Bioevaluation of zinc fortified coated apricots using rabbit experimental model

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DOI: http://dx.doi.org/10.20454/jeaas.2015.950

ABSTRACT

Edible coatings have largely been stemmed out as prospective preservation stratagem to extend shelf life of fruits; additionally these may serve to impart certain textural, nutritional and functional attributes. Recent era has witnessed the use of these novel coatings as a tool to fortify various foods. Purposely, the current research was an endeavor to fortify fresh apricots with zinc (40 ppm ZnO) using sodium caseinate based edible coatings to address zinc deficiency. Accordingly, the zinc fortified coated apricots were prepared and fed to experimental rabbits for a period of six weeks. The sera and organs (liver, kidney & heart) zinc levels depicted a significant increase in zinc content of rabbits. From the findings, it was ascertained that 27.33, 22.47 & 16.25% zinc levels in liver, kidney & heart, respectively were enhanced in zinc fed group (G₁) as compared to control (G₀). Likewise, serum zinc level in G₁ was elevated up to 13.70%. Conclusively, it is summarized that edible coating is a promising strategy to fortify food stuffs with extended shelf life and efficient delivery of zinc fortificant in the living subjects.

KEYWORDS: Edible coatings, zinc deficiency, zinc fortification, zinc oxide, bioevaluation.

INTRODUCTION

The present era has witnessed various diet related maladies. Reliance on monotonous diet, inadequate micronutrient intake and various untold social factors are responsible for malnutrition and allied health incongruities in the developing countries. Among malnourishment, obesity, cardiovascular diseases and especially micronutrient deficiencies involving vitamins and minerals insufficiencies have prevailed to a larger extent. Micronutrients are required by the body on daily basis to sustain smooth survival of life and healthy activities. A slight ambiguity in micronutrient consumption may cause various metabolic dysfunctions (Raine, 2010). In Pakistan, the ubiquitous micronutrient deficiencies are of vitamin A & D, iodine, iron and zinc (NNS, 2011). The mi-
cronutrients are known to regulate various metabolic pathways and their deficiencies lead to severe physiological abnormalities that may hamper the health stratum and life quality of the individuals (Prasad, 2012).

Zinc paucity has long been spread over the globe owing to ignorance and poor dietary practices. This scarcity may also be related to various metabolic malfunctioning and consuming diets meager in zinc. Hypozincemia or zinc deficiency has grabbed global attention as a pinching issue particularly in the developing economies. It is estimated that about 95.4% of South Asian population is deficient in zinc contents due to poor dietary lifestyles (Prom-u-thai et al., 2010).

Zinc is a metal with great nutritional significance and is predominantly necessary in cellular imitation and improvement of the immune systems (Salgueiro et al., 2002). It is indispensable for growth and development. Furthermore, zinc is critical in proliferation, differentiation, maintenance and apoptosis at cellular level. Various enzymes, especially metalloenzymes are zinc dependent for their proper functioning. The functions of zinc include intermediate metabolism, immunity, DNA repair & metabolism, reproduction, taste responses, vision and cognition. Additionally, zinc is essential for brain functioning, neuronal growth, neurogenesis, neurotransmission and synaptogenesis (Maret and Sandstead, 2006). Zinc acts as an excellent anti-degenerative and anti-inflammatory agent (Plum et al., 2010). Likewise, it also participates in the dispensation, packing and consumption of insulin. The zinc is vital in gastrointestinal system owing to its obligatory role in protein metabolism (Bourtoom, 2008). It has been documented that zinc deficiency creates anemia, growth retardation, hepatosple-

omegaly, hypogonadism, rough & dry skin, geophagia and mental lethargy (Raine, 2010; Prasad, 2012).

In an effort to curtail such diet linked maladies, various strategies have been devised. Amongst different approaches, fortification has proved to be one of the vibrant, far reaching and effective choices to eradicate the consequences associated with the menace of under-nutrition. Additionally, this intervention is flexible and socially acceptable to improve the nutrients balance by incorporating certain deficient micronutrients in foods (Prasad, 2012).

Fortification of zinc has been established in the flours of wheat, corn and rice (Prom-u-Thai et al., 2010). For the purpose, different zinc compounds including zinc oxide, zinc chloride, zinc sulfate, zinc gluconate, zinc acetate and zinc stearate have been utilized. Zinc oxide is most frequently used as zinc fortificant in cereal based foods followed by zinc sulfate (Ranum, 2001; Prom-u-Thai et al., 2010). Zinc gluconate is added to the foods at very limited extent. Although, zinc acetate and zinc gluconate is being added in the dietary supplements and weaning foods. Moreover, zinc oxide provides 80% of the zinc to that of its native compound. Additionally, its absorption is as good as any other soluble forms of the zinc salts (Ranum, 2001). Zinc oxide as a fortificant is preferred owing to its low cost and high chemical stability (Prom-u-Thai et al., 2010). It has also been reported that the absorption of zinc is carried out in duodenum and is also related to the gastric pH (Karademir, 2011).

Various researchers have envisioned that edible coatings can be established as a fortification vehicle for micronutrients. These
can be used for the delivery of various additives, nutraceuticals and antioxidants. Moreover, these biodegradable coatings also cover economic, safer and consumer friendly nature making them a paramount tool to use as a fortificant carrier (Falguera et al., 2011; Altamirano-Fortoul et al., 2012; Dhall, 2013).

**MATERIALS AND METHODS**

The fresh apricots were prepared and coated with a mixture of sodium caseinate based edible coating containing 40 ppm zinc from ZnO (Iahtisham-Ul-Haq et al., 2014). The coated apricots were dried at room temperature for 15 minutes after coating and then used for administration.

**Efficacy study**

The zinc coated apricots were administered to rabbits to check the efficacy of zinc fortificant. For the purpose, twenty five young male white rabbits weighing between 1.25-1.475 kg were procured and placed in Animal Room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad. They were acclimatized by feeding basal diet for a period of one week. At the initiation, some of the rabbits were sacrificed to attain baseline value. Afterwards, remaining rabbits were reared for six weeks in two groups (Table 1); 10 rabbits in each group provided with fortified and unfortified apricots along with simultaneous diet provision. At the termination of study, rabbits were slaughtered and their sera and organs (liver, kidney, heart) were collected for various biochemical analyses.

**Organs zinc analysis**

The organs i.e. liver, heart and kidney of both groups were analyzed for zinc contents following the method of Akhtar et al. (2010) by wet digestion followed by zinc assessment using atomic absorption spectrophotometer (Varian AA240, Australia).

**Table 1: Efficacy study plan**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>Control</td>
</tr>
<tr>
<td>G₁</td>
<td>Zinc fed</td>
</tr>
</tbody>
</table>

**Serum zinc analysis**

The sera from the collected blood of rabbits were separated by centrifugation at 10,000 g for 5 minutes followed by zinc analysis using atomic absorption spectrophotometer (Varian AA240, Australia).

**Statistical analysis**

The data obtained for each parameter was subjected to statistical evaluation applying Completely Randomized Design (CRD) to determine the level of significance using Statistix (Version 8.1). Furthermore, Microsoft Excel (version 2007) was used to generate the graphs.

**RESULTS**

The mean squares for the serum and organ zinc analysis are presented in Table 2. From statistical analysis, it is evident that there existed a significant relationship between zinc status and diets fed to both groups.

**Liver zinc**

As obvious from mean squares regarding zinc content in liver that there was found a significant difference amongst the groups of experimental rabbits (Table 2). It can be seen from the mean values (Fig. 1) that there was a clear divergence between the means of G₀ (Control) and G₁ (Zinc fed group). The observed value for the trait was trailed as 43.87±1.36 μg/g for G₀.
However, comparatively elevated levels of zinc in the livers of G1 were recorded i.e. 55.86±1.74 μg/g. It can be deduced that there occurred a significant accumulation of zinc content in the livers of the G1 experimental rabbits with the administration of zinc fortified sodium caseinate based edible coatings. Improved zinc content in liver exhibits a sound signal towards improved efficiency of liver work as zinc is a vital part of metalloenzymes in the liver.

Table 2: Mean squares for zinc content in serum and organs of rabbits

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Serum</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1</td>
<td>714.371**</td>
<td>718.681**</td>
<td>185.806**</td>
<td>39.6493**</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>5.666</td>
<td>1.646</td>
<td>0.597</td>
<td>0.231</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

** = Highly significant (P < 0.01).

Figure 1: Means for zinc content in various organs of control and zinc fed rabbits.
The instant explorations are in accordance with the recent work of Akhtar et al. (2010) who trailed the bioavailability of different sources of zinc in conjunction with iron in rats using whole wheat flour as fortificant vehicle. They used solely the ZnSO$_4$ and ZnO as well as in combination with elemental iron and NaFeEDTA to check the bioavailability of both minerals i.e. Zn and Fe. From their investigations, it was obvious that a significant difference between the organs’ zinc level exist amongst the groups of rats. An increase of 37.48% was found in their research while using 20 ppm of ZnO administered over a period of 30 days. Likewise, in current research 27.33% increased level of zinc in liver was found in zinc fed group as compared to control group (Fig. 2).

![Figure 2: Percent increase in zinc content of serum and organs of zinc fed group as compared to control](image)

**Kidney zinc**

From the mean squares, inference can be made that zinc level in kidney differed highly amongst the groups (Table 2). It can easily be concluded that there exists a significant variation between the mean values of zinc content in kidneys of experimental rabbits of both groups. G0 (Control) group rabbits acquired 27.13±1.04 µg/g of tissue zinc level whilst an elevated zinc content can be found from the mean value for G1 (Zinc fed) group of investigational animals as 33.23±1.21 µg/g of the kidneys (Fig. 1). From the means, it can be deduced that amassing of zinc in kidney tissues enhances its level in the tissues of the organ up to 22.47% (Fig. 2). Moreover, as kidneys are responsible for excretion of the unnecessary metal contents from the body via urine so it can be implicit that the high contact of zinc through blood plasma reaching the kidneys increases the chance of zinc accumu-
lation in the organ as it is continuously remain in contact with the blood for filtration.

The results of instant study are in accordance with the recent work of Akhtar et al. (2010) who used whole wheat flour for cofortification of iron and zinc using different forms of their salts and checked their bioavailability in experimental rats. In their study, they found no significant difference between the zinc absorption from ZnSO4 and ZnO. They observed increased zinc content in kidneys of 20 ppm ZnO fed rats as compared to control. However, zinc interacted with the bioabsorption of iron in the gut where these compete to each other and affect their relative absorption. Earlier, Herman et al. (2002) also narrated that there is no significant difference between absorption of zinc from ZnSO4 and ZnO in dumplings. Moreover, zinc interacts antagonistically with iron rendering it inaccessible to the body.

**Heart zinc**

The zinc content in heart momentously varied amongst the groups as evident from the mean squares (Table 2). It can be deduced that there reside significant difference between the zinc levels of heart in both groups. Mean values regarding zinc content in heart of rabbits are exhibited in Fig. 1. It is palpable from the graph that there was a clear difference between the zinc content of rabbits’ heart in both groups. G1 (Zinc fed) group acquired highest amount of zinc in the organ as 20.16±0.98 μg/g followed by G0 (Control) as 17.34±0.68 μg/g of tissue. Furthermore, it is deducible that administration of zinc fortified edible coated apricots proved promising in enhancing the zinc status of the body as there occurred an increase (16.25%) in heart zinc level of experimental rabbits in G1 (Fig. 2). Additionally, ZnO proved itself a good source to be used as a zinc fortificant because of its bioavailability as various other scientists had also claimed that the absorption of zinc in the body is equal for both ZnO and ZnSO4, commonly used fortificants.

**Serum zinc**

Serum zinc content of rabbits of both experimental groups were found significantly different as evident from mean squares regarding serum zinc content mentioned in Table 2. The administration of zinc fortified apricots was efficient in enhancing the status of zinc in serum of rabbits fed for six weeks as evident from the mean value that corresponds to 99.19±2.53 μg/dL for G1 (Zinc fed group) as compared to G0 (Control) that demonstrated a mean value of 87.24±2.22 μg/dL (Fig. 3). It is deduced that the serum zinc level of experimental animals was enhanced 13.70% in G1 relative to G0 (Fig. 2). Consequently, edible coating proved itself quite valuable as prospective stratagem to deliver the fortificant in the living bodies of investigational rabbits which confirms that these coatings can be utilized as a transportation carrier of zinc to address zinc deficiency.

The results of the instant investigation matched with the trends found in the work of Karademir (2011) who trailed the effect of different pH conditions on the rabbits’ serum zinc levels while administrating zinc sulfate orally. He concluded that there exist a significant correlation between the serum zinc status and the pH at which fortificant is applied. His explorations confirmed the increase in serum zinc levels after zinc sulfate administration. Akhtar et al. (2010) also observed increase in plasma zinc concentration while feeding the rats diet containing 20 ppm zinc oxide. They
also trailed zinc sulfate as fortificant and found no significant difference between the biological absorption of zinc from zinc oxide and zinc sulfate. However, the inclusion of zinc in conjunction with the iron to the diets of rats showed significant antagonistic relation between bioabsorption of these two elements.

Figure 3: Means for zinc content in serum of control and zinc fed rabbits.

CONCLUSION

Globally, the menace of malnutrition has prevailed to larger extent. Nutritional strategies have emerged to tackle this dilemma using food based interventions. Zinc deficiency is pivotal in South Asian countries like Pakistan so there is a dire need to overcome these nutritional problems. The instant findings suggested that the sodium caseinate based edible coatings act as a vital tool to fortify fruits like apricot with zinc. Moreover, the bioefficacy trials for these fortified apricots were found quite satisfactory in improving the overall nutritional status regarding zinc. Nevertheless, there exists a need to extend research in this area using other fortificants to explore the carrier potential of edible coatings.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


