THE UNIVERSITY OF MANITOBA

THE RELATIONSHIP OF PHYTIC ACID CONTENT AND ENDOGENOUS PHYTASE ACTIVITY WITH COOKING TIME IN *VICIA FABA MINOR*

BY
SYLVIA A. ANKRAH

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ABSTRACT

Differences in the cooking time of legumes have been related to their phytic acid content, and to moisture content after soaking. This study attempted to investigate the effects of endogenous phytase activity, phytic acid content and moisture uptake in faba beans (*Vicia faba* L.var *minor*) on their cooking quality.

Phytase activity was found to be present in low amounts in the dry seeds, but increased during soaking at 20 and 35°C. No enzyme activity was evident at higher soaking temperatures, 50 and 65°C. The level of phytic acid remained unchanged after soaking at 20 and 35°C, but decreased with soaking time at 50 and 65°C. Increasing phytase activity during soaking did not actively hydrolyze phytic acid in the intact bean tissue, or in pin-milled faba bean flour. Changes in phytic acid content that occurred were found to be due to leaching effects rather than enzymic action. Water uptake in the faba beans increased with soaking time and temperature, and the effect of increasing soaking temperature on the cookability index was found to be dependent upon the soaking time.

Phytase activity and phytic acid content were found to have no significant effect on the cookability index of soaked faba beans, whereas the effect of water uptake on the cookability index was found to be significant only for short soaking times (eight hours), particularly when the soaking temperature was raised to 35°C from 20°C.

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INTRODUCTION

Legumes are cultivated throughout the world, in both tropical and temperate zones, and have a high protein content ranging from 17 to 38 percent (dry weight basis). They provide an important source of protein and other nutrients for a large proportion of the world population, particularly in areas where animal and fish proteins are relatively expensive or unavailable. Generally legumes are harvested, and stored in the dry state for up to several months before use.

The dried legumes require relatively longer cooking times, than when freshly harvested, to become tender. In addition to being time consuming, the longer cooking time not only increases fuel costs, but reduces the nutritive value of legumes, through the thermal destruction of vitamins and mineral loss into the cooking liquid. Sefa-Dedeh *et al.* (1978) and Al-Nouri and Siddiqi (1982) attributed a reduced consumption of legumes to the long cooking times required. Several researchers have referred to this limiting factor in legumes as the hard-to-cook (HTC) phenomenon or defect, and reports of studies in this area indicate that more than one factor is responsible for this phenomenon. The development of the HTC phenomenon in legumes has been attributed to physical properties of the seed coat (e.g. thickness and permeability), chemical composition (e.g. phytic acid and mineral contents), growing factors (e.g. types of fertilizers applied) and storage factors (e.g. temperature). Variations in cooking time within the same species exist, as in faba beans (*Vicia faba* L. var. *minor*), and have been related to several of the above-men-

tioned factors.

Youssef et al.(1982) have reported that the cooking times of several Canadian faba bean varieties were primarily related to characteristics of the cotyledon and not to seed coat properties. The phytic acid content of peas has been related to their cooking (Mattson,1946), and a similar relationship has been demonstrated in faba beans(Murray et al., 1982). Murray et al.(1982) further reported that the addition of phytase to soft faba beans during cooking made them uncookable. There has been an increased nutritional interest in reducing the phytic acid content in legumes, to make available nutrients such as proteins and minerals which are complexed with phytic acid in a form that is poorly utilized by the digestive system. It has been shown that the activation of endogenous phytase through soaking, germination or by heat treatment is effective in reducing the phytate content of legumes. Chang et al.(1977) noted that incubated samples of California small white beans in water saturated air at 60°C overnight reduced the phytate content by 33 percent. Eskin and Wiebe(1983) reported a phytate reduction of 71.2 percent and 77.3 percent for two faba bean cultivars, Ackerperle and Diana, respectively, over a 10-day germination period.

The objectives of this study were: 1) to follow changes in the levels of activity of endogenous phytase, and concurrent changes in phytic acid content, in faba beans exposed to different soaking times and temperatures, and to determine the effects of such changes, in both enzyme activity and substrate levels, on the cooking times of faba beans, and 2) to investigate the effect

of the same soaking times and temperatures on the water uptake in faba beans, and to determine the effect of such water uptake on the cooking time of faba beans.

LITERATURE REVIEW

The softening of legumes during normal cooking in boiling water has been shown to be accompanied by structural changes, primarily the softening and the breakdown of the middle lamella resulting in the separation of whole cells in the cotyledon tissue (Rockland and Jones, 1974; Sefah-Dedeh et al., 1978; Jones and Boulter, 1983). Rockland and Jones (1974) used the Scanning Electron Microscope (SEM) to observe the structure of the cotyledon tissue of two samples of cooked lima beans; one sample was a fast-cooking lima bean that had been soaked in a solution of inorganic sodium salts and cooked completely in 10 minutes, and the other sample was a slow-cooking lima bean soaked in distilled water that cooked in 45 minutes. They observed the tissue structure of the fast and slow-cooking samples after various cooking times and as a result attributed the difference in the cooking times of the two samples to the differential rates at which cell separation occurred. In the literature reviewed several studies of the factors that may contribute to the cooking time of legumes have been based on the effect those factors may have on cell separation during the cooking process.

Effect of Storage Conditions on the Cooking Time of Legumes

The adverse effects of storage factors (temperature, relative humidity (RH) and time) on the cooking time of some legumes (pinto beans, sanilac beans, lima beans and cowpeas) have been intensively studied by Burr et al., (1969); Sefah-Dedeh et al., (1979), and Jackson and Varriano-Marston (1981), with similar results. In general the cooking time of the legumes increased with storage

time.

Storage under high R.H. conditions that resulted in moisture contents greater than 13 percent in the legumes greatly influenced the cooking time. Burr et al. (1969) and Sefah-Dedeh et al. (1979) reported that legumes stored under those conditions required a much longer cooking time than those samples that were held at lower R.H. conditions or had moisture contents less than 13 percent.

Increasing storage temperature also resulted in longer cooking times. However the combined effect of high temperature (29°C) and high R.H. (85 %) caused an even greater increase in the cooking time as shown by Sefah-Dedeh et al. (1979). Using the SEM, Sefah-Dedeh et al. (1979) observed the microstructure of cowpeas (previously stored at 29°C and 85 % R.H. for 12 months) that had been heated for 90 minutes at 90°C, and detected only a minimal breakdown of the middle lamella. They suggested that during storage under high temperature and high R.H. conditions changes occur in the middle lamella making thermal degradation difficult. It is possible that under those storage conditions minimal enzymatic activity exists.

Ching and Schoolcraft (1968) observed increased amounts of free amino acids leached out of crimson clover and ryegrass seeds with increasing moisture contents and storage temperatures. These findings, they claimed, indicated that proteases were active in stored seeds and activity was related to hydration and temperature. According to Jones and Boulter (1983) a high moisture content during the storage of black beans permits restrictive metab-

olism which leads to the breakdown of their cell membranes. As a result of this, cell constituents (that affect the cooking time of legumes) are released and allowed access to the middle lamella eventually retarding thermal degradation during cooking. The effect of some cell constituents (particularly divalent ions) and their interaction with components of the middle lamella will be discussed in further detail.

Water Absorption in Legumes and its Effect on Cooking Time

The cooking process for legumes involves at least two steps: water absorption to an equilibrium condition with free water, followed by softening of the texture by heat (Jackson and Varriano-Marston 1981). The soaking of legumes for several hours (12-16) prior to cooking is a common practice among domestic users and large-scale processors because it generally reduces the cooking time. Soaking time may be further reduced to 4-5 hours by increasing the soaking temperature of the soaking water (Kon, 1979 and Hsu et al., 1983). Reduced cooking time with soaking may be a result of increasing internal pressure exerted in the cotyledons which aids in cell separation during cooking.

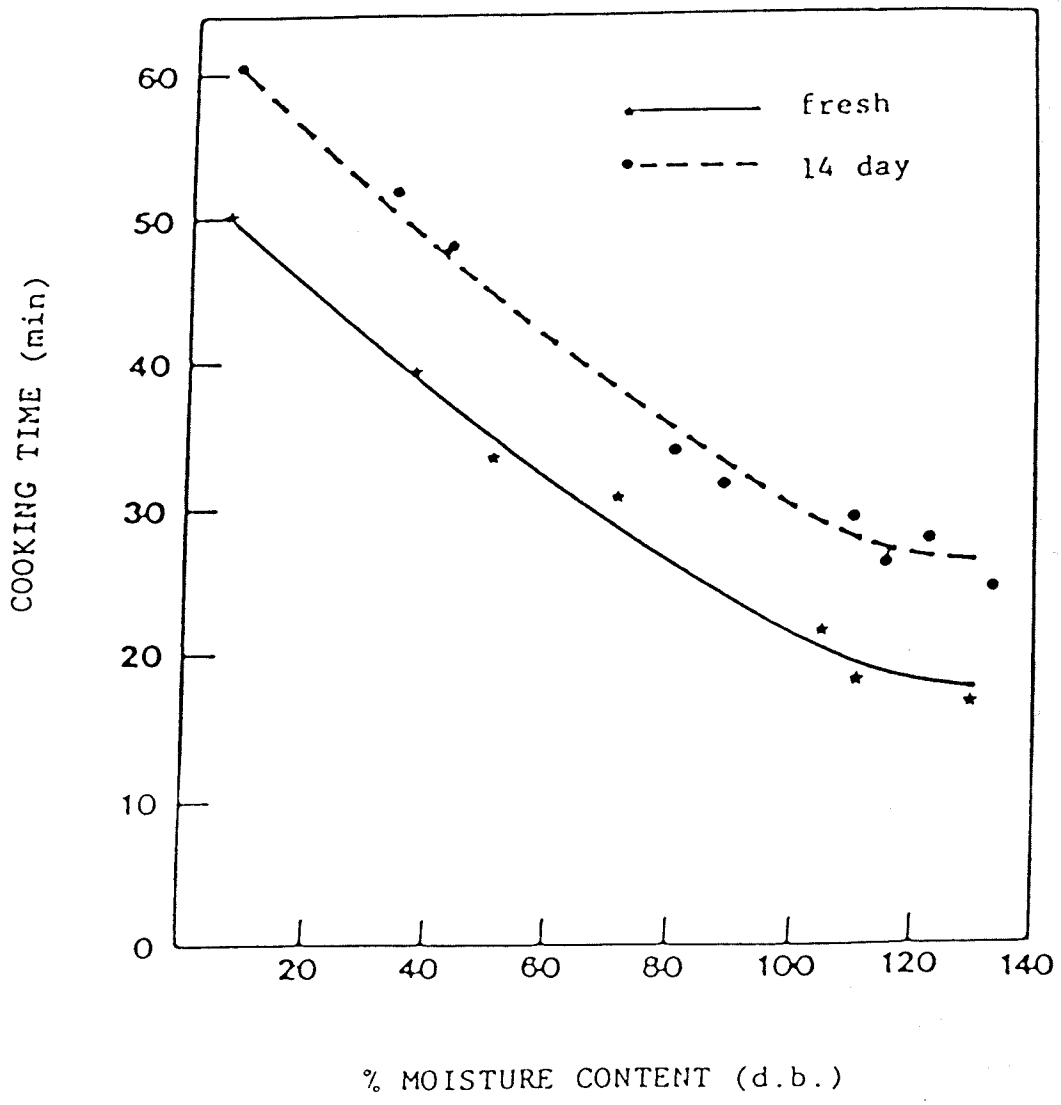
The longer cooking time required by some legumes has been related to the development of "hard-shell", a term used by Bourne (1967) to describe legumes that did not soften during cooking; this was defined as a condition in which seeds failed to imbibe water within a reasonable time when soaked. According to Jackson and Varriano-Marston (1981), two types of hard-shell exist: 1) hard-shell related to seedcoat impermeability, and 2) hard-shell related to cotyledon impermeability. An objective of

their study in 1981 was to determine the roles of seedcoat and cotyledon in water absorption and cooking time of fresh and aged black beans (aged black beans were obtained by storage under accelerated ageing conditions 41°C and 75-100 R.H. for 7,14 and 55 days to parallel the development of the hard-to-cook effect of the long term storage of black beans). Decorticated samples of fresh and aged beans showed significantly reduced cooking times over the intact samples indicating that the seedcoat was the major barrier to bean softening during cooking. Other data from the same study suggested that the seedcoat contribution to cooking time exceeded that of the cotyledon in the fresh samples, but that the cotyledons contribution to the cooking time increased with storage time. This further implied changes occurring in the seedcoat and cotyledon during storage.

Youssef et al. (1982) suggested that one type of hard-shell may have a more marked effect on cooking time than the other. In studying the cookability of Canadian and Egyptian faba beans, Youssef et al. (1981) noted that the cooking time of Egyptian samples was primarily related to the physical properties of the seedcoat (e.g. thickness); during cooking only soft-cooking Egyptian samples showed evidence of seedcoat cracking. On the other hand the cooking time of Canadian samples was related to cotyledon properties (e.g. starch granules); the seedcoat of both soft and hard-cooking Canadian samples cracked during cooking. Although hard-shell effects due to seedcoat properties may be alleviated by dehulling or scarifying it appears that hard-shell effects due to cotyledon properties cannot be easily removed thus

posing a greater problem than the former. Several authors (Burr et al., 1968; Sefah-Dedeh et al., 1979 Jackson and Varriano-Marston, 1981) have shown that the ability to absorb water during soaking is not impaired in legumes that exhibit the hard-to-cook effect. Burr et al. (1968) and Sefah-Dedeh et al. (1979) reported that pinto beans and cowpeas stored under high humidity conditions (high legume moisture content) actually had a higher rate of water absorption at shorter soaking times than those stored under low humidity conditions (low legume moisture content). Sefah-Dedeh et al. (1979) attributed this to the seedcoat achieving equilibration with the high humidity environment and becoming less of a barrier to water penetration during soaking, whereas the reverse was applicable in the latter situation. Results of water absorption studies of black beans by Jones and Boulter (1983) are in disagreement, since they noted that the imbibition value (wet weight/dry weight) of hard beans (1.88) was lower than that of the soft beans (2.17). Parrish and Leopold (1978) had explained that the water absorption data on seeds could be easily misinterpreted if authors recorded water absorption as a percentage of fresh weight without correcting for solids lost during soaking. This could account for the discrepancies that exist for water absorption data of fast and slow-cooking legumes. Although increased moisture content after soaking resulted in shorter cooking times the inherent differences in cooking time between fast and slow-cooking legumes still remained (Fig. 1) (Jackson and Varriano-Marston, 1981). These results suggested that the major differences in cooking time of legumes are not directly related

Figure 1. Cooking times of fresh and 14-day aged black beans as affected by moisture contents. (Jackson and Varriano-Marston, 1981).


fresh
14 day
COOKING TIME (min)
0
10
20
30
40
50
60
20
40
60
80
100
120
140
% MOISTURE CONTENT (d.b.)

to their water absorption properties.

Effect of Solute Leakage From Legumes During Soaking on Cooking Time

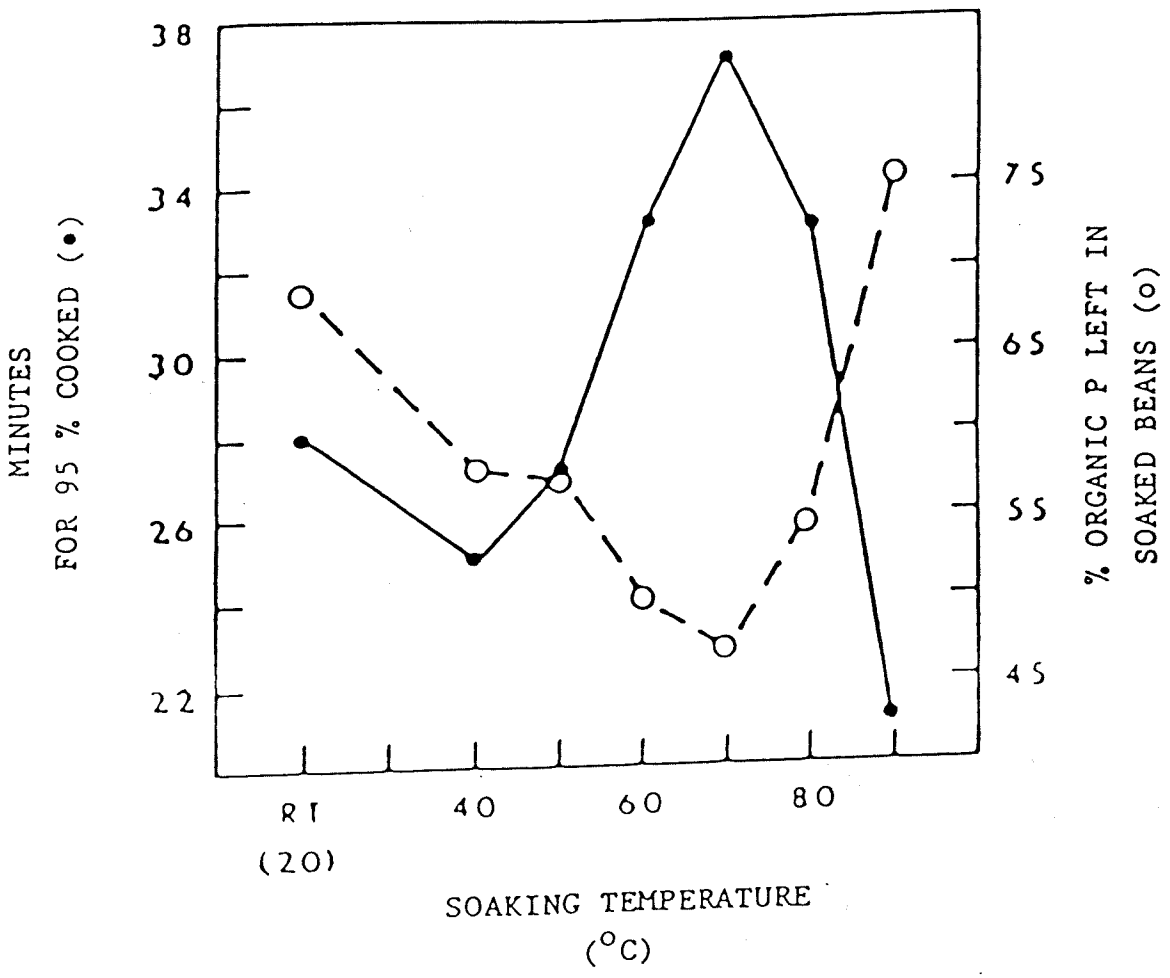
Ching and Schoolcraft(1968) and Parrish and Leopold(1978) reported that an increase in electrolyte leakage from seeds during soaking was an indication of weak, deteriorated and unviable seeds. Increased leakage was a result of the degradation of cellular membranes and a subsequent loss of control of permeability and it increased with the age of the seed. Ching and Schoolcraft cited seed moisture content as a major factor in membrane degradation, since the higher the moisture content the greater the extent of membrane degradation.

Some researchers(Jackson and Varriano-Marston,1981;Jones and Boulter,1983) have related the extent of solute loss from legumes during soaking to their cooking time. A high solute leakage(195 mg g^{-1}) was noted for slow-cooking black beans whilst fast-cooking samples had a much lower value(21 mg g^{-1}) (Jones and Boulter). Earlier,Kon(1979) had correlated the cooking time of California white beans to the amount of organic phosphorus left after soaking(Fig 2); beans that had been soaked at 70°C had the least amount of organic phosphorus and required the longest cooking time. The increased loss of organic phosphorus was not attributed solely to increased membrane degradation but to increasing enzymic(phytase, phosphatase) activity as well.

Effect of Some Chemical Constituents on Cooking Time of Legumes

Some cell constituents including organic phosphorus, phytic

Figure 2. Correlation of cooking time with organic P content of beans soaked at different temperatures (Kon, 1979).


38
34
30
26
22
MINUTES
FOR 95 % COOKED (•)
75
65
55
45
% ORGANIC P LEFT IN
SOAKED BEANS (o)
RT
(20)
40
60
80
SOAKING TEMPERATURE
(°C)

acid, pectins, calcium and magnesium ions have been implicated as factors that affect the cooking time of legumes. Of these, phytic acid has been emphasized as one of the predominant factors affecting the cooking time.

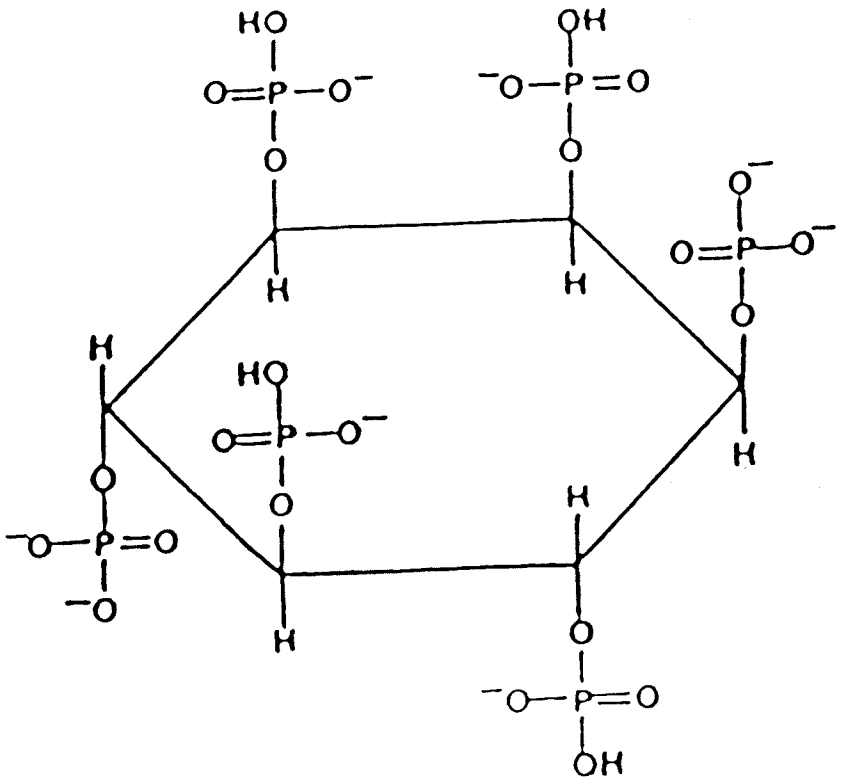
Phytic acid

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) (Fig. 3) and its salts are widely distributed in plant seeds or grains and are considered to be the major storage form of cations and phosphorus, which are utilized during plant germination and growth (Reddy et al., 1982). Reported phytic acid contents are as high as 5.18 % in defatted sesame seed meal and range from 0.28 % to 2.06 % in legumes.

Mattson (1946) carried out an intensive study on the cooking quality of yellow peas and was the first to report a significant relationship between the phytic acid content of the peas and their cooking times. Peas with higher phytic acid content took a shorter time to cook than those samples with lower phytic acid which had a longer cooking time. Since then, other researchers have reported similar findings. Kumar et al. (1978) reported that green gram peas that required a cooking time of 13.6 minutes contained 185 mg phytin phosphorus/100 g, while chickpeas that required a cooking time of 79 minutes contained 80 mg phytin phosphorus/100 g. Similarly Murray et al. (1982) observed that the differences in cooking time for two faba bean cultivars could be related to their phytic acid content.

Mattson (1946) described the role of phytic acid as a divalent ion precipitant with particular reference to calcium and

Figure 3. Possible structure of phytic acid at neutral pH based on the Anderson model (Cheryan, 1980).



magnesium ions. An understanding of the role of phytic acid in this respect requires a brief mention of pectin substances located in the cell wall and middle lamella. The basic structure of pectins consists of long chains of polygalacturonic acid in which the carboxylic groups are partially esterified. The carboxylic groups are able to react with calcium and magnesium ions to form water-insoluble pectins located in the middle lamella (Eskin et al., 1971). However pectin is very soluble when saturated with monovalent cations, such as sodium and potassium ions. During cooking, Mattson (1946) explained that sodium or potassium phytate in the cotyledon reacts with insoluble calcium or magnesium pectate converting the insoluble pectate to soluble sodium or potassium pectate. This reaction renders the middle lamella more soluble during cooking and enables adjacent cells to separate easily.

More recently the role of phytic acid in the cooking time of legumes has been considered from its ability to interact with proteins (Murray et al., 1982). Mechanical stress caused by the swelling and gelatinization of starch granules during cooking may contribute to cell separation (Jackson and Varriano-Marston, 1981), however Olkuu and Rha (1978) have shown that protein is able to interact with starch, retarding gelatinization. Studies of phytic acid interactions in food systems clearly indicate the formation of phytic acid-protein complexes (Cheryan, 1980); based on the results of amylograph viscosity studies with added phytic acid and phytase, Murray et al. (1982) suggested that the formation of a phytic acid-protein complex would reduce the probability of starch and protein interactions during cooking, facilitat-

ing more rapid starch gelatinization. The formation of a phytic acid-protein complex would not necessarily reduce the effect of the reaction between phytic acid and divalent ions as described by Mattson(1946), since it has been shown that a ternary complex involving phytic acid, protein and divalent cations can exist(Cheryan, 1980; Martens, 1981). Thus phytic acid appears to serve two roles at the same time: 1) reducing divalent ion availabilty to pectin, and 2) reducing protein availabilty for interaction with starch, to facilitate the separation of cotyledon cells during cooking as illustrated in Figure 4.

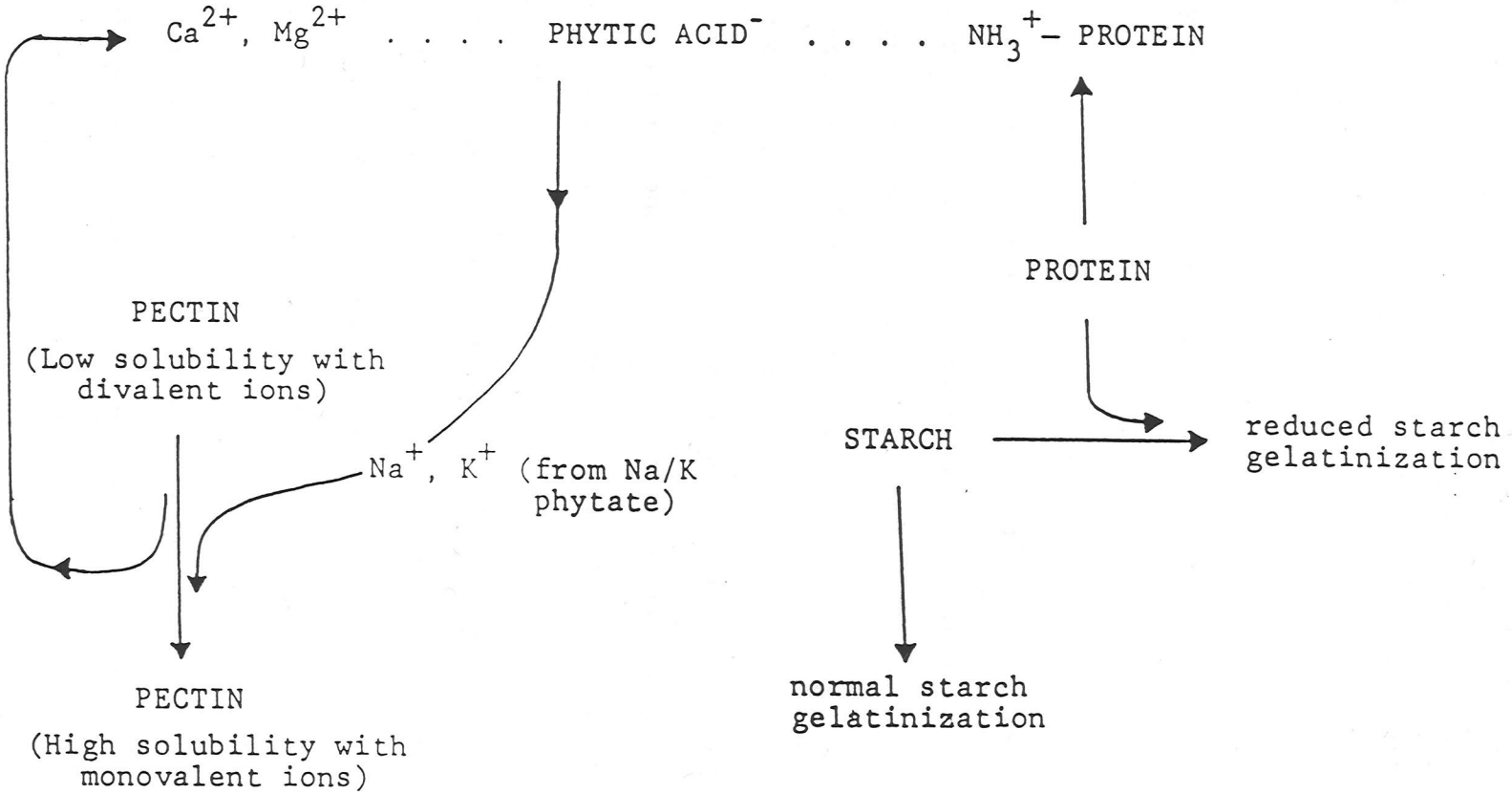
Divalent ions and pectin

Increased calcium and magnesium levels have been associated with longer cooking times in legumes(Mattson, 1946; Quenzer et al.,1978), due to the formation of insoluble pectate with those ions. Muller(1967) adopted a formula expressed as the "PCMP" number relating the contents of phytin, calcium, magnesium and free pectin present in the cell wall:

$$\frac{\text{free pectin} \times (\text{calcium} + \tfrac{1}{2}\ \text{magnesium})}{\text{phytin}}$$

These results indicated a positive relation between hardness after cooking for standard times and the "PCMP" number for almost all 13 varieties used in the study. Although Kumar et al.(1978) had found a correlation between phytic acid content and the cooking time of ungerminated legumes, phytic acid content could not account for the cooking pattern in germinated legumes. They however found the concept of the "PCMP" number more applicable to

Figure 4. Schematic Presentation of the Possible Roles of Phytic Acid During the Cooking of Legumes.


Ca^{2+}, Mg^{2+} . . . . PHYTIC ACID$^-$ . . . . NH_3^+– PROTEIN
PROTEIN
PECTIN
(Low solubility with divalent ions)
Na^+, K^+ (from Na/K phytate)
STARCH
reduced starch gelatinization
PECTIN
(High solubility with monovalent ions)
normal starch gelatinization

both ungerminated and germinated legumes.

Phytase Activity and its Effect on Cooking Time of Legumes

Phytase activity, distribution and characteristics

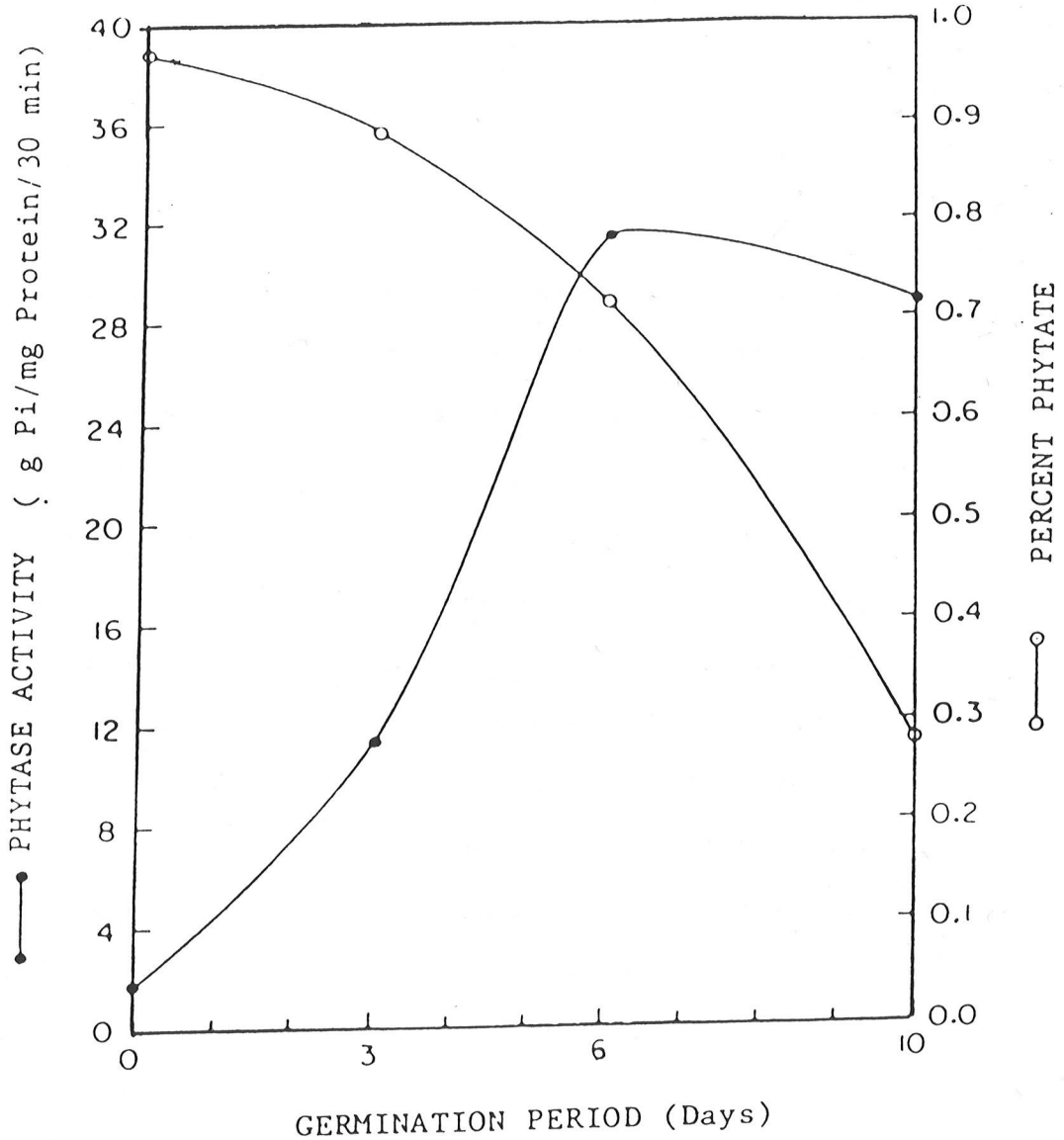
The hydrolysis of phytic acid is catalyzed by the enzyme phytase (myo-inositol hexaphosphate phosphohydrolase), classified by the Enzyme Commission of the International Union of Biochemistry as EC 3.1.3.8. (Dixon and Webb, 1979). Hydrolysis of phytic acid occurs via inositol pentaphosphate to monophosphate as intermediary products, the final products being inositol and inorganic phosphate. The presence of the enzyme was first demonstrated in 1906, and an impure preparation was made in 1907 by Suzuki et al. from an extract of rice bran (cited from Sloane-Stanley, 1961). Since then the enzyme has been widely reported in higher plants, molds, bacteria and fungi and to a lesser extent in animals and humans. Within the last two decades there has been an increase in attempts to demonstrate, purify and characterize the enzyme, particularly in cereals and legumes. This interest is a result of the growing nutritional implications surrounding phytic acid and its ability to react with proteins, vitamins and essential minerals such as zinc, calcium and magnesium. Comparative studies on the distribution of enzyme in wheat and triticale indicated that the bran had a higher phytase activity than flour or whole grain (Singh and Sedeh, 1979), but no information on phytase distribution was presented for legumes.

Dwarf beans (Gibbins and Norris, 1963); mung beans (Mandal and Biswas, 1972); California small white beans (Chang and Schwimmer,

1976); navy beans (Lolas and Markakis, 1979) and faba beans (Eskin and Wiebe, 1983) are some of the legumes that have been observed for phytase activity. The enzyme activity is normally absent, or present in very low levels, in the dry (ungerminated) legumes, the activity increasing after several hours of soaking and on germination (Mandal and Biswas, 1972; Eskin and Wiebe, 1983). Concurrently with increasing phytase activity during a germination period of ten days is a decrease in phytic acid content (Fig.5) Eskin and Wiebe, 1983. According to Mandal and Biswas (1972) there may be three possibilities regarding the appearance of phytase activity in mung bean cotyledon: 1) de novo synthesis, 2) activation of enzyme protein already present in the seeds, or 3) the presence of inhibitors, that could interfere with the activity of phytase, normally present at the earlier stages of germination. Based on experimental results they were able to rule out the latter two possibilities. Experiments with cycloheximide, a potent inhibitor of protein synthesis, suggested that the increase of phytase activity in the cotyledon with an increased period of soaking is due to de novo synthesis. Bianchetti and Sartirana (1967) showed that the synthesis of phytase in wheat embryos was inhibited by inorganic phosphate. A similar effect of inorganic phosphate content on phytase synthesis was demonstrated in a strain of Aspergillus ficuum (Shieh and Ware, 1969). It is possible that as a result of a decrease in the level of inorganic phosphate during soaking, due to leaching and metabolic effects, the inhibitory effect on phytase synthesis is removed.

Chang et al. (1977) reported that phytase activity was also

Figure 5. Changes in phytase activity and percent phytate during germination of Vicia faba L. minor var. Ackerperle (Eskin and Wiebe, 1983).


PHYTASE ACTIVITY (g Pi/mg Protein/30 min)
PERCENT PHYTATE
GERMINATION PERIOD (Days)
0
4
8
12
16
20
24
28
32
36
40
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
3
6
10

initiated under heat treatment(60°C), however they found no evidence to support the de novo synthesis hypothesis and suggested that the initiation of an existing enzyme was a more likely mechanism. In view of conflicting evidence, it is not suprising that a recent review(Oberleas, 1983) states that there is still controversy surrounding the cause of increasing phytase activity during soaking and germination.

Phytase activity is inhibited by high concentrations of phytic acid as well as inorganic phosphate. For several plant phytases, inhibition occurs at substrate concentrations higher than 1.0 mM (Gibbins and Norris,1963; Lolas and Markakis,1977; Chang and Schwimmer, 1977). Inhibition by inorganic phosphate has been shown to be competitive. Chang and Schwimmer(1977) reported 30 % and 50 % inhibition by 0.31 mM and 0.64 mM inorganic phosphate respectively for phytase from California small white beans. Reinhold(1975) further suggested that, in addition to high concentrations of phytate, other factors responsible for slow phytate hydrolysis are: 1) the presence of inhibitors such as calcium and other metal ions, which form stable salts resistant to phytase attack, and 2) the existence of phytate in combination with certain proteins in the form of complexes which may not be vulnerable to phytase attack.

Effect on cooking time

Mattson(1946) observed that if samples of yellow peas were soaked in water for eight days they became entirely uncookable, and if the peas were boiled for 10 minutes after swelling in water for about four hours, further soaking for eight days left

the peas as cookable as in their original condition. On the assumption that the effect of soaking in the former observation was due to the decomposition of phytate by phytase, Mattson added a phytase extract to peas that were cookable, rendering them uncookable. Based on these results, he confirmed that the action of phytase on phytate left the peas uncookable. Murray et al. (1982) later demonstrated a similar effect of phytase on the cooking time of faba beans. When phytase was added to fast-cooking faba beans they became uncookable, whereas the addition of phytic acid to the cooking water prior to cooking increased faba bean softness significantly.

Minimization of the Hard-to-cook Effect and Cooking Time of Legumes

The methods developed by some researchers to minimize the hard-to-cook effect and to reduce the cooking time of legumes all take into account the factors discussed. Based on the results of the effect of storage conditions on the cooking time of legumes, the use of a low storage temperature (4°C) or the practice of storing legumes with a low moisture content (around 8-10 %) in a relatively low humidity environment has been suggested (Burr et al., 1968 and Kon, 1968). Several other methods involve soaking dry legumes in dilute solutions (0.1-1 % concentration) of inorganic sodium salts such as sodium bicarbonate, sodium carbonate and trisodium phosphate. Al-Nouri and Siddiqui (1982) formulated a sodium salt solution that reduced the cooking time of dry broad beans from 110 minutes to 15 minutes. A similar soaking medium had been used by Rockland and Jones (1974) that reduced the cook-

ing time of lima beans from 45 minutes to 10 minutes. The carbonate and phosphate ions precipitate divalent calcium and magnesium ions present in the legumes, and the sodium cations directly displace some of the divalent cations combined with pectic substances (Mattson, 1946). The actual composition and concentration of the soaking medium depends directly on the type of bean and the conditions of processing it will be subjected to later. Al-Nouri and Siddiqui (1982) noted that treatment with 0.25 % sodium carbonate gave the broad bean samples a dark colour and a soapy taste, and treatment with 1.0 % trisodium phosphate resulted in dark beans with a metallic taste. The salt solution that gave a good acceptable colour and flavour contained 0.2 % sodium bicarbonate and 0.1 % trisodium phosphate.

Molina et al. (1976) found that short-heat treatments were effective in controlling the development of the hard-to-cook effect during the storage of black beans. Two minutes in the retort (15 psig, 121°C), and 10 minutes under steam (98°C, without pressure), were the most effective heat treatments. These treatments may have destroyed enzymes present in the legumes during storage that initiate metabolic reactions subsequently affecting the cooking time.

MATERIALS AND METHODS

MATERIALS

Whole faba beans (*Vicia faba* L.var.*minor*), of the Aladin cultivar (1981 crop) were obtained from the Plant Science Department, University of Manitoba, and stored at room temperature (approx. 20°C). Pin-milled faba bean flour of the Ackerperle cultivar (1982 crop) was obtained from the Glenlea Research Station, University of Manitoba, and stored in the coldroom (-20°C). The sodium salt of phytic acid was obtained from Sigma Chemical Company, St. Louis, Missouri, and was of 97 % purity. All other chemicals and reagents used in this study were of analytical grade.

METHODS

Sampling Procedure for Phytase, Phytic Acid and Cookability Index Determinations in Soaked Faba Beans

Whole faba beans (150 g) were soaked in 600 ml of distilled water at 20, 35, 50 and 65°C. Samples (40 g) of the soaked beans were removed after 8, 20 and 24 hours for phytase and phytic acid determinations, and 25 g samples were also removed for cookability index determination. The soaking system was set up in duplicate.

Faba Bean Flour Preparation

Dry faba beans were milled directly whereas samples that had been soaked were freeze-dried prior to milling. Samples were milled in a Chemical Rubber Company (C.R.C.) water-cooled micro-

mill and sieved through 100 mesh to obtain a fine flour. All faba bean flours were stored in the coldroom (2-4°C) in sealed containers.

Phytase Extraction

The method followed was that of Lolas and Markakis (1977). Faba bean flour (5 g) was extracted with 50 ml of 2 % calcium chloride with mechanical shaking, using a Burell wrist-action shaker, for 30 minutes at room temperature. The mixture was centrifuged at 18,800 xg for 30 minutes at 0-5°C. The supernatant was treated with ammonium sulphate to give 30 % saturation, allowed to stand in the coldroom for 30 minutes and centrifuged at 18,800 xg for 20 minutes at 0-5°C. The pellet obtained (fraction 1) was discarded and ammonium sulphate added to the supernatant to give 70 % saturation, then treated as the 30 % saturated sample. The pellet obtained between 30-70 % saturation (fraction 2) was dissolved in 10 ml of 0.01 M Tris-maleate buffer pH 6.5 (50 ml of 0.2 M Tris-maleate +42.5 ml of 0.2 M NaOH diluted up to 200 ml with distilled water; 50 ml of this was diluted to 1L) and dialyzed against the same buffer for approx. 16 hours in the coldroom. The dialysis membrane used had a molecular weight cut-off value of 6,000-8,000. The enzyme extract obtained was stored in glass vials in the coldroom.

Protein Estimation

Protein was determined by the method of Lowry et al. (1951), using crystalline bovine serum albumin as the standard.

Phytase Assay

The method followed was that of Lolas and Markakis (1977). The reaction mixture contained 0.15 ml of 2 mM sodium phytate adjusted to pH 5.3 (8.8 ml of 0.6 M acetic acid + 41.2 ml of 0.6 M sodium acetate diluted to 100 ml with distilled water); 0.65 ml of distilled water and 0.2 ml of enzyme extract. The final phytate concentration in the reaction mixture was 0.25 mM and was incubated at 50±DC for 30 minutes. The reaction was terminated by withdrawing 0.5 ml of reaction mixture and transferring it to 1.2 ml of 1 M trichloroacetic acid in small glass test-tubes, which mixture was allowed to stand for 30 minutes at room temperature before centrifuging for 15 minutes at 12,100 xg at 0-5°C. Enzyme extract boiled for 30 minutes was used as a control.

Determination of Inorganic Phosphorus

The method followed was that of Watanabe and Olsen (1965). Two reagents were required for the determination of inorganic phosphorus and were prepared as follows:

Reagent A: 12 g of ammonium molybdate were dissolved in 250 ml of distilled water, and 0.2908 g of antimony potassium tartrate in 100 ml of distilled water. Both solutions were added to 1 L of 5 N sulphuric acid, thoroughly mixed and diluted to 2 L with distilled water. The reagent was stored in a glass bottle and kept in a cool dark place.

Reagent B: This was prepared daily as required by dissolving 1.056 g of L-ascorbic acid in 200.0 ml of reagent A.

Standard solutions containing 0.5-20μg phosphorus per ml were prepared from a stock solution of 20μg phosphorus per ml

(0.0878 g of potassium dihydrogen phosphate in 1 L of distilled water). A distilled water blank (1 ml), standards and samples were pipetted into large test-tubes and diluted with 19 ml of distilled water. Reagent B (4 ml) was added and mixed thoroughly. The maximum colour (blue) intensity was obtained in 10 minutes. Absorbance was read at 882 nm on a Bausch and Lomb Spectronic 710 Spectrophotometer.

Specific Enzyme Activity

This was expressed as µg inorganic phosphorus released per mg protein in 30 minutes (µg inorganic P/ mg protein/ 30 min.) under the stated conditions for phytase assay.

Phytic Acid Determination

The method followed was that of Latta and Eskin(1980).

A. Sample extraction

Faba bean flour(2.5g) were extracted with 50 ml of 2.4 % hydrochloric acid (54 ml conc. HCl/L) for 1 hour at room temperature (approx. 20°C). The mixture was centrifuged at 18,800 xg for 10 minutes at 0-5°C and the supernatant stored in glass vials in the coldroom.

B. Sample chromatography

A glass column approximately 0.7 cm x 27 cm containing a piece of glass wool at the bottom and 0.5 g of 200-400 mesh AG1 - X8 chloride anion exchange resin was prepared with 15 ml of 5 % hydrochloric acid(112.5 ml conc.HCl/L) and rinsed with 20 ml of deionized distilled water. The sample extract(A) (3 ml) was

diluted to 25 ml with deionized distilled water in a 25 ml volumetric flask and 10 ml of the diluted sample was pipetted onto the column. After the sample was eluted, 15 ml of 0.1 M sodium chloride were added to the column. The eluant collected was discarded and a 25 ml volumetric flask placed under the column. Sodium chloride (15 ml of 0.7 M) was added to the column and the eluant collected. The eluant was diluted to 25 ml with deionized distilled water and poured into large test-tubes.

C. Colour reaction using the Wade reagent

The Wade reagent was prepared by dissolving 0.15 g of hydrated ferric chloride and 1.5 g of sulphosalicylic acid in distilled water and diluting to 500 ml. Phytic acid standards were prepared from a 200 µg phytic acid/ml stock solution (33.9 mg sodium phytate/100 ml) to contain 10-40 µg phytic acid/ml. The standards were stored in the cold room and brought to room temperature prior to use.

A blank of deionized distilled water (3 ml), standards and samples (B) were pipetted into 15 ml tapered centrifuge tubes. Exactly 1 ml of Wade reagent was added and thoroughly mixed on a Vortex mixer. The mixture was centrifuged for 10 minutes at 2,200 xg at room temperature (approx. 20°C). The absorbance was read at 500 nm after zeroing with deionized distilled water.

Calculations

Absorbance readings for the standards and samples were subtracted from the absorbance reading for the blank to give a final absorbance reading. A standard curve was con-

structed (concentration of standard vs. final absorbance reading) and from this the concentrations of phytic acid in the samples was determined. The value obtained was divided by 24 to account for all dilutions in the procedure and to give a result expressed as percent phytic acid values in the original sample. Final percent phytic acid values were reported on a dry matter basis.

Determination of Phytase Activity and Phytic AcidContent in a Faba Bean Slurry

A slurry of the pin-milled flour was prepared by mixing 250 ml of distilled water and 25 g of the flour, and this was followed by continuous stirring for 24 hours at room temperature. After 8, 20 and 24 hours, 54 ml (containing 5 g flour) and 21.6 ml (containing 2 g flour) aliquots of the slurry were removed for phytase and phytic acid determinations ,respectively. Calcium chloride (1 g) was added to the 54 ml aliquot to give a final concentration of 2 % calcium chloride, after which the mixture was shaken for 40 minutes at room temperature using a Burrell wrist-action shaker. The mixture was further treated as outlined under the section "Phytase extraction". Hydrochloric acid (20 ml of 4.8 % HCL) was added to the 21.6 ml aliquot to give a final concentration of 2.4 % HCL, and the mixture was stirred for 1 hour at room temperature. The mixture was further treated as outlined under the section "Phytic acid determination".

Moisture Determination

Moisture in the faba bean flour samples was determined by the air-oven method (14.004, Official Methods of Analysis of the

Association of Analytical Chemists, 1975).

Water Absorption in Faba Beans During Soaking

Water absorption patterns of faba beans were determined after soaking for 8, 20 and 24 hours at 20, 35, 50 and 65°C. The soaked beans were drained for about 90 seconds, blotted dry and weighed. Water absorption was expressed as the percentage increase in the weight of the beans during soaking.

Determination of Cookability Index

Sample preparation

It was necessary to use different cooking times for unsoaked faba beans, and samples that had been soaked for different times, in order to obtain a texture that could be measured with the Ottawa Texture Measuring System (O.T.M.S.) without exceeding the limits of the instrument. Unsoaked faba beans were cooked for 90 minutes, samples soaked for eight hours were cooked for 20 minutes and samples that had been soaked for 20 and 24 hours were cooked for 10 minutes. Unsoaked faba beans (25 g) were cooked in 500 ml of distilled water, and soaked faba beans were cooked in 200 ml of distilled water. After cooking, all samples were drained of excess water and left to cool to room temperature, after which their cookability index was determined.

Procedure

The cookability index was measured on the O.T.M.S. using a Kramer Shear Cell. The Kramer Shear Cell equipment is shown in Fig. 6 and was attached to the O.T.M.S. as shown in Fig. 7.

Figure 6. Kramer Shear Press cell equipment (A. shear-compression cell; B. lower adaptor for Kramer Shear Press cell; C. moving blades).

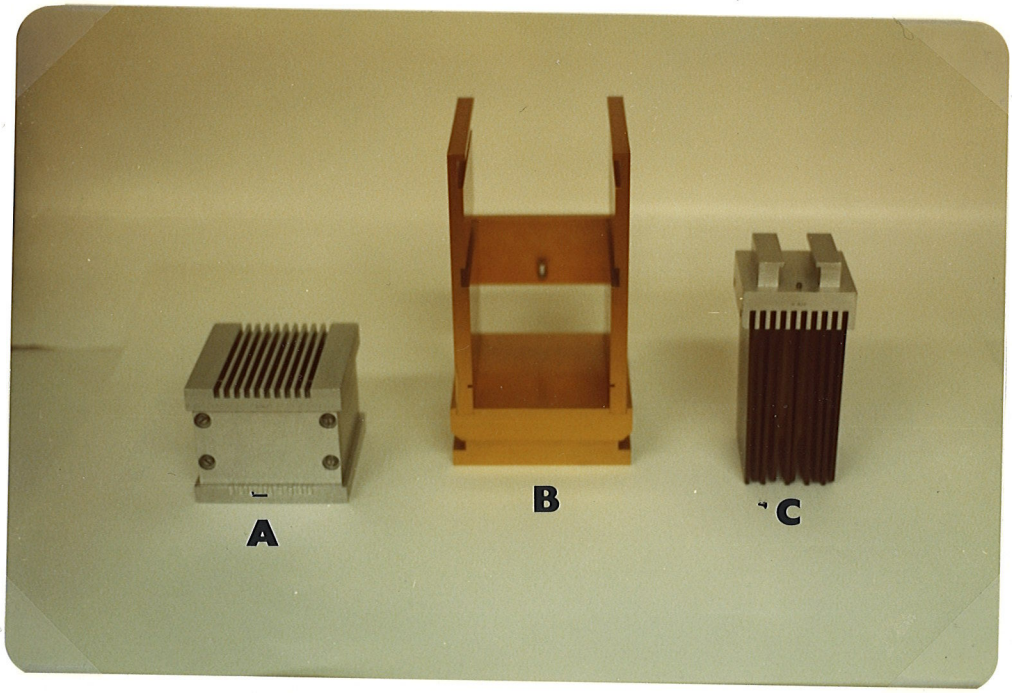
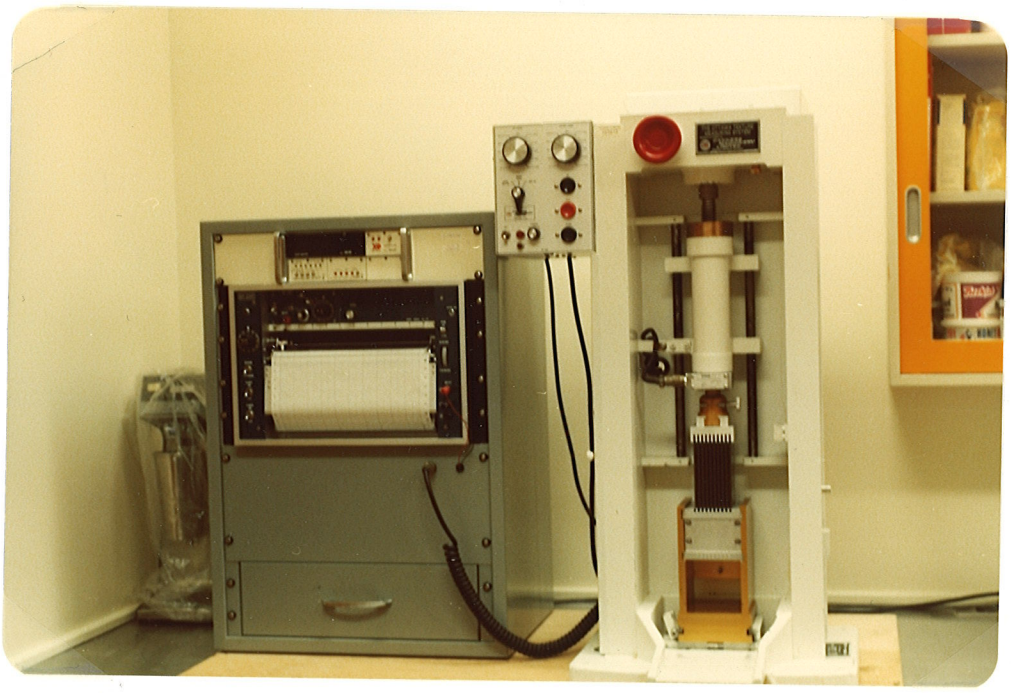

A
B
C

Figure 7. Typical installation in the Ottawa Texture Measuring System.



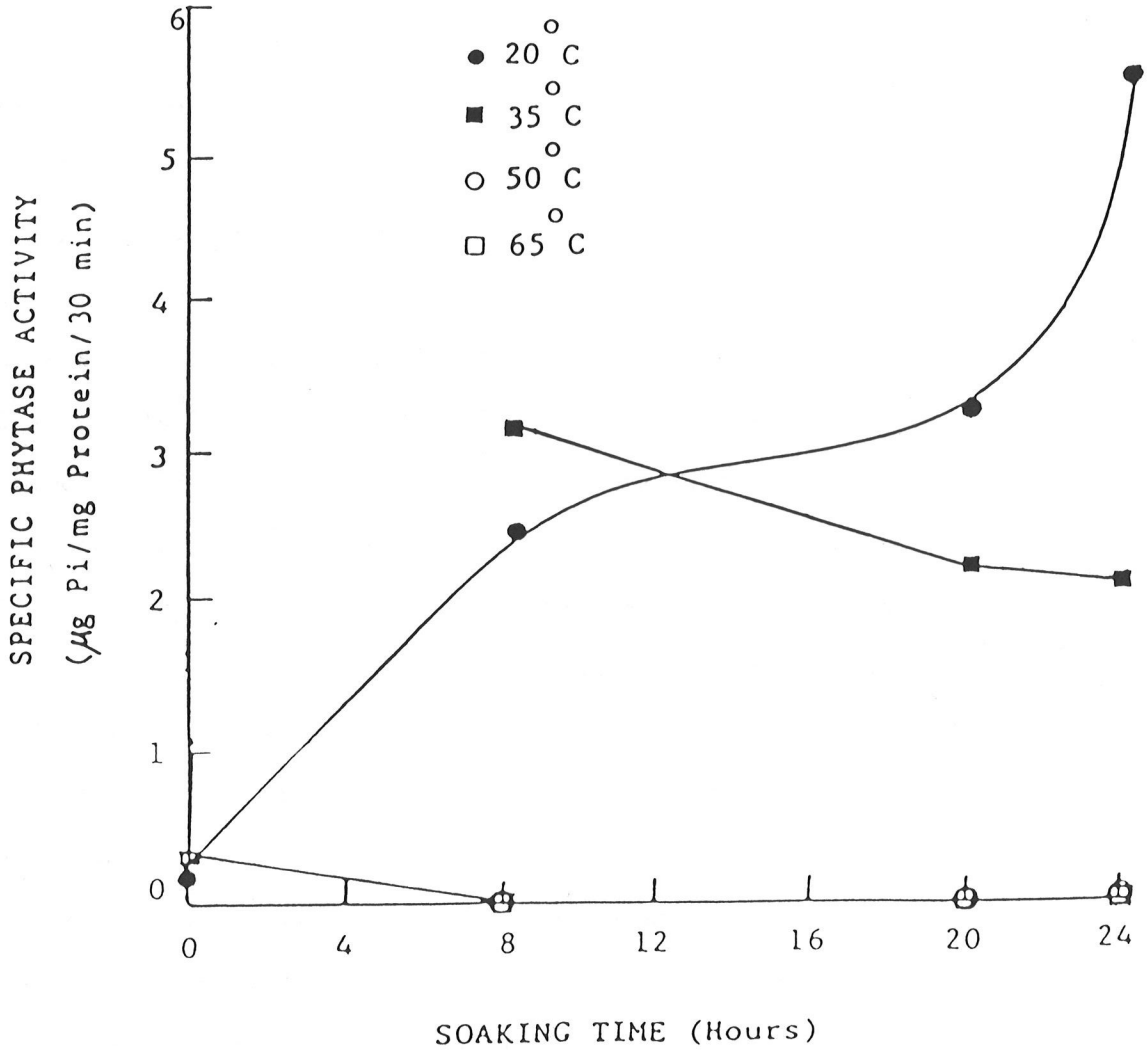
RESULTS AND DISCUSSION

Phytase Activity in Dry Faba Bean and the Effect on the Activity With Soaking Time and Temperature

A low level of phytase activity (0.244 µg P/mg Protein/30 min.) was present in the dry (unsoaked) Aladin cultivar at the beginning of the study, and after about two months the activity was (0.280 µg P/mg Protein/30 min.), which indicated that there was no significant change in the level of enzyme activity in the dry faba beans during the two-month storage period. Phytase activities in two other faba bean cultivars, measured under similar experimental conditions, have been reported to be 1.8 and 1.0 µgP/mg Protein/ 30 min. for the Ackerperle and Diana cultivars respectively (Eskin and Wiebe, 1983), an indication of the variability in phytase levels that exists among faba bean cultivars. Phytase activity, although low, has been detected in dry California small white beans (Chang and Schwimmer, 1977) and navy beans (Lolas and Markarsis, 1977). Mandal and Biswas (1970) however reported that they were unable to detect phytase activity in dry mung beans.

The effect of soaking time and temperature on phytase activity was determined in samples of the Aladin cultivar soaked at room temperature (20°C), and at 35, 50 and 65°C after 8, 20 and 24 hours, and the results are shown in Fig. 8. Phytase activity during soaking at 20°C showed an increase of 10, 13 and 23 fold after 8, 20 and 24 hours ,respectively, over the activity found in the dry seed. An increase in phytase activity was observed in samples soaked at 35°C after 8 hours compared to samples soaked

Figure 8. Effects of Soaking Time and Temperature on Phytase Activity in Vicia faba L. minor (cv. Aladin).


SPECIFIC PHYTASE ACTIVITY
(µg Pi/mg Procein/30 min)
20 °C
35 °C
50 °C
65 °C
0
1
2
3
4
5
6
0
4
8
12
16
20
24
SOAKING TIME (Hours)

at 20°C, after which the activity appeared to decline. After 24 hours at 35°C, the activity was less than half of the corresponding value measured at 20°C. No phytase activity was evident througout the 24-hour soaking period at soaking temperatures of 50 and 65°C.

Similar changes in phytase activity have been observed in legumes germinated at temperatures ranging from 20-35°C (Mandal and Biswas, 1970; Lolas and Markakis, 1977; Eskin and Wiebe, 1983). The cause of increased phytase activity during germination still remains unclear, although both de novo synthesis and the activation of an existing enzyme have been suggested (Mandal and Biswas, 1970; Chang et al. 1977). A recent review (Loewus and Loewus, 1983) suggests that phytase formation and secretion during germination may be controlled to some extent by hormones similar to that involved in the hydrolysis of starch reserves. An earlier study (Clutterbuck and Briggs, 1974) observed that higher levels of phytase and inorganic phosphate were found in barley that had been treated with gibberellic acid, a plant hormone. Inorganic phosphate levels may also play a role in the synthesis of the enzyme as observed by Bianchetti and Sartirana(1967).

The absence of phytase activity at soaking temperatures of 50 and 65°C suggests that the mechanisms involved in the synthesis or activation of the enzyme are fairly heat-sensitive. In addition, the exposure to these soaking temperatures for long periods appears to result in the loss of active enzyme originally present in the dry seed, most probably due to thermal denaturation. The results of this study are not in agreement with the

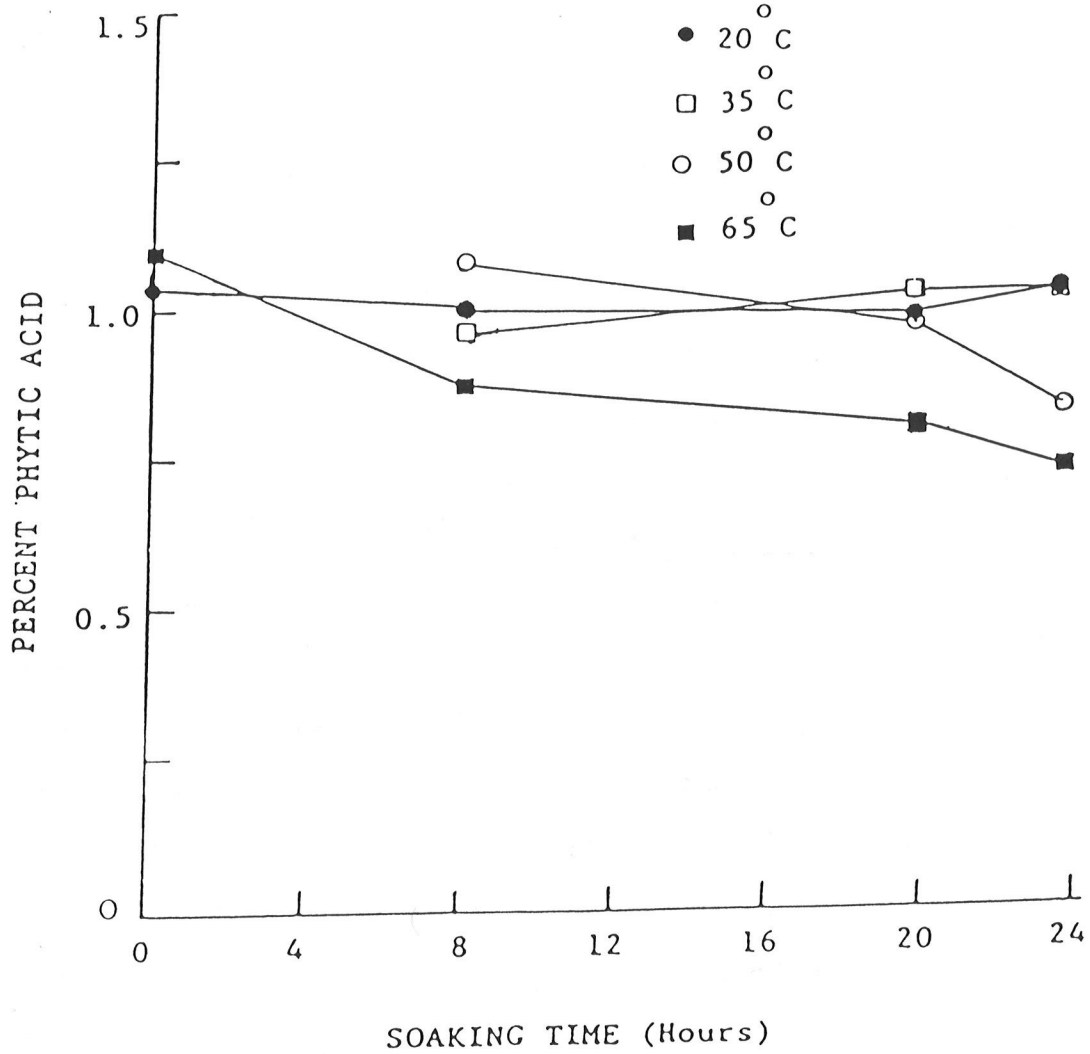
work of Chang et al. (1977) who suggested that the optimum temperature for initiating phytase in California small white beans was 60°C. A better understanding of the effect of temperature on the synthesis of the enzyme will only be possible if the exact mechanisms involved in phytase formation are known, since these mechanisms may also be influenced by temperature. If hormonal control is involved as suggested (Loewus and Loewus, 1983; Clutterbuck and Briggs, 1974) then temperature may very well be a major influence, since the effect of gibberellic acid is known to be reduced or prevented by heat (Briggs, 1973).

Phytic Acid Content in Dry Faba beans and the Effect of Soaking Time and Temperature on Phytic Acid Content

The phytic acid content of the dry seed was determined at the beginning of the study and after a two-month period and the values were 1.02 and 1.10 percent, respectively, which indicated that there was no significant change in phytic acid content during the two-month storage period. Griffiths (1983) reported that the average phytic acid content of cultivars of Vicia faba L. var. minor was 0.99 percent and further observed that considerable variation in phytate content existed both within and between faba bean varieties. Phytic acid content in two faba bean cultivars has been reported to be 0.97 and 1.10 percent in Ackerperle and Diana respectively (Eskin and Wiebe, 1983).

The phytic acid content in faba bean samples was determined after soaking for 8, 20 and 24 hours at 20, 35, 50 and 65°C, and the values obtained are shown in Fig.9. Phytic acid content in the samples remained virtually unchanged during soaking at 20 and

Figure 9. Effect of Soaking Time and Temperature on Phytic Acid Content in Vicia faba L. minor (cv. Aladin).


1.5
1.0
0.5
0
PERCENT PHYTIC ACID
20 °C
35 °C
50 °C
65 °C
0
4
8
12
16
20
24
SOAKING TIME (Hours)

35°C, indicating that the increase in phytase demonstrated under similar experimental conditions did not hydrolyze phytic acid in the intact seeds. At a soaking temperature of 50°C, phytic acid decreased by about 9.5 and 23.5 percent after 20 and 24 hours, respectively, when compared with the phytic acid content measured after eight hours. A 20, 25 and 34 percent decrease in phytic acid was observed after 8, 20 and 24 hours in faba beans soaked at 65 °C. At these soaking temperatures however, phytase activity was not detected, and thus the decreases observed during soaking could not have been due to phytic acid hydrolysis by phytase. In a recent review, Reddy et al. (1982) stated that the amount of phytic acid hydrolyzed during soaking and subsequent germination varied considerably among legumes. For instance, after the first day of germination the phytic acid content in lentils remained unchanged, but decreases of 12.9 and 31.9 percent were reported in pink beans and garden peas, respectively. Lolas and Markakis (1977) also observed no marked change in the phytic acid content of navy beans soaked at 25-27°C for 48 hours. The results of Eskin and Wiebe (1983) indicated decreases in phytate content of 2.6 and 1.0 percent after the first day of germination for Ackerperle and Diana cultivars, respectively. Larger decreases in phytate (71.2 and 77.3 percent) were however observed in the same cultivars at the end of a 10-day germination period.

The apparent lack of phytic acid hydrolysis by phytase in the intact seed could be due to several reasons. Phytase activity is known to be inhibited by inorganic phosphorus (Mandal and Bis-

was, 1972; Chang and Schwimmer, 1976); Ranhotra(1973) also reported that an increased level of inorganic phosphorus could trigger the rephosphorylation of partially-hydrolysed phytic acid. Although phytic acid is a major phosphorus-containing component of plants, other phosphorus-containing compounds, such as phospholipids, phosphoproteins and inorganic phosphates are present in legumes (Lolas and Markakis, 1975) and are a potential source for the release of inorganic phosphorus during soaking and germination, resulting in the possible rephosphorylation of partially-hydrolysed phytic acid that may be present and counteracting the hydrolytic effect of phytase. In addition the formation of complexes of phytic acid with proteins and divalent ions may be more resistant to enzyme attack (Reinhold, 1972). It has been reported that phytase from mung beans and navy beans (Mandal and Biswas, 1972; Lolas and Markakis, 1977) displays a broad substrate specificity which is not unusual for hydrolases. Lolas and Markakis(1977) reported that navy bean phytase was 735 times more active towards pyrophosphate than towards phytic acid. Similar results have been shown with wheat bran phytase (Nagai and Funahasi, 1962), and by Courtois and Perez (1948, cited from Lolas and Markasis, 1977) who found, in all the species of seeds they studied, that phytase hydrolyzed pyrophosphate and glycerophosphate more rapidly than phytic acid. The slow rate of phytic acid hydrolysis observed in some legumes during soaking and the early stages of germination may be due to phytase acting preferentially on phosphorus-containing compounds other than phytic acid, but as germination proceeds and these substrates become depleted, a

faster rate of phytic acid hydrolysis occurs.

At the higher soaking temperatures (50 and 65°C) used in this study, the phytic acid decreases observed were thought to be due to leaching into the soaking medium. Phytic acid was determined in 10 ml aliquots of the soaking medium of beans soaked for 24 hours at 50°C, and 8, 20 and 24 hours at 65°C, and results are shown in Table 1. In general the amount of phytic acid in the soaking medium increased with soaking time at 65°C, and the amount of phytic acid present after 24 hours soaking was about 1.5 times more at 65°C than at 50°C. The presence of phytic acid in the leachate confirms that phytic acid loss (in the experimental procedures employed) was a result of leaching, but not to phytase activity, which was absent. The results of Chang et al. (1977) indicated that after soaking samples of California small white beans for three hours at 60 and 70°C, 14 and 42 percent of phytate initially present in the beans had leached out into the soaking medium, but at 50°C, only trace amounts of phytate were detected.

Changes in Phytase Activity and Phytic Acid Content in a Faba Bean Flour Slurry

As phytase in the intact seed did not appear to hydrolyze phytic acid, a supplementary experiment was designed to determine if the lack of hydrolysis of phytic acid was due to tissue structural organisation which did not allow access of the enzyme to the substrate. Pin-milled faba bean flour (Ackerperle variety) was used in this study, as the effect of mechanical grinding would allow the enzyme to come into closer contact with the substrate.

Table 1

Phytic Acid Present in Soaking Medium[1]

Soaking Temperature (°C)	Phytic Acid(mg) Soaking Time (hours) 8	20	24
50	-[2]	-[2]	9.0
65	6.0	11.0	14.0

1. Values are the amount of phytic acid present in a 10 ml. aliquot, and are the means of two replicates.

2. Indicates no determination was made.

Phytase activity and phytic acid content were determined in a 10 percent slurry of the pin-milled flour, soaked at 20°C, after 8, 20 and 24 hours, and the results are shown in Fig. 10. Phytase activity increased by about 8, 12 and 14-fold after 8, 20 and 24 hours ,respectively. Phytic acid content did not change significantly during the soaking period, although the overall decrease in phytic acid content was about three percent. These results show that the disruption of the tissue structure through pin-milling did not cause any significant increase in phytic acid hydrolysis, and as such, indicates that the tissue stucture in the intact beans did not act as a barrier to prevent phytic acid hydrolysis by phytase. It would seem that the structure of phytic acid complexes, the presence of phytase inhibitors and the broad specificity of the enzyme in the seed would be the major factors retarding phytic acid hydrolysis.

The Effect of Soaking Temperature and Soaking Time on Water Uptake in Faba Beans

Figure 11 shows the results of water uptake measurements (reported as percentage increase in weight) of faba beans during soaking at 20, 35 50 and 65°C after 8, 20 and 24 hours. The results indicate that the soaking temperature had a significant effect on the rate of water uptake in the beans. For all the soaking temperatures, the greatest percentage increase in weight occurred during the first eight hours of soaking. The percentage increase in weight after eight hours was 49.4, 89.7, 94.7 and 90.5 at 20, 35, 50 and 65°C, respectively. After the initial rapid water uptake, a slower rate was observed for the rest of

Figure 10. Changes in Phytase Activity and Phytic Acid Content in a Ten Percent Slurry of *Vicia faba* L. *minor* (cv. Ackerperle).

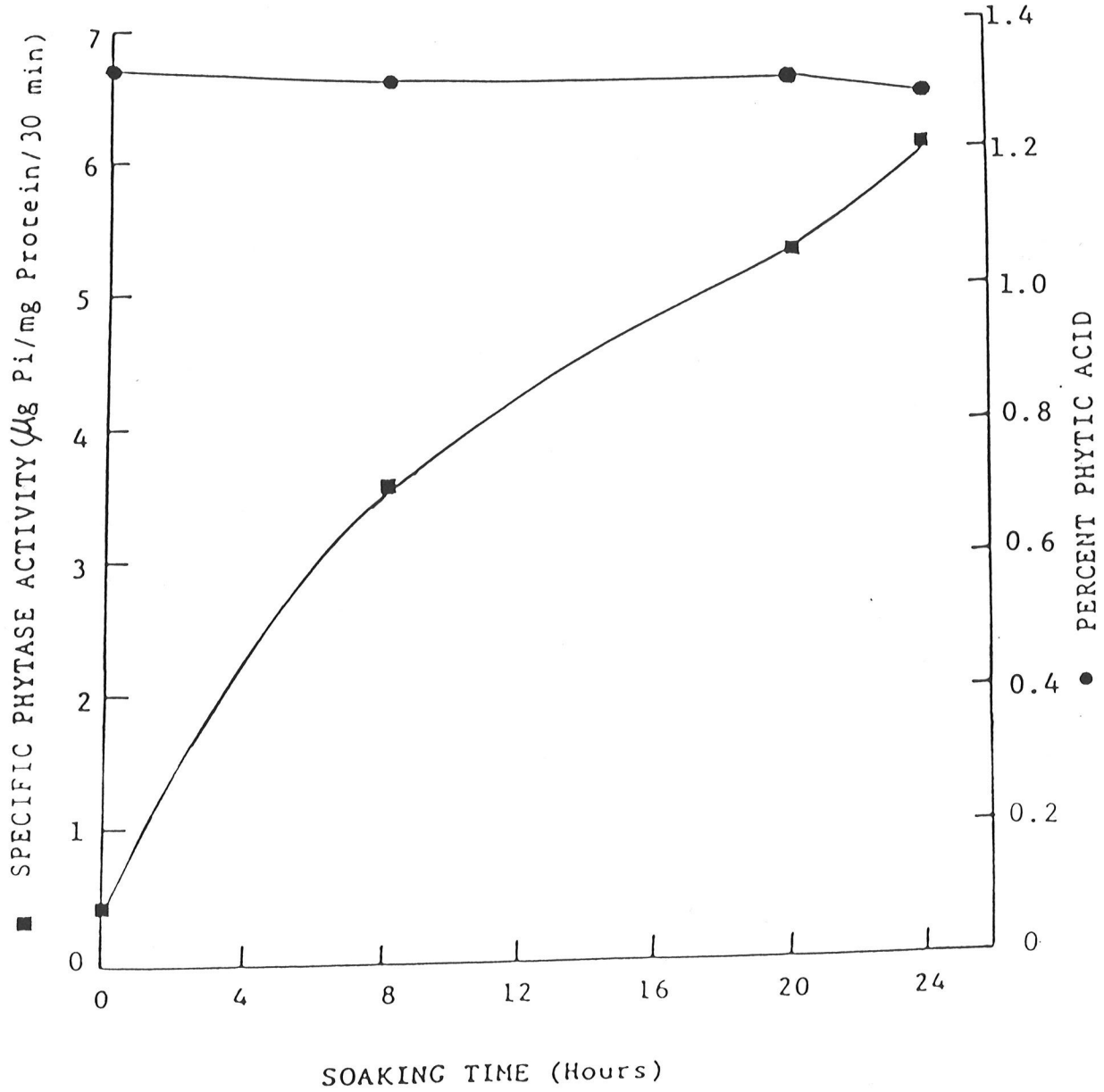
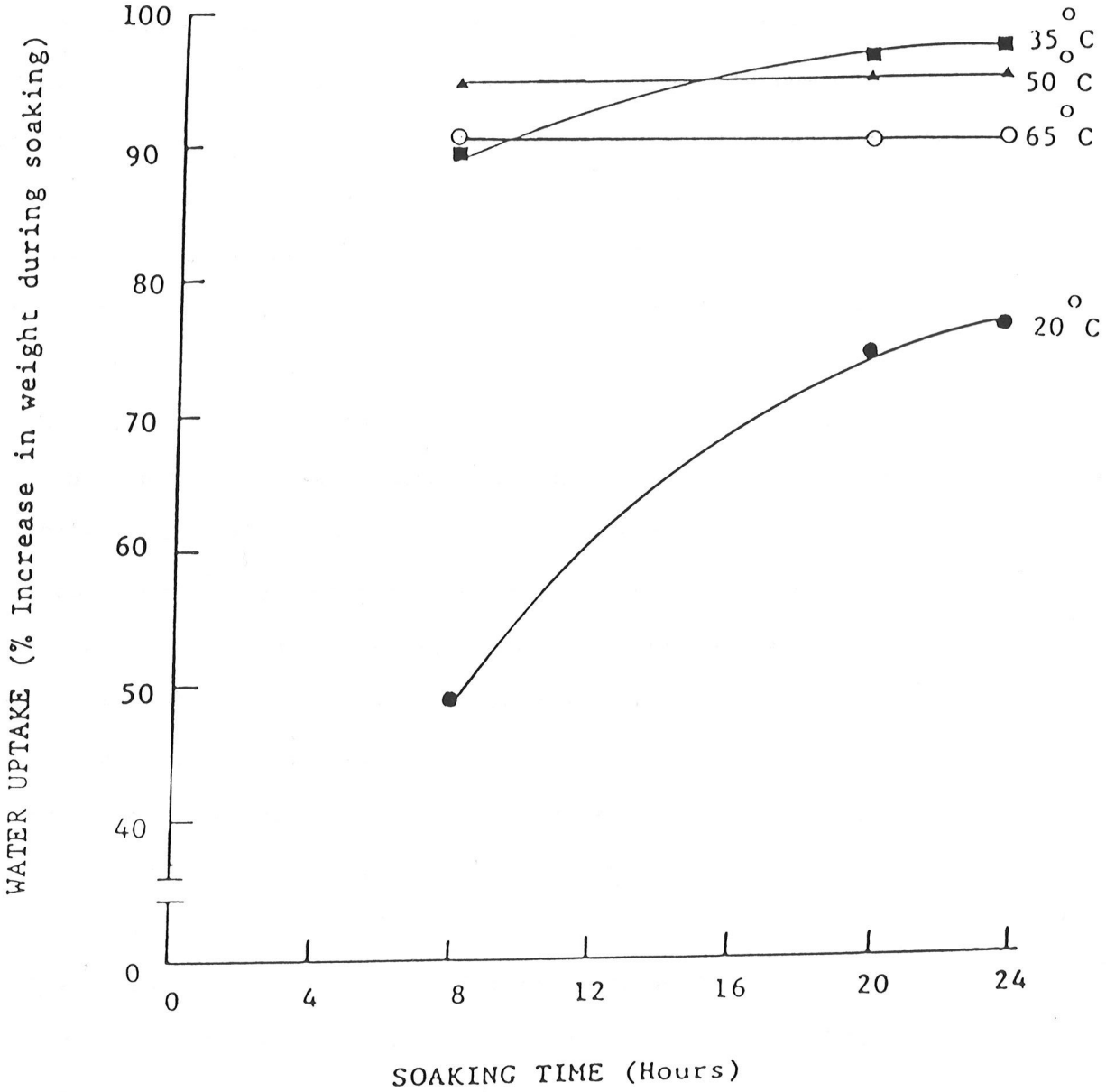

■ SPECIFIC PHYTASE ACTIVITY (μg Pi/mg Procein/30 min)
● PERCENT PHYTIC ACID
SOAKING TIME (Hours)
0
1
2
3
4
5
6
7
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
4
8
12
16
20
24

Figure 11. Effect of Soaking Time and Temperature on Water Uptake in Vicia faba L. minor (cv. Aladin).


WATER UPTAKE (% Increase in weight during soaking)
100
90
80
70
60
50
40
0
0
4
8
12
16
20
24
SOAKING TIME (Hours)
35 °C
50 °C
65 °C
20 °C

the soaking period for soaking temperatures of 20 and 35°C, whereas at 50 and 65°C there appeared to be almost no increase in water uptake.

These results are similar to the results of other workers who studied the water absorption characteristics of cowpeas, black beans and soy beans. Sefa-Dedeh et al. (1978) observed that the amount of water absorbed by cowpeas during soaking increased with soaking time until an equilibrium was attained. At this point, they said, the water absorption components in the beans were saturated resulting in a slower water absorption rate. In black beans, white beans and soy beans the rate of water uptake increased with the soaking temperature (Quast and da Silva, 1983; Kon, 1979; Hsu et al., 1983). For soy beans soaked at 20°C, the beans took about 10.5 hours to reach 90 percent of the total absorption; at 30°C this same level of absorption took about six hours and at 50°C, it took only 2.5 hours (Hsu et al., 1983). In general the increase in water uptake with increasing soaking temperature may be the result of the thermal disruption of the internal structural organisation, facilitating the penetration of water molecules.

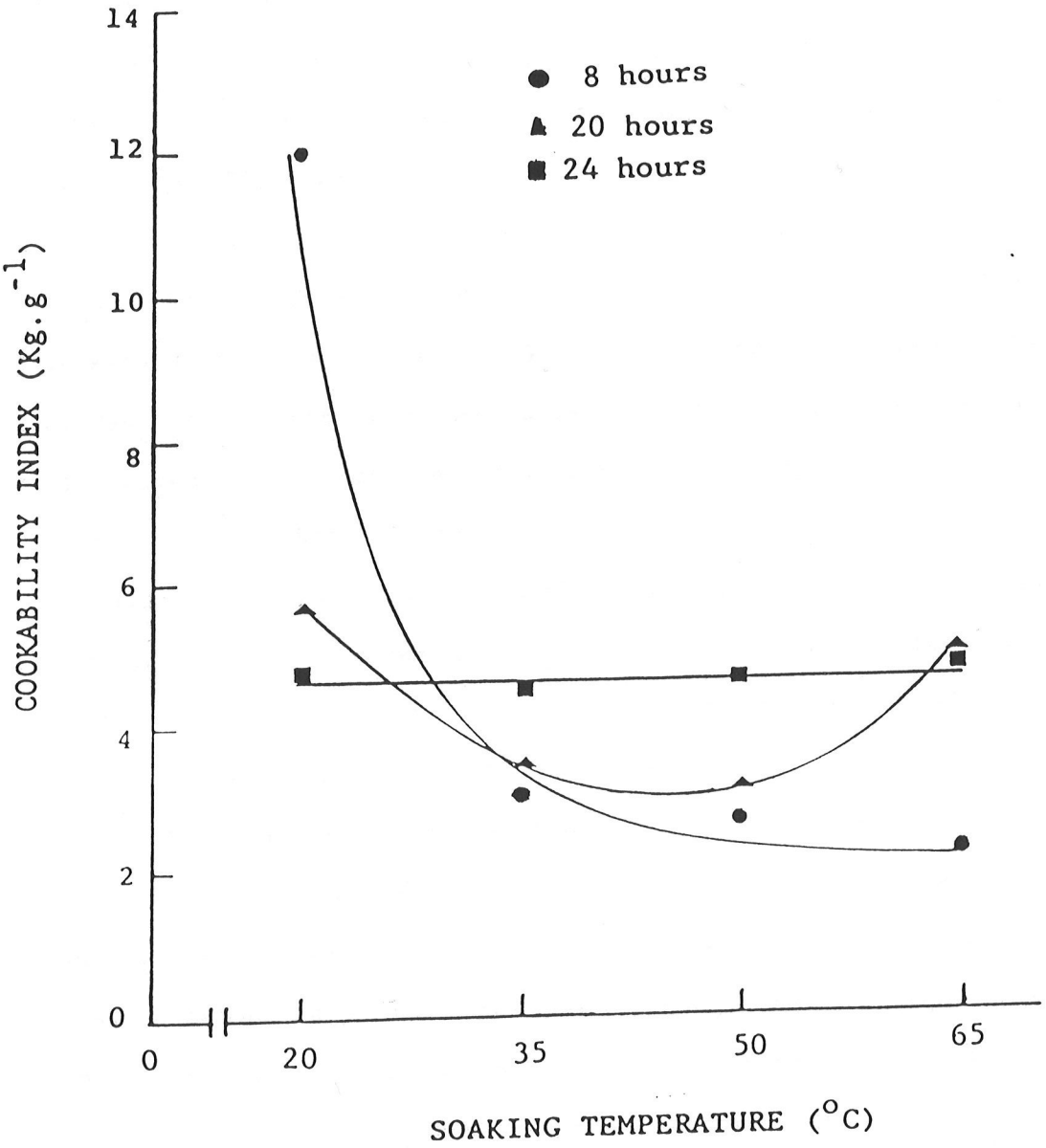
Quast and da Silva (1977) further noted that after a certain time the weight loss due to the loss of soluble solids from black beans was greater than the weight gain. As a result of this the wet weight of the beans reached a maximum during soaking and then declined. The apparent absence of an increase in weight during the soaking of the faba beans at 50 and 65°C after the initial rapid water uptake was most probably a result of solids leached

into the soaking medium. Kon (1979) observed that in general more solids were extracted from white beans at soaking temperatures above 40°C, and said this was an indication that the permeability of the cell membranes were affected enabling more materials to diffuse out.

Cookability Index in Dry Faba Bean Cultivars and the Effect of Soaking Time and Temperature on the Cookability Index

The measurement of the cookability index was an indirect determination of the relative differences in the cooking times of faba bean 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 cookability index values of the dry faba beans were determined at the beginning of the study and after a subsequent two-month storage period. The initial cookability index was 4.667 $Kg.g^{-1}$, and was 6.819 $Kg.g^{-1}$ after the two-month storage period, indicating an increase in cookability index during storage. Figure 12 shows the cookability index values for faba beans soaked for 8, 20 and 24 hours at 20, 35, 50 and 65°C. A preliminary investigation showed that at 20°C, the cookability index decreased with soaking time, but due to the different cooking times used in the study, changes in cookability index with soaking time at the various soaking temperatures could not be fully evaluated. The cookability index values for samples soaked for eight hours decreased with increasing soaking temperature, with the maximum decrease observed when the soaking temperature was increased from 20 to 35°C, for which the cookability index values were 12.065 and 3.112 $Kg.g^{-1}$, respectively. Increas-

Figure 12. Effect of Soaking Time and Temperature on the Cookability Index of Vicia faba L. minor (cv. Aladin).


14
12
10
8
6
4
2
0
0
20
35
50
65
8 hours
20 hours
24 hours
COOKABILITY INDEX (Kg.g^{-1})
SOAKING TEMPERATURE (^{o}C)

ing the soaking temperature above 35°C did not change the cookability index significantly. A different response was observed in samples soaked for 20 and 24 hours. For 20-hour samples, there was a gradual decrease in cookability index with increasing soaking temperature (20-50°C), after which the cookability index increased. The results of samples soaked for 24 hours showed no significant change in cookability index over the soaking temperature range (20-65°C).

The soaking of legumes prior to cooking has been shown to significantly reduce their cooking time (Sefa-Dedeh et al., 1978 Jackson and Varriano-Marston, 1981). One of the most common techniques used in commercial processes for reducing the soaking time of legumes is to increase the temperature of the soaking medium (Kon, 1979). Besides saving time, shorter soaking times also minimize the growth of microorganisms (Quast and da Silva, 1977). The results of this study show that the cooking time of faba bean (measured as the cookability index) can be minimized with increasing soaking time and temperature.

It was however evident from this study that in samples soaked for eight hours, a soaking temperature of 35°C would be adequate in achieving a reduced cooking time, as increasing the soaking temperature above this would not cause any significant change in cooking time. On the other hand, increasing the soaking temperature for samples soaked for 24 hours would not be beneficial.

Increased moisture uptake during soaking has been regarded as the major influence on the cooking time or texture of legumes

(Sefa-Dedeh et al., 1979; Kon, 1979; Jackson and Varriano-Marston, 1981), and this may be the result of increasing internal pressure exerted in the cotyledons, or improved starch gelatinization, which would aid in cell separation during cooking. The relationship between water uptake during soaking and the cookability index of the cooked faba beans will be further discussed.

Effect of Phytic Acid Content on the Cookability Index of Faba Beans

Several researchers have suggested a relationship between the phytic content in legumes and their cooking time; poor cookability in legumes has been associated with a reduction in phytic acid content. Mattson(1946) reported that the phytin content in a soft(fast) cooking pea sample was 557 mg/100 g, while a hard(slow) cooking sample contained less than half as much (220 mg/100 g). Kumar et al.(1978) also observed that samples of greengram beans with a phytin content of 185 mg/100 g cooked in about 13 minutes, whereas samples of chickpeas that cooked in 79 minutes only contained 80 mg/100 g. The cooking times of white beans that had been soaked at different temperatures were correlated with their organic phosphate content after cooking, and the beans that took the longest time to cook were found to contain the lowest amount of organic phosphate (Kon, 1979).

In this study it was observed that the phytic acid content in the faba beans decreased with soaking time, specifically at the higher soaking temperatures (50 and 65°C), consequently these changes would relate to a higher cookability index if a similar

relationship as suggested by Mattson and other workers existed in the soaked faba beans. To determine whether the phytic acid content in faba beans did have any effect on the cooking quality, a linear coefficient analysis was conducted. The linear correlation coefficients determined for samples soaked for 8,20 and 24 hours were 0.229, -0.343 and -0.214, respectively (Appendix 1). These values were found to be insignificant at a 5% probability level, indicating that the phytic acid content in the soaked faba beans did not affect the cookabilty index. It was noted that in the studies of Mattson(1946) and Kumar et al. (1978) the phytic acid content of hard cooking samples was less than half the value of the soft cooking samples. Similarly soft cooking faba beans were reported to contain 0.77 percent phytic acid as compared to 0.20 percent in hard cooking samples (Murray et al., 1982). These results suggest that the effect of differences in the phytic acid content of legumes on their cooking time or quality may only be significant when the phytic acid content in soft cooking samples is more than twice the amount in hard cooking samples. In this study, although the phytic acid content decreased with increasing soaking temperature, these decreases were obviously too low to affect the cookability index. Other researchers have not been able to obtain any correlation between the phytic acid content and cooking time of legumes (Crean and Haisman, 1963; Muller, 1967). Crean and Haisman(1963) had pointed out that although peas with a low phytic acid content were often difficult to cook, a high phytic acid content was an unreliable criterion of good cooking quality. They reported that the maximum formation of

insoluble phytate accounted for only 44 percent of the calcium and magnesium ions available in peas, and generally much less than this was actually sequestered, allowing the free divalent ions to build up at other sites causing a toughening of cell wall tissue, and as such the presence of free phytic acid ions could not prevent the deterioriation of texture during cooking caused by the divalent ions.

The effect of phytic acid content on the cooking time of legumes observed by Mattson (1946), Kumar et al, (1978) and Murray et al. (1982) were all based on dry legumes, whereas a major feature in this study was that the phytic acid changes and the effect on cooking time were observed in legumes that had been soaked prior to cooking. Although Kumar et al. (1978) found a correlation between the phytin content and the cooking time in dry legumes, they were unable to detect a similar correlation in germinated legumes. They monitored changes in divalent ion content (calcium and magnesium), in addition to phytic acid content, during germination but found that no individual factor could account for the cooking patterns in the legumes they used in their study. Muller (1967) concluded that the main factor affecting the cooking quality of several pea and bean varieties , was the composition of the cell wall expressed by a "PCMP" number relating the contents of phytic acid, calcium, magnesium and free pectin. Kumar et al. (1978) found that the concept of "PCMP" number was applicable in their study, where the shortening of cooking time in germinated chickpea and the lengthening of cooking time in germinated greengram and cowpea could be directly correlated with

corresponding changes in "PCMP" number. Although changes in divalent ion content were not monitored in this study, it would be reasonable to expect changes in divalent ion content to occur under the experimental conditions employed, due to leaching. Kon(1979) observed that the amounts of divalent ions that were found in the soaking medium of white beans increased with the soaking temperature. After soaking for four hours at 50°C, two percent of the original calcium content and five percent of the magnesium content were present in the soaking medium, and after soaking for 1.5 hours at 60°C, nine percent of the calcium and 31 percent of the magnesium content were present in the soaking medium. Since the presence of divalent ions in legumes contributes significantly to their cooking quality, such losses that occur as a result of leaching would subsequently improve cooking quality (reducing cooking time).

Effect of Phytase Activity on the Cookability Index of Faba Beans

Phytase activity was detected only at the lower soaking temperatures, 20 and 35°C, and no distinct pattern was observed in the changes in phytase activity within this temperature range with corresponding cookability index. For samples soaked for 8 hours, phytase activity increased and cookability index decreased, for 20 hour samples, both phytase activity and cookability index decreased, and for 24 hour samples phytase activity decreased but the cookability index remained unchanged. Mattson(1946) and Murray et al.(1982) had reported that peas and faba bean treated with added phytase were uncookable, and this may have been due to rapid phytic acid hydrolysis by the enzyme. In

this study it has been shown that despite increasing endogenous phytase activity, phytic acid is not hydrolysed. In addition any changes in phytic acid content that occurred under the experimental conditions employed were too low to affect cookability values.

Effect of Water Uptake on the Cookability Index of Faba Beans

The water uptake results of faba beans soaked for 8, 20 and 24 hours were correlated with the cookability index of the corresponding cooked faba bean samples using linear correlation analysis, to determine whether water uptake had any effect on the cookability index. The correlation coefficients for samples soaked for 8, 20 and 24 hours were -0.99, -0.86 and -0.27, respectively (Appendix 2). The coefficient value obtained for samples soaked for 8 hours was found to be significant at the 5 % probability level, indicating that increasing moisture uptake significantly reduced the cookability index of the faba bean samples. On the contrary, the coefficient values for samples soaked for 20 and 24 hours were not significant, suggesting that the differences in water uptake in those samples did not have any significant effect on the cookability index. These results suggest that the effect of water uptake on cooking time in the faba bean studied may be dependent on the soaking time; although for shorter soaking times cooking time decreases with increasing moisture uptake, this effect appears to decline with longer soaking times.

Sefa-Dedeh et al. (1978) reported a highly significant negative correlation coefficient (-0.98) between the amount of water

absorbed during soaking over a 24-hour period at 25°C, the texture of the cooked cowpeas having been measured with an OTMS cell. A similar correlation was also obtained between water absorption and the texture of the soaked (raw) beans. They found that the texture of the cooked cowpeas could be predicted by an equation involving soaking time, water absorption and the texture of the soaked beans. Very little change in the texture of the cooked beans corresponded with the slow increases in water absorption that were exhibited after water saturation was reached. Jackson and Varriano-Marston (1981) studied the effect of moisture content after soaking on the cooking time of black beans, and reported that the cooking time was inversely proportional to moisture content. Differences in the cooking time of fresh and aged black beans however persisted regardless of bean moisture content. Quast and da Silva (1977) were unable to find a similar correlation, and they reported that hydration prior to cooking did not significantly decrease the cooking times of black beans.

As pointed out, the effect of water uptake on the cooking time of faba beans appears to decline with increasing soaking time. In samples soaked for 8 hours at different temperatures, water uptake did contribute significantly to the cookability of the faba beans. The cookability index values for samples soaked for 24 hours remained virtually unchanged despite differences in water uptake at the different soaking temperatures employed. It seems possible that a water saturation phase had been attained, as suggested by Sefa-Dedeh *et al.* (1978), where the cookability of

the faba beans was independent of the moisture content. At this stage, any differences in cooking time may be attributed to the chemical composition (phytic acid, Ca, Mg and pectin) of the samples rather than water uptake. It can be concluded, from this study, that water uptake during soaking does influence the cooking time of faba beans, to an extent that is dependent on the soaking conditions employed.

Conclusion

The differences in the cooking time of legumes appear to be dependent upon several factors, especially moisture content and the chemical composition of the cotyledon, and this clearly emphasizes the complexity of the actual mechanisms that are involved in the cooking process. Of the three parameters studied, phytase activity, phytic acid content and water uptake, none of these was found to be the sole factor that influenced the cookability index of the soaked faba beans. The amount of water absorbed by the bean samples during soaking at different temperatures for 8 hours had the most significant effect on the cookability index, but water absorption became less significant with longer soaking times. Based on these findings, the cooking time of dry faba beans and the HTC effect can be reduced by a soaking treatment prior to cooking. The time required for cooking will be dependent on the soaking time and temperature employed. For example, in this study, it was evident that soaking faba beans for eight hours at 35°C, rather than at 20°C, would be more beneficial in reducing the cooking time. The use of soaking temperatures above 35°C is not practical as the effect on the cooking

time is not significant, and the use of such temperatures may affect the nutritional quality of the beans. Although these results apply to the Canadian developed Aladin variety of faba beans, they may also be applicable in the cooking practices of dry legumes that are more commonly used in West African countries, such as cowpeas. Apparently a critical moisture content exists, above which other inherent differences among the samples (e.g. chemical composition) are likely to become more important in determining the cooking quality. The phytic acid content may have a relatively minor effect on the cookability index which cannot be ruled out, but under these experimental conditions the effect of phytic acid is less influential, being greatly exceeded by the effect of moisture uptake. It seems probable that the interrelationship of phytic acid with divalent ion content may be a more suitable criterion of assessing the effect of chemical composition, other than moisture content on cookability.

Phytase was present at soaking temperatures of 20 and 35°C, but was unable to hydrolyse phytic acid in the intact seed using the analytical methods in place for this study. The absence of phytic acid hydrolysis still remained when tissue structure was disrupted, indicating that the tissue structure of the intact beans did not act as a barrier to phytic acid hydrolysis by the enzyme. Phytase action on phytic acid may be dependent upon the form of phytic acid that exists in the seed, reflecting the specificity of phytase, or the presence of other potential substrates and the presence of inhibitors in the seed. Phytic acid content decreased at higher soaking temperatures in the absence

of phytase activity, and this was found to be due to the leaching of phytic acid into the soaking medium at these temperatures.

Future work is still required in this area, and should include 1) a determination of the "PCMP" number in faba beans soaked for different times and temperatures, in order to determine if a better correlation exists between this value and the cooking quality, and 2) an investigation of the effects of a longer soaking period(leading to germination) on phytase activity and phytic acid content in faba beans and the correlation with cooking time.

SUMMARY

1. The level of phytase activity in the dry Aladin cultivar was low but increased with soaking at 20 and 35°C. No activity was evident at higher soaking temperatures, probably due to thermal inactivation.

2. The level of phytic acid in the soaked faba beans remained unchanged after soaking at 20 and 35°C despite the presence of phytase. Phytic acid content decreased with soaking time at 50 and 65°C as a result of leaching into the soaking medium.

3. In general, water uptake in faba beans increased with soaking time and temperature.

4. The effect of increasing soaking temperature on cookability index was dependent upon the soaking time.

5. Phytase activity and phytic acid content were found to have no significant effect on the cookability index of soaked samples of the Aladin cultivar of faba beans. The effect of water uptake on the cookability index is only significant for short soaking times (e.g. eight hours).

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Appendix 1

Linear Correlation Coefficient Between Phytic Acid Content And Cookability Index

Soaking Temperature (^{o}C)	Soaking Time (hours)					
	8		20		24	
	X	Y	X	Y	X	Y
20	1.010	12.065	0.990	5.600	1.020	4.712
35	0.976	3.112	0.990	3.504	1.030	4.556
50	1.079	2.750	0.976	3.090	0.825	4.660
65	0.878	2.309	0.816	4.994	0.729	4.956
r	0.229^{ns}		-0.343^{ns}		-0.214^{ns}	

X = % phytic acid
Y = Cookability index (kg. g^{-1})
r = linear correlation coefficient
ns = not significant at 0.05 probability level

Appendix 2

Linear Correlation Coefficient Between Water Uptake and Cookability Index

Soaking Temperature (^{o}C)	Soaking Time (hours) 8 X	8 Y	20 X	20 Y	24 X	24 Y
20	49.43	12.065	74.90	5.600	76.85	4.712
35	89.75	3.112	97.40	3.504	18.25	4.556
50	94.75	2.750	95.23	3.090	15.50	4.660
65	90.47	2.309	90.77	4.994	91.05	4.956
r	-0.99^{s}		-0.86^{ns}		-0.27^{ns}	

X = % increase in weight during soaking
Y = Cookability index (kg. g^{-1})
r = linear correlation coefficient
s = significant at 0.05 probability level
ns = not significant at 0.05 probability level