Corn Food Products Intrinsically Labeled with Zinc-65 for Studies on Bioavailability of Zinc in Humans[1]

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ABSTRACT

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Corn food products intrinsically labeled with radioactive zinc-65 (^{65}Zn) were prepared for use in determining the bioavailability of zinc in humans from corn foods. A defatted endosperm-hull fraction of whole kernel corn, originally derived from corn plants intrinsically labeled with ^{65}Zn, was incorporated with nonradioactive ground corn grits to yield a nonflaked, nontoasted, labeled food product. A second labeled food product was prepared in an identical manner, and this product was further processed into toasted cornflakes. Comparable unlabeled products were also prepared. The abundance ratio of total zinc and ^{65}Zn present in the labeled food products at the start of the feeding studies, as determined by atomic absorption and gamma rays spectrometry, respectively, was about three million to one.

The bioavailability of essential mineral elements including zinc is difficult to establish because of the multitude of complex factors that affect their absorption and retention in humans. Underwood (1977) assessed the physiological role and requirements of zinc in both humans and animals. Erdman (1981) reviewed studies involving zinc and iron bioavailability in cereals and legumes and concluded that considerably more research is needed. Maga (1982) concluded that phytic acid and its derivatives can bind dietary minerals, thus making them unavailable or only partially available. He stressed, however, that food components such as fiber also can play a significant role in mineral utilization. Solomons (1982) described specific factors that affect the bioavailability of zinc. However, studies that delineate specific effects on the absorption of individual mineral elements are difficult to design.

Fox et al (1981) recommended the use of intrinsic or extrinsic labels for bioavailability assessment in humans of naturally occurring or added elements such as zinc. The present study was undertaken to prepare corn products intrinsically labeled with radioactive ^{65}Zn for studies on bioavailability of zinc in humans.

The bioavailability of zinc is expected to be different for the diverse corn products that are currently available, principally because of large differences in the composition of whole kernel corn fractions used in the preparation of these products. For example, researchers studying fractions of the whole kernel have found high levels of the following zinc-binding components: phytin phosphorus, 82% (Hamilton et al 1951); phytate, 90% (O'Dell et al 1972); and phytic acid (Erdman 1979) in the germ fraction but low levels in the endosperm fraction.

MATERIALS AND METHODS

Since most cornflakes and snack foods are derived from dry-milled corn products of endosperm origin, the products labeled with ^{65}Zn were prepared from an endosperm fraction of corn rather than germ. To evaluate the effects of heating and toasting on the availability of zinc, the foods were prepared under both nontoasted and toasted conditions. A total of 0.5 μCi ^{65}Zn per individual human subject were permitted in the subsequent feeding studies. We used commercially available unground and ground corn grits further processed into flaked and nonflaked products. An intrinsically labeled (^{65}Zn) endosperm-hull corn fraction (Garcia et al 1977) was incorporated with the corn grits before being processed into the final products. A portion of this labeled endosperm-hull fraction had been used in a study by Evans and Johnson (1977) to measure the absorption of zinc in corn products by rats.

The ^{65}Zn activity in the labeled corn fractions (defatted endosperm-hull and germ) initially produced (Garcia et al 1977) had decayed to low levels, and their use for incorporation into low-level labeled corn food products for human consumption was now possible. Although lower levels of activity were most likely to be used in the feeding study, a maximum individual dose of 0.5 μCi ^{65}Zn was allowed.

Levels of ^{65}Zn Activity in Corn Fractions

Measurements of the ^{65}Zn in corn fractions were made with a gamma ray spectrometer composed of a sodium iodide (thallium-activated) crystal detector incorporated in a Tracor Analytic model 1185 automatic counting system coupled to a Tracor model TN 1710 pulse height analyzer. We used only those counts from the gamma spectrum that were associated directly with the 1.11 Mev ^{65}Zn photopeak to determine the activity. Table I shows the activity of the labeled corn fractions (defatted germ and defatted endosperm-hull) determined by three alternate techniques: calculation of the decay of the ^{65}Zn from activities previously established for these fractions (Garcia et al 1977); gamma counting of both flours; and solutions of the wet-ashed flours. A newly acquired ^{65}Zn standard was used for calibration of the gamma counting. Even though the ^{65}Zn decay had extended over eight half-lives, there was good agreement with activities established with the new ^{65}Zn standard. Results of these alternate techniques verified the ^{65}Zn activity in fractions that were to be incorporated into food products.

Preparation and Characteristics of Four Food Products

Figures 1 and 2 depict the procedures used in the preparation of the four products. Products identified therein as 1 and 2 are both nonflaked and nontoasted and represent nonlabeled and intrinsically labeled (^{65}Zn) conditions, respectively. Degermed hominy grits obtained from a commercial mill were used as the starting material. As indicated in Fig. 1, 27 kg of the corn grits was ground to pass through 40 mesh, and this ground flour was designated as product 1. Product 2, however, was formulated to incorporate the 30 g of the intrinsically labeled endosperm-hull fraction, which had an activity of approximately 0.1 μCi ^{65}Zn/g at the time of formulation, with 140 g of the ground corn grits flour. The two dry flours were mixed thoroughly in a closed container before distilled water was added and further blended. Water then was removed by evaporation until a moisture content of approximately 9% was attained. Product 2 had a total activity of 3 μCi ^{65}Zn at the time of formulation.

To prepare nonlabeled cornflakes (product 3), distilled water was added to 20 kg of unground degermed corn grits, which were

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[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

then steeped before being autoclaved at 126° C for 3 hr as shown in Fig. 2. After being dried to approximately 20% moisture, the grits were flaked with large rolls to a thickness of 0.064 cm followed by toasting at 260° C. Labeled cornflakes (product 4) were prepared differently; 140 g of the ground corn grits was combined with 30 g of the intrinsically labeled endosperm-hull flour and blended with distilled water, as previously described for product 2. A dough was made and rolled to a thickness of 0.32 cm before being cut into strips 1.3 cm wide for autoclaving at 126° C. The strips were dried to 20% moisture and cut to approximately the size of the unground corn grits used in product 1 before flaking and toasting operations began. The final labeled product 4 weighed 161.3 g and had a moisture content of 3%; however, the total activity (3 μCi ^{65}Zn) was the same as in labeled product 2.

Determination of Zinc-65 and Total Zinc

Approximately 2 g of the labeled prepared products were wet-ashed with concentrated HNO_3 and diluted up to 10 ml in a dilute HNO_3 medium. An aliquot (3 ml) was transferred to a plastic tube and counted in the gamma ray spectrometer.

Replicate 5-g samples of nonradioactive ground corn grits (product 1) and cornflakes (product 3) were also wet-ashed. Total zinc content of all corn fractions and prepared products was then determined by flame atomic absorption (Garcia et al 1974a).

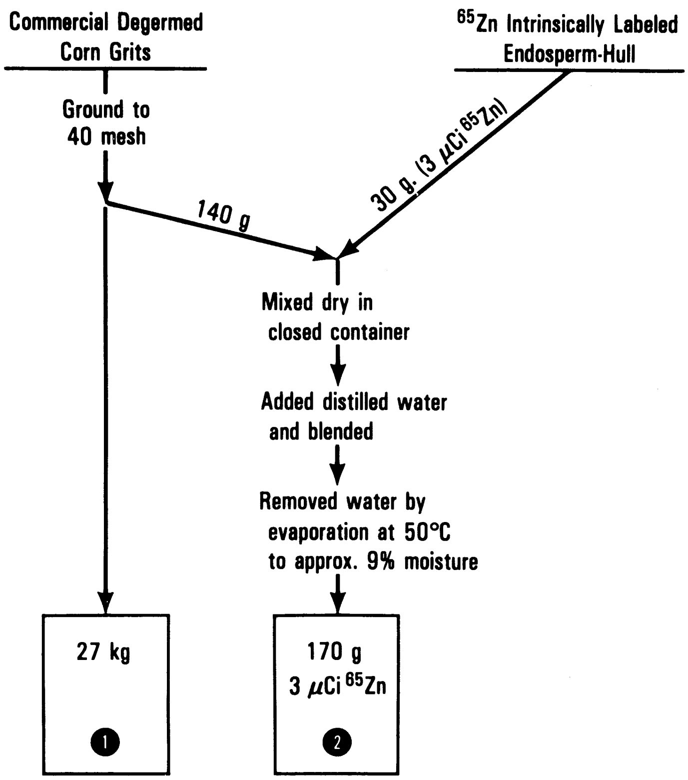

Fig. 1. Procedures and treatments used in the preparation of the nonflaked and nontoasted food products for studies on the bioavailability of zinc in humans. Products 1 and 2 = nonflaked, nontoasted products.

RESULTS AND DISCUSSION

Table II shows the relationship between total zinc and ^{65}Zn in the two labeled corn fractions and in the food products prepared. The labeled endosperm-hull corn fraction had considerably higher levels of total zinc (19.42 μg Zn/g) than did the prepared products because it represented approximately 90% of the entire corn kernel. This fraction included most of the hull and the adhering aleurone layer, which contains higher levels of minerals. Respectively, the aleurone layer and the pericarp (hull and tip cap) represent 2.2 and 6.5% of the total corn kernel (Hinton 1953).

In comparison, commercial corn grits are essentially an endosperm product low in minerals. Thus, the 30 g of the labeled endosperm-hull that was incorporated contributed more

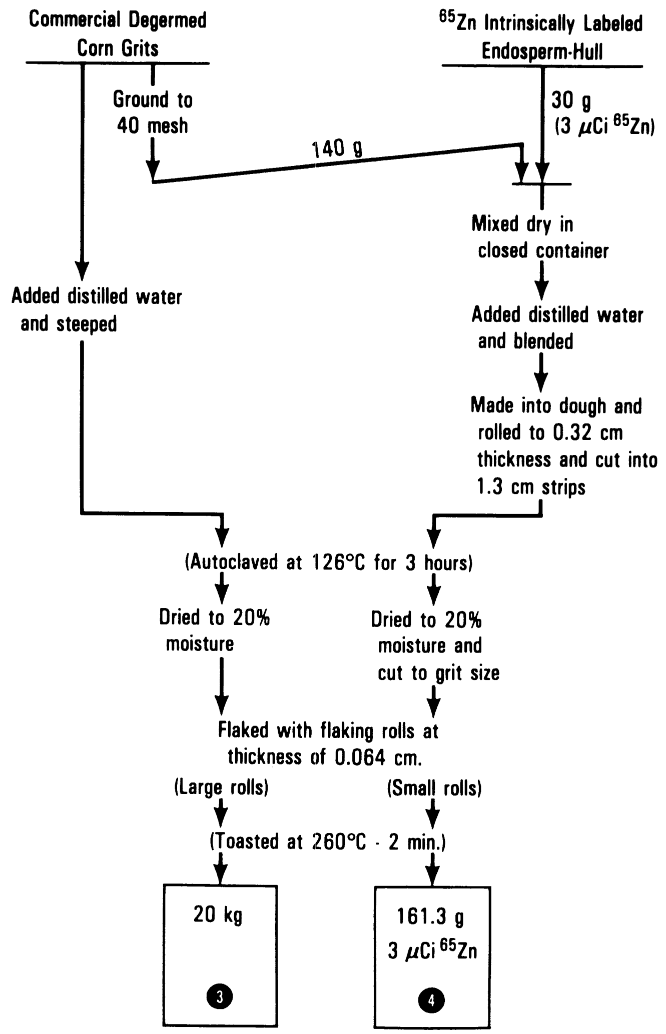

Fig. 2. Procedures and treatments for the preparation of flaked and toasted food products for studies on the bioavailability of zinc in humans. Products 3 and 4 = flaked, toasted products.

TABLE I
Summary of the Activity of Intrinsically Labeled (^{65}Zn) Corn Fractions

Characteristic or Measurement	Elapsed Time from t_0 (days)	Fraction of Initial Activity Remaining on January 23, 1981[b] ($e^{-\lambda t}$)	Activity (μCi ^{65}Zn/g) Defatted Corn Germ: Initial July 1, 1975, t_0	Defatted Corn Germ: January 23, 1981	Defatted Endosperm-Hull: Initial July 1, 1975, t_0	Defatted Endosperm-Hull: January 23, 1981
Decay of ^{65}Zn[a]	2,032 (8.29 T½s)	0.0032	396.17	1.267	24.91	0.0797
Gamma counting[c] of flours	···	···	···	1.214	···	0.0963
Gamma counting[c] of wet-ashed flours	···	···	···	1.309	···	0.0907

[a] T½ = 245 days.
[b] Calculated to date proposed to commence feeding studies.
[c] New ^{65}Zn standard used for gamma counting calibration.

TABLE II
Relationship Between Total Zinc and ^{65}Zn in Labeled Corn Fractions and in Prepared Food Products

Product	Total Zinc μg Zn/g Product	Radioactive ^{65}Zn μCi $^{65}Zn/g$ Product (as of January 23, 1981)	Picograms $^{65}Zn/g$ Product	Weight Ratio Total:Zinc-65
Intrinsically labeled fraction				
Endosperm-hull flour	19.42	0.0907	11.06	1.76×10^6
Corn germ flour	240.3	1.309	159.6	1.51×10^6
Prepared				
No. 2, grits-endosperm-hull	5.73 (5.67)[a]	0.0146 (0.0160)[a]	1.78	3.22×10^6
No. 4, cornflakes	6.18 (5.97)	0.0177 (0.0169)	2.16	2.86×10^6
Nonradioactive				
Prepared				
No. 1, ground corn grits	2.72 ± 0.14[b]			
No. 3, cornflakes	2.51 ± 0.34[c]			

[a] In parentheses are calculated values expected for nonradioactive concentrations and for ^{65}Zn activities in the prepared products, as of January 23, 1981. These calculated values are based on the weights of ground corn grits (140 g) and intrinsically labeled endosperm-hull (30 g) used and the resultant weights of prepared foods 2 and 4.

[b/c] Based on the mean of four and three 5-g samples, respectively.

nonradioactive zinc to prepared products 2 and 4 than did the 140 g of the corn grits. For this reason, the labeled products 2 and 4 contained more than two times the total zinc than did the nonlabeled, nonradioactive products 1 and 3. The zinc content of the nonlabeled cornflakes (2.51 μg Zn/g) that we prepared in these studies compare with commercial cornflakes (2.49 μg Zn/g) (Garcia et al 1974b).

The weight of the ^{65}Zn present in the labeled fractions and prepared products, determined from their established activity on January 23, 1981, was used to calculate the weight ratios between total zinc and ^{65}Zn. The data in Table II show that, although the zinc concentrations were considerably higher in the corn germ than in the endosperm-hull fraction, weight ratios of total zinc to ^{65}Zn were similar. This demonstrated that both forms of zinc were assimilated by the kernel in the same manner. Thus, the bioavailability of zinc in corn foods can be established by radiometric techniques even though compositions of the different corn fractions are different, especially in zinc-binding components such as phytates. At the start of the feeding studies, total zinc was approximately three million times greater than the ^{65}Zn for the two labeled products 2 and 4.

These prepared food products were used to determine the bioavailability of zinc in humans by direct measurement of the ^{65}Zn absorbed by the body, utilizing whole body counting techniques to establish the absorption and retention of zinc (Johnson et al 1983). Johnson et al also investigated the effects of Maillard products (produced during the processing of cornflakes) on the bioavailability of zinc.

In addition, we prepared a formulated defatted corn germ flour (72 g) for use in other studies on zinc bioavailability; the flour was composed of a nonradioactive corn germ flour (69.88 g) plus an intrinsically labeled (^{65}Zn) corn germ flour (2.12 g) with an associated activity of 0.849 μCi $^{65}Zn/g$ as of June 25, 1981. The resultant corn germ flour, which had an activity of 0.025 μCi $^{65}Zn/g$, was used as a partial replacement of wheat flour in the preparation of labeled corn bread.

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