ELSEVIER

Food Chemistry 68 (2000) 295–298

Food Chemistry

www.elsevier.com/locate/foodchem

Folate retention in selected processed legumes

Jennifer Dang, Jayashree Arcot*, Ashok Shrestha

Department of Food Science and Technology, University of New South Wales, Sydney 2052, Australia

Received 28 April 1999; received in revised form 10 July 1999; accepted 10 July 1999

Abstract

The effect of soaking, boiling and pressure cooking on the retention of folates in whole chickpeas (*Cicer arietinum*) and field peas (*Pisum sativum*) was investigated. Pressure-cooking allowed significantly higher ($p < 0.05$) retention of folates compared to boiling in both the legumes. Retention of folates in chickpeas was greater than field peas irrespective of the processing procedure used. Leaching into the cooking medium was identified to be the major cause for losses of folates during soaking and cooking. Leaching losses were greater in field peas compared to chickpeas.

Keywords: Folates; Legumes; Microbiological assay

1. Introduction

Folic acid is a water-soluble vitamin that exists naturally in foods, usually in the polyglutamyl forms. The most common forms are tetrahydrofolic acid, 5-methyl tetrahydrofolic acid, and 5-formyl tetrahydrofolic acid (Gregory, 1989). Pure folic acid or pteroyl glutamic acid (PGA) is not usually found in foods, but is the form that is most stable to chemical degradation, most bioavailable and is used for fortification. The importance of folates in foetal development has received a considerable amount of attention in recent years (Halsted, 1993).

Legumes constitute an important part of the human diet in many parts of the world. They are very good sources of folates which are not readily available due to complex binding with bio-molecules (Kadam & Salunkhe, 1989). Among the grain legumes, pea (*Pisum sativum*) ranks fourth in world production and chickpea (*Cicer arietinum*) ranks fifth (Food and Agriculture Organization, FAO, 1997). Some of the ways in which they are cooked are by soaking and boiling, pressure-cooking and germination. The dried forms of the legumes cannot be consumed directly and need to be processed as they are generally unpalatable, hard to digest and also have some anti-nutritional factors such as haemagglutinins and trypsin inhibitors (Akroyd & Doughty, 1982). However, the long time needed for cooking and softening of legumes may lower the nutritive value by destruction and leaching of valuable vitamins (Vallidevi, Ramanuja, Roa & Nath, 1972). Folic acid is highly sensitive to light, air, heat and extreme pH conditions (Keagy, 1985). It is crucial to have appropriate information on the availability of nutrients in given products and the effect of different processing variables on their retention. Several studies have been reported on the general effects of heat on stability of folates in foods (Chen, Song & Kirsch, 1983; Leichter, Switzer & Landymore, 1978; Lin, Luh & Schweig, 1975). Leaching into the cooking water, boiling temperatures, pH of cooking medium and time of cooking are some of the factors responsible for the loss in folates.

Human plasma was preferred as a folic acid conjugase in this study over other established sources of conjugases like chicken pancreas, hog kidney, rat plasma and rat pancreas. Relatively very few studies have been reported on the use of plasma conjugase despite its advantages over others that are currently available. Human plasma is relatively cheaper, easily available, required in small quantity and no additional purification is needed as it contains very low endogenous folate content and produces monoglutamate as the end product (Keagy, 1985; Lakshmaiah & Ramsastri, 1975).

This study determined the stability of folates during processing of field peas and chickpeas. These legumes were subjected to different processing conditions such as

* Corresponding author. Fax: +61-2-9385-5931.
E-mail address: j.arcot@unsw.edu.au (J. Arcot)

0308-8146/99/$ - see front matter
PII: S0308-8146(99)00202-2

soaking; boiling, pressure-cooking and germination and folate retention was assessed.

2. Materials and methods

One commercial variety each of pea (variety, *Bluey whole blue*) and Chickpea (variety, *Tyson*) was obtained from Allgold Foods, Leeton, NSW, Australia. Raw samples were ground using a Fritsch Pulverisette (pore size, 0.5 mm) mill.

2.1. Soaking

Stainless steel containers were used during soaking and cooking of legumes to avoid the loss of folates due to light. Throughout the process containers were kept covered, except during stirring, for the same reason. One volume of the legume in 3 volumes of water was soaked at room temperature for 16 h. After soaking, excess soak water was measured and samples of soak water and legume were taken and stored at −18°C for folate determination.

2.2. Boiling

One volume of soaked samples in three volumes of water were first heated to boiling temperature and then simmered for 2 h until the peas became soft. The optimum time for simmering required for completion of cooking was established by assessing the softness of the cooked legume.

2.3. Pressure-cooking

Same ratio of sample to water as for soaking was used for pressure-cooking. It was done in a domestic pressure cooker for 20 min. The excess cooking water from boiling and pressure-cooking was measured and samples stored at −18°C for analysis.

2.4. Moisture estimation

Moisture contents of the samples were estimated using a vacuum oven at 70°C overnight (Method no. 32.1.02, Association of Official Analytical Chemists, AOAC, 1995).

2.5. Folate assay

Folate was analysed by the microbiological assay using *Lactobacillus casei*, (ATCC 7469) obtained from Department of Microbiology, University of New South Wales, Sydney. The method suggested by Lakshmaiah and Ramasastri (1975) was generally followed. Unde-conjugated folates (*Lactobacillus casei* activity without human plasma treatment) and total folates (*L. casei* activity after treatment with human plasma) were analyzed. However, a slight modification in the procedure was the amount of conjugase that was increased to 0.25 ml and only 3 h incubation was done (instead of overnight) as recommended by Goli and Vanderslice (1992).

2.5.1. Deconjugation of sample extract

Pooled human plasma obtained from the Prince of Wales Hospital, Sydney was stored at −18°C in 10 ml aliquots. The deconjugation buffer (2.75 ml of 0.1 M phosphate buffer at pH 4.5) and 1 ml of 100 mM 2-mercaptoethanol (BDH) were dispensed into test tubes and incubated at 37°C for 10 min. One millilitre of the sample extract and 0.25 ml of human plasma conjugase were added and mixed in a vortex mixer (M19, Chiltern, Australia). The deconjugation mixture was incubated at 37°C for 3 h in a water bath (Clemco, Sydney) and the enzymatic reaction stopped by boiling for 5 min. The pH of the deconjugated sample was adjusted to 6 and diluted to a volume of 10 ml using an extraction buffer (0.1 M phosphate buffer, 1% ascorbic acid, pH 6). The mixture was centrifuged (Sorvall, Dupont, Connecticut) at 2500 rpm for 10 min to remove any suspended solids and the deconjugated extracts were stored for no longer than a week at −18°C before use.

Preliminary analysis showed that human plasma contained about 15 ng folates per ml, similar to values reported by Keagy (1985) which was 5–15 ng per ml of plasma. All data and nutrient values are reported on a moisture free basis.

2.6. Quality control

Recovery studies were performed by adding known concentrations of pure folic acid (pteroyl glutamic acid) solutions to sample extracts and analysed for unde-conjugated and total folates.

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to determine significant effects of different parameters on the materials using a statgraphics package (Statistical Graphics Corporation, 1993, Manugistics Inc., USA). Least significant differences between treatment means were determined by Duncan multiple range test (DMRT).

3. Results and discussion

3.1. Effect of domestic processing

The folate contents of chickpeas and peas and their respective cooking water are presented in Tables 1 and 2.

Table 1
Folate content (μg/100 g dry weight) of chickpeas and the water used for processing[a]

Samples	Moisture (g/100 g)[c]	Undeconjugated folates[d]	Total folates[d]
Chickpea			
Raw	9.1	121.5 ± 5.5c	149.7 ± 5.2c
Boiled	62.3	63.3 ± 2.7a (52.1)[b]	78.8 ± 4.1a (52.6)
Pressure-cooked	64.6	73.8 ± 4.0b (60.7)	93.0 ± 5.1b (62.1)
In medium			
Soaking water	99.5	21.8 ± 2.3a (17.9)	25.9 ± 2.4a (17.3)
Boiling water	97.6	32.4 ± 3.0b (26.7)	42.2 ± 3.7c (28.2)
Pressure cooking water	98.2	24.6 ± 2.1a (20.2)	29.7 ± 2.1b (19.8)

[a] Values are the mean ± standard deviation of six replicate determinations.
[b] Figures in parentheses represent percent retention of folates (in chickpea) or loss of folates (in medium).
[c] Mean moisture contents of 4 replicate determinations.
[d] Significance of LSD between means at $p < 0.05$. Within columns, means followed by different letters are significantly different.

Table 2
Folate content (μg/100 g dry weight) of peas and the water used for processing[a]

Samples	Moisture (g/100 g)[c]	Undeconjugated folates[d]	Total folates[d]
Peas			
Raw	11.2	87.5 ± 6.7b	101.5 ± 6.7b
Boiled	63.4	38.9 ± 3.1a (44.5)[b]	45.7 ± 4.0a (45.0)
Pressure-cooked	63.7	43.4 ± 4.5a (49.6)	51.1 ± 4.3a (50.3)
In medium			
Soaking water	99.0	18.3 ± 1.4a (20.9)	21.0 ± 1.4a (20.7)
Boiling water	97.2	27.8 ± 2.9c (31.8)	32.3 ± 2.8c (31.8)
Pressure cooking water	96.4	22.7 ± 2.8b (25.9)	27.5 ± 3.0b (27.1)

[a] Values are the mean ± standard deviation of six replicate determinations.
[b] Figures in parentheses represent percent retention of folates (in peas) or loss of folates (in medium).
[c] Mean moisture contents of 4 replicate determinations.
[d] Significance of LSD between means at $p < 0.05$. Within columns, means folowed by different letters are significantly different.

The analyzed folate values for chickpeas and peas (raw and cooked) varied widely with published values (Augustin & Klein, 1989; Holland, Unwin & Buss, 1991; Hoppner & Lampi, 1993; Phillips & Wright, 1983; Tan, Wenlock & Buss; 1985). This variation could be due to the different folate assay procedures used by earlier investigators. Various investigators agreed on the fact that the value of folates in a food is affected by the method employed and deconjugation procedure (Keagy, 1985; Tamura, 1998).

3.2. Retention of folate in the legumes

The undeconjugated and total folate contents of chickpeas on boiling and pressure-cooking were significantly different ($p < 0.05$) (Table 1). Between the two processes, namely, boiling and pressure cooking, the pressure cooked samples had significantly higher ($p < 0.05$) retention compared to the boiled samples. This can be attributed to the shorter exposure to heat during pressure-cooking (20 min) compared to boiling (2 h). There was 52.6 and 62.1% retention of folate in boiled and pressure-cooked samples, respectively. The variability in retention may also be due to differences in oxygen exposure during cooking (Gregory, 1989).

The undeconjugated and total folate contents of peas on boiling and pressure-cooking were significantly different ($p < 0.05$) (Table 2). However, there was no significant ($p < 0.05$) difference in the retention of folates between the two processes namely, boiling and pressure cooking. There was 45 and 50.3% retention of folates in boiled and pressure-cooked samples respectively. Hoppner and Lampi (1993) reported a retention of 66 and 40% folates when chickpeas and peas, respectively were soaked overnight. They also reported a 44% retention of folates when legumes soaked overnight were heated for 150 min.

3.3. Loss of folates in soaking media

Losses in the cooking and soaking waters were seen to be considerable and also indicated that leaching was the predominant reason for loss of folates (Table 1 and 2). In both legumes, there was a significant ($p < 0.05$) difference in the amount of folates (except in undeconjugated folates in the soaking and pressure cooking water for

chickpeas) that leached into the soaking, boiling and pressure-cooking water. The loss was highest in boiling water followed by pressure-cooking and soaking water. Previous studies (Hawkes & Villota, 1989; Leichter et al., 1978; Miller, Guadagni & Kon, 1973) have also shown that the major loss of folates in cooked vegetables can be attributed to leaching into the cooking water that is often discarded, rather than actual destruction of the vitamin. The losses in general were higher in peas than in chickpeas. This observation is similar to that reported by Augustin, Beck, Kalbfleish, Kagel and Matthews (1981) in red kidney beans and small white beans. Retention varied considerably depending on legume species and class. This could also be attributed to the surface area to volume ratio of the legumes (Leichter et al., 1978; Hoppner & Lampi, 1993). In the present study peas were smaller in size, and hence had a larger surface area to volume ratio compared to chickpeas explaining the higher leaching losses in field peas.

Hawkes and Villota (1989) reported that losses of folates in vegetables ranged from 22% in asparagus to 84% in cauliflower. Lin et al. (1975) observed a 6.1% loss through leaching in garbanzo beans during soaking (12 h). This value is lower through leaching in garbanzo beans during soaking (12 h). This value is lower than that observed in this study for chickpeas (17.9%) and peas (20.7%) during the soaking process (16 h).

3.4. Quality control

Recovery of added folic acid during the analysis of the samples was an average of 96% indicating that the results obtained were acceptable.

4. Conclusion

Folate losses were seen in both the legumes irrespective of the processing procedure used. However, soaking followed by pressure-cooking caused more retention compared to boiling suggesting that pressure cooking after soaking could be the best option for maximum retention of folates in the cooked legume.

References

Akroyd, W. R., & Doughty, J. (1982). Legumes in human nutrition. FAO Food and Nutrition paper No. 20, FAO, Rome, Italy.

Association of Official Analytical Chemists (1995). *Official methods of analysis of AOAC*. VA: International Association of Official Analytical Chemists.

Augustin, J., Beck, C. B., Kalbfleish, G., Kagel, L. C., & Mathews, R. H. (1981). Variation in the vitamin and mineral content of raw and cooked commercial *Phaseolus vulgaris* classes. *Journal of Food Science, 46*, 1701–1706.

Augustin, J., & Klein, B. P. (1989). Nutrient composition of raw, cooked, canned and sprouted legumes. In R. H. Mathews, *Legumes: chemistry, technology and human nutrition* (pp. 187–217). New York: Marcel Dekker, Inc.

Chen, T.S, Song, Y. O., & Kirsch, A. J. (1983). Effects of blanching, freezing and storage on folacin contents of spinach. *Nutr. Rep. Int., 28*(2), 317.

Food and Agriculture Organization (1997). *FAO Yearbook: production*. Rome, Italy: Food and Agriculture Organization.

Goli, D. M., & Vanderslice, J. T. (1992). Investigation of the conjugase treatment procedure in the microbiological assay of folate. *Food Chem., 43*, 64–77.

Gregory, J. F. (1989). Chemical and nutritional aspects of folate research, analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. *Advances in Food and Nutrition Research, 33*, 1–101.

Halsted, C. H. (1993). Water-soluble vitamins. In J. S. Garrow, & W. P. T. James, *Human nutrition and dietetics* (9th ed.) (pp. 239–263). Edinburgh: Churchill Livingstone.

Hawkes, J. G., & Villota, R. (1989). Folates in foods: reactivity, stability during during processing and nutritional implications. *CRC Critical Reviews in Food Science and Nutrition, 28*(6), 438–538.

Holland, B., Unwin, I.D., & Buss, D.H. (1991) Vegetables, herbs and spices. Fifth supplement to McCance and Widdowson's, *The composition of foods*. Cambridge, UK: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Hoppner, K., & Lampi, B. (1993). Folate retention in dried legumes after different methods of meal preparation. *Food Research International, 26*, 45–48.

Kadam, S. S., & Salunkhe, D. K. (1989). Minerals and vitamins. In: *CRC handbook of world food legumes: nutritional chemistry, processing technology, and utilization* (Vol. I). Boca Raton, Florida: CRC Press, pp. 117–121.

Keagy, P. M. (1985). Folacin: microbiological and animal assays. In J. Klein, B. P. Klein, & P. B. Venugopal, *Methods of vitamin assay* (4th ed.) (pp. 445–471). New York: John Wiley and Sons.

Lakshmaiah, N., & Ramasastri, B. V. (1975). Folic acid conjugase from plasma-III. Use of the enzyme in the estimation of folate activity in foods. *Intern. J. Vit. Nutr. Res., 45*, 262–272.

Leichter, J., Switzer, V. P., & Landymore, A. (1978). Effect of cooking on the folate content of vegetables. *Nutrition Reports International, 18*(4), 475–479.

Lin, K. C., Luh, B. S., & Schweig, B. S. (1975). Folic acid content of canned garbanzo beans. *Journal of Food Science, 43*(3), 562.

Miller, C. F., Guadagni, D. G., & Kon, S. (1973). Vitamin retention in bean products: cooked, canned and instant bean powders. *Journal of Food Science, 38*, 493–495.

Phillips, D. R., & Wright, A. J. A. (1983). Studies on the response of *Lactobacillus casei* to folate vitamin in foods. *The British Journal of Nutrition, 49*(2), 181–186.

Tamura, T. (1998). Determination of food folate. *Methods of Nutritional Biochemistry, 9*, 285–293.

Tan, S.P., Wenlock, R.W., & Buss, D.H. (1985). Immigrant foods. Second supplement to McCance and Widdowson's, *The composition of foods*. London: Elsevier/North-Holland Biomedical Press.

Vallidevi, A., Ramanuja, M. N., Rao, N. A. N., & Nath, H. (1972). Effect of processing and storage on thiamin, riboflavin and nicotinic acid content of four varieties of Indian pulses. *Ind. J. Nutr. Dietet., 9*, 129.