

## Fortification of precooked maize flour with coarse defatted maize germ<sup>1</sup>

*Francisco Rivero<sup>2</sup>, Enzo Racca<sup>2</sup>, Carlos Martínez-Torres<sup>3</sup>, Peter Taylor<sup>3</sup>, Irene Leets<sup>3</sup>, Eleonora Tropper<sup>3</sup>,  
María Nieves García-Casal<sup>3</sup>, José Ramírez<sup>3</sup>, Miguel Layrisse<sup>3</sup>,*

**SUMMARY.** The possibility of improving the dietary value of precooked maize flour through fortification with 11% coarse defatted maize germ was investigated. The results of tests in humans presented here show that the total iron absorption from the fortified preparation is similar to that from the precooked maize alone, but with the advantage of being richer in several nutrients: protein (25%), zinc (61%), potassium (47%) and magnesium (112%), as well as fiber (34%). Fortification lowers (by 20%) rather than raises the cost of the flour, and may be an important contribution to the diet of those populations where maize bread is a major component of the diet.

**RESUMEN.** Fortificación de la harina precocida de maíz con germen de maíz semirrefinado y desgrasado. Fue investigada la posibilidad de mejorar el valor dietético de la harina precocida de maíz mediante la fortificación con 11% del germen de maíz semirrefinado y desgrasado. Los resultados de las pruebas en humano presentados aquí, mostraron que la absorción total del hierro de las preparaciones fortificadas fue similar a la absorción del maíz precocido administrado solo, pero con la ventaja de estar enriquecido con varios nutrientes: proteínas (25%), zinc (61%), potasio (47%), magnesio (112%), como también con fibra (34%). La fortificación disminuye el precio de la harina en 20% y puede ser una importante contribución a mejorar la dieta de aquellas poblaciones en las cuales el pan de maíz es un componente importante en la dieta.

### INTRODUCCION

Precooked maize flour has almost replaced maize grain and polished maize as a foodstuff in the diet of the socioeconomic populations of Central America and the northern parts of South America. There are several advantages of using precooked maize flour instead of the whole maize grain in the preparation of food, including greater convenience, a reduced phytate content, and improved iron bioavailability. The only major disadvantage is the reduction of its iron content from about 3 mg to 1 mg/100 g (1). Considering the high incidence of iron deficiency in Latin America (2), it should be beneficial to replace the iron lost during the industrial preparation of the precooked flour due to removal of the germ. This article

presents data showing iron absorption from precooked maize flour fortified with products derived from maize germ which also contain relatively large amounts of protein, as well as other minerals besides iron.

### MATERIAL AND METHODS

#### Subjects

The data on iron absorption were obtained from 56 adult subjects living in rural areas of Venezuela who volunteered to participate in this study. This type of study has been approved by the Ethical Committee for Clinical Investigation of the Venezuelan Institute for Scientific Research (IVIC). The blood hemoglobin concentration (3), serum iron concentration (4), unsaturated serum transferrin concentration (5), and serum ferritin concentration (6), were determined in each subject.

#### Maize processing

During the first stage of the industrial processing of the maize grain at Refinadora de Maíz Venezolana, the endosperm is separated from the germ and hull by dry-milling. The endosperm is first steam cooked in tunnels, then milled, to give

1 Part of the studies presented here were supported by REMAVENCA and Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT).

2 Refinadora de Maíz Venezolana C.A. (REMAVENCA), Venezuela.

3 Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela.

the final precooked maize flour preparation. In the second stage of the process, oil is extracted from the germ and hull by solvent extraction with hexane, and the remaining material milled and screened into three fractions: bran, coarse germ and refined germ.

#### **Fortification of precooked maize flour with defatted coarse and refined maize germ**

Whole precooked maize grain is enriched with 11% coarse maize germ (particle size: 250-595 µm diameter), then milled on an industrial scale to produce a flour called «Pronto» to be consumed by the lower socioeconomic strata of the population, at a 20% lower cost than the precooked maize flour. The flour has a slight brown color, but surveys carried out by the manufacturers have shown that the taste of the bread and other organoleptic properties are very similar to the bread prepared with precooked maize flour alone.

The same process was repeated in the laboratory with the difference that the dough from each flour was extrinsically labeled with a different radioisotope of iron before mixing to prepare the bread. A similar blend was also prepared using 11% refined germ instead of the coarse form. For one study Pronto was also labeled with radioiron. In all cases the dough was shaped into disc-shaped «arepas» and baked in an oven at 200°C for 45 minutes. With the addition of water to produce the dough, 100g of flour produce an arepa of approximately 250 g. Since the consistency of the germ does not permit the preparation of a dough, when refined germ was given alone, it was simmered in a saucepan for about 15 minutes with enough water to give a final semisolid preparation, which was then labeled with radioiron.

#### **Radioactive labeling**

The  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  as ferric chloride was obtained from New England Nuclear, Boston. Approximately 0.7 µCi of  $^{59}\text{Fe}$  or 2 µCi of  $^{55}\text{Fe}$  were used to extrinsically label each meal. The radiolabel was added to each portion of dough with a syringe, then carefully mixed in before shaping into an arepa. As in previous studies, a reference dose containing 3 mg of iron as ferrous sulfate mixed with ascorbic acid at a 2:1 ascorbate-iron molecular ratio was also administered (7).

#### **Iron absorption**

All the meals were given in the morning after an overnight fast. No food or drink was allowed for 3 h after the administration of the meal. On day 1, the laboratory blended bread was administered. In the first study only the precooked flour portion was labeled ( $^{59}\text{Fe}$ ) whereas in the other two studies, the germ fraction was also labeled ( $^{55}\text{Fe}$ ). Blood was drawn 15 days later to determine the hematological profile of the subjects tested, and to measure the radioactivity in the blood samples. The subjects were then given another meal on day 15 and the reference dose of ferrous sulfate on day 16. Blood was drawn on day 30 to measure incorporation of radioactivity.

Duplicate 10 ml aliquots of blood together with samples of the radioactive food and the reference dose were prepared for counting following the technique of Dern and Hart (8,9). The iron absorption from food was calculated using an estimate of blood volume based on sex, weight and height (10). No correction was made to determine the total iron utilization.

#### **Chemical analysis of foods.**

The macronutrient determinations were performed according to standard methods: protein (11), fat (12) and fiber (13). The micronutrients were determined by atomic absorption spectroscopy, except iron which was determined by the digestion method (14). The antinutrients phytate (15) and tannin (16) were also determined.

#### **Statistical analysis.**

The means and standard errors for the iron absorption and serum ferritin concentration data were calculated using logarithms. Absorption results were compared using Student's test for paired data, except in the one case that the test for unpaired data was applied to the absorption from meals given to different subjects.

## **RESULTS**

#### **Chemical analysis**

Table 1 shows the nutrient and antinutrient contents of the different maize products. Several important macronutrients and micronutrients are found at significantly higher concentrations in the defatted germ compared to the precooked flour, especially protein, iron, potassium, magnesium and zinc. The enrichment of Pronto due to the addition of coarse germ to precooked maize may be appreciated in the last column. However, the addition of germ also raises the levels of the antinutrients, particularly the phytate content which being 19 times higher in the coarse germ than in the precooked flour resulted in a 2.5-fold increase in the Pronto preparation.

#### **Iron absorption**

Table 2 shows the results of three studies which compare the iron absorption from different fractions derived from maize which may be used in the preparation of the bread. The mean iron absorption from the reference dose of ferrous sulfate and the serum ferritin concentration results indicated that the three groups were significantly different in terms of their iron status. Therefore, their iron absorption results could not be compared directly. In order to normalize the absorption results, the iron absorption from each individual was multiplied by the mean of the total ferritin concentration of all individuals studied and divided by the serum ferritin concentration of that particular subject. Normalization was not based on the reference dose absorption results, as in previous studies, since experience has shown that large daily physiological variations in iron absorption make the ferritin concentration normalization method more reliable.

**TABLE 1**  
NUTRIENT AND ANTINUTRIENTS IN MAIZE PRODUCTS

	Precooked maize flour	Defatted germ		
		Refined	Coarse	Pronto
<b>Macronutrients</b> (g/100g)				
Protein	8.02±0.55	23.13±1.09	29.48±1.11	10.05±0.45
Fat	1.26±0.20	0.45±0.20	0.70±0.20	0.78±0.21
Total Fiber	0.78±0.11	N.T.	5.40±0.10	1.17±0.11
<b>Micronutrients</b> (mg/100g)				
Ca	1.5±0.03	5.9±0.50	8.6±0.30	2.9±0.03
Co	N.D.	N.D.	N.D.	N.D.
Cu	0.12±0.01	0.60-0.03	0.70±0.03	0.22±0.01
Fe	0.12±0.33	18.1±3.43	12.27±0.42	2.10±0.35
K	110±30	1227±200	1060±60	200±10
Mg	17±0.4	422±30	298±20	51±4
Mn	0.13±10.01	1.70±0.30	1.62±0.01	0.12±0.01
Na	0.15±0.1	0.9±0.02	0.6±0.02	0.3±0.04
Zn	0.74±0.04	9.2±1.0	2.3±0.2	1.29±0.03
<b>Antinutrients</b> (mg/100g)				
Phytate	198±18	4172±241	3740±72	544±49
Tannin	17	80	107	21

N.D. = Not detected

N.T.= Not tested

The means and standard deviations were obtained from 5 random samples with the exception of the tannate for which only 2 samples were tested.

In the first study, the mean iron absorption by 10 subjects from precooked maize bread given alone was 5.3% (A), and 29% from the reference dose (D). Line B shows the low percentage absorption (2.8%) from 11 g of refined germ alone which contains a relatively high concentration of phytate (37 mg/g). When precooked maize flour and refined germ were combined (C), the iron absorption from the radiolabeled precooked fraction was slightly less than from the precooked flour (A), alone but significantly greater than from the refined germ (C) alone ( $p < 0.02$ ). In this preparation the phytate in the germ has been diluted to 6 mg/g, but is not as low as the 2 mg/g found in precooked maize flour.

In the second study, the mean absorption from precooked maize flour alone was 6.5% (A), not significantly different, according to Student's test for unpaired data from the 5.3% measured in the first study ( $p > 0.7$ ). In the second test, iron absorption from the two fractions of the doubly labeled

**TABLE 2**  
IRON ABSORPTION FROM DIFFERENT MAIZE GRAIN PRODUCTS

	STUDY		
	1	2	3
<b>Subjects-Hematological Profile</b>			
Number/sex	3M/7F	5M/11F	15M/15F
Hemoglobin (g/dL)	12.9±0.5 <sup>a</sup>	12.4±0.4	13.0±0.3
Serum transferrin saturation (%)	28±5	28±4	31±2
Serum ferritin (µg/L)	21 (17-28)	20 (15-26)	34 (30-39)
<b>Absorption results<sup>bc</sup></b>			
<b>A.</b>			
Precooked maize flour (100g)	5.3 <sup>a</sup> (3.0-9.2)	6.5 <sup>a</sup> (4.3-9.9)	
<b>B.</b>			
Refined germ (11g)	2.8 <sup>b</sup> (1.7-4.4)		
<b>Blended flour</b>			
<b>C.</b>			
Precooked maize flour (89g)	4.6 <sup>a</sup> (2.9-7.2)	4.5 <sup>ba</sup> (2.8-7.4)	2.8 <sup>a</sup> (2.2-3.5)
<b>D.</b>			
Germ fraction (11g)	—	3.4 <sup>bd</sup> (2.2-5.3)	3.2 <sup>ac</sup> (2.4-4.1)
<b>E</b>			
Pronto (100g)			3.5 <sup>a</sup> (2.6-4.6)
Reference dose FeSO <sub>4</sub> (3 mg Fe)	29.3 (22.5-38.2)	20.3 (16.5-25.0)	17.5 (14.4-21.3)

<sup>a</sup> The hemoglobin and transferrin results shown are arithmetic means ±S.E. The figures for the ferritin concentration and the absorption results represent the geometric mean, with the standard error range in brackets below.

<sup>b</sup> The iron absorption results were normalized according to the ferritin concentration of the individual. See RESULTS.

<sup>c</sup> Means in the same column followed by a common letter in italics are not significantly different according to Student's test for paired data at the 5% level.

<sup>d</sup> Refined germ

<sup>e</sup> Coarse germ.

blended bread (precooked flour and refined germ) was measured (C and D). The absorptions from the two fractions were different, 4.5% and 3.4% respectively, but not significantly. The absorption from the germ fraction, but not the precooked flour fraction, was significantly lower than that from the precooked maize bread (A) given alone. However, since the total amount of iron in the blended flour is higher, the actual amount absorbed may be calculated to be 55% higher (113 µg) than from the precooked flour (73 µg).

In the third study, the percentage of iron absorption from the two fractions of the doubly labeled bread was measured, as in the previous study, with the exception that coarse germ was added instead of refined. The mean absorptions from the precooked (C) and germ (D) fractions were similar, 2.8% and 3.2% respectively ( $p > 0.3$ ). This percentage of absorption is comparable to the 3.5% obtained with Pronto ( $p > 0.2$ ) which contains the same proportion of coarse germ, and approximately

the same level of phytate (see Table 1). Based on the weights of the fractions, their iron content, and percentage iron absorption, absolute amounts of 71 µg and 74 µg of iron absorbed may be calculated for the laboratory blended flour and Pronto respectively.

### DISCUSSION

Defatted corn germ meal or cake, the residue obtained after industrial oil extraction, has been destined in the past to animal feed. However, its transformation into defatted corn flour of good organoleptic and nutritional quality, by applying a milling and screening process, has been of value in the elaboration of several food products due to its high content of protein (20-30 %) (17), lysine (5.9 g/100g proteins) (18) and several minerals (potassium, magnesium, iron and zinc). Defatted germ has been used, up to 25%, in the preparation of baked cookies and muffins (19), to fortify wheat bread adding 12%-24% (17), and in pasta products by blending commercial durum semolina with 10-30% of germ flour (20).

During the industrial milling and screening of maize germ, two fractions are obtained: the coarse and the refined fractions. Both are characterized by a higher protein and iron content than the flour prepared from the endosperm, but the former is also high in fibre. Refined germ could be used to fortify precooked maize flour; study 2 showed that the absorption of iron was 55% higher than from the precooked flour given alone. However, this product already has a use in the food industry and its utilization in combination with the precooked maize flour would increase the cost of the final product.

The studies presented here demonstrate that the coarse germ may also be used for fortification. Although the figures are not significantly different, the absorptions from the two fractions of the fortified flour in the second study (precooked flour and refined germ) were somewhat different (4.5% and 3.4% respectively). In the third study using coarse germ and with a larger number of subjects, the absorptions from the two fractions were very similar (2.8% and 3.2%) suggesting that they were equally affected by the absorption inhibitors and constitute a common iron pool.

There are limits to the proportion of germ which may be added. More than about 11% makes the fortified flour difficult to form into a dough, and may be expected to be counterproductive because of the increased phytate concentration. The iron absorption from whole maize which contains about 100 mg phytate/100 g has been shown to be low (1,7,21).

Fortification of precooked maize flour with 11% coarse germ has been found by the manufacturers to fulfill consumer requirements with regards to appearance and taste. Our results show that iron absorption from bread prepared from the fortified flour is similar to that from unfortified flour, but with the advantage of a higher concentration of protein (25%), fiber (34%), zinc (16%), potassium (47%) and magnesium (112%).

### REFERENCES

1. Martínez-Torres C., Taylor P., Leets I., Tropper E., Ramfrz J & M. Layrisse. Iron absorption from maize bread. *Food Nutr Bull* 9:64-69, 1987.
2. Layrisse M. Iron deficiency in Latin America -causes and prevention. In: *International Symposium of Vitamin and Nutrition. Int J Vit Nutr Res* 27:105-116, 1985.
3. Crosby WH., Minn JL & FW Furth. Standardizing a method for clinical hemoglobinometry. *US Armed Forces Med J* 5:693-703, 1954.
4. International Committee for standardization in hematology. Recommendations for measurement of serum iron in human blood. *Brit J Haematol* 38:291-294, 1978a.
5. International Committee for standardization in hematology. The measurement of total and saturated iron-binding capacity in serum. *Brit J Haematol* 38:281-290, 1978b.
6. Flowers CA., Kuizon M., Beard JL., Skikne BS., Covell AM & JD Cook. A serum ferritin assay for prevalence studies of iron deficiency. *Am J Hematol* 23:141-151, 1986.
7. Layrisse M., Cook JD., Martínez-Torres C., Roche M., Kuhn IN & CA Finch. Food iron absorption: a comparison of vegetable and animal foods. *Blood* 33:430-443, 1969.
8. Dem JR & WL Hart. Studies with doubly labeled iron. II. Separation of iron from blood samples and preparation of ferrous perchlorate for liquid scintillation counting. *J Lab Clin Med* 57:460-467, 1961b.
10. Nadler SB., Hidalgo JV & T. Bloch. Prediction of blood volume in normal human adults. *Surgery* 51:224-232, 1962.
11. Lowry O., Rosenbrough N., Farr L. & R Randal. Protein measurement with the phenol reagent. *J Biol Chem* 193:265-275, 1951.
12. American Association of Food Chemists. *Approved Methods of Analysis. Method 30-26*, approved April 1961, revised October 1976, reviewed October 1982. The Association: St. Paul, MN, USA, 1983.
13. Prosky L., Asp I., Schweizer RF, De Vries JW & I. Furda. Determination of insoluble, soluble and total dietary fiber in food and foods products. Interlaboratory study. *J Assoc Anal Chem* 71:1017-1023, 1988.
14. Bothwell TH., Charlton RW., Cook JD & CA Finch. *Iron Metabolism in man*. Oxford, England, Blackwell Scientific Publications, 1979.
15. Haug W. & HJ Lantzsch. Sensitive method for the determination of phytate in cereals and cereal products. *J Sci Food Agric* 34:1423-1426, 1983.
16. Price ML & L. Butler. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. *J Agric Food Chem* 25:1268-1273, 1977.
17. Barbieri R. & EM Casiraghi. Production of a food grade flour from defatted corn germ meal. *J Food Tech* 18:35-41, 1983.
18. Tsen CC., Mejibian CN & GE Ingle. Defatted corn germ flour as a nutrient fortifier for bread. *Cereal Chem* 51:262-273, 1974.
19. Blessin CW, García WJ, Deatherage WL, Calvin JE & HGE Ingle. Composition of three food products containing defatted corn germ flour *J Food Sci* 38:602-606, 1973.
20. Lucisano M., Casiraghi EM & R. Barbieri. Use of defatted corn germ flour in pasta products. *J Food Sci* 49:482-484, 1984.
21. Cook J., Layrisse M., Martínez-Torres C., Walker R., Monsen E. & CA Finch. Food iron absorption measured by an extrinsic tag. *J Clin Invest* 51:805-815, 1972.

Recibido : 03-07-1993

Aceptado : 10-02-1994