Food Chemistry **17** (1985) 1–11

The Relationship of Endogenous Phytase, Phytic Acid and Moisture Uptake with Cooking Time in *Vicia faba minor* cv. Aladin

H. Michael Henderson & Sylvia A. Ankrah

Department of Food Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

(Received: 11 September, 1984)

ABSTRACT

Fababeans (cv. Aladin) were soaked at 20, 35, 50 and 65 °C for 8, 20 and 24 h and endogenous phytase activity, phytic acid content, moisture uptake and the cookability index (an indirect determination of cooking time) were determined, with corresponding determinations in dry fababeans. Phytase activity was low in the dry beans but increased during soaking at 20 and 35 °C, with no activity after soaking at 50 and 65 °C. The phytic acid content was constant during soaking at 20 and 35 °C, but decreased at the higher temperatures due to leaching into the soaking medium. Moisture uptake increased with soaking time and temperature. No relationship was demonstrated between the cookability index and either phytase or phytic acid. The effect of moisture uptake on the cookability index was significant in fababeans soaked for 8 h ($r = -0{\cdot}99$, $p < 0{\cdot}05$), especially when the soaking temperature was increased from 20 °C to 35 °C.

INTRODUCTION

Legumes are an important source of protein for a high proportion of the world's population, particularly in areas where animal and fish proteins are relatively expensive or unavailable. Harvested legumes are stored in the dry state, often for up to several months, before use. Dried legumes

Food Chemistry 0308-8146/85/$03·30 © Elsevier Applied Science Publishers Ltd, England, 1985. Printed in Great Britain

require relatively longer cooking times, compared with freshly harvested legumes, to attain an acceptable tenderness. The longer cooking time increases fuel costs and reduces the nutritive value of the legumes: this limiting factor is termed the hard-to-cook (HTC) phenomenon or defect. Commercially, dry legumes are soaked for 12–16 h at room temperature prior to processing in order to decrease the time required for cooking (Quast and da Silva, 1977; Hsu *et al.*, 1983). The soaking step is lengthy and ways to shorten it, especially by increasing the temperature of the soaking water, have been devised (Kon, 1979).

The reduction in the level of phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate), the major storage form of phosphorus, in legumes is important, especially with respect to its antinutrient properties, but such reduction has been associated with poor cookability in legumes (Mattson, 1946; Kumar *et al.*, 1978; Kon, 1979). The activation of endogenous phytase (*myo*-inositol-hexakisphosphate phosphohydrolase, EC 3.1.3. (8,26) through soaking, germination and increased temperature has been effective in reducing the phytate content of legumes (Chang *et al.*, 1977; Eskin & Wiebe, 1983). Phytase hydrolyses phytic acid to *myo*-inositol and free orthophosphate ion, through inositol phosphate intermediates by the progressive release of inorganic phosphate. With respect to soaking and the early stages of germination, Tabekhia & Luh (1980) have suggested that the first two days represent a latent period with minimal phytase activity and a slight decrease in the phytic acid content. Ologhobo & Fetuga (1984) demonstrated, in cowpeas, lima beans and soybeans, soaked in deionised water at 27 °C for 3 days, decreases in phytate content which were, however, lower than in the corresponding germinated beans.

The fababean (*Vicia faba* L. var. *minor*), a high protein legume, is cultivated in Europe, the Middle East, North Africa and, relatively recently, in the Canadian Prairies. The purpose of the investigation reported in this paper was to determine practical conditions for the soaking of fababeans, in terms of minimum time and temperature, in order that the subsequent cooking time could be reduced. Concurrent determinations were therefore made on moisture uptake, phytic acid content and endogenous phytase activity in the soaked fababeans in order to determine the influence of these three factors on the subsequent cooking time. The fababeans used in this study were the first cultivar of Canadian origin, Aladin, released in 1981. Cookability studies on several other varieties of fababeans available in Canada and in Egypt indicated

that the HTC phenomenon was related to seed coat and cotyledon characteristics (Youssef *et al.*, 1982).

MATERIALS AND METHODS

Source material

Whole fababeans (*Vicia faba* L. var. *minor* cv. Aladin), harvested in 1981, were obtained from the Department of Plant Science, University of Manitoba, and were stored at room temperature (approximately 20 °C) until required.

Soaking procedure and sample preparation

This study was undertaken on two separate occasions. Whole fababeans (150 g) were soaked in 600 ml of distilled water at 20, 35, 50 and 65 °C (duplicate batches) and samples of the soaked beans were withdrawn after 8, 20 and 24 h of soaking. Dry fababeans were milled directly, while soaked beans were freeze-dried prior to milling. Samples were milled in a Chemical Rubber Co. water-cooled micro-mill and sieved through 100-mesh to obtain a fine flour. The flours were stored at 2–4 °C in sealed containers.

Analytical procedure

Phytase was extracted and partially purified from fababean flour following the procedure of Lolas & Markakis (1977), except that 30% and 70% ammonium sulphate fractionation was used. Phytase activity was determined by the method of Lolas & Markakis (1977) and the rate of increase of inorganic phosphate was followed using the ascorbic acid method of Watanabe & Olsen (1965). The specific activity of phytase was expressed as micrograms of inorganic phosphate released per milligram of protein in 30 min, and the values were corrected against a boiled enzyme control. Protein was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as the standard. Phytic acid was determined by the method of Latta & Eskin (1980), and values were reported as the percentage of phytic acid on a dry matter basis. Moisture in the fababean flours was determined by the air-oven method

(AOAC, 1975). The soaked fababeans were drained for 90 s, blotted dry and weighed. Moisture uptake was expressed as the percentage increase in weight of the beans during soaking.

Determination of cookability index

The measurement of the cookability index was an indirect determination of the relative differences in the cooking times of the fababean samples, a higher cookability index indicating that a longer cooking time would be required by a sample to attain a desired tenderness when cooked. The index was measured on the Ottawa Texture Measuring System (OTMS) using a Kramer Shear Cell, and was calculated as the maximum force required to shear the cooked fababeans (kilograms per gram of sample).

Dry fababeans (25 g per 500 ml of distilled water) were cooked for 90 min, samples soaked for 8 h were cooked for 20 min and samples soaked for 20 and 24 h were cooked for 10 min (25 g per 200 ml of distilled water). After cooking, all samples were drained of excess water and allowed to cool to room temperature before cookability index determination. The different cooking times were necessary to obtain textures which could be measured on the OTMS without exceeding the limits of the instrument.This meant, however, that the effect of soaking time on changes in the cookability index, at each of the soaking temperatures, could not be fully evaluated.

RESULTS AND DISCUSSION

Phytase activity, phytic acid content and the cookability index were determined in dry (unsoaked) fababeans both at the commencement and at the termination of this study, and the results are shown in Table 1.

A low level of phytase activity was demonstrated. Corresponding phytase activities in the Ackerperle and Diana fababean cultivars, determined under similar experimental conditions, were found to be 1·8 and 1·0 μg of inorganic phosphate released per milligram of protein in 30 min, respectively (Eskin & Wiebe, 1983). Low phytase activity has been detected in dry California small white beans (Chang & Schwimmer, 1977) and navy beans (Lolas & Markakis, 1977), but Mandal & Biswas (1970) did not detect activity in dry mung beans. The phytic acid content of the dry fababeans used in this study was comparable with that found

TABLE 1
Analysis of the Dry (Unsoaked) Fababeans (*Vicia faba* L. var. *minor* cv. Aladin)

	Experimental period	
	Commencement	*Termination*
Phytase[a]	0·24	0·28
Phytic acid[b]	1·02	1·10
Cookability index (kilograms per gram)	4·67	6·82

[a] Specific activity is expressed as micrograms of inorganic phosphate released per milligram of protein in 30 min.
[b] Percentage on dry weight basis.

(0·80–1·04%) in five fababean varieties by Griffiths (1983), and in the Ackerperle and Diana varieties (0·97 and 1·10%, respectively) by Eskin & Wiebe (1983).

The effect of soaking time and temperature on phytase activity was determined in fababeans soaked at 20, 35, 50 and 65 °C for 8, 20 and 24 h and the results are shown in Table 2.

Phytase activity was found to increase markedly during soaking at 20 °C, and increased activity was observed in samples soaked at 35 °C for 8 h compared with samples soaked at 20 °C for the same time period, but a longer soaking time at 35 °C resulted in decreasing activity. No activity was demonstrated throughout the 24-h soaking period at soaking temperatures of 50 and 65 °C. Similar changes in phytase activity during

TABLE 2
Effect of Soaking Time and Temperature on Phytase Activity[a] in Fababeans cv. Aladin

Soaking temperature	*Soaking time (h)*		
(°C)	*8*	*20*	*24*
20	2·49*a*	3·32*a*	5·62*b*
35	3·15*a*	2·25*a*	2·12*a*
50	0	0	0
65	0	0	0

[a] Specific activity units.
a and *b* are assigned to indicate significant differences at the 0·05 level according to Tukey's test.

TABLE 3
Linear Correlation Analysis of Phytic Acid Content, Moisture Uptake and Cookability Index

Soaking temperature (°C)	*Soaking time (h)* 8			20			24		
	Moisture uptake[a]	*Phytic acid*[b]	*Cookability index*	*Moisture uptake*	*Phytic acid*	*Cookability index*	*Moisture uptake*	*Phytic acid*	*Cookability index*
20	49·4*a*	1·01*a*	12·07*a*	74·9*b*	0·99*a*	5·60*b*	76·9*b*	1·02*a*	4·71*b*
35	89·8*b*	0·98*a*	3·11*b*	97·4*b*	1·03*a*	3·50*b*	98·3*b*	1·03*a*	4·56*b*
50	94·8*b*	1·08*a*	2·75*b*	95·2*b*	0·98*a*	3·09*b*	95·5*b*	0·83*b*	4·66*b*
65	90·5*b*	0·88*b*	2·31*b*	90·8*b*	0·82*b*	4·99*b*	91·1*b*	0·73*c*	4·96*b*
r	−0·99 (s)	−0·23 (ns)		−0·86 (ns)	−0·34 (ns)		−0·27 (ns)	−0·21 (ns)	

[a] Percentage increase in weight during soaking.
[b] Percentage on dry weight basis.
r = Correlation coefficient with cookability index (kilograms per gram).
ns = Not significant. s = Significant at $p < 0{\cdot}05$.
a, *b* and *c* are assigned to indicate significant differences at the 0·05 level according to Tukey's test.

the first day of the germination period, at the lower temperatures, were observed in fababeans (Eskin & Wiebe, 1983), navy beans (Lolas & Markakis, 1977), California small white beans (Kon, 1979), and in mung beans (Mandal & Biswas, 1970). Becker *et al.* (1974) demonstrated that the optimum temperature for phytase activity in whole California small white beans, incubated up to 50 h, was 35–45 °C. The absence of phytase activity at soaking temperatures of 50 and 65 °C suggests that there may be heat-sensitive mechanisms involved in the synthesis or activation of the enzyme, such as hormonal control of phytase formation during germination (Loewus & Loewus, 1983). In addition, thermal denaturation may account for the loss of the phytase activity originally present in the dry seed.

The effect of soaking time and temperature on the phytic acid content was determined in fababeans in the stated conditions and the results are shown in Table 3.

The phytic acid content remained virtually constant during soaking at 20 and 35 °C, indicating that the concurrent phytase demonstrated did not appear to hydrolyse phytic acid in the intact seeds. There was a decrease in the phytic acid content, after soaking at 50 °C for 24 h, which became more marked on soaking the beans at 65 °C. At these two temperatures, phytic acid loss could not have been due to phytase activity and phytic acid was found to have leached out into the soaking medium (Table 4).

The apparent lack of phytic acid hydrolysis by phytase in the intact fababeans may have been due to one or more factors, for instance, inhibition by inorganic phosphate (Mandal *et al.*, 1972; Chang & Schwimmer, 1977), or the preference of phytase for phosphorus-containing substrates other than phytic acid (Lolas & Markakis, 1977)

TABLE 4
Phytic Acid[a] Present in the Soaking Medium

Soaking temperature (°C)	*Soaking time (h)* 8	20	24
50	nd	nd	9·0
65	6·0	11·0	14·0

[a] Milligrams of phytic acid present in a 10-ml aliquot of soaking medium.
nd = Not determined.

during soaking and the early stages of germination. In this study, a supplementary experiment, using pin-milled fababean flour, was designed to determine whether or not the lack of phytic acid hydrolysis was due to the tissue structural organisation of the intact seed which may have limited enzyme–substrate proximity. Phytase activity and phytic acid content were determined in a 10 % slurry of the flour, soaked at 20 °C at 0, 8, 20 and 24 h. During the entire soaking period the phytic acid content did not change significantly, while there was a concurrent marked increase in phytase activity. Therefore, the tissue structure of the intact beans did not act as a barrier to prevent phytic acid hydrolysis by phytase.

Moisture uptake was determined on the soaked fababeans (Table 3). At all soaking temperatures, the greatest percentage increase in weight occurred during the first 8 h of soaking. After the initial rapid moisture uptake, a slower rate was observed for the remainder of the soaking period at 20 and 35 °C, whereas, at 50 °C, there was practically no further increase in moisture uptake. At 65 °C, the percentage increase in weight was slightly less than at 50 °C. Moisture absorption characteristics have been studied in cowpeas by Sefa-Dedeh *et al.* (1978) who demonstrated that the amount of water absorbed per unit weight of cowpeas increased with soaking time, to reach an equilibrium after approximately 12 h of soaking. The effect of temperature on moisture uptake by soybeans was studied by Hsu *et al.* (1983), who demonstrated both a rapid initial moisture uptake at all temperatures studied and an inverse relationship between soaking temperature and the time taken for the beans to reach 90 % of total absorption. Quast & da Silva (1977) observed, in black beans, that after the initial 12–14 h of water uptake, at 23 °C and above, there was a net decrease in weight due to the loss of soluble solids.

Cookability index values were determined on the soaked fababeans (Table 3). The maximum decrease between adjacent values was shown within samples soaked for 8 h, on increasing the soaking temperature from 20 to 35 °C. Otherwise, the cookability index did not vary greatly among fababeans subjected to the other soaking treatments.

Linear correlation analysis (Table 3) indicated that the phytic acid content of the soaked fababeans did not affect the cookability index and, therefore, in this study, a relationship between phytic acid content and cooking time of fababeans was not demonstrated. The observed decreases in phytic acid content may have been too small to have had any measurable effect on the cookability index. Also, in spite of the presence of endogenous phytase activity, phytic acid was not hydrolysed and

therefore no relationship between the enzyme and the cookability index was demonstrated.

A correlation coefficient of $-0{\cdot}99$ (significant at $p < 0{\cdot}05$) was found between moisture uptake and the cookability index in fababeans soaked for 8 h (Table 3), whereas the coefficients for samples soaked for 20 and 24 h were not significant. Sefa-Dedeh *et al.* (1978) found a high negative correlation ($-0{\cdot}97$, $p < 0{\cdot}05$) between the hardness of soaked cowpeas and the amount of water absorbed over the same soaking times, and also a coefficient of $-0{\cdot}9832$ between water absorption and cooked cowpea texture measured with an OTMS test cell. Jackson & Varriano-Marston (1981) demonstrated in black beans that the cooking time was inversely proportional to the moisture content.

This study has demonstrated that, in fababeans, soaking for 8 h at 35 °C significantly reduced the subsequent cooking time, compared with soaking for 8 h at 20 °C. Increasing the soaking temperature beyond 35 °C did not bring about any marked change in cooking time. No net practical advantage would appear to have been gained by increasing the soaking time. The effect of moisture uptake on the cookability index may be dependent upon the soaking time: the relationship between the two former factors became less pronounced at longer soaking times. A water saturation phase may have been attained where the cookability index is independent of the moisture content and may therefore become proportionately more dependent upon other fababean chemical constituents, possibly the interaction of phytic acid, pectin, calcium and magnesium ions, expressed by the 'PCMP' number (Muller, 1967).

ACKNOWLEDGEMENT

Financial assistance from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

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