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Influence of food acidulants on bioaccessibility of zinc and iron from selected food grains

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Four common acidulants in Indian dietary, *i.e.*, citric acid, tamarind (*Tamarindus indica*), amchur (*Mangifera indica*), and kokum (*Garcinia indica*) were examined for a possible influence on the bioaccessibility of zinc from selected food grains. Among the four acidulants examined, amchur and citric acid generally enhanced the bioaccessibility of zinc and iron from all the food grains studied. The increase in zinc bioaccessibility produced by citric acid was around 40% in rice and chickpea, while amchur produced around 60% increase from decorticated green gram. This positive influence of acidulants on zinc bioaccessibility from food grains was seen both in the raw and cooked form. Tamarind and kokum, the other two acidulants tested, generally did not have a favourable influence on zinc and iron bioaccessibility. This lack of positive influence of these two acidulants on mineral availability could be attributable to the presence of significant amounts of tannin in them. Citric acid and amchur also generally enhanced the bioaccessibility of iron from these food grains.

Keywords: Bioaccessibility / Food acidulants / Food grains / Iron / Zinc

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1 Introduction

Zinc is an essential trace element playing a vital role in several physiological processes, including cell division and growth, and foetal and brain development. Zinc is essential in the composition or function of over 70 enzymes involved in digestion and other major metabolic functions. Deficiency of this element, although not completely assessed, is believed to be as widespread as that of iron and is a cause for concern especially in the developing countries [1]. Although animal foods are the rich sources of zinc in our diet, a majority of the population in developing countries derive this micronutrient mainly through food grains. In general, the average zinc intakes of adult vegetarians tend to be lower than those of their omnivorous counterparts. Cereals are the primary sources of zinc in most vegetarian diets, secondary sources being legumes [2]. Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of haemoglobin, myoglobin and cytochromes. Despite large scale intervention programmes, iron-deficiency anaemia remains the most widely prevalent nutritional problem in the world. Although many factors are responsible for the onset of iron deficiency, the most likely cause of this nutritional problem in developing countries is the poor bioavailability of dietary iron.

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Bioavailability of micronutrients, particularly zinc and iron is low from plant foods [2, 3]. Bioavailability of trace minerals such as iron is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Although not exhaustively evidenced, it is possible that the bioavailability of zinc from food grains is similarly influenced by such diverse factors coexistent in them. Organic acids are known to promote the absorption of iron from plant foods [4]. Acidulants such as lime, tamarind, kokum and amchur are commonly used in Indian culinary to impart a desirable sour taste to certain food preparations. In the absence of any information on the influence of food acidulants on the bioaccessibility of zinc from food grains, it would be relevant to examine this. The present investigation was undertaken to study the influence of common food acidulants, citric acid (acid constituent of lime), tamarind (*Tamarindus indica*), amchur (*Mangifera indica*) and kokum (*Garcinia indica*), on the bioaccessibility of zinc from selected food grains, both in the raw and cooked forms. The comparative influence of the same acidulants on iron bioaccessibility from these food grains was evaluated.

2 Materials and methods

2.1 Materials

The cereal rice (O*ryza sativa*) and legumes chickpea (*Cicer arietinum*; whole and decorticated), green gram (*Phaseolus aureus*; decorticated), and red gram (*Cajanus cajan*; decorticated) were procured locally, cleaned and used for bioaccessibility studies. The food acidulants examined in this study, tamarind (*Tamarindus indica*) powder, amchur (dried raw mango, *Mangifera indica*) and kokum (*Garcinia indica*) were also procured locally. The citric acid used was of analytical grade. Pepsin (from porcine pancreas), bile extract (porcine) and pancreatin (porcine) were procured from Sigma Chemical Company, USA. All other chemicals used were of analytical grade. Triple distilled water was employed throughout the study. Acid-washed glassware was used during the entire study.

2.2 Total zinc and iron

Food samples were ground finely and ashed in a muffle furnace at 550°C for 5 h and the ash was dissolved in concentrated HCl. Zinc and iron content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out with standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.

2.3 Bioaccessibility of zinc and iron

Bioaccessibility of zinc and iron was determined in selected food grains (rice, chickpea, red gram and green gram) in the absence and presence of individual acidulants as listed. The acidulants were included at levels that produced a decrease in pH by 1 unit.

1. Food grain (10 g) + no acidulant
2. Food grain (10 g) + citric acid (0.05 g)
3. Food grain (10 g) + tamarind powder (0.75 g)
4. Food grain (10 g) + amchur powder (0.75 g)
5. Food grain (10 g) + kokum powder (0.75 g)

Two parallel sets of above samples were examined for mineral bioaccessibility in raw and cooked forms. Determination of zinc and iron bioavailability in these variations of food samples, as well as all other chemical analyses were carried out in quadruplicate.

Bioaccessibility of zinc and iron in various food grain samples was determined by the *in vitro* method described by Luten *et al.* [5] involving simulated gastrointestinal digestion with suitable modifications. The samples were subjected to gastric digestion by incubation with pepsin (pH 2.0) at 37°C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest, by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin-bile extract mixture (1 L of 0.1 M sodium bicarbonate containing 4 g pancreatin + 25 g bile extract). The titratable acidity was defined as the amount of 0.2 M sodium hydroxide required attaining a pH of 7.5.

To simulate intestinal digestion, segments of dialysis tubing (molecular mass cut-off: 10 kDa) containing 25 mL sodium bicarbonate solution, being equivalent in moles to the NaOH needed to neutralize the gastric digest (titratable acidity) determined as above, were placed in Erlenmeyer flasks containing the gastric digest and incubated at 37°C with shaking for 30 min or longer until the pH of the digest reached 5.0. The pancreatin-bile extract mixture (5 mL) was then added and incubation was continued for 2 h or longer until the pH of the digest reached 7.0. At the end of simulated gastro-intestinal digestion, zinc and iron present in the dialysate were analysed by atomic absorption spectrometry. The dialysable portion of the total mineral present in the sample (expressed as percent) represented the bioaccessible mineral.

2.4 Heat processing of food grains

To examine the influence of acidulants on zinc and iron bioaccessibility from heat-processed food grains, the selected food grains (10 g) were pressure-cooked in 30 mL water for 10 min (15 psi), in the presence of the acidulants. The cooked samples were homogenized and used for the determination of mineral bioaccessibility as above.

2.5 Estimation of tannin

Tannin was estimated by the modified vanillin assay of Price *et al*. [6], using catechin as the standard.

2.6 Statistical analysis

Statistical analysis of analytical data was done employing Student's *t*-test according to Snedecor and Cochran [7].

3 Results and discussion

3.1 Influence of citric acid on zinc and iron dialysability

Organic acids such as citric, malic, tartaric, and ascorbic acid are well documented to have a significant enhancing influence on iron bioavailability [4]. Information on such possible influence of organic acids on the bioavailability of zinc is, however, lacking. Several acidulants are commonly employed in Indian dietary, which, being a source of one or the other organic acid, could possibly alter the bioavailabil-

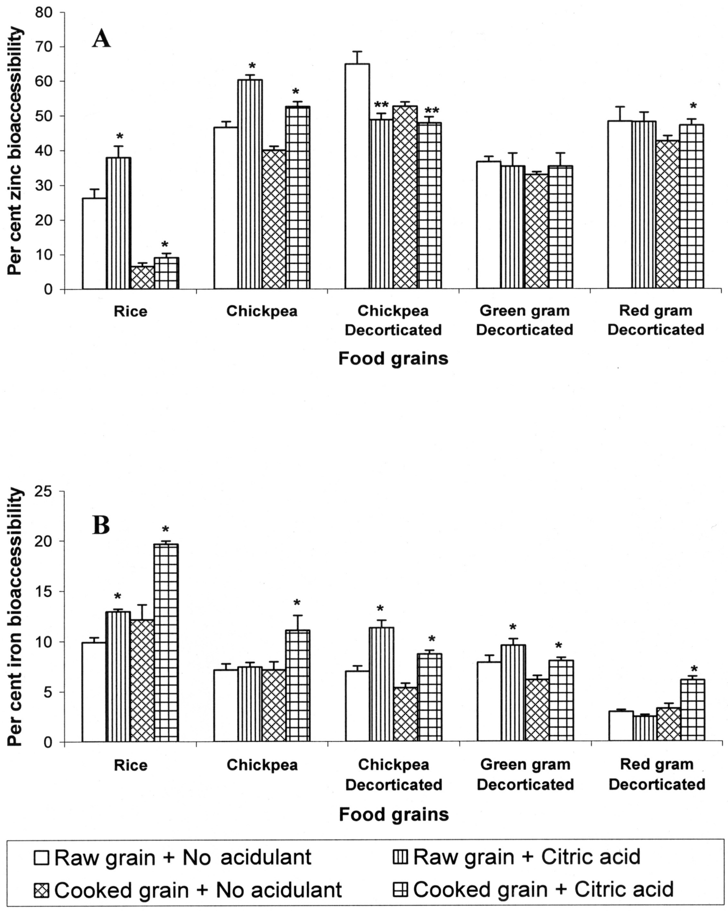

Figure 1. Effect of citric acid on zinc and iron bioaccessibility from raw and cooked grains. Values represent mean ± SEM of four independent determinations. *Significant increase; **Significant decrease.

ity of the trace mineral zinc from food sources. The present study, which has examined the bioaccessibility values of zinc and iron from selected food grains both in presence and absence of individual acidulants, has shown differences that provide an insight into the contribution of the acidulant component to the availability of trace minerals.

The influence of citric acid on bioaccessibility of zinc from rice, chickpea, green gram and red gram is shown in Fig. 1 A. Presence of citric acid considerably increased the bioaccessibility of zinc from rice and chickpea, by 44% and 40% from raw and cooked rice, and by 29% and 31% from raw and cooked chickpea, respectively. The zinc bioaccessibility from cooked red gram was enhanced by 11%. Citric acid did not have a similar positive influence on zinc bioaccessibility from the other food grains examined; it had a negative influence on raw decorticated chickpea (23% decrease).

The influence of citric acid on bioaccessibility of iron from food grains is presented in Fig. 1B. Citric acid generally enhanced iron bioaccessibility from the tested food grains. The increase in bioaccessibility was 30% and 62% from raw and cooked rice; around 62% from both raw and cooked decorticated chickpea; and 22% and 31% from raw and cooked decorticated green gram. The increase in iron bioaccessibility in presence of citric acid was 86% and 55% from cooked decorticated red gram and whole chickpea, respectively, while this acidulant had no effect on iron bioaccessibility from the two raw grains. Thus, the beneficial influence of citric acid on iron bioaccessibility was relatively higher when the grains were cooked along with the acidulant. This trend, however, was not evident in the case of zinc bioaccessibility. Citric acid, the acidic constituent of lime, has been employed in this study to represent the parent acidulant, for the sake of convenience.

3.2 Influence of amchur on zinc and iron dialysability

Bioaccessibility of zinc from rice, chickpea, green gram and red gram in presence of the acidulant amchur is shown in Fig. 2A. Amchur enhanced the bioaccessibility of zinc from all the food grains examined. The increase in zinc bioaccessibility from rice was more than 100% from raw and cooked grain. Zinc bioaccessibility from chickpea

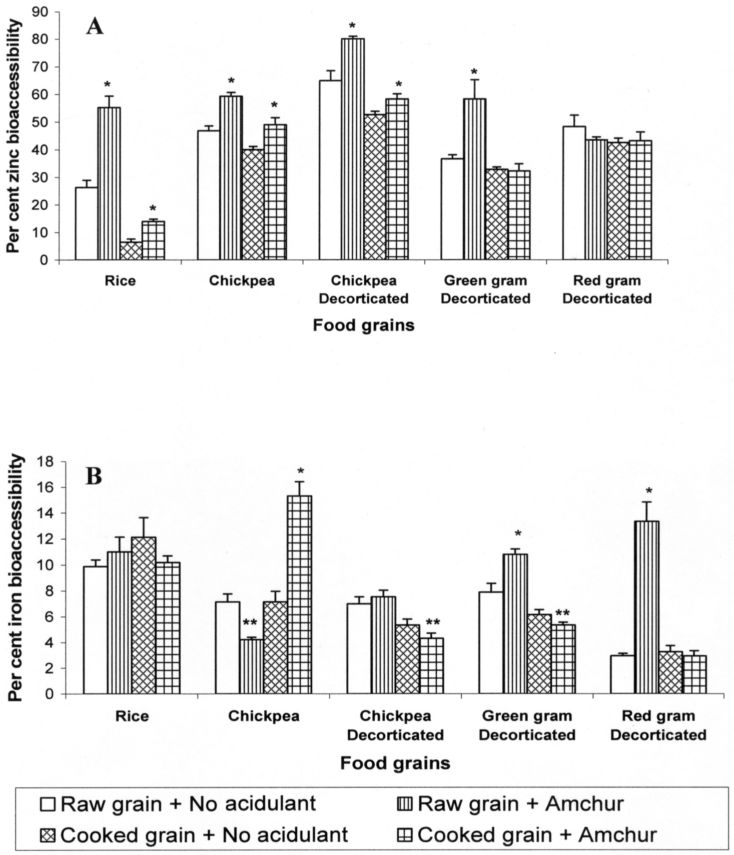

Figure 2. Effect of amchur on zinc and iron bioaccessibility from raw and cooked grains. Values represent mean ±SEM of four independent determinations. *Significant increase; **Significant decrease.

(whole grain) was increased by 27% (raw grain) and 22% (cooked grain), and that from decorticated chickpea was increased by 23% and 11% in the raw and cooked grains, respectively. An increase in zinc bioaccessibility of 59% was observed from raw decorticated green gram. No influence of amchur on zinc bioaccessibility was evident in red gram, either raw or cooked, and in cooked green gram.

Figure 2B presents the iron bioaccessibility from food grains as influenced by the acidulant amchur. Amchur had a general enhancing influence on iron bioaccessibility from the raw food grains. It increased the bioaccessibility value by 11%, 37%, and 350% in rice, decorticated green gram and decorticated red gram, respectively. On the other hand, iron bioaccessibility from raw chickpea was decreased by 41%. Amchur significantly enhanced the bioaccessibility from cooked chickpea (as much as 114%), while it generally had a negative influence on iron bioaccessibility from the other cooked grains.

3.3 Influence of tamarind on zinc and iron dialysability

The effect of tamarind on the bioaccessibility of zinc from food grains is shown in Fig. 3A. Inclusion of tamarind produced an increase of 18% in the bioaccessible zinc concentration of raw chickpea. In all other cases, tamarind did not have any effect, or even produced a negative effect (a decrease by 10–57%). Whereas a negative influence of tamarind was seen in cooked rice (57% decrease), with no effect in the raw grain, the opposite trend was observed in the case of decorticated chickpea, where a decrease by 32% was observed in the raw grain.

Figure 3B presents the influence of tamarind on the bioaccessibility of iron from food grains. Tamarind similarly showed a negative influence on iron bioaccessibility from all the food grains examined, and this ranged from a decrease by 28% in raw chickpea to 58% in cooked decorticated chickpea. The only exception to this was in the case of cooked whole chickpea, where tamarind enhanced the iron bioaccessibility (by 22%).

3.4 Influence of kokum on the dialysability of zinc and iron

The effect of kokum on the bioaccessibility of zinc from food grains is shown in Fig. 4A. Kokum generally decreased the bioaccessibility of zinc from the food grains studied, except in the case of whole chickpea, where it pro-

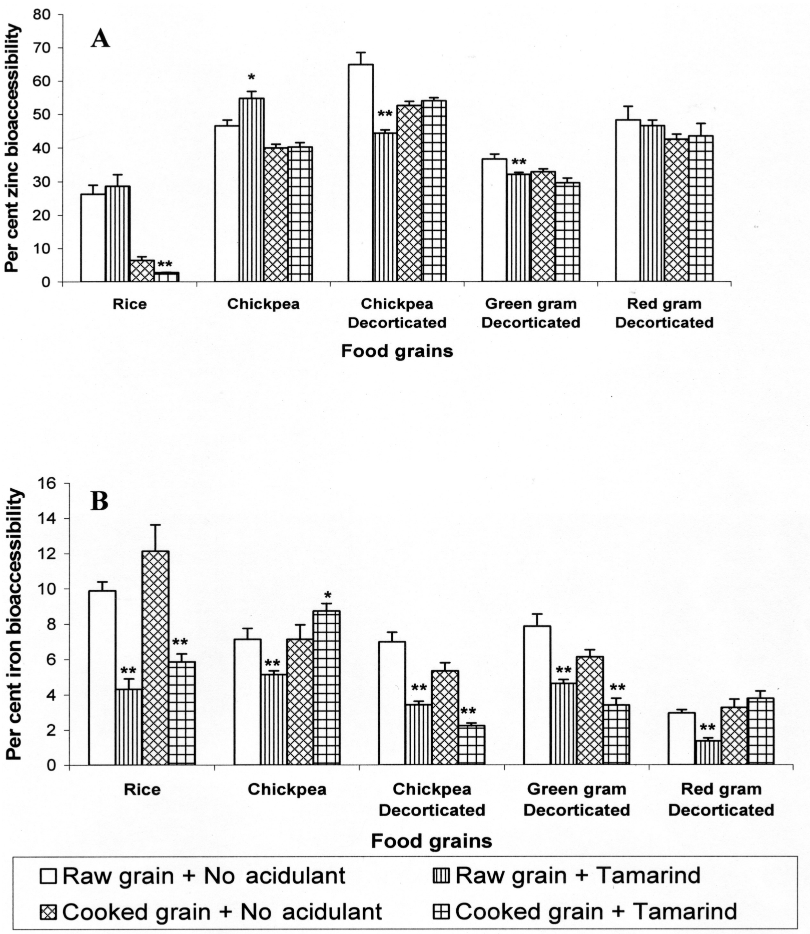

Figure 3. Effect of tamarind on zinc and iron bioaccessibility from raw and cooked grains. Values represent mean ± SEM of four independent determinations. *Significant increase; **Significant decrease.

duced an increase of 18% in the cooked grain. The decrease in bioaccessibility value produced by kokum ranged from 10% in raw decorticated chickpea to 39% in cooked rice. Influence of kokum on the bioaccessibility of iron from food grains is presented in Fig. 4B. Kokum had a negative influence on the bioaccessibility of iron from all the food grains tested, this effect being as high as 88% in cooked decorticated green gram. As in the case of tamarind, the only exception was cooked whole chickpea, where an increase in iron bioaccessibility (by 60%) was observed.

Thus, among the four common food acidulants examined, amchur and citric acid exerted a positive influence on mineral bioaccessibility in a larger number of samples. The observation that citric acid and amchur had a similar effect in enhancing the bioaccessibility of zinc could be due to the fact that citric acid is also the main acidulant in amchur, with minor amounts of malic acid. Even though the other two acidulants, tamarind and kokum, have been included in amounts that produce the same level of acidity as amchur and citric acid, they did not exhibit a similar positive effect on mineral availability. Tamarind has tartaric acid as the main acid, while kokum has hydroxycitric acid and minor quantities of tartaric acid. Thus, among various organic acids, citric acid has the maximum beneficial effect with regard to enhancing the mineral bioaccessibility. This absence of a comparable positive influence of the other two acidulants, tamarind and kokum, may also be attributable to the high concentrations of tannin present in them (Fig. 5). These two acidulants contributed significant amounts to the total tannin content of the food samples examined. Tannin is known to strongly inhibit mineral absorption [8]. Although there are reports that organic acids (citric, malic, ascorbic and tartaric) enhance the absorption of iron from foods, the commonly used food acidulants have not been examined in this context. The present study is the first report on the influence of amchur, tamarind and kokum (as sources of organic acids) on mineral bioaccessibility.

The beneficial influence of citric acid on zinc bioaccessibility observed here roughly correlated with the concentration of phytic acid inherent in the food grain (our unpublished data). Thus, pronounced enhancement in the zinc bioaccessibility value was evident in rice and chickpea, which had lower concentrations of phytic acid (150 and 263 mg/100 g,

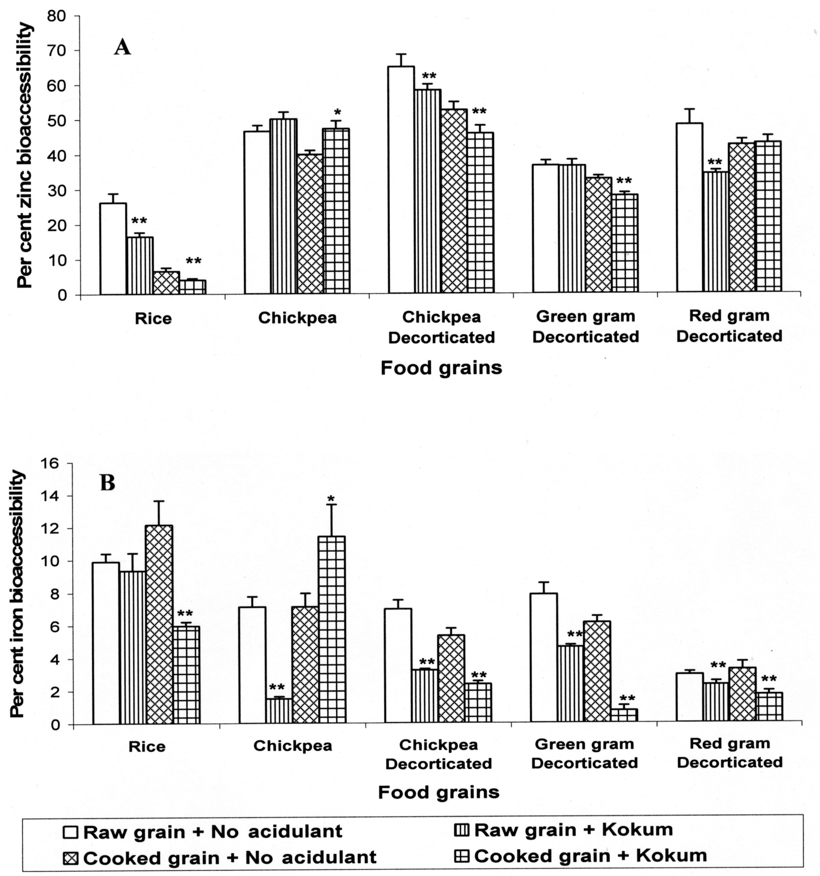

Figure 4. Effect of kokum on zinc and iron bioaccessibility from raw and cooked grains. Values represent mean ±SEM of four independent determinations. *Significant increase; **Significant decrease.

respectively) compared to the other grains studied (324 mg/100 g in decorticated chickpea to 630 mg/100 g in decorticated green gram). Phytate is known to be a potent inhibitor of trace element absorption [9]. Citric acid was thus unable to counter the inhibitory effect of phytate on zinc absorption, where the concentrations of the latter in the grains were higher. However, amchur significantly enhanced zinc availability even from those food grains that had higher concentration of phytate. Such an inverse relationship between the beneficial influence of acidulant and inherent phytate concentration was not evident in the case iron bioaccessibility. Citric acid enhanced the bioaccessibility of iron from all the grains tested, irrespective of their phytate concentration. We have earlier documented that iron bioaccessibility from food grains, especially pulses, was not negatively influenced by the inherent phytate (our unpublished data).

Citric acid (of lime) and amchur have been shown here to significantly enhance the absorbability of both zinc and iron from rice. The usage of lime and raw mango in combination with rice in preparations such as "lemon rice" and "mango rice" is actually in vogue in Southern India. Such a practice could thus prove advantageous in terms of zinc absorption. The particularly higher magnitude of positive influence of most of the acidulants on mineral bioavailability from rice observed in this study relative to other food grains could be attributed to the low titres of inherent factors such as phytate, tannin and calcium interfering with mineral absorption.

The *in vitro* method employed here for the estimation of mineral (Zn and Fe) availability is based on simulation of gastro-intestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that dialyses through a membrane. The dialysability of a mineral gives a fair estimate of its availability for absorption *in vivo*. Such *in vitro* methods are rapid, simple and inexpensive, and offer the possibility of optimally controlling the assay conditions, which can lead to high accuracy. However, it should be remembered that the results from *in vitro* methods are relative rather than absolute estimates of mineral absorbability, since they are not subjected to the physiological factors that can affect bioavailability [10]. Such relative estimates of the mineral availability obtained in the present investigation on the comparative zinc bioavailability values for different food grains, and the influence of

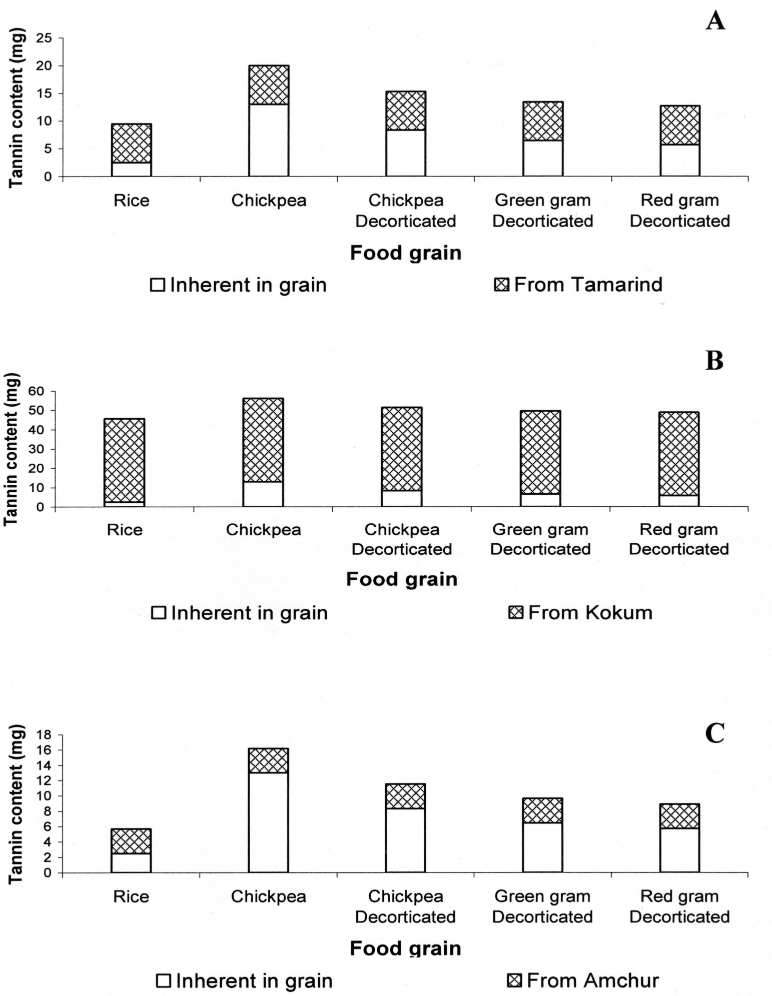

Figure 5. Tannin concentration in food sample contributed by acidulants, tamarind (A), kokum (B) and amchur (C).

cooking on the same are still valid and suffice to form a strategy to derive maximum mineral availability.

4 Concluding remarks

The present study has revealed that citric acid has a similar enhancing effect on both zinc and iron bioaccessibility from the food grains examined. Amchur too had a similar positive influence on bioaccessibility of zinc and iron in most of the food grains. However, in the case of cooked food grains, this effect was more evident on zinc bioaccessibility. The other two food acidulants, tamarind and kokum, generally had a negative influence on both zinc and iron bioaccessibility from all the food grains studied, both in the raw and cooked forms. Among the food grains examined, whole chickpea seems to have derived the maximum beneficial effect from all the four food acidulants, especially when cooked, with reference to bioaccessibility of these two trace minerals.

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