

FOLATES IN PORTUGUESE RICE - INFLUENCE OF COOKING

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Abstract – Naturally forms of folates are described as an unstable nutrient, the retention in processed and stored food could be lower than that in raw. With this work the folate content in rice raw and cooked was evaluated. For quantification of five different forms of folate was performed in a UPLC-MS/MS. In rice the predominant form was 5-MTHF. The highest value found in the raw samples are 96.6 - 98.2 µg/100 g. Boiling process does not significantly affect ($p < 0.05$) the content of any forms of folates. The consumption of one portion of rice supplies 11.4 to 11.9% of the dietary reference values for folates.

Keywords: Cooking methods; Retention factor; Folate content; Recommended nutrient intake; *Oryza sativa*; Food analysis; Food composition

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the second largest cereal crop in the world, being responsible for feeding about half the world's population. Recent data shows that Portugal has a rice consume of 15.8 kg/capita/year, the largest in Europe, producing 168 300 tons of rice per year being the fourth largest producer in Europe.

Folate is a generic term used for different water-soluble vitamins of the B-complex group. It is an essential micronutrient needed for optimal health,

and development. Dietary folate deficiency is common around the world, and deficiency of this vitamin is linked to various metabolic disorders [1]. Nutritional deficiency is common in those that have a low folate consumption diet. This can be exacerbated by malabsorption conditions, including celiac disease. Pregnancy and lactation increases the risk of folate deficiency due the high requirement, to support optimal growth and development of the fetus [2].

Naturally occurring folate is present in a wide range of foods. Staple diets consisting largely of cereal grains and tubers are very low in folate but can be improved by the addition of legumes or green leafy vegetables [3]. According to the most recent edition of European Food Safety Authority, a Population Reference Intake (PRI), for healthy adult men and women, should be 330 µg of dietary folate equivalent (DFE) for day. These findings are in close agreement with controlled studies showing that folate intakes of around 200–300 µg/day may be sufficient to maintain serum and red blood cell folate concentrations [4].

Since folate is an unstable nutrient, the amount of folate available in processed and stored food could be considerably lower than that in raw food. In large-scale service systems, for example hospitals, knowledge of the content of folate in food can be critical because of special or increased requirements for many patients. This must be taken

into consideration when calculating the real total folate intake from food.

2. MATERIALS AND METHODS

2.1. Sample preparation and cooking method

Samples of Portuguese Indica and Japonica rice were purchased from local markets, in Lisbon, Portugal, according to the designed sampling plan. Boiled samples were cooked in a Termomix® TM31 food processor (Vorwerk, Germany) [5].

2.2. Folate quantification

The determination of folic acid (FA), 5-methyltetrahydrofolate (5-MTHF), tetrahydrofolate (THF), 5-formyltetrahydrofolate (5-CHOTHF) and 10-formyltetrahydrofolate (10-CHOTHF) was performed using a UPLC-MS/MS. The chromatographic conditions used in a Acquity UPLC –TQD was a HSS T3 1.8 µm 2.1 x 150 mm column, from Waters at 45°C, 0.35 ml/min flow rate of 0.1% formic acid solution (A) and acetonitrile (B) in gradient elution. The extraction of folates was performed with tri-enzyme treatment (α -amylase, protease and rat serum) in a 7.2 pH bicarbonate buffer 0.5 mM with 0.5% of dithiothreitol (DTT) and 1% of ascorbic acid. After centrifuging the resulting solution, the supernatant was ultrafiltrated on a 5kDa filter. The method include the use of reference internal standards isotopically labeled of 13C5 FA and 13C5 5-MTHF.

2.3. Cooking yield

The cooking yield (Y_c) of rice was calculated, this quantifies the gain or weight loss during processing and was calculated according to equation 1 [6]. In this work it was assumed that food weight variations were due to losses or gains water.

$$Y_c = \frac{\text{Weight of cooked sample (g)}}{\text{Weight of raw sample (g)}} \quad (1)$$

2.4. Determination of retention

The retention of amino acids was calculated according to reference [7], using the following equation:

$$TR\% = (A \times B \div C \times D) \times 100 \quad (2)$$

where A - nutrient content (g) of cooked sample; B - sample weight (g) after cooking; C - nutrient content (g) of raw sample and D - weight (g) of raw sample.

2.5. Calculation of the equivalent portion of raw food

To calculate the equivalent portion (EP) for each cooked food was used as a base table equivalent to a portion published by the reference [8]. This provides for raw cereals and derivatives equivalent portion 35g. Thus, to calculate the equivalent portion of cooked food was applied to equation 3.

$$EP \text{ raw food (g)} = 35 \text{ g} \times Y_c \quad (3)$$

2.6. Statistical analysis

Data were reported as mean and standard deviation (SD). Analyses of variance (ANOVA) were used to evaluate results. When the overall result was statistically significant, the TukeyKramer multiple comparison test was conducted to identify each of the significantly different cooking processes.

Homogeneity of variances was tested by Cochran's and Levene's test and the results were undertaken using Kruskal-Wallis non-parametric test for heterogeneous variances. Differences were considered significant at a p-value below 0.05. All statistical tests were conducted by Statistica v.8 software (Statsoft Ibérica, Lisboa, Portugal).

3. RESULTS AND DISCUSSION

The quantification method was performed using a calibration curve with six levels between 7 ng/ml and 100 ng/ml. with correlation coefficients ≥ 0.9985 to all endogenous folates. The matrix match LoD ranged between 0.1 ng/mL (FA, 5-MTHF; 5-CHOTHF) and 3.0 ng/mL (THF). Laboratory competence is demonstrated through satisfactory participation in proficiency testing schemes FAPAS 2186 and 2191, breakfast cereal, where the Z-Score obtained was 1.6 and -1.9 for folic acid respectively. Also a NIST SRM 1849a Infant/Adult Nutritional Formula was used to assess the accuracy of method.

The cooking yield for cooked indica and japonica rice samples was 2.54 and 2.50, respectively.

Regarding folates in rice, the predominant form in all samples was 5-MTHF. The highest value found in the raw samples, 96.6 - 98.2 µg/100 g (table 1).

Values are expressed as average ± standard deviation of triplicates, µg/100 g dry weight (n=3).

¹ Different letters in each parameter for each sample indicate significant differences at $p < 0.05$.

Table 1 – Folate content in rice

	Indica Rice		Japonica Rice	
	Raw	Boiled	Raw	Boiled
FA	1,5 ± 0,4	0,5 ± 0,5	1,2 ± 0,2	0,2 ± 0,2
THF	<LoQ	<LoQ	<LoQ	<LoQ
5-MTHF	98,2 ± 0,8	36,5 ± 3,2	96,6 ± 0,9	40,4 ± 5,5
10-CHOTHF	9,8 ± 0,9	4,4 ± 0,9	9,1 ± 0,4	3,6 ± 0,7
5-CHOTHF	<LoQ	<LoQ	<LoQ	<LoQ
Total folates	110 ± 1,3	41,4 ± 2,3	107 ± 1,0	44,2 ± 5,3

LoQ: limit of quantitation (THF < 0,56; 5-CHOTHF < 0,24)

Values are expressed as average ± standard deviation of triplicates, µg/100 g dry weight (n=3).

The 5-CHOTHF and THF forms were below limit of quantitation in all analyzed samples. Identical profile of the different forms of folate has been described by reference [9] for rice.

These results are proportionally in accordance with [10]. LC-MS/MS method provide sensitive, specificity and accurate separation of several folate forms [11] than the microbiological assay method used by [10]. Also the use of trienzyme treatment which generally provides the highest detectable value of food folate concentration could explain the differences found [12].

Concerning the effect of cooking on the folate content in rice, results are expressed in dry basis (table 2). These results show that boiling does not significantly affect ($p < 0.05$) the content of any forms of folates.

Table 2 - Folates content in cooked rice¹

	Indica Rice		Japonica Rice	
	Raw	Boiled	Raw	Boiled
FA	1,7 ^a ± 0,4	1,4 ^a ± 1,2	1,3 ^a ± 0,2	0,6 ^a ± 0,5
5-MTHF	112 ^a ± 0,93	99,7 ^a ± 8,9	110 ^a ± 1,0	110 ^a ± 14,9
10-CHOTHF	11,2 ^a ± 1,1	12,1 ^a ± 2,5	10,3 ^a ± 0,5	9,8 ^a ± 1,9
Total Folates	115 ^a ± 1,4	113 ^a ± 6,3	122 ^a ± 1,1	121 ^a ± 14,6

During cooking, there is a reduction FA content in japonica rice with a retention factor of 0.45 (Fig.1), although without significant difference and with no impact on content of total folates.

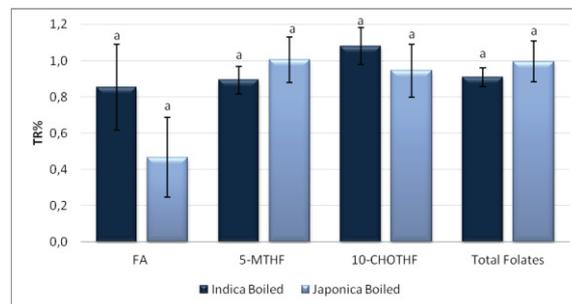


Fig. 1. True retention (%TR) for folates in boiled rice. Different letters in each folate indicate significant differences at $p < 0.05$

To calculate the equivalent portion to the raw food the Equation 3 was applied. The portions for the boiled indica and japonica rice were 89 and 87 g, respectively. For raw samples was 35 g.

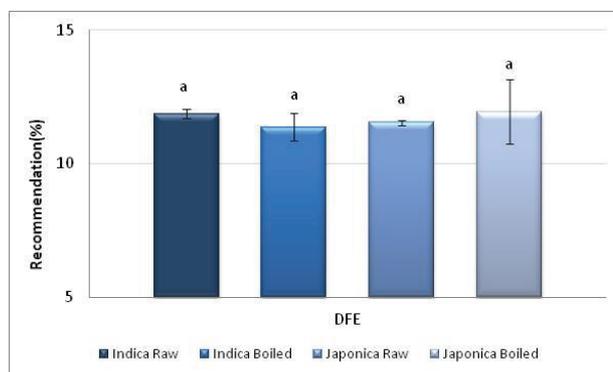


Fig. 2. Folate Recommendation (%) by portion of cooked and raw rice. Different letters in each folate indicate significant differences at $p < 0.05$

Figure 2 show that the consumption of one portion of rice supplies 11.4 to 11.9% of the dietary reference values for folates (Fig. 2).

4. CONCLUSIONS

This study provided information on folate content in one of the major cereal consumed in

world. As labelled internal standards are used and two MRM transitions per compound are monitored, our UPLC–MS/MS method is very sensitive and specific. It has been successfully applied to determine five different low-concentrated folate monoglutamates in rice seeds. By analysing polished rice seeds raw and cooked, we found 5-methyltetrahydrofolate to be the main folate form in rice.

In conclusion this work demonstrate that folates in rice are not affected by boiling and a consumption of an equivalent portion provide more than 10% of the dietary reference values for folates.

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