid concentration in the fruits (Ballot *et al.,* 1987; Rossander & Hallberg, 1979; Layrisse, Martinez- Torres & Renzi, 1976). The organic acids present in combination (as that in fruits) showed a better improvement in iron availability than each of them in isolation.

A study on the influence of dietary determinants on the non-heme iron absorption in humans and rats showed that rats were less sensitive either in influencing/inhibiting the availability by respective exogenous additives. The authors have suggested that the *in vitro* digestion methods to be a more reliable guide to non-heme iron absorption in humans (Benito & Miller, 1998), which is also less time consuming and economical.

With more and more research followed by industrialisation in the area of fruit processing and beverages, a wide range of synthetic juices and soft drinks are getting popularised and the consumption of processed fruit beverages is increasing especially among younger generations. Though the enhancing effect of fruit and fruit juices on the available iron in various foods is well established, studies on the effect of commercially processed juices and soft drinks on the iron availability are scarce. Hence, the present investigation was undertaken with the objective of analysing the effect of various forms of processed juices on the bioavailable iron in rice based meals with different green leafy vegetables. In addition, effects of four varieties of fresh fruit pulp and juice were also studied for comparison.

MATERIALS AND METHODS

Rice (*Oryza sativa*), dehusked red gram (*Cajanus cajan*) and four green leafy vegetables namely *Chenopodium album* ('Bathua' leaves), *Trigonella foenum graecum* (fenugreek leaves), S*olanum nigrum* ('Manthakkali' leaves) and *Peucedanum graveolens* ('Shepu' leaves); four fruits i,e. tomato (*Lycopersicon esculentum*), apple (*Malus sylvestris*), blue seedless grapes (*Vitis vinifera*) and orange (C*itrus aurantium*) were procured from a local market. Two brands each of 4 types of processed juices were procured from a local supermarket for the study (Table 1).

Table 1. Types of processed beverages and their organic acid composition

Processed juice	*Organic acid indicated on the label*	
	Brand A	*Brand B*
Fruit beverage that needs reconstitution or reconstituted juice powder (RJP)	Ascorbic acid	Citric acid, Ascorbic acid
Fruit squashes that requires reconstitution with water (processed fruit concentrate)	Citric acid	Citric acid, Ascorbic acid
Ready to drink (RTD) fruit juices	Citric acid, Ascorbic acid	Ascorbic acid
Carbonated beverages (CB)	Citric acid	–

Preparation of meal/ diet

The test meal comprised of rice (150 g), red gram (30 g), and a green leafy vegetable (150 g). These were separately pressure-cooked using double glass distilled water, homogenised together to give a fine puree in a blender. Test meal with four different green leafy vegetables formed four experimental meals. A fruit (as pulp), fruit juice (extracted in a juicer and filtered), or a processed juice (reconstituted using distilled water as per the instruction on the pack) was added at a level of 400 g or ml per meal and blended well.

Chemical analysis

In vitro availability (dialysable iron) of iron of the homogenised diets with fruit additive was determined by the method of Equilibrium dialysis (Luten *et al.,* 1996) with suitable modifications in a representative sample of the above meal. In brief, the diets were first subjected to simulated gastro-intestinal digestion by adjusting the pH to 2.0 followed by addition of pepsin (3ml of 16% pepsin in 0.2M HCl) and incubated in a shaker at 37°C for 2 hours. Then the digests were frozen for 90 min. Titratable acidity was determined in an aliquot containing pancreatin-bile extract mixture (5ml of 400mg pancreatin and 2.5g bile extract in 1L of 0.1M $NaHCO_3$) with 0.2M NaOH until a pH of 7.5 was attained.

The above frozen digests were thawed and subjected to simulated intestinal digestion by placing the dialysis tubing (molecular cut off: 10kda) in Erlenmeyer flasks. The dialysis tubing contained 25ml of $NaHCO_3$ (equivalent to moles of NaOH determined by titratable acidity). The flasks were incubated in a shaker water bath at 37°C for 30 min (until the pH reached 5.0), pancreatin – bile extract mixture was added and shaken for another 2 hours (until pH reached 7.0). Then the dialysates were carefully transferred to graduated tubes and volume was measured. The dialysates representing the bioavailable iron was analysed for iron (AOAC, 1965) using a UV-VIS spectrophotometer 108 type (make-Systronics).

Ascorbic acid in fruits and fruit juices was estimated by 2,6, - dichlorophenol –indophenol visual titration method (Ranganna, 1986). Total acidity – in fruits and fruit juices were determined by titration with dilute NaOH and expressed as percent prominent acid present in the juice (Ranganna, 1986). The diets were dried and ashed in a muffle furnace and ash solution was prepared by dry ashing. Total iron were estimated colorimetrically by a-a-dipyridyl method (AOAC, 1965). All chemicals used for the analysis were of analytical grade, double glass-distilled water was used for entire analysis. All analysis were done in duplicates, the data presented here represent the mean value of 4 replicates.

Statistical analysis

The effect of fruit additives on the available iron were analysed by subjecting the analysed data to Paired T test using the statistical package SPSS 10.0. The probability level was fixed to $P<0.05$.

RESULTS

A meal consisting of staple cereal rice, red gram and a green leafy vegetable was prepared and analysed for total and dialysable iron. Results as presented in Table 2 shows that total and dialysable iron was higher in diets containing *Peucedanum graveolens* and *Solanum nigrum.* The percent bioavailable iron ranged from 3.67 – 5.79 in diets with different green leafy vegetables. The differences between the dialysable iron content of diets with different leafy vegetables was found to be extremely significant (F = 342.44***). Ascorbic acid content was

Table 2. Total and dialysable iron of diets with different greens

Diets with different green leafy vegetables	*Total iron (mg/diet)*	*Dialysable iron (μg/diet)*	*Percent dialysable iron*
Trigonella foenum	0.536±0.007	19.68±0.353	3.67
Peucedanum graveolens	1.032±0.028	54.90±0.169	5.32
Chenopodium album	0.699±0.031	34.40±1.186	4.92
Solanum nigrum	1.096±0.013	63.47±1.011	5.79
F Ratio	284.3***	342.44***	

*** P<0.001, extremely significant

found to be higher in orange and tomato and lower in grapes and apple (Table 3). Total acidity was higher in grapes, moderate in orange and tomato and lower in apple. Ascorbic acid content of the analysed RJP's was 1.1 mg and 28.7 mg/100ml in Brand A and Brand B respectively, the total acidity content was almost similar in both the brands. Brand A and Brand B squash contained 1.9mg and 7.4mg of ascorbic acid per 100ml respectively. Total acidity of squash was found to be higher in Brand A than in Brand B. The ascorbic acid content of RTD juice of Brand A was 16.1mg and 5.5mg/100ml in Brand B, while total acidity of both the brands was similar. Ascorbic acid content of both the brands of carbonated beverages was very low and similar and total acidity was also comparable between the brands analysed.

The results of analysed iron bioavailability from different diets with added fruits are presented in Figure 1. On addition of orange fruit to the diets, the diet with *Chenopodium album* showed a highest increase (104%) in dialysable iron, while diets with other three greens showed 33-43% increase. The extent of increase was found to be significantly (t = 3.609, P = 0.037) higher than the control diets. Addition of orange juice to the diets tremendously increased the dialysable iron, it ranged from 100% in the diet containing *Solanum nigrum* to 263% with the diet having *Chenopodium album*. The extent of increase was found to be highly significant (t = 6.397, P = 0.008).

A similar trend was observed with the addition of apple wherein the diet with *Chenopodium album* witnessed a highest increase of 55% as against approximately 30% increase with the diets containing other greens (t = 4.592, P = 0.019). Apple juice brought about a higher increase in dialysable iron in diet with *Chenopodium album* (121%) than diets with other greens (76-90%) and their respective controls (t = 3.133, P = 0.057).

Of the fruits added, tomato showed the least increase in the dialysable iron, however the increase was found to be statistically significant (t = 3.547, P = 0.038). On addition of tomato juice a higher increase was observed with the diet having *Chenopodium album* (137%), moderate in the diet with *Solanum nigrum* (75%), and lower in diet with other two greens (45 and 55%).

Addition of grapes to the diet brought about 23-35% increase in dialysable iron with diets containing different greens (t = 4.020, P = 0.028). Grape juice enhanced the dialysable iron by 52-75% in diets having different greens. The enhancing effect of dialysable iron by grape juice in the diets with different greens was found to be statistically significant (t= 4.013, P = 0.028).

Table 3. Ascorbic acid and total acidity of the fruit, fruit juices and processed beverages per 100ml*

Beverage	*Ascorbic acid (mg)*		*Total acidity (%)*	
	Pulp	*Juice*	*Pulp*	*Juice*
Orange	18.1±0.003	25.2±0.004	1.84±0.002	2.52±0.003
Tomato	11.7±0.001	17.7±0.003	1.24±0.00	1.84±0.001
Apple	1.8±0.06	2.7±0.09	0.41±0.01	0.68±0.02
Grapes (seedless, blue variety)	3.1±0.01	4.5±0.02	2.48±0.004	3.61±0.007
	Brand A	*Brand B*	*Brand A*	*Brand B*
Reconstituted juice powder (RJP)	1.1±0.00	28.7±0.00	1.02±0.0	1.34±0.001
Squashes	1.9±0.04	7.4±0.00	2.00±0.0	1.15±0.00
Ready to drink Juices (RTD)	16.1±0.07	5.5±0.00	2.00±0.0	2.00±0.00
Carbonated beverages	0.3±0.00	0.3±0.00	1.18±0.0	1.41±0.00
F ratio	0.5933^{ns}	0.0550^{ns}		

ns= not significant
* Values represent means of four determinations

Addition of RJP's to the diets increased the dialysable iron by 31-65% with Brand A (t = 4.923, P = 0.016) and 30-81% with Brand B (t = 4.379, P = 0.022) over their respective control diets (Figure 2). The extent of enhancement of increase that occurred was found to be significant at 5% level. However the differences between the brands were non-significant.

Addition of RTD Brand A juices to the diets accounted for 79-96% increase in dialysable iron from three diets and 36% from one diet, whereas Brand B brought about an increase of 33-58% in three diets and a 91% increase in dialysable iron in one diet. The enhancement in dialysable iron brought about by both the RTD juices were highly significant (P < 0.01).

Addition of Brand A squash increased the dialysable iron by 20-30% with different diets, while Brand B brought about 86-138% increase with different greens. The enhancement in dialysable iron brought about by both the squash samples were highly significant (P < 0.01).

Addition of CB to meals from Brand A, increased the dialysable iron by 56 and 66% in diets with *Chenopodium album* and *Peucedanum graveolens*, while it was 107 and 131% in diets with *Trigonella foenum graecum* and *Solanum nigrum* respectively. The increase in the dialysable iron was found to be statistically significant (P<0.05). Unlike Brand A, CB from Brand B did not exhibit any marked enhancing effect on the iron dialysability (P>0.05).

Analysed ascorbic acid to iron ratios were computed in diets having different green leafy vegetables and the added fruit juice, pulp and the processed beverage (Table 4). Of the fresh form of additives, the ratios were higher for the pulp than juice in all the four fruits which paralleled their ascorbic acid content. The ratios with different fruits followed the order – orange>tomato>grapes>apple. Among

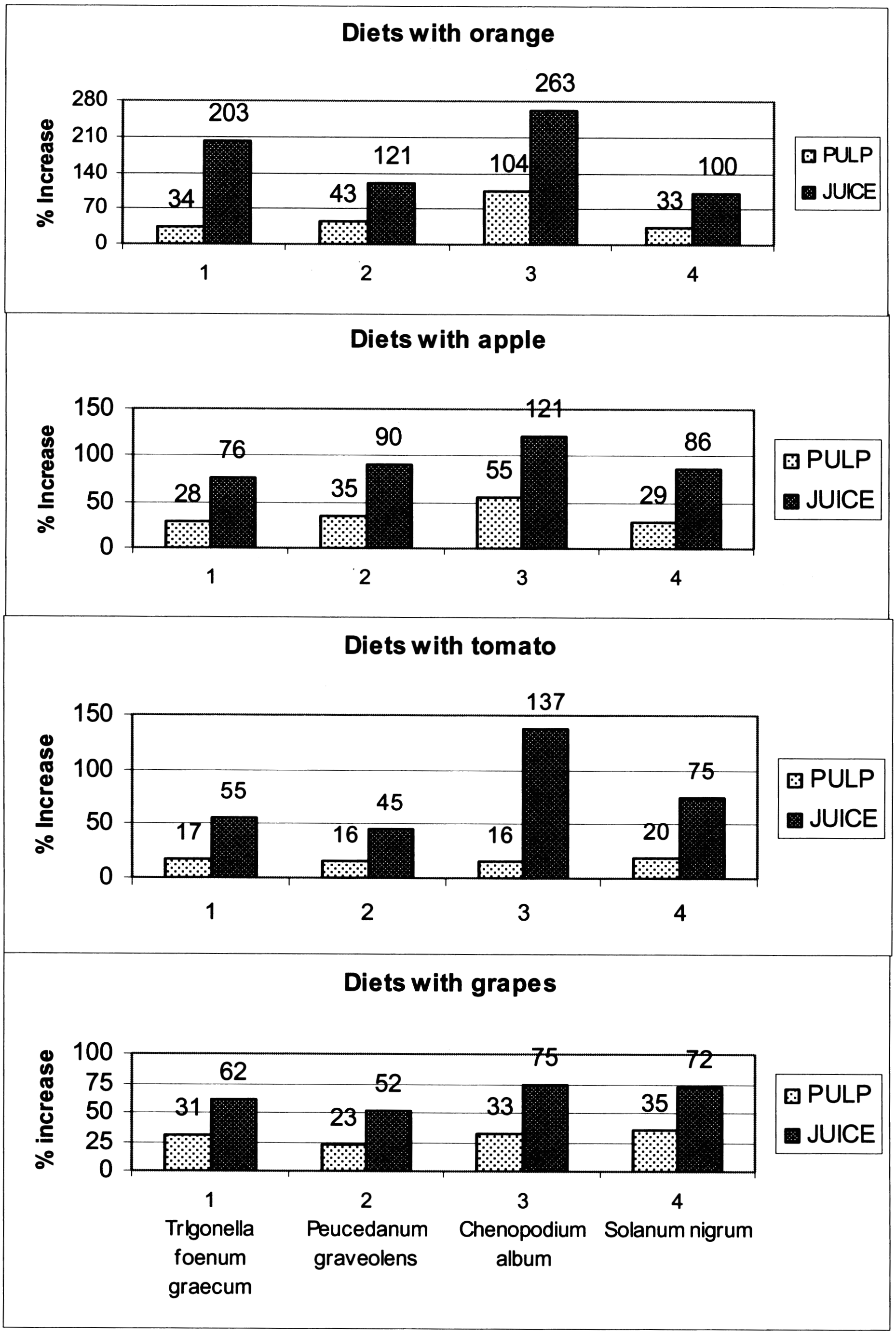

Figure 1. Percent increase in dialysable iron from different diets with added fruit pulp and juice

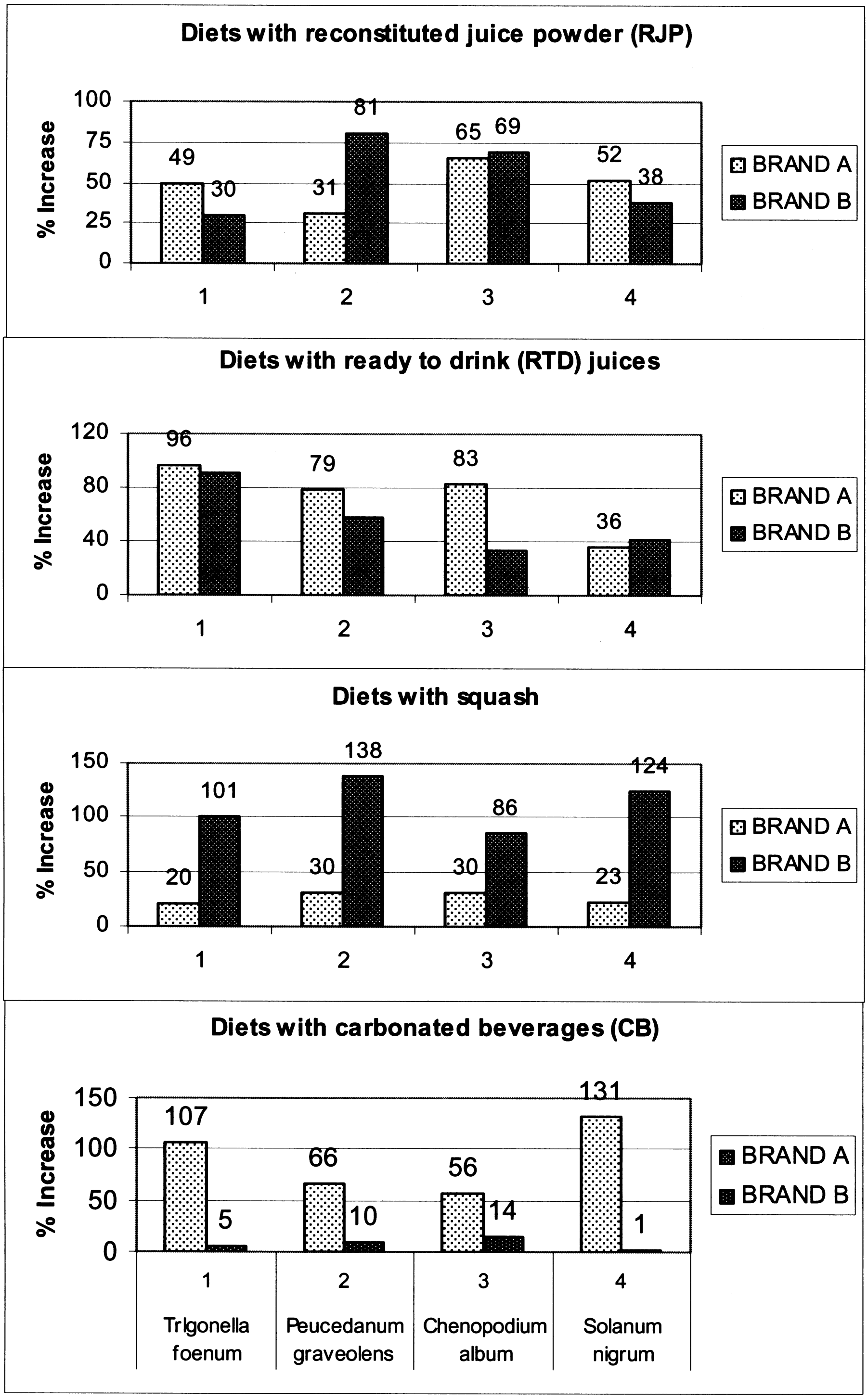

Figure 2. Percent increase in dialysable iron from different diets with added two brands of processed beverages

Table 4. Ratio of ascorbic acid: iron in rice based diets with fresh fruits and processed beverages

Fruit or processed beverage	*Diets with different green leafy vegetables*			
	Trigonella foenum	*Peucedanum graveolens*	*Chenopodium album*	*Solanum nigrum*
Orange pulp	33.8	17.5	25.9	16.5
Orange juice	47.0	24.4	36.1	23.0
Tomato pulp	21.8	11.3	16.7	10.7
Tomato juice	33.0	17.2	25.3	16.1
Apple pulp	3.4	1.7	2.6	1.6
Apple juice	5.0	2.6	3.9	2.5
Grape pulp	5.8	3.0	4.4	2.8
Grape juice	8.4	4.4	6.4	4.1
F ratio	85.7642***			
RJP brand A	1.9	1.1	1.6	0.6
RJP brand B	53.5	27.8	41.1	26.18
Squash brand A	3.5	1.8	2.7	1.7
Squash brand B	13.8	7.2	10.6	6.8
RTD brand A	30.0	15.6	23.0	14.7
RTD brand B	10.3	5.3	7.9	5.0
CB brand A	0.6	0.3	0.4	0.3
CB brand B	0.6	0.3	0.4	0.3
F ratio	12.1536***			

the processed beverages ratios were maximum for RJP (brand B) and RTD (brand B), followed by squash (brand B). The ratio with CB's were least and similar among brands.

DISCUSSION

Dialysable iron, a measure of biologically available iron is a reflection of the interaction of the components of the diet. Enhancement of iron dialysability on addition of fruits to cereal-based diets was moderate, it varied by the fruit's acidity and ascorbic acid contents and also by the type of green leafy vegetable added to the diet. As can be seen from Figure 1, orange, apple and grape fruits showed a 30% increase with all diets except the one with *Chenopodium album*. With orange and apple fruits, diet with *Chenopodium album* showed a 3- and 1.5-fold higher increase in dialysable iron respectively, while tomato juice brought about half of the increase brought by other fruits. Despite a higher ascorbic acid content in tomato than the two other fruits, the lower increase of dialysable iron observed could be attributed to the presence of fibrous seeds and peel with iron absorption inhibitory property. This is in confirmation with the findings of other workers (Hazell & Johnson, 1987) who have reported that removal of peel in grapes and pears enhanced iron diffusability. Contrary to this, there are also observations that peeling of skin in grapes had no additional enhancing effect on iron diffusability (Ballot *et al.*, 1987).

The observations of Ballot *et al* (1987) have revealed that 100g of fruit/200g rice meals fed to women showed no significant improvement in the hemoglobin and transferrin saturations. The promoting effect in the diffusible iron correlated well with the organic acid composition of the fruits as well as their polyphenol and fiber contents.

The effect of pear, apple, grape fruit, orange and white grape juices on the iron absorption from foods is well documented (Hazell & Johnson, 1987; Ballot *et al.,* 1987; Boato *et al.,* 2002). The increase in dialysable iron on addition of orange juice was very high (2-5 fold) with different greens, higher with *Chenopodium album* and lower with *Solanum nigrum* diets. A higher availability of iron observed with the diet having *Chenopodium album* can be attributed to the comparatively low dietary fiber content (Kojima, Wallace & Bates, 1981).

A comparatively high dialysability/solubilisation of iron in the diets brought about by the addition of orange juice is a reflection of its highest ascorbic acid content and a moderate acidity. This confirms the findings of earlier workers in beans (Kojima, Wallace & Bates, 1981) wherein they have reported that solubilisation of iron with orange juice was equal to a combination of 10mM citric acid and 10mM ascorbic acid. Hence it is said that orange juice has a well balanced ratio of citric acid and ascorbic acid to provide high iron availability. The long term effect of high ascorbic acid on iron status may be less than that predicted from single meal studies. The effect of organic acids is dependent on the source of iron, type and concentration of organic acid, pH, processing methods and the food matrix (Teucher, Olivares & Cori, 2004).

Tomato juice possessed a higher ascorbic acid next to orange juice but its effect in increasing dialysable iron was very much lower. Of all the organic acids present in fruit juices only oxalic acid is known to inhibit iron absorption. Tomato contains 8mg (per 100g) of oxalic acid, but the presence of citric acid (390mg/100g) being higher along with ascorbic acid counteracts the inhibitory effect to a certain extent that is reflected in the iron dialysability in the diets (Ballot *et al.,* 1987). Though the ascorbic acid content of apple juice was lower than grape juice, a comparatively higher dialysability brought about by apple juice could be attributed to the presence of a very high content of maleic acid, a high citric acid and a lesser oxalic acid (Ballot *et al.,* 1987). Juices brought about a 3-6 fold increase in the dialysable iron in the diets over the addition of respective fruits (in the same quantity) as the juices are devoid of fiber and also have higher content of organic acids.

Though it is well established that the ability of fruit juices to enhance the iron dialysability correlates with the ascorbic acid content, the role of other organic acids in influencing the iron availability cannot be overlooked. Apart from the organic acid content of the enhancer/fruit juice, the composition of the meal especially the greens included in the diet play a role in deciding the extent of available iron of the diet. Research on available iron in greens have indicated that availability varies from vegetable to vegetable and its composition, and it is the function of either the total iron and ascorbic acid content or the presence of inhibitors as oxalic acid, polyphenols etc. (Chawla, Saxena & Seshadri, 1988;).

On an average, RJP from Brand B increased the dialysable iron by 54% in the diets with different greens, the extent of increase attained on addition of Brand A was 49%. However these differences in the enhancement observed between the brands of RJP's were found to be non-significant (P = 0.760) as per ANOVA. This difference in the extent of increase could be due to the differences in the concentration of ascorbic acid content of the juices, in addition the label of Brand B indicated the presence of citric acid that was not found in Brand A. These observations are similar to results of Kojima, Wallace & Bates (1981) who reported a 60% increase in dialysable iron in beans by a similar RJP.

The differences encountered in the enhancing ability of two different squashes varied widely. On an average Brand B squash exhibited a 4-fold higher increase than Brand A for which its high ascorbic acid content is a clear answer. The label of Brand B indicated the presence of ascorbic acid (added) while the label of Brand A indicated the presence of only citric acid. This indicated that ascorbic acid was a more effective enhancer than citric acid.

RTD juice of Brand A on an average resulted in a 74% increase and Brand B, in a 55% increase in dialysable iron, this related well with their ascorbic acid concentrations. The label on Brand A indicated a higher concentration of ascorbic acid along with citric acid but Brand B contained only ascorbic acid, hence a higher increase was seen on addition of Brand A.

On an average, CB from Brand A promoted iron dialysability of 90% while Brand B neither possessed a promotive or an inhibitory effect. Labels on CB indicated the presence of citric acid in Brand A indicating the essentiality of an organic acid for enhancing iron dialysability in the diets.

In the case of fresh fruit and fruit juices, diet with *Chenopodium album* showed a higher increase irrespective of the form and fruit added but no such trend was observed with the processed fruit products. This could be due to the presence of other components, which may have interacted with the dietary greens to alter the extent of iron dialysability.

The associations between the ascorbic acid and the dialysable iron content of diets were found to be extremely significant implying ascorbic acid content to be an important factor modifying the dialysable iron content of diets irrespective of the fruit base. The trend looked similar and good even in the case of processed beverages exhibiting an extreme level of association between ascorbic acid and dialysable iron contents. The enhancing ability of dialysable iron in the diets by the fruits, fruit juices and different types of processed beverages were done. It was found that the association of fresh juices versus RJP's ($F=0.7254^{ns}$), squashes ($F= 0.1157^{ns}$) , RTD's ($F= 0.4294^{ns}$) and CB's ($F=0.8896^{ns}$) were found to be non significant indicative of a similar enhancing ability by both fresh and processed beverages.

CONCLUSION

Addition of fruits and fruit juices to the cereal diets with greens (that have moderate iron content with low bioavailability) can definitely enhance the availability of iron by several fold. Of the fruits and fruit juices, orange proved to be a superior/potent enhancer followed by apple, while tomato and grapes were poor enhancers. Since orange is a seasonal fruit, other fruits containing high ascorbic acid/organic acids as citric and maleic can also be advocated to get maximum iron availability out of the consumed diets. Processed fruit juices of any form will definitely enhance the dialysable iron but the extent of enhancement is dependent on the added organic acid and freshness of the product. The role of carbonated beverages that do not contain any fruit powders/ components depend only on the presence of added organic acid, hence there is every possibility of them being either an enhancer or a neutral additive. From the iron availability point of view, which is a prime determinant of iron deficiency anaemia, consumption of a fruit or a fruit juice along with meals is healthy, promising and a preventive measure for iron deficiency anaemia. Our results indicate that consumption of a processed fruit juice is also beneficial in this regard.

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