Ascorbyl palmitate enhances iron bioavailability in iron-fortified bread[1–3]

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ABSTRACT

Background: One of the strategies to control iron deficiency anemia is the fortification of food with iron. A mechanism for improving the bioavailability of iron is to add an iron absorption promoter.

Objective: The objective was to determine the effect of ascorbyl palmitate (AP) on the bioavailability of iron in fortified bread made from refined wheat flour.

Design: The iron bioavailability of wheat flour fortified with either ferrous sulfate alone or ferrous sulfate plus AP was studied with the use of double radio iron (^{55}Fe and ^{59}Fe) erythrocyte incorporation in 14 women.

Results: Geometric mean (± range of 1 SD) iron absorption from the bread fortified with ferrous sulfate was 10.5% (4.1–27.0%). The addition of AP at molar ratios of AP to Fe of 2:1 and 4:1 significantly increased iron absorption [14.6% (5.9–36.1%) and 20.2% (10.6–38.6%), respectively; $P < 0.001$].

Conclusion: AP is a strong promoter of iron absorption from fortified bread because of its thermoresistant properties.

KEY WORDS Iron bioavailability, iron fortification, ascorbyl palmitate, wheat flour

INTRODUCTION

Bread made from refined wheat flour is consumed extensively throughout the world, especially by populations in developing countries. Several countries have passed legislation for the addition of iron and vitamins to wheat flour as a strategy to decrease the high prevalence of iron deficiency (1). Up to now, most of these efforts were ineffective because of several factors: *1*) flours of various levels of refinement are used to make bread; those flours that are less refined have higher contents of phytic acid (*myo*-inositol-6-phosphate) which is a strong inhibitor of iron absorption; *2*) low consumption of bread; *3*) type of iron fortificant used; *4*) health status and environmental factors that affect iron metabolism; and *5*) low level of fortification (1–3). Many studies have shown that iron is well absorbed from bread fortified with iron in the form of soluble salts (4–6). However, at high levels these salts produce organoleptic changes in the flour, especially when stored for long periods of time. Flours fortified with elemental iron do not undergo the same organoleptic changes because the iron compound is inert; nevertheless, it is absorbed in lower percentages (7). However, a recent study showed the efficacy of electrolytic iron in bread (8).

One of the recommended strategies to improve the bioavailability of iron is to add an absorption promoter such as ascorbic acid (AA) (9). However, because AA is thermolabile, it is destroyed during the process of baking (10, 11). In contrast, ascorbyl palmitate (AP), a synthetic ester composed of palmitic acid and L-ascorbic acid, is thermostable, and its reductive and vitamin properties are maintained even when exposed to baking temperatures (12). The objective of this study was to determine the effect of AP on the bioavailability of iron in bread made from iron-fortified refined wheat flour.

SUBJECTS AND METHODS

Subjects

Fourteen healthy women (35–45 y old) were selected to participate in this absorption study. None of the women was pregnant at the beginning of the study (confirmed by a negative human gonadotrophin chorionic urine test), and all were using intrauterine devices as their method of contraception at the time of the study. Informed consent was obtained from all volunteers before this study began. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology, and the doses of radioactive isotopes used were approved by the Chilean Commission of Nuclear Energy.

Bread

All breads were prepared by study personnel at the micronutrient laboratory of the Institute of Nutrition and Food Technology. Unfortified wheat flour of 70% extraction produced by an industrial mill was used to prepare the breads used for the iron absorption tests (Granotec SA, Santiago, Chile). Four batches of iron-fortified bread dough of ≈2 kg each were prepared by mixing 1.05 kg flour, 21 g yeast, 11 g sugar, 5 g salt, and 0.157 g

[1] From the Institute of Nutrition and Food Technology (INTA) of Universidad de Chile, Santiago, Chile (FP, MO, EH, SN, MT, and DLdR); the US Department of Health and Human Services, Washington, DC (FP, MO, EH, SN, MT, and DLdR); and DSM Nutritional Products Ltd, Basel, Switzerland (HC).

[2] Supported by a grant (no. 1030633) from Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Chile. DSM Nutritional Products Ltd, Basel, Switzerland, provided the ascorbyl palmitate.

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$FeSO_4 \cdot 7H_2O$ (30 mg Fe/kg; Merck, Darmstadt, Germany) per batch. The ferrous sulfate was added first to the water to allow for homogenization. AP (222, 445, and 890 mg; 6-palmitoyl-L-ascorbic acid; DSM Nutritional Products Ltd, Basel, Switzerland) were added directly to the flour in 3 of the batches of dough, resulting in molar ratios of AP to Fe (AP:Fe) of 1:1, 2:1, and 4:1. All ingredients, including iron isotopes, were first mixed by hand in polyethylene recipients during 30 min, and the mixture was then fermented at 22 °C for 60 min. Afterward, the bread dough was shaped into buns and left to ferment for 30 more minutes. Finally, the buns were baked at 440 °F for 25 min. The final product offered to the subjects was French-type bread buns of ≈100 g each. Five-gram aliquots were taken from each individual bun and pooled to measure the water content of the breads. The total iron content of the final product was measured by atomic absorption spectrometry (Perkin Elmer Model 2280; Perkin-Elmer Corporation, Norwalk, CT) after wet digestion (13).

Isotopic studies

Iron radioisotopes (^{55}Fe and ^{59}Fe), as ferric chloride (Du Pont de Nemours & Co Inc, Wilmington, DE), of high-specific activity were used as tracers. Isotopes were added to the ferrous sulfate solution. This solution was added to wheat flour as previously mentioned. All breads were prepared the day before administration. Bread was consumed after an overnight fast, and no food or beverages other than water were allowed during the following 4 h. The amount of bread intake was calculated by differential weight of the plate with bread before and after the intake. For the calculation of total radioactivity ingested, radioactivity from bread aliquots was counted in sextuplicate as standards. Measurement of blood radioactivity was performed from duplicate venous samples following the method by Eakins and Brown (14). Samples were counted the sufficient number of times to ensure <3% counting error. A liquid-scintillation counter (Beckman LS 5000 TD; Beckman Instruments Inc, Fullerton, CA) was used for all the isotope measurements. Percentage of absorption was calculated based on the blood volume, as estimated from height and weight (15), and assuming 80% red cell use of radioactive iron (16).

Study protocol

The study was designed to determine the effect of incremental quantities of AP on the absorption of iron from bread fortified with 30 mg Fe as ferrous sulfate/kg wheat flour. On day 1, the subjects received ≈100 g bread that was fortified with iron only and was labeled with 44 kBq ^{59}Fe (control bread), and on day 2 they received 100 g bread fortified with iron and 222 mg AP (molar AP:Fe of 1:1) and which was labeled with 148 kBq ^{55}Fe. A venous blood sample was obtained on day 14 to measure circulating radioactivity and to determine the iron status of the subjects. Subjects were then given 100 g bread fortified with iron and 445 mg AP (molar AP:Fe of 2:1) and labeled with 44 kBq ^{59}Fe. The following day (day 15) they received 100 g bread fortified with iron and 890 mg AP (molar AP:Fe of 4:1) and labeled with 148 kBq ^{55}Fe. A final venous sample was obtained on day 28 to measure the increase in red blood cell radioactivity.

Ascorbyl palmitate recovery

Four more batches of unlabeled iron-fortified bread buns were prepared specifically to determine the effect of baking on the concentration of AA and AP. Two of the 4 batches of bread buns were prepared with 190 and 580 mg AA/kg flour, and the other 2 were prepared with 880 and 2520 mg AP/kg flour. The final products were analyzed for iron content with the use of atomic absorption spectroscopy (Perkin Elmer Model 2280; Perkin-Elmer Corporation). Reduced AA (17), total AA (12), and AP were analyzed with the use of HPLC (model 440; Waters Association, Milford, MA) (12).

Blood analyses

Hemoglobin and mean cell volume (MCV) (CELL-DYN 1700; Abbott Diagnostics, Abbott Park, IL), serum iron, total iron binding capacity, transferrin saturation (Sat) (18), zinc-protoporphyrin (FEP) (ZP Hematofluorometer Model 206D; AVIV Biomedical Inc, Lakewood, NJ), and serum ferritin (SF) (19) were assessed to evaluate the iron status of the subjects. All women with hemoglobin concentrations < 120 g/L were classified as having anemia. Those who had normal hemoglobin concentrations with ≥2 abnormal biochemical measurements of iron status were classified as having iron deficiency (MCV < 80 fL or FEP > 70 μg/dL red blood cells or Sat < 15 or SF < 12 μg/L). Iron deficiency anemia was defined as hemoglobin < 120 g/L plus ≥2 abnormal biochemical measurements of iron status.

Statistics

Because the percentages of iron absorption, Sat, and SF concentrations all have skewed distribution, these values were first transformed to their logarithms before calculating means and SD and performing statistical analyses. The results were then retransformed into antilogarithms to recover the original units and expressed as geometric means ± 1 SD range. Analysis of variance for repeated measures was used to establish significant differences in iron absorption. (SAS Online Doc 8.0; SAS Institute Inc, Cary, NC).

RESULTS

The mean (±SD) hemoglobin, MCV, and FEP of the participating subjects was 124 ± 17 g/L, 84 ± 12 fL, and 81 ± 46 μg/dL red blood cells, respectively (**Table 1**). Furthermore, the geometric mean (± range of 1 SD) for Sat and SF was 12.4 (3.9–39.3) and 14 (6–34), respectively. Three of the women who participated in the study had iron deficiency anemia, one woman had anemia without iron deficiency, and another subject had iron deficiency without anemia.

Bread buns used in the iron absorption studies had mean iron concentrations of 47 ± 5 mg/kg, 40 ± 5 mg/kg, 48 ± 2 mg/kg, and 45 ± 2 mg/kg for days 1, 2, 14, and 15, respectively. The mean water content of the buns was 34.4%, 37.3%, 36.8%, and 37.3%, respectively. Geometric mean (range of 1 SD) of iron absorption from the bread fortified with ferrous sulfate was 10.5% (4.1–27.0%) (Table 1). The addition of AP at a molar ratio of 1:1 (AP:Fe) did not modify iron absorption [9.3% (3.6–24.3%); P = NS]. However, a 39% and 93% increase in iron absorption was observed when AP was added at molar ratios of 2:1 and 4:1 relative to iron [14.6% (5.9–36.1%) and 20.2% (10.6–38.6), analysis of variance for repeated measures F = 13.3, $P < 0.001$ (Scheffé post hoc test: molar AP:Fe of 0:1 compared with 4:1, 1:1 compared with 2:1, and 1:1 compared with 4:1; $P < 0.01$)] (Table 1 and **Figure 1**).

TABLE 1
Iron nutritional status and iron absorption from iron-fortified wheat flour with or without ascorbyl palmitate (AP) in 14 women[1]

	Iron nutritional status					Iron absorption by AP: Fe molar ratio[2]				Ratio[3]		
Subject	Hemoglobin	MCV	FEP	Sat	SF	0:1 (A)	1:1 (B)	2:1 (C)	4:1 (D)	B:A	C:A	D:A
	g/L	*fL*	*μg/dL RBC*	*%*	*μg/L*			*%*				
1	127	92	66	27.0	51	1.5	1.7	2.0	10.1	1.14	1.29	6.55
2	129	83	89	3.6	6	2.3	1.6	6.3	7.7	0.7	2.7	3.3
3	127	91	49	10.9	15	5.3	12.3	17.1	19.7	2.32	3.22	3.70
4	126	92	60	14.8	25	6.3	6.6	12.7	14.3	1.04	2.00	2.27
5	131	88	57	36.6	16	7.4	2.9	5.1	13.4	0.40	0.69	1.81
6	141	95	63	41.3	21	8.9	8.5	10.2	25.2	0.95	1.15	2.85
7	137	95	54	33.0	37	9.4	11.3	15.6	9.5	1.20	1.66	1.01
8	138	90	54	31.8	33	11.2	7.0	15.4	14.5	0.62	1.37	1.30
9	148	89	46	21.0	25	15.7	16.3	13.2	14.8	1.04	0.84	0.95
10	99	63	157	2.1	2	22.2	18.8	39.8	43.8	0.85	1.80	1.97
11	95	68	140	2.0	10	26.2	32.8	55.4	60.7	1.25	2.11	2.31
12	129	83	54	26.1	13	27.1	14.3	13.4	29.1	0.53	0.49	1.07
13	119	91	60	16.7	6	27.2	21.0	37.3	37.2	0.77	1.37	1.37
14	92	57	191	2.0	7	34.4	27.7	40.1	43.7	0.80	1.17	1.27
Mean	124 ± 17[4]	84 ± 12	81 ± 46	12.4 (3.9–39.3)[5]	14 (6–34)	10.5 (4.1–27.0)	9.3 (3.6–24.3)	14.6 (5.9–36.1)	20.2 (10.6–38.6)	0.89 (0.58–1.37)	1.39 (0.83–2.33)	1.93 (1.10–3.40)

[1] MCV, mean cell volume; FEP, zinc-protoporphyrin; RBC, red blood cell; Sat, transferrin saturation; SF, serum ferritin.
[2] ANOVA for repeated measures: $P < 0.001$ (Scheffé post hoc test: A compared with D, B compared with C, and B compared with D, $P < 0.01$).
[3] ANOVA for repeated measures: $P < 0.001$ (Scheffé post hoc test: B:A compared with C:A and B:A compared with D:A, $P < 0.01$).
[4] $\bar{x} \pm$ SD (all such values).
[5] Geometric $\bar{x}$; 1-SD range in parentheses (all such values).

Regarding AA recovery, from the unlabeled, iron-fortified bread buns prepared with 190 and 580 mg AA/kg flour, only 11.0% and 13.5% of the originally added AA was recovered, respectively. In contrast, the recovery of AP was 78.9% and 86.3% from buns prepared with 880 and 2520 mg AP/kg flour, respectively.

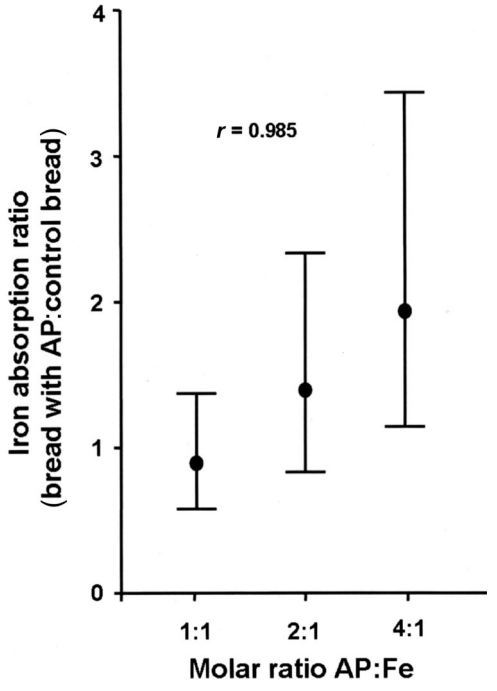

FIGURE 1. Ratio of iron absorption of bread fortified with ascorbyl palmitate (AP) to iron absorption of control bread; shown are the geometric mean ± range of 1 SD. ANOVA for repeated measures: $F = 16.4$, $P < 0.001$. Scheffé post hoc test for 1:1 ratio compared with 2:1 and 1:1 ratio compared with 4:1, $P < 0.01$.

DISCUSSION

The results from this study show that AP is a good promoter of iron absorption, and, that similar to AA, its effect has a direct relation with the molar AP:Fe (20–22). In samples of dried, milled bread, ≈80% of the AP originally added to the flour was recovered. Mauro et al (12) obtained comparable recovery of AP (82%) from bread made in similar conditions. Because the AP was recovered from both crumb and crust, it can be deduced that, because the crust is subjected to more extreme conditions during baking, survival from crumb alone may be higher (12). The high percentages of recoverability confirm that AP is resistant to high temperatures. It is known that after ingestion, the surviving AP is attacked by intestinal lipase, releasing the AA with its entire original reductive properties still intact (23). The mechanisms of the promoting properties of AA on the absorption of iron at the intestinal level were described many decades ago (24).

It has been shown that the low bioavailability of iron in the diet is the principal cause of the high prevalence of anemia in third-world countries (25). Cereals are the principal component of the diet for most of these populations and, therefore, can be used as a vehicle for iron fortification. However, high levels of iron absorption inhibitors such as phytates and tannins present in the diet are one of the most important reasons that have made fortification ineffective.

One approach to reducing the inhibiting effect of phytates and tannins on iron absorption has been to include an enhancing substance such as AA (9). Unfortunately, AA is thermolabile and, in as much, is ineffective as an iron absorption promoter in bread. In addition, it is oxidized during the kneading and baking processes (10–11). Another strategy is to add EDTA, a more stable compound that protects nonheme iron from the effects of iron absorption inhibitors such as phytates and polyphenols (26–28). However, the use of EDTA in iron fortification must be well controlled to not surpass the recommended human intake limits (29, 30). Recently, Fidler et al (31) described erythorbic acid in

infant cereal as a strong promoter of nonheme-iron absorption. The addition of erythorbic acid to an infant cereal with 4.1% iron absorption increased the absorption to 2.6 and 4.6 times when it was added at a ratio of erythorbic acid to iron of 2:1 and 4:1, respectively. Unfortunately, those same researchers identified limitations for the use of erythorbic acid in fortification programs because this AA stereoisomer does not have antiscorbutic properties (31).

It should be considered that our study used highly refined wheat flour (70% extraction). Although the phytic acid (*myo*-inositol-6-phosphate) content was not analyzed in the present study, similar flours usually obtain concentrations of ≈100 mg/100 g flour (32). The inhibitory effect of phytic acid on the absorption of nonheme iron (33–35) and the inverse relation between the amount of phytic acid in the diet and iron absorption are both well established (33).

Hallberg (35) has shown that the presence of AA counteracts the inhibitory effect of phytic acid. Diets that provide 25 mg phytate are sufficient to inhibit the absorption of iron, but, by adding 50–100 mg AA, it is possible to counteract the inhibitory effect and even promote the absorption of iron. In diets of third-world populations, which may provide ≤250 mg phytate, >500 mg AA would be needed to counteract the inhibitory effects of the phytic acid (35). In the present study, whereby 80% of AP was recovered, the quantities added to the flour were equivalent to 75, 150, and 300 mg AA/kg flour and were sufficient not only to balance the inhibitory effect of phytic acid but also to have a strong protective effect on iron at the intestinal level. AP did not modify iron absorption when it was added at AP:Fe of 1:1. However, when added to the flour at AP:Fe of 2:1 and 4:1, it increased iron absorption by 1.4 and 1.9 times, respectively. This means that iron intake from bread could be significantly increased without increasing the consumption of this food item. The net amount of iron supplied by 100 g bread fortified with 30 mg Fe/kg flour would increase from 0.3 mg to 0.42 and 0.57 mg if the flour is also enriched with 445 and 891 mg AP, respectively.

The current cost of iron fortification with ferrous sulfate at a level of fortification of 30 mg Fe/kg is ≈US$0.2/ton flour. The addition of AP at 445 mg/kg would raise the cost to US$29/ton flour. Although the current costs of fortification with AP may be high, if fortification were to be conducted on a mass scale, the costs could be significantly reduced. Furthermore, AP serves as dough conditioners and as antistaling agents in bread making (36).

In conclusion, this study shows that AP is a strong promoter of iron absorption as a result of the large percentage of AP recovered after the bread-making process. Hence, the addition of AP to iron-fortified wheat flour should be explored in those countries where the bread consumption is low. Furthermore, research is needed to assess the efficacy of adding AP to iron-fortified flour on the iron status or prevalence of anemia in a population.

We appreciate the collaboration of all the participants of the study and thank Maria Angélica Letelier for her valuable help with laboratory analyses.

FP, MO, and EH contributed to the study design. FP, MT, and SN were responsible for the implementation of the study. The statistical analysis was done by FP and MO. The manuscript was written by FP, MO, EH, DLdR, and HC. HC belongs to the staff of DSM Nutritional Products Ltd, Basel, Switzerland.

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