IMPROVING THE TEXTURAL PROPERTIES AND STORAGE STABILITY OF COWPEAS:

PROCESS DEVELOPMENT AND EVALUATION

by

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(Under the direction of Manjeet S. Chinnan)

ABSTRACT

The hard-to-cook defect is a well-known problem in stored legumes, which reduces the consumption and production of these nutritious foods. It is a generally held view that phytase is involved in the development of the defect. The main objectives of this dissertation were to develop a heat process targeted at inactivating phytase and consequently improving the textural and storage qualities of cowpeas by preventing/reducing hardening.

The inactivation kinetics of phytase was determined at different moisture contents (10-35% d.b.) in the temperature range of 70-95°C. Phytase exhibited a high thermo-stability, retaining 63-95% activity at 10% moisture. Increasing temperature and moisture content enhanced enzyme inactivation. Inactivation kinetics was expressed by a fractional conversion model, which was successfully used in predicting phytase activity in cowpea flour. The estimated $E_a$ were 33.29, 37.87 and 43.4 KJ/mol at 10, 25 and 35% moisture, respectively. Phytase inactivation in cowpea seeds was spatially dependent; the regions close to the surface showed a higher inactivation than the interior. Phytase
inactivation predicted using a finite element model was slightly lower than experimental values.

Twelve treatments were designed by varying moisture content, steaming temperature and time. Their potential in preventing hardening of stored cowpeas was investigated. The effect of the drying conditions applied after steaming was also studied. Changes in textural and physicochemical indices were measured after storage at either 4°C or 42°C/80%RH. Generally, steaming significantly reduced the cooked texture before storage; however, only steaming at 121°C for 4 or 6 min was successful in preventing/reducing hardening during storage at 42°C/80%RH.

Significant decreases in water absorption, phytate content and phytase activity were recorded whereas increases in solids loss, electrolyte leakage and pectin loss were noted. Drying conditions contributed significantly in maintaining the textural characteristics of stored cowpeas. Cooked texture, phytase activity and phytate content significantly decreased as drying temperature increased. Increasing drying temperature and humidity had positive effects on water absorption and electrolyte leakage. Pectin loss during soaking and cooking was however not significantly influenced by any of the drying parameters considered.

INDEX WORDS: Cowpeas, Hard-to-cook, Steam treatment, Phytase, Inactivation kinetics, Moisture content, Storage, Texture, Fractional conversion technique, Drying conditions.
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DEDICATION

This work is dedicated to my husband and dearest friend, Kwabena Kessie.

_Whatever our souls are made of, his and mine are the same_ (Emily Bronte).
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My most sincere thanks and praise go to my heavenly Father, who reminded me daily: "My grace is sufficient for you, for my power is made perfect in weakness." Therefore I will boast all the more gladly about my weaknesses, so that Christ's power may rest on me (2 Cor. 12:9, NIV). His grace was always more than sufficient.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................................................................ v

LIST OF TABLES ........................................................................................................................ vii

LIST OF FIGURES ........................................................................................................................ ix

CHAPTER

1 INTRODUCTION ......................................................................................................................... 1

2 LITERATURE REVIEW .......................................................................................................... 6

3 HEAT-MOISTURE TREATMENTS OF COWPEA FLOUR AND THEIR EFFECTS ON PHYTASE INACTIVATION .......................................................................................................... 45

4 MODELING THE THERMAL INACTIVATION OF PHYTASE IN STEAMED COWPEA SEEDS ....................................................................................................................... 69

5 THE INFLUENCE OF PRETREATMENTS ON THE DEVELOPMENT OF THE HARD-TO-COOK DEFECT IN COWPEA SEEDS ................................................................................................. 93

6 THE EFFECT OF DRYING CONDITIONS ON THE DEVELOPMENT OF THE HARD-TO-COOK DEFECT IN STEAM-TREATED COWPEA ................................................................................. 136

7 SUMMARY AND CONCLUSIONS ............................................................................................. 184

APPENDICES ............................................................................................................................ 187
**LIST OF TABLES**

| Table 3.1 | ANOVA summary table for phytase inactivation ................................................. 62 |
| Table 3.2 | Kinetic parameters for phytase inactivation in cowpea flour ................................. 63 |
| Table 3.3 | Predicted and measured relative phytase activity (%) in heated cowpea flour .......... 64 |
| Table 4.1 | Estimated kinetic parameters for the inactivation of phytase in steamed cowpea seeds ......................................................................................................................... 85 |
| Table 4.2 | Comparison of predicted and experimental residual phytase activity (%) in different seed fractions of cowpeas steamed at 100°C ................................................................. 86 |
| Table 5.1 | Process variables and their corresponding levels used in the experimental design .......................................................................................................................... 124 |
| Table 5.2 | Analysis of variance for moisture content, cooked texture, water absorption, solids loss, electrolyte leakage, phytic acid, phytase activity and pectin loss of steam treated cowpeas for moisture content, steaming temperature, steaming time and storage condition ........................................................................................................ 125 |
| Table 6.1 | Process variables and levels ................................................................................... 165 |
| Table 6.2 | Treatment combinations from the Box-Behnken experimental design .......................... 166 |
| Table 6.3 | The effect of drying condition and storage on the moisture content of steamed cowpeas ........................................................................................................................................ 167 |
| Table 6.4 | Regression coefficients and analysis of variance of selected second-order models for textural and physicochemical characteristics of steamed cowpeas before storage ........................................................................................................ 168 |
Table 6.5  Regression coefficients and analysis of variance of selected second-order models for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under mild conditions.......................................................... 169

Table 6.6  Regression coefficients and analysis of variance of selected second-order models for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under severe conditions....................................................... 170
LIST OF FIGURES

Figure 3.1  Thermal inactivation of phytase in cowpea flour as a function of heating time at various temperatures and moistures................................................................. 65

Figure 3.2  Thermal dependence of rate constant for thermal inactivation of cowpea phytase ..................................................................................................................... 66

Figure 3.3  Effect of moisture content on loss of phytase activity in heated cowpea flour...... 67

Figure 3.4  Changes in inactivation energy of phytase activity with moisture content in heated cowpea flour ........................................................................................................... 68

Figure 4.1  Fractionating cowpea seeds using a specially designed device equipped with a high speed drill and suction ..................................................................................... 87

Figure 4.2  Cross-sectional representation of the three layers of a cowpea cotyledon.......... 88

Figure 4.3  Thermal inactivation of phytase in cowpea seeds steamed at 100 or 121°C .......... 89

Figure 4.4  Thermal inactivation of phytase in the outer, middle and inner layers of cowpeas following steam treatment at 100 or 121°C .......................................................... 90

Figure 4.5  Predicted phytase inactivation in cowpea seeds steamed for 60, 120, 180s........... 91

Figure 4.6  Simulated moisture profile of steamed cowpea seeds after 60 s of steaming. ...... 92

Figure 5.1  Moisture content of steam-treated cowpeas under different storage conditions ... 126

Figure 5.2  Changes in cooked hardness of steamed cowpeas ............................................. 127

Figure 5.3  Changes in water absorption of steamed cowpeas ............................................ 128

Figure 5.4  Solids lost during soaking of steamed cowpeas ............................................... 129

Figure 5.5  Electrolyte leakage during soaking of steamed cowpeas .................................. 130
Figure 5.6 Changes in phytate content of cowpeas following steam treatment and storage. 131

Figure 5.7 Changes in phytase activity in cowpeas following steam treatment and storage. 132

Figure 5.8 Changes in pectin loss in cowpeas following steam treatment and storage. 133

Figure 5.9 SEM images of the effect of different heat-moisture pretreatments on cowpea cotyledons. 134

Figure 5.10 SEM images of the effect of different heat-moisture pretreatments on cowpea cotyledons after storage at 42°C/80% RH. 135

Figure 6.1 Effect of drying conditions on the cooked texture of steam-treated cowpeas. 171

Figure 6.2 Relative cooked hardness of steam-treated cowpeas after storage under mild or severe conditions. 172

Figure 6.3 Response surface plot for texture of steam-treated cowpeas cooked for 60 min, after storage under severe conditions and as a function of drying temperature and humidity. 173

Figure 6.4 Effect of drying conditions on the phytase activity in steam-treated cowpeas. 174

Figure 6.5 Response surface plot showing effect of drying temperature and humidity on phytase activity in steam-treated cowpeas (Initial samples). 175

Figure 6.6 Effect of drying conditions on the phytate content of steam-treated cowpeas, showing samples analyzed before storage (initial) and after storage under severe or mild conditions. 176

Figure 6.7 Response surface plot of the effect of drying temperature and humidity on phytate content in steam-treated cowpeas, showing initial samples and after storage under severe conditions. 177
Figure 6.8  Changes in water absorption capacity of control and steam-treated cowpeas with storage.......................................................................................................................................... 178

Figure 6.9  Response surface plots of the effect of drying temperature and humidity on water absorption capacity of steam-treated cowpeas, showing initial samples and after storage under severe conditions.......................................................... 179

Figure 6.10 Changes in electrolyte leakage of control and steam-treated cowpeas .............. 180

Figure 6.11 Response surface plots of the effect of drying temperature and humidity on electrolyte leakage in steam-treated cowpeas, showing initial samples and after storage under severe conditions.......................................................... 181

Figure 6.12 Effect of drying conditions on pectin loss from steam-treated cowpeas during soaking showing samples analyzed before and after storage under severe or mild conditions.......................................................... 182

Figure 6.13 Effect of drying conditions on pectin loss from steam-treated cowpeas during cooking showing samples analyzed before and after storage under severe or mild conditions.......................................................... 183
CHAPTER 1

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are an important source of protein in the diets of many populations in the world especially in regions where animal protein is relatively expensive. The cowpea seed is one of the most widely adapted, versatile and nutritious grain legumes. About two-thirds of the production and more than three-fourths of the area of production is spread over Sudan Savanna and Sahelian zones of sub-Saharan Africa (Ehlers and Hall 1997). The dry grain is the principal product for human consumption but the leaves, fresh peas and fresh green pods are also consumed (Ehlers and Hall 1997).

The nutritional profile of cowpeas is comparable to most edible legumes such as soybean, common bean and faba beans. Cowpeas are high in protein (23-30%), carbohydrate (56-60%) and also contain thiamine (0.74 mg/100g), riboflavin (0.42 mg/100g), niacin (2.81 mg/100g) and folic acid (0.6mg/100g) (Bressani 1985; Phillips and McWatters 1991). Cowpeas contain anti-nutritional factors such as lectins, trypsin inhibitors and condensed tannins that are known to decrease protein digestibility and therefore nutritional quality. They contain high amounts of the essential amino acid, lysine but lacks sufficient amounts of sulfur containing amino acids. An important characteristic of cowpeas, and other food legumes, is their high capacity to complement cereal grains that have high concentration of sulfur amino acids; the legumes contribute lysine and the cereals contribute methionine to the blend.

In spite of the obvious nutritional benefits of cowpeas, their utilization is not optimal due to major constraints that include the hard-to-cook defect, insect infestation and flatulence. The
hard-to-cook (HTC) defect associated with stored dry beans refers to the failure of the seeds to soften enough to be edible after cooking for a reasonable length of time (Aguilera and Rivera 1992). The extended cooking period required is of economic and nutritional importance due to higher fuel consumption, increased leaching of nutrients and unacceptable quality. The underlying mechanism of the defect is still not clearly defined or understood but it is believed to be due to a combination of factors including enzymatic and non-enzymatic pathways. The earliest and most widely held hypothesis was proposed by Mattson (1946) who suggested a relationship between the increased hardness of stored legumes and phytate content after observing that hard-to-cook peas contained 50% less phytate than fresh peas. He proposed that in fresh peas, phytate chelates Ca and Mg ions reducing the formation of cross-links with pectate in the middle lamella. During storage under high temperature and humidity, phytase is activated and hydrolyzes phytic acid, causing a decrease in the chelating potential of phytic acid and enhancing cross-linking of pectic substances in the middle lamella. The Ca and Mg pectates that are formed do not dissolve readily when subjected to heating and therefore restricts cell separation leading to a significant increase in cooked bean hardness.

The most effective method of preventing the development of the hard-to-cook defect in legumes is storage of low moisture seeds under low temperature and low humidity conditions. However, this is not always possible or practical and therefore there is the need to explore the use of other methods. The most commonly investigated preventive method is the application of either dry or moist heat treatments prior to storage. Varying levels of successes have been achieved depending on the treatment combination used. Molina and others (1976) heated black beans at 98 and 121°C for various times before storing at 25°C and 70% RH for 9 months. They found that the shortest heat treatments evaluated (2min/121°C and 10 min/98°C) were the most
effective in preventing the defect over the storage period. In their study, Plhak and others (1987) used methods including sun-drying, solar drying, microwave heating, dry roasting and irradiation. They noted that only irradiation was effective in reducing the rate of hardening. High temperature-short time and medium temperature-long time heat treatments resulted in samples that were significantly softer than the control (Aguilera and Steinsapir 1985).

In spite of supporting evidence in literature detailing the role of phytase in the development of the hard-to-cook defect, no attempts have been made to investigate the inactivation of phytase as a strategy for preventing the hard-to-cook defect. Changes in phytase activity should be reflected in the rate of developing the hard-to-cook defect. The use of indicator enzymes in heat treatments provides a natural margin of safety in that if the enzyme is inactivated then it is reasonable to assume the associated quality-related problems have been eliminated (Anthon and Barrett 2002). In this dissertation, we hypothesized that a heat process capable of inactivating the phytase enzyme would have a positive effect on the textural characteristics and therefore storage stability of cowpeas. The development of a steaming process and its consequent effects on some quality characteristics of stored cowpeas are discussed in this dissertation.

**OBJECTIVES**

The overall objective of this study was to investigate the feasibility of improving the textural and storage qualities of cowpeas using various heat pre-treatments. The specific objectives studied were:

1. To model and estimate the inactivation kinetics of phytase as a function of heating temperature and moisture content.
2. To determine the adequacy of a finite element model in predicting the degree of phytase inactivation at different locations in a cowpea seed.

3. To design and assess the potential of steam and moisture treatments in preventing the hardening of cowpea seeds during storage under different conditions.

4. To monitor the quantitative changes in phytase activity and other physicochemical indices and determine their contribution to the storage stability of cowpeas.

5. To evaluate the effect of drying conditions applied after steaming on the textural and physicochemical characteristics of stored cowpeas.

REFERENCES


CHAPTER 2

LITERATURE REVIEW

Comparing the Hard-shell and Hard-to-cook Defects

According to Stanley and Aguilera (1985), the textural defects associated with grain legumes falls into one of two categories; either hard-shell or hard-to-cook. In the hard-shell state, seeds do not hydrate enough during soaking and therefore do not become tender when cooked. Alternatively, hard-to-cook seeds hydrate normally during soaking but do not soften within normal cooking times (Shehata 1992). Another distinguishing feature is related to the storage condition under which the defects occur. Hard-shell usually occurs in the northern latitudes where bean seeds are stored at high temperature and low humidity, as opposed to hard-to-cook seeds which are typically associated with storage under high temperature and high humidity (Liu 1995). Thirdly, the hard-shell problem can be eliminated or reversed by the removal of the seed coat or by transferring the seeds to an environment of intermediate humidity (Liu 1995). Since the hard-to-cook defect is not merely due to seed coat impermeability but rather due to biochemical changes occurring in the seed, its reversal is not as simple as removal of seed coat. Hentges and others (1990) however reported that the hard-to-cook defect in dry beans and cowpeas could be reversed by storing the seeds for an additional period at 6.5°C and 71% RH.

Hard-shell seeds are fairly common; of 260 legumes species examined, 85% had varying amounts of impermeable seeds (Shehata 1992). The seed viability and germination rate are reduced in the hard-shell condition, making it a problem also for seed-growers (Rolston 1978).
There are two forms of the hard-shell which occur in legumes. One type is completely reversible and is usually associated with freshly harvested crops whereas the other type is reversible to some extent and develops during storage (Shehata 1992). Mechanical scarification of hard-shell seeds renders them hydratable during soaking and they easily soften with cooking (Shehata 1992). This confirms the suggestions that the hard-shell problem is mainly due to the seed coat being impermeable to water. The reversible hard-shell condition is believed to be caused by a combination of factors including genetics, climatic conditions, crop husbandry, seed size and degree of ripeness (Shehata 1992).

The water impermeability of some *Pisum* sp. is possibly due to the presence of a continuous and very hard pectinaceous layer of the caps of the palisade cells as well as the presence of quinines in a continuous layer of cells around the seed both in the lumen and the cell wall (Werker and others 1979). It has been reported that the lower the original seed moisture content, the more apparent the hard-shell problem becomes after soaking (Deshpande and others 1984; Castellanos and others 1995). Results indicated that common beans with moisture content of 90 g/kg seed readily exhibited the hard-shell problem, however when the moisture content was 120 g/kg seed or greater, all the bean varieties studied showed similar water absorption capacity (Castellanos and others 1995). The decrease in seed coat permeability at low moisture content was thought to be due to reduced pore and fissure sizes in the cuticle layer covering the seed surface (Tang and others 1994).

Rodriguez and Mendoza (1990) reported that the hardness and thickness of the seed coats of hard-shell mung beans were twice that of normal seeds. In addition, the seed coats of the hard seeds had 12% higher fiber content, 7 times more lignin and 23% more silica than the normal seeds. The proximate analysis was comparable except for fiber content which was 9-25%
greater in the hard seeds. They also reported that the amino acid composition and pectic substances content were similar.

The hard-to-cook defect is typically characterized texturally by the restricted softening of the cotyledon after cooking. This has been suggested to be as a result of a lack of cell wall separation due to changes in the lamella-cell wall complex (Rockland and Jones 1974). The critical factors influencing the development of the hard-to-cook defect are seed moisture content, storage temperature and the relative humidity of the environment (Antunes and Sgarbieri 1979, Sefa-Dedeh and others 1979). Unlike hard-shell seeds, the hard-to-cook defect is promoted by storing seeds at high moisture content, typically greater than 13%. A significant deterioration in flavor and texture of beans after 12 months storage at 25°C and 13% moisture content has been reported (Morris and Wood 1956). Although it is believed that the hard-to-cook defect of beans is due to structural changes in the cotyledons, the chemical reactions responsible are not completely understood (Hohlberg and Stanley 1987).

The nutritional quality of seeds that have developed the hard-to-cook defect is significantly lower compared to normal seeds. Antunes and Sgarbieri (1979) reported a decrease in protein quality as well as the availability of essential amino acids. Tuan and Phillips (1992) however observed that although protein digestibility was significantly reduced in the hard-to-cook seeds, starch digestibility was not significantly affected. Significant decreases in the tannin content of the seed coat and an increase in the cotyledon, of three varieties of chickpea was observed by Reyes-Moreno and others (2000) following storage at 33-35°C and 75 % RH for 160 days. In all samples, the levels of phytic acid in cotyledons as well as the in vitro protein digestibility decreased with the seed hardness.
Development of the Hard-to-cook Defect

Various investigators have discussed the deterioration of legume seed quality as a function of time and storage condition. The factors responsible for this change in quality are mainly high levels of seed moisture content, and humidity and temperature of the storage environment. The changes in quality as a result of seed deterioration include increased hardness of the cotyledons, loss of cookability, deterioration of texture and flavor, loss of nutritive value and decreased consumer acceptance (Cunha and others 1993). This deterioration in seed quality has been generally referred to as the hard-to-cook defect and it is mainly characterized by the failure of seeds to soften after a reasonable cooking time. The restricted softening of the cotyledon after cooking has been suggested to be due to insufficient cell wall separation as a result of changes in the lamella-cell wall complex (Rockland and Jones 1974).

The mechanisms through which stored beans develop this hard-to-cook defect are still under investigation. However, various hypotheses have been proposed to explain the cause of the hardening of the bean. These include lignification of the middle lamella, protein denaturation in relation to starch gelatinization, formation of insoluble pectates, lipid oxidation and/or polymerization and the pectin-cation-phytate model (Moscoso and others 1984; Hincks and Stanley 1986; Reyes-Moreno and Paredes-Lopez 1993; Liu 1995).

Non-enzymatic lignification of the middle lamella has been proposed to explain the development of the hard-to-cook defect in legumes (Molina and others 1976). Stanley and Aguilera (1985) suggested that the inability of heat treated beans to germinate indicated a lack of active enzymes; therefore the noticeable increase in hardness of the heated beans following storage under unfavorable conditions strongly argued for a non-enzymatic process. According to Molina and others (1976) cotyledons of beans stored at 25°C showed an increase in the lignified
protein fraction with concomitant increase in hardness values. They reported a high correlation ($r = 0.91$) between the two indices. However, they observed no changes in the amount of lignified protein fraction in either the seed coat or cotyledons of seeds stored at 4°C. Lignin is formed following the oxidation and polymerization of polyphenolic compounds through a process mediated by the enzyme peroxidase and also partly through non-enzymatic means (Hincks and Stanley 1986). The enzyme peroxidase is both highly heat stable and prone to regeneration (Schwimmer 1981).

According to Liu and others (1992) the cell-wall pectin-cation model did not completely account for the development of the hard-to-cook defect, and in addition, results from their study suggested the involvement of intracellular protein and starch interactions. They hypothesized that the defect was partly due to protein insolubilization and thermal destabilization or to reversible denaturation, which occurred during storage under high temperature and high humidity. In their opinion, these changes led to the possible formation of a protein network that resulted in limited starch gelatinization during cooking (Liu and others 1992).

Multiple mechanisms have also been proposed which involved the loss of phytate during initial storage in addition to phenol metabolism during extended periods of storage. Additionally, both enzymatic and non-enzymatic reactions, occurring either together or sequentially leading to increased hardness have been suggested (Hincks and Stanley 1986; Aguilera and Ballivan 1987). Another hypothesis involved the hydrolysis of storage proteins following the observation of the production of lower molecular weight and more reactive proteins during extended storage (Stanley and Aguilera 1985).

A majority of the studies to elucidate the mechanism of the hard-to-cook defect in legumes have focused on changes in pectin and phytate concentration during soaking and
cooking (Jones and Boulter 1983a; Shehata and others 1985; Hentges and others 1991). Overall, there seems to be a general consensus that cell wall pectin is a key element in the hardening of legumes during storage. It is believed that during storage, soaking and/or cooking, pectin exchanges its bound monovalent cations with divalent cations released from phytates. This results in the insolubilization of the cell wall pectins, which has an overall effect of strengthening the cell wall (Liu and others 1993). In their study, Liu and others (1993) observed that pectin loss increased slightly after soaking but decreased after cooking and also that it may have occurred mainly through the $\beta$-elimination reaction. Mattson (1946) was the first to suggest a relationship between the increased hardness of stored legumes and phytate content after the observation that hard-to-cook peas contained 50% less phytate than fresh peas. He proposed that in fresh or control peas, phytate chelated Ca and Mg ions and reduced the formation of cross-links between Ca and/or Mg and pectate in the middle lamella. During storage under high temperature and humidity, phytase is activated and hydrolyzes phytic acid. This hydrolysis decreases the chelating potential of the phytic acid and enhances cross-linking of pectic substances in the middle lamella. The Ca and Mg pectates that are formed do not dissolve readily when subjected to heating and therefore restricted cell separation leading to a significant increase in cooked bean hardness.

Jones and Boulter (1983a, b) proposed that the enzyme pectin methylesterase (PME) was also involved in the phytate-pectin mechanism. They suggested that in conjunction with the hydrolysis of phytic acid occurring within the cell, PME hydrolyzed pectin in the middle lamella to pectinic acid and methanol. Magnesium then migrated from within the cell to bind with pectinic acid in the middle lamella to form magnesium pectinate. This hypothesis was supported by an apparent decrease in pectin solubility, pectin esterification and phytic acid concentration in
seeds that had developed the hard-to-cook defect. Similar results have been reported by other investigators (Moscoso and others 1984; Vindiola and others 1986; Hentges and others 1991).

Aguilera and Rivera (1992) noted that an impediment to understanding the mechanism of the hardening of dry beans was due to the complexity associated with in situ determination of biochemical changes.

**The role of phytase/phytic acid in the development of the hard-to-cook defect**

A dual-enzyme mechanism involving phytase and PME has been proposed. It is hypothesized that the action of phytase on phytic acid releases phosphate, magnesium and calcium ions within the cotyledons cells. This occurs in tandem with the hydrolysis of pectin by PME producing pectinic acid and methanol within the middle lamella (Mafuleka and others 1993). The calcium and magnesium ions thus formed traverse the cotyledons to the middle lamella to form calcium and magnesium pectates that subsequently restrict cell separation during cooking. Several studies have examined the relationship between the development of the hard-to-cook defect and phytate content. Kon and Sanshuck (1981) observed a negative correlation between the phytic acid/calcium ratios and cooking time in dry beans. They further reported that infiltrating these slow cooking beans with phytic acid resulted in a reduction in cooking time to values comparable to that of the control sample.

A low but significant correlation was observed between losses in phytate content and cooking time in black beans leading to the suggestion that phytate was a contributor but possibly not the sole operating mechanism in the hardening defect (Hincks and Stanley 1986). Hincks and Stanley (1986) monitored the phytase activity in regular dried and heat treated black beans during storage under two conditions: high temperature, high humidity and low temperature, low
humidity. The samples stored under high temperature and high humidity conditions generally displayed increasing activity over the storage period. In low temperature and humidity storage there was an initial drop in activity followed by an increase in activity to original levels. They suggested that the lower activities observed in the heat treated samples indicated a partial denaturation or disruption of the enzyme, however since phytase activity increased over time under both storage conditions it implies the enzyme was still active and eventually recovered.

Mafuleka and others (1993) recorded elevated phytase activities in decorticated Malawian red and white bean genotypes stored at different temperatures, water activities and for varying time periods. A strong positive correlation between phytase activity and cooked white bean hardness after storage for 4 and 8 months was recorded. They concluded that the mechanism involving phytic acid hydrolysis by phytase seemed to be the dominant system influencing the hard-to-cook defect in white beans during storage for 0-8 months. Longe (1983) reported that phytic acid was the only chemical component among four others that showed a correlation with the cooking time of 13 varieties of cowpeas.

Bernal-Lugo and others (1990, 1991) obtained results that contradict the above studies. The role of phytic acid in the softening of cooked fresh beans was investigated by measuring its diffusion during cooking (Bernal-Lugo and others 1991). They observed no leaching of phytic acid into the cooking liquor or a change in solubility in either of the two varieties of beans studied. Their conclusion was that phytic acid did not appear to be the chelating agent during the thermal softening of beans. Bernal-Lugo and others (1990) monitored changes in cooked hardness, phytic acid, phytase activity and integrity of protein bodies in two bean cultivars stored for 33 days at 75% RH and 41°C. Results indicated that the Michigan cultivar showed a higher tendency to harden even though its initial and final phytic acid contents were higher than the Ojo
de cabra cultivar. Bernal-Lugo and others (1990) therefore concluded that neither phytate content nor its rate of hydrolysis played a major role in the development of the hard-to-cook defect in stored beans.

**Prevention and Reversal of the Hard-to-Cook Defect**

Due to the economic and nutritional losses associated with the development of the hard-to-cook defect, several methods have been investigated to determine their usefulness in reversing the hard-to-cook defect in legumes. The most effective method for preventing the defect is storage under controlled conditions of low moisture, temperature and humidity. However, this is not always a practical approach especially in tropical countries where the majority of the problem is manifested due to cost and possibly lack of the requisite technology.

There is a general consensus that the storage quality of beans is significantly affected by the moisture content, storage temperature, storage humidity and storage time. High moisture content usually above a critical value of 13% greatly accelerates the hardening process (Aguilera and Stanley 1985). Morris and Wood (1956) reported no changes in flavor, lipid acidity and texture of beans with 10% moisture content stored at 25°C for 2 years. The importance of low temperature and humidity was demonstrated by Berrios and others (1999) who reported that common beans maintained their quality when stored at temperatures of 20°C or lower and relative humidity of less than 75%. Several investigators have all reported that low temperature storage (0-20°C) resulted in practically no textural changes in stored beans (Burr and others 1968; Molina and others 1976; Garruti and Bourne 1985). Hentges and others (1990) showed that the defect was not a permanent condition as generally presumed. They observed that seeds which had undergone the hardening process could be softened by storing at 6.5°C/71%RH.
Molina and others (1976) employed heat in developing a simple process to reduce textural defects in stored black beans. When the samples were subjected to the treatment (retorting or heating under atmospheric steam), it was found that hardness was retarded during 9 months of storage at 25°C and 70% RH. However, these samples were still relatively harder than similarly treated samples stored at 4°C. In general, brief heat treatments of legumes have all produced an immediate increase in hardness. This is thought to be due to thermal activation of phytase and/or a case hardening with concomitant reduction in water imbibition (Aguilera and Stanley 1985).

Aguilera and Steinsapir (1985) investigated the feasibility of using a variety of heat treatments – irradiation, high temperature-short time roasting and medium temperature-long time heating as a means of reversing or preventing the hard-to-cook defect. They reported that although the pretreatments were not fully successful in preventing seed hardening during storage, some treatments resulted in seeds with significantly lower hardness values as compared to the control.

According to Aguilera and Rivera (1990) storage under controlled atmospheres of CO₂ and N₂ had no major effect on the hardness of both roasted and untreated beans. They also noted that beans stored in packages impermeable to moisture losses hardened at a rate far lower than that of beans stored in conventional woven polypropylene bags. Berrios and others (1999) monitored changes in selected physical and chemical characteristics of black beans after 2 years storage under different conditions. Results from their study indicated that storage under refrigerated hypobaric conditions (4.5°C, 50-60% RH and at atmospheric pressure of 125 mmHg) resulted in seeds which were comparable to fresh beans in terms of cooking time, amount of solids leached during soaking and percentage of hard-shell. However, beans stored
under ambient conditions (23-25°C, 30-50% RH) showed increases in all measured indices. Overall, the beans stored under ambient conditions exhibited changes in quality indices characteristic of hard-to-cook beans. The storage condition did not alter the electrophoretic pattern of the main protein, globulin G1. They concluded that refrigeration and hypobaric storage could be used to maintain the quality of beans up to 2 years after harvest.

Cunha and others (1993) also investigated the possibility of preventing the hard-to-cook defect in dry beans by treating with gamma rays and microwaves prior to storage. They however reported that the pretreatment was not successful in preventing the beans from hardening. Other methods used to prevent seed hardening include chelation, which involves the removal of divalent cations and replacement with monovalent cations; and use of quick-cooking beans (Aguilera and Stanley 1985). Chelating agents such as ethylenediaminetetra-acetic acid (EDTA) capable of removing calcium from calcium pectate have been used to reverse the defect. Kon and Sanshunck (1981) observed that soaking hard-to-cook beans in phytic acid or EDTA reduced cooking time such that previously hard-to-cook beans were fully cooked in the same time as a control sample.

**Phytase Activity and Sources**

The phytases (*myo*-inositol hexakisphosphate 3- and 6-phosphohydrolases) are a subfamily of high molecular weight histidine acid phosphatases that catalyze the hydrolysis of phytic acid to inositol and free orthophosphate (Liu and others 1998; Viveros and others 2000). The main end products of the reaction are phosphoric acid and *myo*-inositol with intermediate compounds such as phosphotidyl inositols representing various degrees of dephosphorylation from inositol.
hexakisphosphate to inositol (Liu and others 1998). The enzyme is widely distributed both in plants and animals as well as various fungi and bacteria species (Cosgrove 1966).

Phytases are classified into two groups: 3-phytases and 6-phytases, depending on the method of degrading inositol hexakisphosphate (i.e. site of initial attack of the susceptible phospho-ester bond) and the subsequent products formed. Dephosphorylation involving the 3-phytases is initiated at the 3 position of the inositol ring resulting in the formation of 1,2,4,5,6-pentakisphosphate and inorganic phosphate ($P_i$). Alternatively, 6-phytases attack the 6 position forming the 1,2,3,4,5-pentakisphosphate and $P_i$. Another difference between the two enzyme classes is that the action of 3-phytases does not always result in complete dephosphorylation whereas that of 6-phytase does (Angel and others 2002). In general, the 3-phytase type is characteristic of phytases derived from microorganisms whereas those obtained from seeds of higher plants are mainly 6-phytase (Cosgrove 1966). However, 3-phytase activity was observed in a purified phytase fraction from lupine seeds and 6-phytase activity has also been reported in *Paramecium* and *E. coli* (Greiner and others 2002). According to the enzymatic nomenclature recommended by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB), these two enzymes are classified as EC 3.1.3.8 and 3.1.3.26 respectively (Cosgrove 1966).

Phytases have generally not been fully characterized biochemically (Wyss and others 1999). They are naturally found in microorganisms, particularly fungi, and in a number of seeds including cereals, legumes, byproducts and other feedstuff (Eeckhout and DePaepe 1994). Phytases are generally thermostable enzymes active in a broad pH range (Dvorakova 1998). Phytase activity has been extensively studied in a variety of plant seeds including barley, bean, corn, wheat, lettuce, pea, potato, mungbean, rice, soybean, sorghum and navy beans. Activity
has also been measured in some animal tissues specifically calf liver and calf blood, however, it has not been successfully identified/measured in the blood of mammals including humans. Fungi specifically *Aspergillus* accounts for the majority of the activity in microorganisms although bacteria, yeasts, soil microorganisms and micorrhizal microorganisms also exhibit phytase activity.

Conflicting reports are present in the literature concerning the presence of phytase activity in dry plant seeds. According to some authors, phytase activity is absent in dry seeds appearing only during germination (Mandal and others 1972; Walker 1974). However, other studies have found the presence of the enzyme in dry seeds with an increase in activity during germination (Paul and others 1970; Yoshida and others 1975). Maga (1982) reported that although phytase is present in mature seeds, it appears to have little effect on phytate in dry or dormant seeds. Glass and Geddes (1959) however observed an increased level of inorganic phosphate along with lower phytate levels in wheat stored under elevated temperature and moisture conditions. Based on this finding, they proposed that the level of inorganic phosphate could be used to indicate adverse storage of wheat. Studies have shown a decrease in phytate content of germinating seeds with a corresponding increase in phytase activity and concentration of inorganic orthophosphate. There are two opposing views on the cause of the increase in activity during germination. One school of thought proposes that the increase is due to *de novo* synthesis of phytase during germination whereas the other results point to the activation of pre-existing phytase, and in some cases there is evidence against *de novo* synthesis. Nayini and Markakis (1986) however, concluded that seeds contain both constitutive and germination-induced phytases.
Phytase activity is largely determined or affected by environmental conditions under which the measurements are made. The conditions that result in optimum activity for extracted phytase, may not necessarily be the same as those under which phytate is degraded in the whole seed. This is because the latter situation depends on accessibility between enzyme and substrate which is also affected by pH and microstructure of the seed. Some phytases have been characterized based on the optimum pH and temperature necessary for activity including phytases from lupine, soybean, faba bean, oat and barley (Greiner 2002; Gibson and Ullah 1988; Greiner and others 2001; Greiner and Alminger 1999; Greiner and others 2000). In general, phytase show a remarkable thermal stability and are active within a wide pH range. The optimum pH for activity varies depending on the origin of the enzyme with microbial phytases exhibiting optimum activity between pH of 4.5 – 7.5. Phytase from plant sources have pH optima between 4.0 and 5.6 (Dvorakova 1998). However, an alkaline phytase with a pH optimum of 8 was extracted from legume seeds (Scott 1991) and mature lily pollen (Scott and Loewus 1986).

The temperature dependency of phytases has also been determined. Enzyme activity is maximized at temperatures ranging from as low as 37°C for Klebsiella sp. to 77°C for Schwanniomyces castelli (Dvorakova 1998). The optimum temperature for plant phytases is typically located in the middle of this range (about 55°C). Ferreira and others (1998) observed that the thermal stability and temperature optimum of four (4) cytoplasmic isoforms of soybean phytase were dependent on the presence and type of substrate added.

There are several publications on the purification and characterization of phytases from cereals and microbial sources, however, not much is known about phytases from legume origins. Currently data is available on phytases purified from soybean (Gibson and Ullah 1988), faba
beans (Greiner and others 2001), mungbean (Mandal and others 1972), lupine (Greiner 2002) and scallion leaves (Phillippy 1998). Greiner (2002) purified 3 phytases from germinated lupine seeds. They were characterized as monomeric proteins with molecular masses of 57, 57 and 62 kDa, optimum temperature and pH of 50°C and 5 respectively. The phytases are strongly inhibited by substrate levels greater than 8 mmol/L and by its reaction end product, inorganic phosphate (Schwimmer 1981). Multiple forms of phytases have been identified from plant sources including barley (Greiner and others 2000), wheat (Nagai and Funahashi 1962; Nakano and others 1999), rapeseed (Houde and others 1990), soybean (Hamada 1996) and maize (Hubel and Beck 1996).

In most cases, phytase from legume sources behaves like a single chain or monomeric protein of molecular mass approximately between 57 to 160 kDa. They are not metalloenzymes since their activities are not significantly enhanced by the presence of metal ions with some ions having a slight to significant inhibitory effect (Greiner 2002). Additionally, enzyme activity was inhibited by the presence of Cu, Zn, fluoride, phosphate, molybdate and vanadate. Kuvaeva and Kretovich (1978) reported that phytase existed as a single molecular form in dry pea seeds. However, during germination a second molecular form was synthesized de novo.

Phytases generally show a wide specificity for substrate though the highest affinity is for phytate. Activity is inhibited by both excess substrate and reaction products usually inorganic monophosphate. The degradation of phytate occurs in a stepwise manner with the removal of a phosphate group resulting in the formation of a myo-inositol intermediate which then becomes a substrate for further hydrolysis. Phytase activity is typically estimated by measuring the amount of inorganic phosphate released during incubation of the plant material as whole flour or by extracting the phytase. Greiner and Egli (2003) concluded that the determination of phytate-
degrading activity by quantification of the liberated inorganic phosphate was more robust and precise than quantifying residual phytate content.

Earlier studies had reported that phytase was readily inactivated (partially or totally) at temperatures of 70°C or higher (Peers 1953; Pointillart 1988; Jongbloed and Kemme 1990). Ma and Shan (2002) however observed a high thermal stability of phytase in 3 cereal seeds. After heating for 1 hour at 100°C, rye2, wheat NEAU123 and triticale 5305 retained 104.6, 89.5 and 86.4% respectively of their original phytase activity. The conflicting results could be due to differences in heating conditions since phytase is more stable in dry conditions than in moist conditions.

**Phytic Acid in Plant Food Products**

Phytic acid or *myo*-inositol 1,2,3,4,5,6-hexakisphosphate is the principal storage form of phosphate in cereals and legumes, and is usually deposited together with proteins and minerals in aleurone storage vacuoles (Raboy 1990). Despite being the main storage form of phosphorus in cereals and legumes, phytate is considered an antinutritional factor since it binds minerals such as zinc and iron. It is considered the single most important antinutritional factor for the availability of minerals particularly iron, calcium and zinc. Phytic acid contains 70-80% of all bound seed phosphorus. It occurs naturally as a salt with monovalent and divalent cations and as phytate. Phytate occurs more commonly as the calcium salt, the main exception being associated with sesame seeds where the Mg phytate form is found (O’Dell and Boland 1976). Phytin refers to a Ca-Mg salt of phytic acid whereas phytate implies the mono to dodeca anion of phytic acid (Maga 1982). It possesses several physiological functions during hydration and germination and chelates a variety of minerals including Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Mg$^{2+}$, Fe$^{3+}$ and Ce$^{2+}$ forming phytate-
mineral and/or protein-mineral-phytate complexes which are not readily absorbed in the intestinal tract (Liu and others 1998). Excessive consumption of dietary phytate leads to mineral deficiency symptoms (Maga 1982). Thus the degradation of phytate by phytase for example is of nutritional significance.

The cation chelating properties of phytate serves an important role in the softening of legumes during cooking. During cooking phytate exchanges its monovalent cations for divalent cations hydrolysed from pectin. Cereals and legumes contain up to approximately 5% by weight of phytate (Boland and others 1975; Maga 1982). Hentges and others (1991) reported a value of 0.06-0.97 g/100g phytate in cowpeas stored for about 24 months.

Phytic acid is dephosphorylated by the action of phosphatase enzymes or phytases and also by heating in acid or alkaline solutions. According to Maga (1982) phytate is fairly heat stable, although this stability appears to be dependent on the source and possibly structural differences. Destruction of a large proportion of soy isolate phytate occurred only after prolonged autoclaving (4h at 115°C) whereas only 20% or the phytate content of sesame meal was degraded (Lease 1966; Rackis 1974). Moist heat hydrolyses phytate to different end products based on the severity. Soybean flakes heated for 2h resulted in the formation of the penta-phosphate form. After further heating for 4h, there was an increase in the tetra-phosphate level and approximately equal amounts of the penta- and hexa-phosphate forms (Boland and others 1975).
**Enzyme Inactivation Kinetics**

A kinetic model of the activity of an enzyme is useful in combination with a process model to design or optimize the heat treatment of foods. The reaction rate of most heat inactivation processes at constant temperature follows a first order reaction according to the equation:

\[ N = N_0 e^{-kt} \]  

where \( N \) – actual level of a quality attribute, \( N_0 \) – initial level of the quality attribute, \( k \) – reaction rate constant at constant temperature (T) and \( t \) – processing time (Hendrickx and others 1995).

Two parameters are needed to characterize the thermal stability of a given enzyme. One is the rate of inactivation at a specified temperature, expressed either as a rate constant or a D value, and the other is a measure of how the rate of inactivation varies with temperature, given either by the activation energy (Ea) or a z value. With these two parameters the rate of enzyme inactivation at any temperature and accordingly the expected level of residual activity remaining after a given heat treatment can be calculated (Anthon and Barrett 2002).

The rate constant is usually a function of both intrinsic (e.g. pH), and extrinsic (temperature) factors, and also interactions between the two (Hendrickx and others 1995). The effect of temperature on the rate constant is expressed by the Arrhenius equation:

\[ k = k_o e^{-E_a / RT} \]  

where \( k \) - rate constant, \( k_o \) - pre-exponential factor, \( E_a \) – activation energy, \( R \) – universal gas constant, \( T \) – absolute temperature (K).

The activation energy is generally derived from the slope of the plot of ln k vs. 1/T and depends on composition factors such as water activity, moisture content, solid concentration, pH and others (Saguy and Karel 1980). Additionally, the activation energy may vary substantially when the reaction mechanism changes with temperature.
The inactivation rate constant has been found to be very dependent on moisture content of the sample under consideration. DiPetro and Liener (1989) observed that the rate of inactivation of trypsin inhibitors in soy flour at 95°C was significantly influenced by the moisture content (5-15%) of the flour. Similar findings were reported by Buera and others (1984) who studied the inactivation kinetics of trypsin inhibitors in *Phaseolus vulgaris* beans. They reported that the rate constant reached a maximum value at moisture content of 30% (dry basis).

Micro-environmental factors such as the relative amount of free and bound water, substrate binding, pH and presence of salts have a significant effect on the heat stability of enzymes (Adams 1991). Ponne and others (1996) observed a strong dependence of the inactivation kinetics on the physical environment. They reported that the rate of inactivation of lipase at the reference temperature (90°C) was 5-50 times higher in the aqueous environment (rapeseed extract) as compared to the seed matrix. Van Zuilichem and others (1993) also found an increased heat resistance during the inactivation of trypsin inhibitor in soy bean with low moisture content (13%) as compared to beans with higher moisture content (43%) (cited in Ponne and others 1996). Meerdink (1993) gave an overview of models that described the dependence of the inactivation constant k of enzymes on temperature and water concentration. The activation energy of enzyme inactivation (E) and the rate constant (k) increased at higher water contents and resulted in increased temperature sensitivity to enzyme inactivation. However, most models described in literature are not usually built on fundamental knowledge of enzyme inactivation mechanisms at different water contents (Ponne and others 1996). Usually, the variation of the inactivation rate at high temperatures follows the empirical law of Arrhenius.
It has been previously indicated that the inactivation of enzymes can be described by a first order kinetic model and the inactivation rate constant (k) can be estimated using linear regression analysis according to equation (1). Ly-Nguyen and others (2002a) described a special case of the first order model known as the fractional conversion model, (f). This is simply the first order reaction modified using the fractional conversion technique which accounts for the nonzero activity \( A_{\infty} \) after prolonged heating. The fractional conversion model applies when the enzyme under study contains a stable fraction that is not affected by the processing conditions (Ly-Nguyen and others 2003). The model can be expressed as

\[
f = \frac{(A_0 - A_i)}{(A_o - A_{\infty})}
\]

For most irreversible first order reactions, \( A_{\infty} \) approaches zero, and Eq. 3 can be reduced to

\[
f = \frac{(A_0 - A_i)}{A_o}
\]

A plot of the logarithm of \((1-f)\) versus time results in a straight line with a rate constant expressed by the negative slope value:

\[
\ln\left(\frac{A_i}{A_o}\right) = \ln(1 - f) = -kt
\]

Thus, when \( A_{\infty} \) approaches zero, eq. 1 and eq. 5 are identical.

To account for the non-zero activity after prolonged heating, the following form of the fractional conversion is employed:

\[
\ln(1 - f) = \ln\left[\frac{(A_i - A_{\infty})}{(A_o - A_{\infty})}\right] = -kt
\]

Rearranging eq. 6 yields eq. 7

\[
A_i = A_{\infty} + (A_o - A_{\infty})\exp(-kt)
\]
By plotting $A_t$ versus inactivation time at constant temperature conditions, the inactivation rate constant, $k$ and the remaining activity, $A_{\infty}$ can be estimated using nonlinear regression analysis. A non-linear estimation is made using the sum of square of the relative error between measured and calculated enzyme activity as the optimization criterion.

The rate constants determined are re-plotted in Arrhenius plots and the activation energy ($E_a$) calculated from the slope according to eq. 8:

$$\ln(k) = -\left(\frac{E_a}{RT}\right) + c$$

(8)

The inactivation rate constant at a reference temperature ($k_{\text{ref}}$) can be determined from the value of $\ln(k_{\text{ref}})$ at this temperature given by the regression line in the Arrhenius plot. The inactivation rate constant at any temperature ($T$) can then be calculated using the two known parameters, $E_a$ and $k_{\text{ref}}$ from eq. 9:

$$\ln(k) = \ln(k_{\text{ref}}) - \frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)$$

(9)

The fractional conversion model has been applied to the inactivation kinetics of PME from carrots and strawberries (Ly-Nguyen and others 2002a; 2002b; 2003).

**Chemical and Physical Changes induced by Drying Processes**

One of the most widely practiced food preservation method is drying in which water is removed or reduced to very low levels. This eliminates microbial deterioration and minimizes chemical and physical changes during storage (Singh and Heldman 2001; Lewicki and Jakubczyk 2004). In general, the drying process involves a simultaneous heat and mass transfer. The driving force for heat transfer is the temperature gradient between the surface of the material and ambient air, whereas the moisture gradient between the interior and surface of the food drives the mass
transfer (Prothon and others 2003). Removal of water using hot air proceeds by means of convective evaporation resulting in a variety of physical and chemical changes in the particular plant tissue (Lewicki and Jakubczyk 2004; Lewicki 1998). These changes following the removal of water include destruction of natural structures, loss of semi-permeability of membranes, increased rigidity and mechanical strength of outer layers of the dried material and protein aggregation (Lewicki and Jakubczyk 2004; Lewicki and others 1997; Chapman 1994).

According to Krokida and Maroulis (1997) the quality of dehydrated products and the corresponding changes in physicochemical characteristics are influenced or determined by the method and variables of drying. Various studies have focused on the changes, beneficial and detrimental, occurring after drying or application of high temperatures to different food products. The results of a few of these studies are reviewed below.

High temperature pre-decortication treatment of cowpeas was found to be very effective in aiding seed coat removal; however it resulted in unfavorable or undesirable changes in seed functionality in food applications (Hudda 1983). Hung and others (1988) observed that the use of a pre-decortication drying temperature resulted in more brittle cowpea seeds as compared to the control. The drying temperature especially high temperature also significantly affected the quality of products (akara) prepared using the treated cowpea seeds. The most significant change was observed in the microstructure of the cowpea seeds where the severe heat treatment resulted in damages to the middle lamella (Hung and others 1990). Additionally, changes in birefringent properties of starch granules and decreases in the amount of air incorporated into the whipped paste were reported.

The effect of steaming time, drying temperature and humidity on water absorption capacity, dehulling efficiency and cooked bean hardness of cowpea seeds was investigated by
Komey (1999). Steaming resulted in decreased water absorption capacity, improved dehulling efficiency and increased cooked bean hardness in all samples. On the other hand, increased water absorption capacity, improved dehulling efficiency and decreased cooked bean hardness were recorded with increasing drying temperature and humidity.

Enwere and others (1998) evaluated the effect of drying temperature on the functionality of cowpea seeds used in the preparation of akara. Changes in starch content, water extractable proteins as well as microstructural observations of cowpea cotyledons, flour, starch and akara crumb were investigated. They reported the absence or decrease in quantity of protein fractions observed in samples dried at 30°C as compared with samples dried at 80 or 120°C. High temperature drying caused the loss of starch granules, protein matrix or even entire cellular contents in the cowpea cotyledons whereas the only differences noted in the flour samples were in the size and shape of starch granules, protein particles and cell wall material.

Hung (1989) studied the effect of drying conditions on equilibrium moisture content (EMC) and textural properties of roasted peanuts. He reported that high drying temperature resulted in lower EMC of peanuts and lower shear-compression force and energy.

Champagne and others (1998) monitored the differences in texture profile analysis of cooked rice following post-harvest processing. They observed that only cohesiveness was significantly impacted by the drying conditions employed. Drying at lower temperatures (ambient or 18°C) resulted in less cohesive samples as compared to rice dried at a higher commercial temperature. This was attributed to the development of fine, hair-line fissures at high temperatures which allows quicker absorption of water during cooking.

Changes in the ultimate tensile strength and modulus of elasticity of two parboiled rice varieties were evaluated as a function of steaming time, drying temperature and post-drying
tempering conditions (Saif and others 2004). It was reported that the ultimate tensile strength and modulus of elasticity significantly increased with increasing steaming time and decreased at drying temperatures greater than 40°C.

Del Caro and others (2004) investigated the changes in the main chemical constituents of two varieties of stored prunes after low (65°C) and high (85°C, then 70°C) temperature drying. The parameters measured were ascorbic acid and polyphenol content, and antioxidant activity. Ascorbic acid content was better retained in samples dried at the lower temperature as compared to the high temperature throughout the storage period. Although the drying temperature had a significant effect on the polyphenol content, the effect was largely dependent on the class of polyphenol (anthocyanins, cinnamates or flavonols) measured. Antioxidant activity was higher in the samples dried at the higher temperature for one of the prune varieties studied, whereas the other variety showed no significant difference in the effect of drying temperature.

A three-stage drying method at 38-50-38°C reduced the overall drying time of ginseng roots by 40% relative to the time required under conventional drying procedures at a maximum temperature of 38°C (Davidson and others 2004). In general, there were no significant differences in the effect of the two drying methods on quality changes such as color and ginsenoside concentrations.

The temperature of the drying air, volume of air, sample weight and pre-treatments all significantly affected the drying characteristics and quality of pea samples dried in a fluidized bed dryer (Kaur and Bawa 2002). Losses in vitamin C content were directly proportional to the drying temperature used and in addition, the equilibrium moisture content was dependent on the pre-treatments and drying temperature.
The above results underscore the importance of regulating or monitoring the drying process used for any food product. According to Singh and Heldman (2001), the structural configuration of fruits and vegetables require that the drying process must be accomplished in a fashion that results in the least detrimental changes in product quality.

Applications of the Finite Element Method in Food Processing

The appropriate processing parameters for the thorough analysis of a food process may be determined using mathematical modeling. This helps in reducing the number of experiments required to understand the influence of processing variables on the final products and therefore saves time, cost and effort (Bakshi and Chhinnan 1984). The finite element method (FEM) is a numerical procedure employed for the solution of differential equations using minimizations (Segerlind 1976; Hong and others 1986), and has been applied in various fields including structural analysis, fluid mechanics and heat transfer (Segerlind 1976).

FEM is based on the concept that any continuous factor such as temperature, pressure or displacement, can be approximated by a discrete model composed of a set of piecewise continuous functions defined over a finite number of sub-domains (Segerlind 1976). The finite element method involves dividing the region of interest into small elements; typically, the triangle is the shape of choice for 2-dimensional elements though other shapes including rectangles and quadrilaterals are also used (Baerdemaeker and others 1977). According to Segerlind (1976) the following steps are required to construct a discrete model:

1. A finite number of points, called nodal points or nodes, in the domain are identified.
2. The value of the continuous factor at each node is denoted as a variable and determined.
3. The domain is divided into a finite number of sub-domains called elements which are connected at common nodal points and collectively approximate the shape of the domain.

4. The continuous quantity is approximated over each element by a polynomial equation that is defined using the nodal values of the continuous quantity. Although a different polynomial is defined for each element, they are selected such that continuity is maintained along the element boundaries.

Puri and Anantheswaran (1993) conducted a review of the applications of the finite element method in food processing. They observed that it has been used successfully in a variety of processing operations. The studies could be broadly classified into heating and cooling, heat and mass transfer, and freezing and thawing. They also suggested areas for future applications including extrusion, microwave heating, aseptic processing and optimization of food quality. A few of the food applications of the finite element method are summarized below.

Ngadi and others (1997) used the finite element method to model moisture transfer in a chicken drum during deep fat frying. Minimizing moisture loss from the food during deep fat frying is a key factor in maintaining the quality. They used quadrilateral elements to solve the moisture content equation in the muscle, bone, bone marrow and cartilage. The predicted values compared favorably with experimental data. Additionally, the simulated model revealed a region of rapidly decreasing moisture content near the surface of the chicken.

FEM was used to model the heat transfer in meat patties during single-sided pan frying (Ikediala and others 1996). The symmetrical half portion of the patty was discretized into 4-node quadrilaterals yielding 209 nodes and 180 elements. They reported a close agreement between the predicted and observed temperature profiles during pan frying at 140 and 180°C
with standard deviations of between 0.59-3.24°C. They indicated that the rate of moisture loss, cooking time and crust formation were all a function of pan temperature and/or turn-over frequency and time.

The heat and mass transfer occurring during steaming of cowpea seeds was modeled using FEM by Fang and others (2003). They divided a longitudinal section of cowpeas into 3 layers which were further divided into 3-node linear triangular elements to obtain a total of 203 elements. The numerical solution for temperature and moisture content during steaming and in each layer was then estimated. They reported that the predicted values were comparable to the experimentally measured data.

Jiang and others (1987) also used FEM to study the pre-cooling of broccoli stalks. Pre-cooling vegetables is an important step in maintaining their freshness. They applied a quadratic quadrilateral element to generate cooling curves. They observed that the difference between predicted and measured values was within 1.1°C, however, the predicted values deviated from measured values in the lower part of the stalk.

A quantitative methodology using FEM was developed to evaluate the thermal process schedules for low-acid foods containing particulates of any shape (Sastry 1986). The effect of particle size, residence time distributions with the heat exchanger and holding tube, and convective heat transfer coefficients on temperature distribution was investigated. He reported that all the factors had significant influences on the thermal process required to attain commercial sterility. Additionally, comparable results for measured and predicted cold-spot temperatures during processing of mushrooms at 121°C in a can were recorded.

Hong and others (1986) developed a finite element model capable of predicting the moisture transfer in mixed food products during storage. The texture, storage stability and
susceptibility to microbial spoilage of food products are largely determined or influence by the moisture content. In addition, the final moisture content of each component of a multi-component product will affect the quality and shelf-life of the product. In developing their model, it was assumed that the limiting factor determining the rate of moisture transfer was the component with the lowest effective diffusion coefficient divided by the square of half of the smallest dimension. Raisins were therefore judged to be the rate limited component and their geometry was divided into 63 nodes and 48 elements. They found that the predicted volume average moisture content was in satisfactory agreement with experimental values.

The extensive applications of finite element method in foods and other areas of study are mainly due to the advantages associated with its use. These advantages as outlined by Segerlind (1976) and Puri and Anantheswaran (1993) include:

1. The method can be applied to bodies composed of several materials.
2. The element sizes can be varied and therefore be expanded or refined as needed.
3. Irregular regions can be modeled with greater accuracy.
4. Mixed boundary conditions are easily handled.

REFERENCES


CHAPTER 3

HEAT-MOISTURE TREATMENTS OF COWPEA FLOUR AND THEIR EFFECTS ON PHYTASE INACTIVATION

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3.1 ABSTRACT

Samples of finely ground cowpea flour with moisture content adjusted to 10, 25, 35\% (dry basis) were heated in sealed retort pouches at 70-95 °C for periods of 2 to 32 min. Phytase showed a high thermal resistance with residual activity ranging between 50 and 95\%. Thermal inactivation of cowpea phytase was adequately described by a fractional conversion model based on a first order rate equation. Overall, increasing temperature and initial moisture content resulted in increased enzyme inactivation. Estimated activation energies between 70 and 95 °C were 33.3, 37.9, and 43.4 kJ/mol at 10, 25, and 35\% moisture, respectively. The kinetic models generated were successfully used to predict phytase activity in cowpea flour.

Keywords: phytase, cowpeas, inactivation kinetics, moisture content, fractional conversion
3.2 INTRODUCTION

Cowpeas are an important source of protein in the diets of many populations around the world providing a less expensive alternative to animal protein. Despite the high nutritional quality of cowpeas and other legumes, limitations such as the hard-to-cook defect decrease consumption and production. The basis of the defect is still under debate; however, a dual-enzyme mechanism involving phytase and pectin methylesterase has been suggested (Jones and Boulter 1983). It is hypothesized that phytase acts on phytic acid in the cotyledon releasing inorganic phosphate, magnesium and calcium ions whiles pectin methylesterase hydrolyzes pectin to pectinic acid and methanol in the middle lamella (Mafuleka and others 1993). The calcium and magnesium ions move to the middle lamella forming calcium and magnesium pectates that subsequently restrict cell separation during cooking.

The hard-to-cook defect is mainly characterized by a significant increase in the time required to achieve softening of the cotyledons during cooking. Several studies have examined the relationship between the development of the hard-to-cook defect and phytate content. Kon and Sanshuck (1981) reported a negative correlation between the phytic acid/calcium ratios and cooking time in dry beans. A low but significant correlation \((r = -0.716)\) was observed between losses in phytate and cooking time in black beans suggesting that phytate is a contributor but perhaps not the sole operating mechanism in the hardening defect (Hincks and Stanley 1986). Longe (1983) also reported that phytic acid was the only chemical component among four others that showed a correlation with the cooking time of 13 varieties of cowpeas. Mafuleka and others (1993) recorded increased phytase activity and a strong positive correlation with cooked texture in decorticated Malawian red and white bean genotypes stored under different temperature, water activity and time periods.
Phytases are a subfamily of high molecular weight histidine acid phosphatases which catalyzes the hydrolysis of phytic acid to inositol and free orthophosphate (Liu and others 1998; Viveros and others 2000). The main end products of the reaction are phosphoric acid and myo-inositol with intermediate compounds such as phosphotidyl inositols representing various degrees of dephosphorylation from myo-inositol 1,2,3,4,5,6-hexakisphosphate to inositol (Liu and others 1998). The enzyme is widely distributed both in plants and animals as well as various fungi and bacteria species (Cosgrove 1966). Phytases are generally thermostable enzymes with pH optima between 4.0 and 5.6 (Dvorakova 1998). Although Jongbloed and Kemme (1990) previously showed that phytase was readily inactivated at temperatures of 70 °C or higher, Ma and Shan (2002) reported a high thermal stability in 3 cereal seeds. After heating for 1 h at 100 °C, rye2, wheat NEAU123, and triticale 5305 retained more than 80% of their original phytase activity.

Based on the reported relationship between phytase activity and cooking time of various legumes, we are hypothesizing that inactivating phytase could be used as a strategy in controlling the hard-to-cook defect. There is however a scarcity of information on the behavior of phytase following heat treatment. Thus understanding the inactivation kinetics would help in designing heat processes targeted at preventing or reducing the development of the hard-to-cook defect in legumes. The objectives of this study were to determine the effectiveness of heat treatments in inactivating phytase in cowpeas and to model and estimate the kinetics of thermal inactivation as a function of moisture content.
3.3 MATERIALS AND METHODS

Freshly harvested cowpea seeds were obtained from SeedGrow, LLC (Meridian, Calif., U.S.A.) and stored at 4 °C until used. All chemicals used were of analytical grade and obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo., U.S.A).

Conditioning of cowpea flour

Cowpea seeds were finely ground in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve. The moisture content of the flour was adjusted by adding a pre-determined amount of water to the flour in a mixer. The amount of water required was calculated using the following equation;

\[ W = \frac{(B * C)}{D} - A \]  

where, W is amount of water required (g/100 g), A is initial moisture content (g/100 g), B is dry matter in original sample (g/100 g), C is final moisture content (g/100 g), D is dry matter in final sample (g/100 g).

The experiments were performed at moisture contents of 10, 25, and 35% (d.b.). About 300 g flour was conditioned at each moisture content, sealed in high density polyethylene bags and stored for 24 h at 4 °C to allow for equilibration. The sealed bags were kept at 4 °C until their use within a period of about 7 d. Moisture content was determined by a standard AOAC method (AOAC 1980).
Thermal Inactivation Experiments

A 6 x 5 factorial design with 6 levels of heating temperature (70 – 95 °C) and 5 levels of heating time (2 – 32 min) was used. Retort pouches (15.5 cm x 6.5 cm) obtained from CLP Packaging Solutions Inc. (Fairfield, N.J., USA) were used for the thermal inactivation study. About 3 g flour at the desired moisture content was thinly dispersed in the pouch to a thickness of 3.6 mm and vacuum sealed. The pouches were heated in an ethylene glycol bath with controlled temperature (T_{desired} ± 3 °C). Thermal inactivation was carried out at heating temperatures ranging from 70 to 95 °C and exposure times between 2 to 32 min. The temperature in the pouch was estimated by inserting a 30 gauge k-type thermocouple with a 0.01 inch bead (Omega Engineering, Stamford, Conn., USA) in a similar pouch and recording the change in temperature over the heating time. The come-up time, which was the time required to reach 99% of the desired temperature, was between 10 and 20 s depending on the final temperature desired. The pouches were removed from the heating bath as a function of the inactivation time and were immediately cooled in a water-ice mixture for 5 min. The residual phytase activity in the flour sample was determined soon after using the method described below. A control sample which was not subjected to any heat treatment was included for comparison. The phytase activity in this control sample was considered as the initial phytase activity (A_o). Thermal treatments of the flour were performed in triplicate.

Phytase Activity Assay

The phytase activity was measured by direct incubation with sodium acetate buffer according to the method described by Greiner and Egli (2003) with some slight modifications. The heat treated sample (1 g) was suspended in 20 ml of 0.1 M sodium acetate buffer, pH 5.0
containing 100 µmol of sodium phytate pre-incubated at 45 °C. The reaction mixture was incubated at 45 °C for 30 min after which the amount of phosphorus released was determined using the method described by Eeckhout and De Paepe (1994). A 2 ml portion of the reaction mixture was added to a test tube containing 2 ml of 10% TCA to arrest the reaction and then centrifuged at 10,000 g for 5 min. One ml of the supernatant was then added to 1 ml of a color forming reagent. The color reagent was a mixture of four parts of solution A (15 g of ammonium heptamolybdate in 55 ml of 36 N H2SO4, made up to 1 L) and one part of solution B (27 g of FeSO4.7H2O, a few drops of 36 N H2SO4, made up to 250 ml). The blue color formed was measured at 700 nm in a diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, Calif., USA) after centrifuging at 10,000g for 3 min to remove any cloudiness formed. A calibration curve was produced over the range of 1 – 4 µmol of phosphate and used to estimate the enzyme activity. The analysis was performed in triplicate and reported as units/kg flour. One unit of phytase activity was defined as the amount of phytase which liberates inorganic phosphorus from a 0.001 M Na-phytate solution at a rate of 1 µmol/min at pH 5 and 45 °C.

**Kinetic Data Analysis**

Inactivation of enzymes can be described by a first order kinetic model and the inactivation rate constant (k) can be estimated using linear regression analysis (Wiley 1994; Whitaker 1994).

\[
A_t = A_o \exp (-kt)
\]

Where \(A_o\) and \(A_t\) are the initial activity and residual activity at time \(t\), respectively.

Ly-Nguyen and others (2002) described a special case of the first order model known as the fractional conversion model which was applied in this study. Their paper provides an outline
of the basis and details of the model, as shown in the following summary. Fractional conversion 
\((f)\) accounts for the nonzero activity \((A_\infty)\) after prolonged heating and can be expressed as

\[
f = \frac{(A_0 - A_f)}{(A_o - A_\infty)}
\] (3)

For most irreversible first order reactions, \(A_\infty\) approaches zero, and Eq. 3 can be reduced to

\[
f = \frac{(A_0 - A_f)}{A_o}
\] (4)

A plot of the logarithm of \((1-f)\) versus time results in a straight line with a rate constant 
expressed by the negative slope value:

\[
\ln\left(\frac{A_f}{A_o}\right) = \ln(1 - f) = -kt
\] (5)

Thus, when \(A_\infty\) approaches zero, Eq. 5 and 2 are identical.

To account for the non-zero activity after prolonged heating, the following form of the 
fractional conversion is employed:

\[
\ln(1 - f) = \ln\left(\frac{(A_f - A_\infty)}{(A_o - A_\infty)}\right) = -kt
\] (6)

Rearranging Eq. 6 yields Eq. 7

\[
A_f = A_\infty + (A_o - A_\infty)\exp(-kt)
\] (7)

By plotting \(A_t\) versus inactivation time at constant temperature conditions, the inactivation rate 
constant, \(k\) and the remaining activity, \(A_\infty\) can be estimated using nonlinear regression analysis.
The non-linear estimation function in STATISTICA (StatSoft, Tulsa, OK) was used with the 
sum of squares of the relative error between measured and calculated enzyme activity used as the 
optimization criterion.
The rate constants determined above were re-plotted in Arrhenius plots at the moisture contents studied and the activation energy ($E_a$) calculated from the slope according to Eq. 8 (Anthon and Barrett 2002).

$$\ln(k) = -\left(\frac{E_a}{RT}\right) + c$$

(8)

### 3.4 RESULTS AND DISCUSSION

Phytase proved to be fairly heat stable. The enzyme retained almost 63% of its initial activity after subjection to the highest temperature (95 °C) and time (32 min) combination at the lowest moisture content (10%). The reduction in activity was determined to a large extent by the moisture content of the flour prior to heat treatment. Overall, the reduction in phytase activity relative to initial activity ranged between 5-37% for samples heated at 10% moisture content whereas heating at 25 and 35% moisture resulted in 20-56% and 29-64% reduction in phytase activity respectively. Other studies have also reported that plant phytase (dry flour) showed a high heat resistance. According to Ma and Shan (2002), wheat and rye phytase retained 89 and 105% respectively of their activity after heating at 100 °C for 1 h. All the factors considered in the present study (moisture content, temperature and time) had significant effects on the residual phytase activity following the heat treatments (Table 1). Increasing temperature has a general effect of increasing enzyme activity up to an optimum after which the activity decreases as the enzyme is progressively inactivated (Parkin 1993). It was therefore expected that residual phytase activity would decrease with increasing temperature. Additionally, the sensitivity of an enzyme to thermal inactivation is dependent on the water content of the environment such that
enzymes in dry or semi-moist food systems tend to be more heat stable (Parkin 1993). This is because water is needed to promote the unfolding of proteins during thermal denaturation.

A plot of residual phytase activity as a function of heating time resulted in nonlinear curves irrespective of heating temperature or initial moisture content (Figure 1). The thermal inactivation of phytase in cowpea flour could not be adequately described by a simple first order kinetic model. Generally, the plots showed an initial drop in phytase activity during the first few minutes of heating at each temperature. Beyond this, there appeared to be a gradual leveling in activity as the residual activity apparently stabilized. The results suggest that the decrease in phytase activity may be more dependent on heating temperature with the effect of heating time not being as pronounced especially for samples treated at high temperatures (80-95 °C). None of the treatment combinations studied resulted in complete inactivation of the enzyme. Although it is not known if isozymes of cowpea phytase exist, multiple enzymes have been reported for some plant seeds including lettuce (Shannon 1968), lupine (Greiner 2002) and soybean (Hamada 1996). The use of whole flour rather than a purified enzyme fraction could have introduced isozymes which could account for the deviation from simple first order kinetics. It has also been reported that there is an association between phytase and a non-specific acid phosphatase also capable of hydrolyzing phytic acid (Lolas and Markakis 1977).

The data were adequately described by a fractional conversion model in the temperature range studied (70-95 °C). This suggests the presence of a stable enzyme fraction that was apparently not significantly affected by the processing conditions under study. This behavior was observed at all the three moisture contents studied. The fitting of this model to the experimental data is shown in Figure 1. The fractional conversion model was used to estimate the kinetic inactivation parameters, namely; the initial phytase activity ($A_o$), the inactivation rate
constant \( k \) and the amount of enzyme remaining after treatment \( A_{\infty} \). These parameters were estimated by fitting individual data points to the model using non-linear regression and are summarized in Table 2.

The initial phytase activity estimated using non-linear regression was found to be between 81.4-84.0 units/kg. This was comparable to the average initial activity (82.3 units/kg) measured in the samples. The fraction remaining after the longest heating time varied with the heating temperature and initial moisture content. The values ranged between 32 and 72 units/kg representing approximately 38 and 88% of the initial activity, and decreased with increasing temperature and moisture content (Table 2). The high values obtained are indicative of the high thermal stability of the phytase enzyme. The variation in \( A_{\infty} \) values implies that the susceptibility of the stable enzyme fraction to heat inactivation is dependent on treatment combinations. This further suggests that under the appropriate conditions of heating temperature and moisture content, it might be possible to completely inactivate the enzyme or reduce it to negligible levels. The effect of temperature on the inactivation rate constants was described using the Arrhenius equation and is shown in Figure 2. The inactivation rate constants increased with increasing temperature as expected. This temperature dependence of the inactivation rate constant is explained by the concept of activation energy (Morales-Blancas and others 2002). The calculated activation energies, \( E_a \) in the temperature range 70 to 95 °C were 33.3 ± 0.4, 37.9 ± 0.6, and 43.4 ± 0.3 kJ/mol, respectively at 10, 25, and 35% moisture. Peers (1953) reported an inactivation energy value in the temperature range 55-65 °C of 171.5 kJ/mol for partially purified phytase in wheat. The typical range for inactivation energy for enzymes is 209-628 kJ/mol (Parkin 1993).
Enzyme activity relative to the unheated control was significantly reduced by heating moistened cowpea flour. Increasing moisture content generally facilitated the thermal inactivation of phytase. The variation of the inactivation rate constant and inactivation energy with moisture content are shown in Figures 3 and 4 respectively. There was a general increase in the rate constant with increasing moisture content confirming the hypothesis that the heat stability of enzymes increases at low moisture contents. At higher moisture contents, the increased availability of solvent water results in an increased opportunity for denaturation of the enzyme. The thermal stability of phytase at different initial moisture content according to k values would therefore be as follows: 35 < 25 < 10%. The inactivation energy, $E_a$ was highly correlated with the moisture content ($R^2 = 0.972$). This dependence on the moisture content is also obvious from Figure 4 where increasing moisture content resulted in an increase in $E_a$. Since $E_a$ decreases at the lower moisture contents, it suggests that the susceptibility of phytase to thermal inactivation decreases at the lower moisture content. Several results in literature have also shown this dependence of the activation energy for deteriorative reactions on the moisture content of the particular food product (Labuza 1980; Buera and others 1984). The non-linear curves obtained for the inactivation of phytase could have resulted from thermal inactivation being a water catalyzed reaction dependent upon successive sorption of water molecules onto the protein (Multon and Guilbot 1975). The temperature sensitivity of enzyme inactivation is directly related to the water content of the system since water is needed to facilitate the unfolding of the protein during denaturation. Enzymes in dry or semi-moist foods tend to be more heat stable (Parkin 1993). A similar effect of moisture content on the enzyme inactivation has been reported in several studies on other enzymes. These include the inactivation of phospholipase in
soy beans (List and others 1990), myrosinase in canola seeds (Owusu-Ansah and Marianchuk 1991), and lipase in rapeseed (Ponne and others 1996).

**Experimental Validation of Results and Predictions**

To test the validity of the proposed model, determinations of phytase activity were carried out experimentally in cowpea flour heated at 2 temperatures (70 and 95 °C) for 5 and 15 min for all the moisture contents studied previously. The residual phytase activity in the sample was then determined and compared to the values predicted by the model using the same factors. From the results (Table 3), it is observed that there is a close agreement between predicted and experimental values, suggesting that the fractional conversion model used is both reliable and valid under the experimental conditions.

### 3.5 Conclusions

Phytase from cowpea flour exhibits a high heat resistance between moisture contents of 10 and 35% (dry basis). The thermal inactivation of the enzyme is highly dependent on the heating temperature and moisture content of the medium. Although the inactivation did not follow typical first order kinetics, a modified first order model based on the fractional conversion technique was used to successfully describe and predict the residual phytase activity in cowpea flour.

This study was carried out to provide information on the factors which significantly impact the inactivation of cowpea phytase and further apply this knowledge in developing heat processes for cowpea seeds. Overall, the results indicated that the highest degree of inactivation was achieved under conditions of high temperature, high moisture content and within the first 4
min of exposure to heat. In order to strike a balance between achieving a sufficient degree of phytase inactivation (to affect its contribution to cowpea hardening), and retaining as much of the physicochemical characteristics of the treated cowpeas; the intermediate moisture content (25%) used in this study would be selected for further study. Additionally, the cowpea seeds would be briefly exposed to heat treatment since the decrease in phytase activity occurs fairly quickly.

**Acknowledgments:** This study was supported by a grant from the Bean/Cowpea Collaborative Research Support Program, U.S. Agency for International Development (Grant # GDG-G-00-02-0012-00). We thank CLP Packaging Solutions Inc. (Fairfield, N.J., USA) for their kind donation of retort pouches and Mr. Glenn Farrell for his assistance in setting up the thermal inactivation experiments.

### 3.6 References


Table 3.1: ANOVA summary table for phytase inactivation

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>2</td>
<td>90515.20**</td>
</tr>
<tr>
<td>Temperature (Temp)</td>
<td>5</td>
<td>13065.00**</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>2733.65**</td>
</tr>
<tr>
<td>Moisture x Temp</td>
<td>10</td>
<td>440.75**</td>
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<td>Temp x Time</td>
<td>20</td>
<td>8.01**</td>
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<tr>
<td>Moisture x Time</td>
<td>8</td>
<td>105.96**</td>
</tr>
<tr>
<td>Moisture x Temp x Time</td>
<td>40</td>
<td>26.76**</td>
</tr>
</tbody>
</table>

** Significant at p< 0.01
Table 3.2: Kinetic parameters for phytase inactivation in cowpea flour

<table>
<thead>
<tr>
<th>Moisture Content (%)</th>
<th>Temp (°C)</th>
<th>( A_0 ) (units/kg)</th>
<th>( A_\infty ) (units/kg)</th>
<th>( k ) (min(^{-1}))</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>70</td>
<td>81.95 ± 0.19(^a)</td>
<td>72.66 ± 0.17</td>
<td>0.169 ± 0.012</td>
<td>0.937</td>
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<td></td>
<td>75</td>
<td>82.24 ± 0.15</td>
<td>71.80 ± 0.08</td>
<td>0.229 ± 0.007</td>
<td>0.978</td>
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<tr>
<td>10</td>
<td>80</td>
<td>81.56 ± 0.19</td>
<td>68.60 ± 0.17</td>
<td>0.237 ± 0.029</td>
<td>0.815</td>
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<tr>
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<td>85</td>
<td>81.78 ± 0.37</td>
<td>65.68 ± 0.37</td>
<td>0.240 ± 0.019</td>
<td>0.877</td>
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<tr>
<td></td>
<td>90</td>
<td>81.97 ± 0.22</td>
<td>60.24 ± 0.27</td>
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<td>0.951</td>
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<td>82.22 ± 0.19</td>
<td>57.03 ± 0.39</td>
<td>0.407 ± 0.016</td>
<td>0.926</td>
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<tr>
<td>25</td>
<td>70</td>
<td>81.84 ± 0.18</td>
<td>58.96 ± 0.20</td>
<td>0.421 ± 0.024</td>
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<td></td>
<td>75</td>
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<td>46.76 ± 0.19</td>
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<td>81.53 ± 0.17</td>
<td>42.89 ± 0.25</td>
<td>0.663 ± 0.032</td>
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<td>90</td>
<td>83.98 ± 0.17</td>
<td>39.90 ± 0.23</td>
<td>0.782 ± 0.021</td>
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<td>38.65 ± 0.16</td>
<td>1.104 ± 0.023</td>
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<tr>
<td>35</td>
<td>70</td>
<td>82.18 ± 0.17</td>
<td>51.67 ± 0.16</td>
<td>0.678 ± 0.022</td>
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<td>75</td>
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<td>1.136 ± 0.024</td>
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<td>1.290 ± 0.016</td>
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</tr>
<tr>
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<td>36.00 ± 0.02</td>
<td>1.596 ± 0.068</td>
<td>0.988</td>
</tr>
<tr>
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<td>90</td>
<td>82.37 ± 0.00</td>
<td>33.49 ± 0.02</td>
<td>1.849 ± 0.083</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>82.38 ± 0.00</td>
<td>31.57 ± 0.03</td>
<td>2.052 ± 0.125</td>
<td>0.994</td>
</tr>
</tbody>
</table>

\(^a\) – asymptotic standard error
Table 3.3: Predicted and measured relative phytase activity (%) in heated cowpea flour

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Moisture Content (% dry basis)</th>
<th>10</th>
<th>25</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Measured</td>
<td>Predicted</td>
<td>Measured</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
<td></td>
<td>93.22</td>
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<td>15</td>
<td></td>
<td>89.06</td>
<td>91.44</td>
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<tr>
<td>95</td>
<td>5</td>
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<td>73.27</td>
<td>74.97</td>
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<td>46.80</td>
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<tr>
<td>R²</td>
<td></td>
<td></td>
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</table>
Figure 3.1: Thermal inactivation of phytase in cowpea flour as a function of heating time at various temperatures and moistures
Figure 3.2: Thermal dependence of rate constant for thermal inactivation of cowpea phytase
Figure 3.3: Effect of moisture content on loss of phytase activity in heated cowpea flour
Figure 3.4: Changes in inactivation energy of phytase activity with moisture content in heated cowpea flour

$R^2 = 0.972$
CHAPTER 4

MODELING THE THERMAL INACTIVATION OF PHYTASE IN STEAMED COWPEA SEEDS²

² Nicole S. Affrifah, Manjeet S. Chinnan and Chun Fang
To be submitted to Lebensmittel-Wissenschaft und -Technologie (Food Science and Technology)
4.1 **ABSTRACT**

Cowpeas at 20% moisture content were steamed at 100 or 121°C for 0-15min. Seeds were milled whole or fractioned into 3 layers (inner, middle, outer) using a customized drill. Residual phytase activity was assayed by measuring the amount of inorganic phosphate produced using an external source of phytate. A combined kinetic and heat/mass transfer model was employed to predict phytase inactivation in the three layers. Seeds milled whole showed residual phytase activity between 59.5–63.4%, which was generally lower than the activity measured in the layers (60.3-81.4%) for the same treatment combination. Phytase was not uniformly inactivated throughout the seed but was spatially dependent. The measured residual activity in the outer layer (66%) was lower than in the middle (73%) and inner (75%) layers after 15min steaming. The combined model predicted the spatial distribution of residual phytase activity at any given time. Predicted inactivation values were lower than experimental values. The average relative error for phytase inactivation was 5.8, 12.3 and 14.2% respectively for outer, middle and inner layers. Predicted and measured residual activities were closer in the outer layer as compared to the inner and middle layers.

Keywords: Cowpeas, Steaming, Phytase, Inactivation kinetics, Finite element model
4.2 INTRODUCTION

Phytases are a collective group of enzymes responsible for catalyzing the hydrolysis of phytic acid to myo-inositol pentakisphosphate and orthophosphate (Konietzny & Greiner, 2002). They are widely distributed in plants and animals and are also found in various fungi and bacteria species (Cosgrove, 1966). Phytases are generally thermostable enzymes with a pH optimum between 4 and 7.5 depending on the origin of the enzyme (Dvorakova, 1998). They have been reported to be involved in the underlying mechanism of the hard-to-cook defect through their action on phytates (Jones & Boulter, 1983; Mafuleka, Ott, Hosfield & Uebersax, 1993). Inactivating phytase could therefore be a possible control measure against the development of this major defect associated with stored legumes. A number of studies have focused on the effectiveness of applying dry or moist heat prior to storage and varying levels of success in preventing hardening has been achieved (Molina, Baten, Gomez, King & Bressani, 1976; Aguilera & Steinsapir, 1985; Plhak, Stanley, Hohlberg & Aguilera, 1987).

Enzyme activity is affected by the environmental conditions under which the measurements are made. The conditions that result in optimum activity of an extracted enzyme may not necessarily be the same as those prevailing during in vivo activity. This is because the latter situation depends on accessibility between enzyme and substrate which is largely determined by factors such as the microstructure of the seed. Ponne, Möller, Tijskens, Bartels & Meijer (1996) observed that the degree of thermal inactivation of rapeseed lipase was dependent on the physical environment of the enzyme i.e. aqueous extract versus seed matrix. This was attributed to the presence of high moisture in the rapeseed extract where a higher level of inactivation was achieved as compared to the whole seeds. Other studies have also highlighted
the positive effect of increased moisture content on enzyme inactivation (List, Mounts, Lanser & Holloway, 1990; Owusu-Ansah & Marianchuk, 1991).

The inactivation of phytase in cowpea flour was measured as a function of moisture content, heating temperature and time (Affrifah, Chinnan & Phillips, 2004). Results indicated that the inactivation process was highly dependent on the initial moisture content of the flour used. Additionally, increasing heating temperature resulted in a higher degree of inactivation and inactivation increased with heating time up to a point after which it leveled off. Overall, none of the treatment combinations resulted in complete phytase inactivation; phytase activity was reduced by 64% after heating cowpea flour with 35% moisture (d.b.) at 95°C for 32 min. The inactivation kinetics was adequately described by a model obtained by modifying a typical first order reaction using the fractional conversion technique.

According to Fang & Chinnan (2004) starch gelatinization following steaming of cowpea seeds was mostly concentrated in the regions closest to the surface of the seed. This was due to an unequal distribution of moisture in the seed with a higher concentration being located in the outer layer as compared to the interior of the seed. Currently, research on the hard-to-cook defect has been focused on the whole seed; however in light of the previous statements, a study on the physicochemical changes in different layers of the seed might be useful in understanding the defect and possibly, prevent its development. The objective of this study was to predict and validate residual phytase activity profile in whole cowpea seeds using a combined kinetic and heat/mass transfer model.
4.3 MATERIALS AND METHODS

A 6 x 2 factorial design with six levels of heating time (0-15 min) and two levels of temperature (100, 121°C) in a completely randomized design was used. Cowpea seeds were graded with a standard 16/64 circular sieve and the seeds retained on the screen were used in the study. Twenty-four samples of dry cowpeas each weighing 1 kg were placed in labeled low-density polyethylene Ziploc™ bags and randomly assigned to the treatment combinations. The initial moisture content of the cowpea seeds was adjusted to 20% (w.b.) before they were subjected to the treatments and all the treatments were done in duplicate. The contents of each bag was evenly spread out (1 layer thick) on a wire tray and placed in a Dixie steam retort (Model RDSW-3, Dixie Canner Equipment Co., Athens, GA). The seeds were then exposed to steam at either 100 or 121°C for the desired time period.

Steamed cowpeas were then dried 5h at 60°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA), split into individual cotyledons and dehulled. One batch of dehulled cotyledons was milled whole in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve. Seeds measuring approximately 3.22 ± 0.34mm in thickness were selected from the remaining cotyledons. These were then fractioned into 3 layers; outer, middle and inner each approximately 1mm in thickness, using a customized drill (Figure 4.1). The drill was a Dremel engraving tool (Model 275) with a 2mm round ball attached by a 3 foot flexible shaft to a variable speed DC driving motor. A vacuum line was connected in parallel with the shaft; and a collection bottle and tip were placed adjacent to the vacuum line to collect the fine flour produced. The residual phytase activity was determined for samples milled whole or as fractions using the method described below.
Simulation of Phytase Inactivation using a Finite Element Model

Fang, Chinnan & Thai (2003) developed a finite element model which describes the simultaneous heat and mass transfer occurring during the steaming of cowpea seeds. The heat transfer component of their model was based on Fourier’s equation and Fick’s equation was the basis of the mass transfer. To construct the model, one half of a longitudinal section of an individual cowpea cotyledon was divided into 3 layers, with each layer being further divided into 3-node linear triangular elements (Figure 4.2). The temperature and moisture content of the 213 elements obtained were calculated and averaged within each of the three layers. The model equation was solved using the Newton-GRMES method yielding a set of nodal values for temperature and moisture content at each time.

Residual phytase activity was then modeled in the seeds by combining the heat/mass transfer model developed by Fang, Chinnan & Thai (2003) with the kinetic model for phytase inactivation generated in our previous study (Affrifah, Chinnan & Phillips, 2004). The remaining phytase activity $A_t$ as a function of time and moisture content was estimated at each node using eq. (1) at inactivation time $t$.

$$A_t = A_e + (A_0 - A_e) \exp(-kt)$$  \hspace{1cm} (1)

Kinetic Model for Phytase Inactivation

The kinetic model for thermal inactivation of phytase at varying moisture content (9, 20, 26% w.b.) was determined by fitting experimental data to a fractional conversion model as previously described (Affrifah, Chinnan & Phillips, 2004). The reaction rate constant, $k$ as a function of moisture content, $M$ (w.b.) was expressed by:

$$k = 0.0014M - 0.0087$$  \hspace{1cm} (2)
During the generation of kinetic data, heating flour samples with moisture content of 26% (w.b.) at 100°C led to the release of a high amount of moisture. This increased the pressure in the retort pouches used in the thermal inactivation resulting in rupture. Consequently, it was not possible to conduct the experiments under these conditions and the reaction rate constant for samples at 26% and 100°C was estimated as follows. The estimation was based on a reference temperature (82.5°C) following the procedure of Anthon, Sekine, Watanabe & Barrett (2002). The reference temperature selected (82.5°C) was within the temperature range (70-95°C) used for the determination of inactivation kinetics (Affrifah, Chinnan & Phillips 2004). The rate constant at the reference temperature (k_{ref}) was estimated from the value of ln(k_{ref}) at this temperature given by the regression line in the Arrhenius plot for the corresponding moisture content. The rate constant for the inactivation (k) at 26% moisture and 100°C was then calculated using the following equation:

$$\ln(k) = \ln(k_{ref}) - \frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T_{ref}} \right)$$

(3)

**Phytase activity**

The phytase activity was measured by direct incubation with sodium acetate buffer according to the method described by Greiner & Egli (2003) with slight modifications. Cowpea seeds were finely ground in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve. About 1g of the flour was suspended in 20ml of 0.1M sodium acetate buffer (pH 5.0) containing 100µmol of sodium phytate (Sigma-Aldrich Chemical Co., St. Louis, Mo., U.S.A) and pre-incubated at 45°C. The reaction mixture was incubated at 45°C for 30min after which the amount of phosphorus released was determined using the method
described by Eeckhout & DePaepe (1994). A 2ml portion of the reaction mixture was added to a tube containing 2ml of 10% trichloroacetic acid to arrest the reaction and then centrifuged at 10,000g for 5min. One ml of the supernatant was then added to 1ml of a color forming reagent. The color reagent was a mixture of four parts of solution A (15g of ammonium heptamolybdate in 55ml of 36 N H₂SO₄, made up to 1L) and one part of solution B (27g of FeSO₄·7H₂O, a few drops of 36 N H₂SO₄, made up to 250ml). The blue color formed was measured at 700nm in a diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, CA) after centrifuging to remove any cloudiness present. A calibration curve was produced over the range of 1 – 4 µmol of phosphate and used to estimate the enzyme activity. The analysis was performed in duplicate and reported as units/kg flour. One unit of phytase activity was defined as the amount of phytase which liberates inorganic phosphorus from a 0.001 M Na-phytate solution at a rate of 1 µmol/min at pH 5 and 45 °C.

4.4 RESULTS AND DISCUSSION

Phytase Inactivation in Intact Cowpea Seeds

When cowpea seeds were steamed at 100 or 121°C and milled as whole cotyledons, the phytase activity was reduced to between 61.04 – 63.9 units/kg which represented about 59 – 63% of the original activity. It was obvious that the inactivation data obtained was not adequately described by a simple first order kinetic model. The plots showed an initial rapid drop in activity, and then there was a very gradual decrease in activity as heating time progressed (Figure 4.3). This trend was also observed in heated flour samples (Affrifah, Chinnan & Phillips, 2004). The experimental values were compared to values predicted using the fractional conversion model as described in our previous study (Affrifah, Chinnan & Phillips, 2004) and the fitting parameters
used are as shown in Table 4.1. Statistical analyses showed that the residual activity in samples steamed for 3 and 6 min was significantly different from the other steaming times. Additionally, the reduction in activity was significantly higher in the samples treated at 121°C. Comparing data on phytase inactivation in cowpea flour and seeds, it was observed that heating moist flour samples (20% moisture) at 100°C for 16 min resulted in a 50% reduction in phytase activity (data not shown). However, when whole cowpea seeds were steamed under similar conditions, only a 40% reduction in activity was recorded. This is probably due to the larger surface area and more uniform temperature distribution in the flour samples as compared to the seeds.

**Phytase Inactivation in Fractions of Cowpea Seeds**

Changes in phytase activity in different sections of cowpea seeds were also monitored after steaming at 100 and 121°C. The seed was fractioned into three layers which were designated as outer, middle and inner layers (Figure 4.3). The activity was significantly affected by the steaming temperature resulting in a progressive decrease in activity (Figure 4.4). Heating at 121°C resulted in a higher reduction in phytase activity as compared to 100°C irrespective of the layer under consideration. The inactivation of phytase did not occur uniformly throughout the seed. In general, the outer layers showed a higher decrease in activity followed by the middle layer with the inner layer exhibiting the smallest percentage change in activity after any steaming time or at any temperature (Figure 4.4). The increase in residual activity with increasing distance from the surface of the seed was possibly due to the combination of insufficient heat and moisture penetration into the interior of the seed. This uneven response to heat treatment at different points in the seed was also observed for starch gelatinization during
steaming of cowpeas (Fang & Chinnan, 2004). They also reported that a greater percentage of starch gelatinization occurred in the outer layers as compared to the underlying layers.

A comparison of the phytase activity measured in the whole seeds with the activity determined in the three(3) individual layers showed that the residual activity was significantly lower when the seeds were ground whole prior to analysis. For any treatment combination, the residual activity measured in the whole seeds was significantly lower than either the residual activity in each layer or the average of residual activity in the three layers. Heat generated in the mill used for grinding could have contributed to the decrease in phytase activity recorded in the whole seeds. According to Aguilera & Rivera (1992) a major impediment to understanding the biochemical nature of the hard-to-cook phenomenon has been the difficulty in determining biochemical changes in situ and therefore most data are based on assays of extracts of the seed. This study has however shown that there are differences in the results obtained for phytase inactivation depending on the method employed for sample preparation. This effect of sample preparation may also significantly affect the results obtained for other physicochemical characteristics. Although the changes in the different layers do not necessarily approximate the changes in situ, it might be a more useful approach in investigating the specific changes associated with the hardening phenomenon.

*Simulation of Phytase Inactivation*

A simulation study on the inactivation of phytase in cowpeas was undertaken by combining the above inactivation kinetics (eq. 2) with the heat and moisture transfer model developed by Fang, Chinnan & Thai (2003). Details on the heat and moisture transfer model, which was generated using the finite element method, can be found in their report. The
simulation study was only done for seeds steamed at 100°C because this was the highest temperature used in developing the kinetic model and also, the heat and moisture transfer models were generated at this temperature. For simulation purposes, each cowpea cotyledon was considered as being composed of 3 longitudinal layers (Figure 4.2). The phytase inactivation in each layer was then predicted as a function of steaming time and moisture content and plotted as contours as shown in Figure 4.5 for 60 – 180s of steaming. Predicted phytase activity decreased rapidly with steaming time to approximately 70% in the layer closest to the surface whereas the interior of the seed retained 77% of the initial activity after only 60s of steaming (Figure 4.5A). This rapid decrease in activity progressed with increasing steaming time. However in cowpeas steamed for 180s, the residual activity in all the layers reached a constant value of approximately 67% after which there was no measurable effect of heating on phytase inactivation (Figure 4.5C).

Results from our previous study (Affrifah, Chinnan & Phillips 2004) has suggested the presence of two phytase fractions - heat sensitive and heat stable in cowpeas. In general, the heat sensitive fraction of the enzyme disappeared rapidly in all the layers especially those closest to the surface.

The moisture profile in the seed as predicted using the heat and transfer models was directly related to the degree of inactivation achieved in a particular layer. A representative plot is shown for 60s steaming in Figure 4.6. In general, high moisture content was associated with a higher level of phytase inactivation (or low residual activity). As previously observed by Fang & Chinnan (2004), the moisture content of the seed during steaming was highest in the layers closest to the surface and decreased with increasing distance from the surface. The moisture content near the seed surface increased rapidly reaching a value of 34% (w.b.) within 50s of steaming. This high moisture content probably contributed to the higher reduction in phytase activity recorded after 50s in the layer closest to the surface.
The predictive power of the combined models used in the simulation study was validated by heating seeds with moisture content of 20% (w.b.) at 100°C and measuring residual activity in the three layers. Due to practical limitations, the experimental data were determined for steaming times between 180-900 s whereas the predicted values were generated from 60 to 900 s. The application of moist heat to cowpea seeds resulted in a reduction in phytase activity as expected. The decrease in activity was however not as severe as predicted in the simulation study. From the experimentally measured results, phytase activity decreased to approx. 76, 79 and 81%, respectively, in the outer, middle and inner layers of cowpeas steamed for 180s as compared to a predicted value of approximately 67% in the three layers for the same steaming time (Table 4.2). The percentage relative error calculated between the experimental and predicted activity for 180s ranged between 12.38 to 17.31%. A further decrease in activity was measured following steaming for 900s to 66, 73 and 75% in the outer, middle and inner layers, respectively. However, the values predicted at the same steaming time were between 65-66% with a percentage relative error of 1.6-11.76%. In general, a better prediction was made for residual activity in the outer layers as compared to the predictions in the inner and middle layers. This was particularly obvious as steaming time increased (Table 4.2). The percentage relative error especially for the outer layer, decreased as the predicted and measured residual activity became comparable. In general, the difference in the measured and predicted values increased towards the interior of the seed.

A possible explanation for the observed differences between measured and predicted activity could have resulted from the calculation of the residual activity. The residual activity was defined as the ratio of activity after any specific time to the initial activity, i.e. the activity in an unheated control. For the experimental data, the initial phytase activity in each corresponding
layer of unheated cowpea seeds was used. However, the initial activity of whole cowpea flour was used to calculate the residual activity for the predicted values since the kinetic data was based on whole cowpea flour. Furthermore, in this study, seeds were dehulled before being fractioned into layers whereas whole flour was used in the heat-moisture treatments to generate data for the inactivation kinetics. The presence of phytase activity has been reported in the seed coat fractions of wheat and lentil seeds (Brinch-Pedersen, Olesen, Rasmussen & Holm, 2000; Bose & Taneya, 1998). Thus dehulling the seeds prior to fractioning resulted in a decrease in initial activity and therefore the experimental samples were compared to a sample with a lower initial phytase activity.

Contrary to the results from the simulation study, the reduction in phytase activity in the individual layers did not show the gradual leveling to a constant value with increased steaming time. There was rather a progressive decrease in activity with increasing steaming time. This trend was also observed when phytase activity was measured in steamed whole seeds, although the decrease in the whole seeds was relatively slower with increasing steaming time. It is possible that the total processing time (15 min) used in this study was not long enough to ensure the complete inactivation of the heat stable fraction and therefore the activity continued decreasing slowly with time. A longer steaming time was not used in order to prevent the possible cooking of the cowpeas.

### 4.5 Conclusions

Phytase inactivation in steamed cowpeas was significantly affected by the location in the seed. Inactivation decreased with increasing distance from the seed surface resulting in between 76-81% and 66-75% residual phytase activity, after 3 and 15 min, respectively. The residual
activity measured in whole seeds was significantly lower than the activity in the individual layers. The combined kinetic and heat-mass transfer model yielded predicted phytase inactivation values which were slightly lower than the experimentally determined values. Comparable predicted and measured residual activity was observed in the outer layer in contrast to the inner and middle layers.

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### 4.6 REFERENCES


<table>
<thead>
<tr>
<th>Temperature (°C)</th>
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<th>121</th>
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</thead>
<tbody>
<tr>
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<td>102.06</td>
</tr>
<tr>
<td>$A_\infty$ (units/kg)</td>
<td>62.60</td>
<td>61.88</td>
</tr>
<tr>
<td>$k$ (min$^{-1}$)</td>
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<td>1.1961</td>
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</table>
Table 4.2: Comparison of predicted and experimental residual phytase activity (%) in different seed fractions of cowpeas steamed at 100°C

<table>
<thead>
<tr>
<th>Time (s)</th>
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<th>Middle Layer</th>
<th>Inner Layer</th>
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<tbody>
<tr>
<td></td>
<td>Expt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Predicted&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Rel. error (%)</td>
</tr>
<tr>
<td>60</td>
<td>nd&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.01</td>
<td></td>
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<tr>
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<td>nd</td>
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</tr>
<tr>
<td>360</td>
<td>71.56</td>
<td>66.09</td>
<td>7.64</td>
</tr>
<tr>
<td>540</td>
<td>69.61</td>
<td>65.62</td>
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</tr>
<tr>
<td>720</td>
<td>66.33</td>
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<td>1.78</td>
</tr>
<tr>
<td>900</td>
<td>65.72</td>
<td>64.67</td>
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<sup>a</sup> – experimental values; <sup>b</sup> – predicted using combined model; <sup>c</sup> – not determined.
Figure 4.1: Fractionating cowpea seeds using a specially designed device equipped with a high speed drill and suction
Figure 4.2: Cross-sectional representation of the three layers of a cowpea cotyledon
Figure 4.3: Thermal inactivation of phytase in cowpea seeds steamed at 100 (○) or 121°C (●). Straight lines represent predicted values calculated based on the fractional conversion model and the parameters in Table 4.1.
Figure 4.4: Thermal inactivation of phytase in the outer (□), middle (○) and inner (△) layers of cowpeas following steam treatment at 100 (solid line) or 121°C (dashed line)
Figure 4.5: Predicted phytase inactivation in cowpea seeds steamed for 60 (A), 120 (B), 180s (C).
Figure 4.6: Simulated moisture profile of steamed cowpea seeds after 60 s of steaming.
CHAPTER 5

THE INFLUENCE OF PRETREATMENTS ON THE DEVELOPMENT OF THE HARD-TO-COOK DEFECT IN COWPEA SEEDS

3 Nicole S. Affrifah, Manjeet S. Chinnan, and R. Dixon Phillips
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5.1 **Abstract**

The effectiveness of different treatments in preventing the characteristic hardening of cowpeas stored under unfavorable conditions was investigated. The factors considered were steaming temperature (100 and 121°C), steaming time (2, 4, 6 min) and initial seed moisture content (13 and 20%). The treated cowpeas were stored under two different conditions of 4°C and 42°C/80% RH. The cooked texture following treatment was comparable to the control; before storage, peak force values for treated samples were between 504-639N and 616N for the control. Although the treatments reduced the cooked hardness of all the samples prior to storage, only steaming at 121°C for 4 and 6 min was effective in preventing hardening during storage. Steaming reduced the phytase activity of cowpeas to 53-64% of original activity; however, storage resulted in a significant increase in activity. Steaming and storage significantly decreased water absorption from 91.28% to 75.68-88.02% after 24h soaking. A decrease in phytate content, and increases in electrolyte leakage and pectin loss were also recorded. None of the measured indices showed a particularly significant correlation with the cooked hardness even though they had been previously shown to be important in the development of the hard-to-cook defect. It is therefore suggested that the changes in the cooked hardness of cowpeas following the pretreatments occurs through a pathway different from that typically associated with the hard-to-cook defect in cowpeas.

*(Keywords: cowpeas, steam treatment, moisture content, storage, hard-to-cook)*
5.2 INTRODUCTION

Storage of legumes under adverse conditions of high temperature and high humidity has been associated with a hardening phenomenon characterized by extended cooking times for cotyledon softening (Hengtes et al., 1991; Hincks and Stanley, 1986). This hardening effect, referred to as the hard-to-cook defect, is one of the major limiting factors affecting the increased utilization of grain legumes, with the *Phaseolus* and *Vigna* cultivars being most susceptible (Aguilera and Stanley, 1985; McWatters et al., 1987).

Although various studies have been conducted to understand the biochemical nature of the hardening phenomenon, a major impediment has been the difficulty in determining biochemical changes *in situ* and therefore most data are based on assays of extracts of the seed (Aguilera and Rivera, 1992). A clearer understanding of the underlying mechanism(s) is needed to help design treatments for its prevention, unfortunately to date no firm conclusions have been drawn on the mechanism. Several hypotheses have been proposed to explain the hard-to-cook defect. Formation of insoluble pectates (Mattson, 1946; Jones and Boulter, 1983a), lignification of the middle lamella (Hincks and Stanley, 1986), protein denaturation in relation to starch gelatinization (Liu et al., 1992b) and lipid oxidation and/or polymerization (Richardson and Stanley, 1991) have all been implicated.

Hentges et al. (1990) showed that the previously held view that the hard-to-cook defect was a permanent condition was false. They observed that the defect was very easily reversed by storing hard-to-cook dry beans and cowpeas for an additional period at 6.5°C and 71% RH. Pretreatments including partial processing using both moist and dry heat prior to storage of black beans (Molina et al., 1976; Plhak et al., 1987); storage of black beans under controlled atmospheres (Aguilera and Rivera, 1990; Berrios et al., 1999); and soaking lima beans in
solutions of chelating agents and monovalent cations (Rockland and Jones, 1974; Kon and Sanshuck, 1981) have all been used in an attempt to reverse or prevent the hard-to-cook defect. Molina et al. (1976) heated black beans in a retort (121°C) or under steam (98°C) for different time periods before storing at 25°C and 70%RH for 9 months. They reported that the shortest heat treatment significantly decreased hardening during storage and also recorded a positive correlation \((r = 0.91)\) between the cooked bean hardness and the lignified protein content of the cotyledon. In their study, Plhak et al. (1987) used different heating methods including solar drying, microwave heating, dry roasting and irradiation prior to storage of black beans at 30°C/80% RH or 15°C/30% RH for 12 months. They observed that only irradiation and storage at low temperature and low humidity effectively reduced the rate of hardening.

A dry heat process using ceramic beads in a large scale, continuous roasting of beans has also been investigated (Aguilera et al., 1982). Although the high temperatures (approx. 100°C) attained were successful in inactivating trypsin inhibitors, it was inadequate in preventing bean hardening. Aguilera and Steinsapir (1985) used irradiation, high temperature-short time (HTST) and medium temperature-long time treatments on beans before storing at 22°C for a total of 10 months. According to their results, most of the HTST treated and irradiated samples were significantly softer than the control after 12-15 min of autoclaving.

In spite of the wide variety of heat treatments applied as a control measure against the hard-to-cook defect, very few studies have been directly focused on the enzymatic pathways which are thought to be involved in the development of the defect. Plhak et al. (1987) monitored total extractable phenol content and peroxidase activity which contributes to lignification, to investigate if changes in their relative amounts could be related to hardening of black beans.
Results from their study however indicated that neither index was a reliable indicator of the hardening process.

There is a lot of evidence in literature supporting the role of phytase in the development of the hard-to-cook defect through the formation of insoluble pectates (Jones and Boulter, 1983a; Shehata et al., 1985; Hentges et al., 1991). However, no attempts have been made to explore the inactivation of phytase as a strategy for preventing the hard-to-cook defect. A preliminary study conducted to study the inactivation of cowpea phytase indicated that the initial moisture content and heating temperature were significant factors (Affrifah et al., 2004). Furthermore, heating time was significant up to a cut-off point after which increasing exposure time did not appear to make a difference on the degree of inactivation achieved.

This paper describes the effect of different steaming and moisture treatments on quantitative changes in phytase activity and other physicochemical indices and their contribution to the storage stability of cowpeas. Additionally, the potential of the treatments in preventing the hardening of cowpea seeds during storage under different conditions was investigated.

5.3 MATERIALS AND METHODS

Freshly harvested cowpea seeds were obtained from SeedGrow, LLC (Meridian, Calif., U.S.A.) and stored at 4 °C until used. All chemicals used were of analytical grade and obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA).

Experimental design and sample preparation

The experimental design was a 4-factor factorial model in a completely randomized design with the factors being initial moisture content, steaming temperature, steaming time and
storage condition. Steaming times were selected within the region where the greatest proportion of phytase inactivation occurred as observed from a preliminary study in our lab (Affrifah et al., 2004). The levels of moisture were also chosen to represent the original seed moisture (13%) and the intermediate level used in the preliminary study (20%). Downie et al. (1997) reported that storing runner beans at elevated temperature (42°C) and high relative humidity (80%) for 5 weeks was sufficient to induce the hard-to-cook defect. Preliminary work using cowpea seeds stored under these conditions for a total of 5 weeks confirmed their results and these conditions were therefore used in the current study. Based on these findings, the following levels for each factor were used: initial moisture content – 13, 20%; steaming temperature – 100, 121°C; steaming time – 2, 4, 6 min and storage condition – Initial (no storage), Mild (4°C) and Severe (42°C/80% RH). The treatment combinations were replicated twice. Two controls, representing unsteamed cowpeas, stored under mild or severe conditions were also analyzed and compared with the treated samples. These were designated Mild-control and Severe-control respectively. Table 5.1 shows a summary of the variables and their corresponding levels used in this study.

Cowpea seeds were size-graded using a standard 16/64 circular sieve with seeds retained on the screen being used in the study. This was necessary to avoid including small size seeds which have been reported to be susceptible to developing the hard-shell defect resulting in poor water imbibition (Bourne, 1967). Twenty-four samples of dry cowpeas each weighing 2 kg were placed in labeled low-density polyethylene Ziploc™ bags and randomly assigned to the treatment combinations. The seeds were conditioned to the desired moisture content by adding a pre-determined amount of water calculated using the following equation (Scanlon et al., 1998);

$$W = \frac{A(B - C)}{100 - B}$$
where, $W$ is amount of water required (g), $A$ is weight of cowpeas (g), $B$ is final moisture content (%), and $C$ is initial moisture content (%).

The required amount of water was added to each bag and mixed by shaking and rolling the bags until the liquid was evenly distributed. The bags were stored overnight at 4°C to ensure the equilibration of water absorption by the seeds (Scanlon et al., 1998).

For each treatment combination, the entire content of a labeled bag was evenly spread out (1 layer thick) on a wire tray and steamed in a Dixie steam retort (Model RDSW-3, Dixie Canner Equipment Co., Athens, GA). This was followed by drying at 60°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA) for about 5 h. The dried seeds were sprayed with a solution of potassium sorbate in absolute methanol (1:8 w/v per kg) to control mold growth, and spread out in a single layer overnight to allow the methanol to evaporate before storage (Garruti and Bourne, 1985). The steamed and dried samples were divided into 3 equal batches of about 0.6 kg each. One set was analyzed within a few days and was labeled as ‘initial’. The other 2 batches were placed into mason jars partially covered with cheese cloth. These jars were stored either in an environmental growth chamber (Environmental Growth Chamber, Model NQ2, Chagrin Falls, OH) set at 42°C and 80% RH or at 4°C in a walk-in cooler for a total of 9 weeks. The storage period was extended from the 5 weeks used in the reference study (Downie et al., 1997) to 9 weeks to ensure that there was sufficient time for any hardening to occur.

**Moisture content**

Samples (3g) were dried at 130°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA) to a constant weight (Rivera et al., 1989).
Cooked bean hardness

A slight modification of the method described by Liu et al. (1993a) was used. Cowpea seeds weighing about 30g were tied in cheese-cloth and cooked in deionized water for a total of 2 h with a sample being removed after 30min intervals. The samples were allowed to cool to room temperature prior to measuring hardness (approx. 30min). An Instron Universal Testing Machine (Model 1122, Instron, Canton, MA) with a 500-kg load cell fitted with a Kramer Shear test cell was used to determine the hardness of the cooked beans. The test cell was filled with the entire content of the cheese-cloth and the test performed at a crosshead speed of 50 mm/min. The maximum force required to compress and shear the beans was determined from the force deformation curve. The peak force (N) was recorded as an average of duplicate measurements.

Water absorption

Ten grams of cowpea seeds were soaked in about 100ml of deionized water for 1, 3, 6, 12, 18 and 24 h. After each soaking period, the seeds were removed, blotted to remove the surface water and weighed. The amount of water absorbed was determined from the increase in weight. All the determinations were performed in duplicate and the ‘corrected’ water absorption capacity (WAc) was calculated as described by Jackson and Varriano-Marston (1981):

\[ WA_c(\%) = \frac{Wt.\ after\ soaking - Initial\ Wt. + Solids\ Loss}{Dry\ Wt.} \times 100 \]

Solids loss and electrolyte leakage

The amount of solids lost and electrolyte leakage during soaking in deionized water was determined (Liu et al., 1992a; Berrios et al., 1999). The electrolyte leakage was determined by measuring the conductivity of soaking liquors after removal of seeds. The volume of the soaking
liquor was made up to the original volume with deionized water and the conductance measured using a digital conductivity meter (Fisher Scientific, Pittsburgh, PA). The conductivity was reported as mS/cm at 25°C. The soaking liquor was partially dried at 90°C, then dried overnight at 40°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA) and weighed to determine solids lost. This was reported as g/10g dry seeds.

**Phytate content**

The colorimetric method described by Vaintraub and Lapteva (1988) was used. Cowpea seeds were finely ground in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve. About 1g of the flour was extracted by stirring for 1h at room temperature with 20ml of 2.4% HCl. The slurry was centrifuged in a Beckman centrifuge (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA) at 20,000 g for 15min at 15°C, decanted and 5ml of the supernatant diluted to 25ml. About 3 ml was pipetted into a centrifuge tube and 1ml of Wade reagent (0.03% FeCl$_3$.6H$_2$O and 0.3% sulfosalicyclic acid in distilled water) was added and vortexed for 5s. The mixture was then centrifuged for 5 min and the absorbance of the supernatant read at 500nm in a diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, CA). A standard curve was prepared using sodium phytate. The phytate concentration was calculated from the difference between the absorbance of the blank and that of the assayed sample and reported as the average of duplicate determinations.

**Phytase activity**

The phytase activity was measured by direct incubation with sodium acetate buffer according to the method described by Greiner and Egli (2003) with slight modifications.
Cowpea seeds were finely ground in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve. About 1g of the flour was suspended in 20ml of 0.1M sodium acetate buffer (pH 5.0) containing 100µmol of sodium phytate and pre-incubated at 45°C. The reaction mixture was incubated at 45°C for 30min after which the amount of phosphorus released was determined using the method described by Eeckhout and DePaepe (1994). A 2ml portion of the reaction mixture was added to a tube containing 2ml of 10% trichloroacetic acid to arrest the reaction and then centrifuged at 10,000g for 5min. One ml of the supernatant was then added to 1ml of a color forming reagent. The color reagent was a mixture of four parts of solution A (15g of ammonium heptamolybdate in 55ml of 36 N H\textsubscript{2}SO\textsubscript{4}, made up to 1L) and one part of solution B (27g of FeSO\textsubscript{4}.7H\textsubscript{2}O, a few drops of 36 N H\textsubscript{2}SO\textsubscript{4}, made up to 250ml). The blue color formed was measured at 700nm in a diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, CA) after centrifuging to remove any cloudiness present. A calibration curve was produced over the range of 1 – 4 µmol of phosphate and used to estimate the enzyme activity. The analysis was performed in duplicate and reported as units/kg flour. One unit of phytase activity was defined as the amount of phytase which liberates inorganic phosphorus from a 0.001 M Na-phytate solution at a rate of 1 µmol/min at pH 5 and 45 °C.

**Pectin loss**

The method described by Liu et al. (1993b) was used. About 20g of seeds tied in cheese-cloth were soaked in 100ml deionized water for 6h, drained and the soaking liquor saved. Fresh deionized water (100ml) was added and seeds heated at 100°C for 30 min, drained and the cooking liquor saved. The soaking and cooking liquors were then separately made up to 100ml
and centrifuged at 20000g for 15min. The supernatant (3 ml) was pipetted into 50ml centrifuge tube and 16ml of 95% ethyl alcohol added to obtain a final concentration of 80% alcohol. The tubes were stored at 4°C for 48h and then centrifuged at 20000g for 15min. The precipitate was dissolved in 1ml water and 2 drops of 1N NaOH for 2 hours. A total of 9 ml of 78% sulfuric acid was added (4.5ml at a time) with each addition followed by vortexing and chilling in an ice bath. The tubes were sonicated for 3min, heated at 50°C for 10min and cooled in an ice bath. A 1ml aliquot was mixed with 2ml cold 6N NaOH, then 0.5ml of this mixture was added to a tube containing 0.5ml of 2% NaCl. This was followed by the addition of 4 ml of cold concentrated H2SO4, heating at 70°C in a water bath for 10min and then cooling in tap water for 10min. The color was developed by adding 0.1ml of 3, 5-dimethylphenol (0.1% in glacial acetic acid). The absorbance was read after 15min at 400 and 450nm. The difference in absorbance at the two wavelengths was used to calculate the galacturonic acid content. A standard curve was prepared using 0-125nmol galacturonic acid. The pectin loss was expressed as mg galacturonic acid/g dry seeds.

**Scanning Electron Microscopy**

Dried cowpea seeds were dipped into liquid nitrogen and then fractured cross-sectionally with a razor blade. The sections were mounted on aluminum stubs using conductive carbon and then sputter-coated with a 15.3nm layer of a mixture of gold-palladium under vacuum. The coated material was then viewed and photographed in a Leo 928 scanning electron microscope (LEO Electron Microscopy Inc., Thornwood, NY) at an accelerating voltage of 5kV. The cotyledon cells were viewed based on their position relative to the seed coat. Photographs were
then taken of cells located either close to the seed coat (outer cells) or far from the seed coat (inner cells).

5.4 RESULTS AND DISCUSSION

For ease of discussion, the samples are grouped based on their storage condition and are referred to as follows: INITIAL – analyzed immediately following steam treatment; MILD – stored at 4°C; SEVERE – stored at 42°C/80% RH.

Representative graphs are shown for cooked texture, water absorption capacity, solids loss, and electrolyte leakage since similar trends were observed for all the treatment combinations. To show the effect of storage condition, the selected data shown corresponds to cowpeas steamed with 13% moisture at 121°C for 4 min. The effect of moisture content and steaming temperature is illustrated using cowpeas steamed for 4 min and stored at 42°C/80% RH. The effect of steaming time is shown for cowpeas steamed with 13% moisture at 100°C and stored at 42°C/80% RH.

**Moisture Content**

It has been reported that high moisture content of grain legumes during storage has a significant effect on the hardening process associated with storage (Antunes and Sgarbieri, 1979). Since the steaming process results in an increase in moisture content, the steamed cowpeas were dried approx. 5 hours at 60°C to reduce their moisture content. The moisture content of INITIAL samples ranged between 10.67-13.14 % (Figure 5.1). Stored samples showed moisture content between 10.53-12.58% for MILD samples and 11.86-12.72% for SEVERE samples. The moisture content of the seed before steaming and storage conditions were observed to have significant
effects on the moisture content of the steamed cowpeas (Table 5.2). As expected, samples steamed with a moisture content of 13% generally had moisture content after drying which was lower than that of the samples steamed at 20%. The change in moisture content during storage was very dependent on the storage temperature as well as the steaming temperature. Cowpeas steamed at 100°C generally showed an increase in moisture content during storage under severe conditions, whereas those steamed at 121°C showed no change or a very slight decrease in moisture with storage (Figure 5.1).

**Cooked Texture**

The effect of steam and moisture treatments on the development of the hard-to-cook defect in cowpeas was determined. Representative graphs are shown to illustrate the general trend observed for the effect of storage condition (Figure 5.2A), steaming temperature and moisture content (Figure 5.2B) and steaming time (Figure 5.2C).

The peak force under compression in a Kramer shear cell decreased in a curvilinear manner with increasing cooking time. Scanlon et al. (1998) reported similar results for lentils. The peak force values were used to evaluate the effectiveness of the treatments since the peak force is inversely proportional to bean softness.

Storage under severe conditions for 9 weeks was effective in inducing the hard-to-cook defect in control seeds which showed a drastic increase in cooked hardness (Figure 5.2A). It was therefore concluded that any differences observed in the treated seeds were due to the treatments applied prior to storage. The peak forces of *Initial* and *Mild* samples were all comparable to the mild-control. Differences were observed between treated seeds stored under severe conditions, where all samples steamed at 100°C and those steamed 2 min at 121°C showed a
significant increase in peak force during storage. However cowpeas steamed for 4 and 6 min at 121°C did not harden with storage, even under severe conditions. Increasing steaming time resulted in significantly softer beans however there were no significant differences between 4 and 6 min. Overall, cowpeas steamed for 4 or 6 min at 121°C at a moisture content of 13% were most comparable to the control.

Statistical analysis of the data indicated that the moisture content of the seed before steaming did not have a significant effect on the peak force (p>0.05), however, all the other main factors considered were significant. There were also significant interactions between storage conditions and each of the other three factors considered (moisture, steaming temperature, steaming time). This suggests that changes in hardness during storage were very much dependent on the treatment applied prior to storage. The results of the statistical analysis of peak force data obtained after 60 min cooking are shown in Table 5.2 and in Appendix 1 for 30, 90 and 120 min cooking.

Cowpeas were judged to be optimally cooked upon yielding to slight pressure when individually squeezed between the forefinger and thumb (Jones and Boulter, 1983a). It was observed that mild-control cowpeas were optimally cooked after 60 min and the peak force value was approximately 616N. This is shown as the horizontal line in Figure 5.2 and was used to evaluate the effectiveness of the steam and moisture treatments. It was apparent that irrespective of the initial moisture content, cowpeas steamed at 100°C and stored under severe conditions generally had higher values than the optimal even after cooking for 2 hours (Figure 5.2B). Thus steaming at 100°C did not appear to be effective in reducing the cooking time of cowpeas stored under unfavorable conditions. It must be however noted that although an increase in hardness was observed in cowpeas steamed at 100°C and stored under severe conditions, it was
significantly lower than the increase observed in the severe-control. Conversely, steaming at 121°C resulted in cowpeas which showed peak force values comparable to the mild-control and this was still true after storing these steamed cowpeas even under severe conditions (Figure 5.2A). This observation was more pronounced in cowpeas treated with an initial moisture content of 13%. Molina et al. (1976) reported that the shortest heat treatments (2 min at 121°C or 10 min at 98°C) were the most effective in preventing the development of the hard-to-cook defect in black beans. In this study, however, significant differences were noted in steaming time such that, steaming for a longer time (4 and 6 min) was more effective in preventing the defect (Figure 5.2C). It therefore appears that the effect of heat treatment on development of the hard-to-cook defect may be species related.

Although both steam treatments resulted in seeds that were either comparable to or softer than the mild-control, the samples treated at the lower temperature (100°C) eventually showed an increase in hardness with storage. A comparison of studies on heat treatment of different legumes suggests that in most cases a more intense heat treatment seems to be more effective in preventing future hardening during storage (Molina et al., 1976, Aguilera and Steinsapir, 1985, Hincks and Stanley, 1986). If the hardening process is mediated by enzymes as proposed, the lower temperatures may not have a complete lethal effect on the enzymes which subsequently recover during storage and initiate the hardening process (Hincks and Stanley, 1985).

**Water Absorption Capacity**

Figure 5.3 illustrates changes in water absorption capacity for steamed and control cowpeas at different soaking times. The plots shown are representative and illustrate the effect of storage
condition (Figure 5.3A), steaming temperature and moisture content (Figure 5.3B), and steaming time (Figure 5.3C) for selected data are as described earlier.

All the samples showed an initial rapid uptake of water followed by a progressively slower rate as the seeds became saturated. Although some authors have reported a beneficial effect of pretreatment (moist heat and infrared) on water absorption capacity (Molina et al., 1976, Abdul-Kadir, 1990), data from this study showed that the treatments significantly (p<0.05) decreased the amount of water absorbed at all the soaking times. However, a similar decrease in water absorption capacity following steam treatment of cowpeas was reported by Saalia (1995) and Komey (1999). The observed decrease in water absorption exhibited by the steamed seeds could be due to microstructural changes following the heat treatment. Scanning electron microscopic studies of steamed cowpeas have previously shown that cells in the cotyledon appear to be fused (Saalia, 1995). Thus it is possible that the reduction in intercellular spaces resulted in less water penetrating into the seeds. The decrease could also be a result of hydrophobic aggregation of denatured proteins reducing the number of available sites for water binding (Zheng et al., 1998).

Water absorption capacity also decreased with storage under severe conditions for both steamed cowpeas and the severe-control (Figure 5.3A). Results on the effect of storage at high humidity and high temperature on water absorption capacity is conflicted. Some investigators have reported an increase in water absorption by aged seeds compared to soft seeds (Jackson and Varriano-Marston, 1981, Hentges et al., 1991, Hincks et al., 1987) whereas a decrease was reported in other studies (Jones and Boulter, 1983a; Liu et al., 1992a; Reyes-Moreno et al., 2000). Variations in the results of the different studies may be due to differences related to
species, initial moisture content, storage conditions and whether corrections were made for solids loss.

In general, all the factors considered had a significant effect on the rate of water absorption. The effect of the factors on water absorption capacity after 12 h of soaking is shown in Table 5.2 since this trend was similar to that of the other soaking periods. The results for the other soaking periods are summarized in Appendix 1. A higher initial moisture content and steaming at 121°C resulted in lower water absorption (Figure 5.3b). It was generally observed that the more severe the steam treatment the greater the reduction in water absorption. Water absorption was also significantly decreased by storage with the decrease being more marked at the higher temperature and humidity.

**Solids Loss**

A general increase in solids loss was observed as the soaking time of cowpeas increased from 1 to 24 h (Figure 5.4). All the factors considered in the study had a significant effect on the solids loss from samples soaked for 1 h (Table 5.2). The data for the other soaking times are shown in Appendix 2. It was observed that when the samples were soaked for longer than 3 h, only the effect of steaming temperature was significant. From figure 5.4B, it can be seen that the samples steamed at the higher temperature (121°C) lost more solids. Liu et al. (1992a) reported that the leakage of solids in cowpeas was temperature dependent, increasing with incubation temperature. High steaming temperature and low initial moisture content resulted in significantly increased solids loss. Furthermore, storage under mild conditions resulted in slightly lower solids loss. According to Parrish and Leopold (1978), solids loss is an indication of seed deterioration. The higher loss of solids in hard-to-cook seeds has been attributed to
membrane impairment induced by unfavorable storage conditions (Simon, 1974, Jones and Boulter, 1983b). This allows the free release of cytoplasmic solutes including sugars, amino acids and inorganic salts into solution (Harman and Granett, 1972). Llano et al. (2003) also observed a loss of membrane integrity in kiwifruit tissue following steam blanching. Thus the application of high temperatures could have resulted in loss of membrane integrity, accounting for the increased solids loss observed.

**Electrolyte Leakage**

The concentration of electrolytes leached during soaking as measured by the specific conductance of the soaking liquor increased with soaking time. Figure (5.5) shows the characteristic trend in the changes in electrolyte leakage following steam treatment and storage. The plots shown are for treatment combinations similar to those described above for cooked texture. Cowpeas steamed at an initial moisture content of 13% had a significantly higher loss of electrolytes compared to those steamed at an initial moisture content of 20%. Additionally, seeds steamed at a higher temperature leaked significantly more electrolytes. Storage, irrespective of the conditions, resulted in an increase in electrolyte leakage as compared to the leakage measured in the initial samples. Other studies have also reported that fresh beans exhibited lower concentration of electrolyte leakage compared to hard-to-cook beans (Jackson and Varriano-Marston, 1981; Plhak et al., 1989; Hentges et al., 1991, Berrios et al., 1999).

The conductivity of the soaking liquors was significantly correlated (p < 0.01) to solids loss at all the soaking periods used in the study with correlation coefficients ranging from 0.8425 to 0.9443. Statistical analysis of the data showed that the changes in electrolyte leakage were dependent on the soaking period under consideration. After 1 h soaking, all the factors measured
significantly influenced the electrolyte leakage from cowpea seeds (Table 5.2). However, when
the soaking period was longer than 3 h, steaming temperature and storage condition were
significant. Additionally, moisture content was significant but only for samples soaked for 12,
18 and 24h (Appendix 2).

**Phytic Acid Content**

The phytic acid content of seeds was determined following steam treatment and after storage
(Figure 5.6). Steaming at 121°C drastically reduced the phytate content by almost 50% of the
initial amount (Figure 5.6C, D). Steaming seeds with a higher initial moisture content also
resulted in further reduction in phytate content (Figure 5.6D). This could be beneficial to the
nutritive value of the treated cowpeas since phytic acid acts as a strong cation chelator, binding
minerals such as zinc and iron (Sandberg, 2002). Storage of samples did not appear to have any
particular effect on the phytate content. Additionally, increasing steaming time generally
resulted in a decrease in phytic acid concentration. The decrease in phytate content following
treatment was significantly affected by the steaming temperature, steaming time and moisture
content as indicated by statistical analysis (Table 5.2). However, the effect of storage condition
was not significant.

Phytic acid content was significantly (p < 0.05) correlated to the texture of cowpeas cooked
for 30-120 min. The slight positive correlation (0.3459-0.4759) obtained is in contrast to reports
available where high phytate content is usually associated with softer cooked beans (Kon and
Sanshuck, 1981; Longe, 1983). However in this study, cowpeas with low phytate content
generally exhibited lower peak force values indicating that they were softer. This observation
was not surprising because the application of steam resulted in hydrolysis of phytic acid thereby
reducing its concentration in the seed. The data on cooked texture indicated that treatments at high temperature and longer times resulted in softer beans. This explains the positive correlation observed between phytate content and cooked texture. The results suggest that the changes occurring during the steam treatment may be distinct from the biochemical changes associated with the development of the hard-to-cook defect. The consequences of the steam treatment appear to be greater than the previously reported relationship between phytate content and cooked texture. A similar observation was made by Arntfield et al. (2001) in a study on the effect of micronization of lentils in reducing cooking time. They concluded that there did not seem to be a correlation between the level of phytic acid in the lentils and the firmness resulting from the micronization process. According to Kon and Sanshuck (1981) the ratio of % phytic acid to % Ca present in the seeds may be a better indicator of the relationship between phytic acid and cooking time. This ratio was however not determined in this study.

**Phytase Activity**

The changes in phytase activity following steam treatment are shown in Figure 5.7. The application of steam to cowpeas reduced the phytase activity to approximately 53-64% of the original activity. The data showed an increase in phytase activity in the samples during storage. This increase in activity, up to about 60-74%, may explain the decrease observed in phytate content during storage. From Table 5.2, it can be seen that the effect of storage condition as well as the steaming temperature were significant. The strong dependence of phytase inactivation on the initial moisture content previously observed in cowpea flour (Affrifah et al., 2004) was not manifested in intact beans. This is possibly due to the protective role of the cellular structure where enzymes and substrates are usually not in direct contact. Similar results were reported by
Rivera et al. (1989) who studied peroxidase in black beans. Significant differences in the effect of steaming temperature were observed, with the higher temperature resulting in higher inactivation. However there were no significant differences in the effect of steaming time, a previously observed effect in cowpea flour. Storage resulted in significant increases in phytase activity with seeds stored at a higher temperature showing a higher level of activity. This is consistent with the findings of Hincks and Stanley (1986) and Mafuleka et al. (1993) who also reported an increase in phytase activity in stored beans.

Phytase activity had a very low but significant correlation ($r = 0.46$) with the changes in phytate content of cowpeas but no correlation was observed with the hardness of cooked cowpeas. The inactivation kinetics of phytase was determined in a previous study (Affrifah et al., 2004) to understand the behavior of phytase during heat treatment. This was also necessary because we hypothesized in this dissertation that based on its role in the hardening process, phytase can be used as an index of the efficiency of a heat treatment designed to prevent the hard-to-cook defect. Thus a heat treatment capable of inactivating or at least significantly reducing phytase activity would be possibly effective in preventing the hard-to-cook defect. It has been previously suggested that an increase in phytase activity during storage of legumes under high temperature and humidity conditions plays a key role in promoting the hardening phenomenon (Jones and Boulter, 1983a). This was contradicted by the results obtained in this study because phytase activity increased during storage even in steamed cowpeas which showed either a decrease in or constant peak force values. This suggests that the overall changes induced in the cowpea seeds as a result of the steaming process negated the expected effect of phytase activity. This observation was also reflected in the relation between phytate content and the
cooked texture of the steamed cowpeas as discussed above. Overall, the changes in phytase activity did not adequately reflect the changes in texture of the cowpeas.

**Pectin Loss**

*Soaking:* Pectin loss from control and steamed cowpeas during a 6h soaking period was determined before and after storage (Figure 5.8). In general, there was no noticeable change in pectin loss from seeds steamed at 100°C for different times. Conversely, seeds steamed at 121°C showed an increase in pectin loss with increasing steaming time. Albersheim (1959) observed that the stability of pectin solutions was highly dependent on the temperature and pH of the medium. He reported that pectin became less stable with increasing temperature at neutral or slightly acidic conditions. Liu et al. (1993b) reported that the loss of pectin from soaked cowpeas exhibited a temperature dependent pattern. Kon (1968) also confirmed earlier observations that heat treatment converted some of the water-insoluble pectins into water-soluble pectins. These reports support the observation that the solubility of pectin increased at the higher treatment temperature.

All the factors considered had a significant effect on the amount of pectin lost during soaking in water (Table 5.2). There were also some significant interactions between the factors. Steaming at 121°C resulted in a significantly higher soluble pectin content compared to steaming at 100°C. The amount of pectin lost during soaking increased with storage and also storage temperature. Liu et al. (1993b) also recorded an increase in pectin loss in cowpeas aged by storing at 30°C/64% RH. Steaming for a longer period also resulted in higher losses. The effect of temperature was the same irrespective of the moisture content prior to steaming.
Cooking: The effect of cooking at 100°C for 30min on pectin loss in control and treated cowpeas was determined before and after storage (Figure 5.8). Cooking at 100°C for 30min resulted in almost a ten-fold increase in pectin loss as compared to soaking for 6h. Although there was no conclusive trend in the loss of pectin, cowpeas steamed at 100°C showed a general decrease whereas steaming at 121°C resulted in a general increase in pectin loss with increasing steaming time. With the exception of the initial moisture content, all the factors considered had a significant effect on the amount of pectin lost during soaking in water (Table 5.2). Seeds treated at 121°C generally had a higher soluble pectin content compared to those treated at 100°C. Despite the fact that storage resulted in an increased loss of pectin, there was no significant difference in the effect of storage temperature. Furthermore, steaming for 2 and 6 min resulted in higher losses than steaming for 4 min. It has been suggested that decreased pectin solubility and decreased pectin esterification during storage may contribute to the increased cooking time of legume seeds (Hengtes et al., 1991). This was only evident in the untreated control whereas the data for treated samples showed that storage increased the solubility of pectin even under unfavorable conditions.

No significant correlation was observed between pectin loss during either soaking or cooking and the cooked hardness of treated cowpeas. It suggests that the changes in compression force after cooking as a result of the steam treatment were possibly not due to differences in pectin loss.

Microstructural Changes

The microstructure of the steamed and untreated cowpeas was examined with scanning electron microscopy to observe any changes following the steam treatment. Examination of the cross-
sectional view of fractured cotyledons showed some differences as a result of the steam treatment. Both treated and untreated cotyledons fractured along the cell walls and therefore it was not possible to observe changes in cellular contents. The most notable difference was in the number and appearance of intercellular spaces. The untreated cotyledons showed more intercellular spaces when compared to any of the treated cotyledons where cells appeared to be more fused together (Figure 5.9). Since it was not possible to quantify the change in the number of intercellular spaces, it was difficult to conclusively comment on the effect of the different treatments on the microstructure of the samples. However, it was quite obvious for the 100°C steamed seeds that cells located toward the interior of the cotyledons of the had slightly more spaces compared to cells located in the outer layers. In addition, the inner cells from the 100°C seeds had more spaces than the cells from both locations in the 121°C samples (Figure 5.9C-H). This fusion of cells following steam treatment has been previously reported by Saalia (1995) and could account for the decreased water absorption capacity observed in this study.

Another observation made involves the appearance of starch granules. In spite of the fact that the fracturing of the samples along the cell wall prevented observation of the cellular contents, it was clear that the control seeds had more visible protrusions of starch granules. These granules seem to disappear with increasing moisture content and steaming temperature possibly due to starch gelatinization.

The cotyledons of samples stored under severe conditions were also observed to compare any difference in the effect of storage. However no clear differences were observed between the stored cells (Figure 5.10). Liu et al. (1993c) and Sefa-Dedeh et al. (1979) all reported a similar structure for dry control and dry aged cowpea seeds.
5.5 CONCLUSIONS

Steaming cowpeas at 121°C for 4 and 6 min was effective in preventing the increase in cooked hardness following storage under unfavorable conditions. Although the other treatments resulted in a reduction in cooked hardness, they were not as effective in preventing the increase in hardness during storage.

It is important to note that none of the measured physicochemical indices appeared to be significantly correlated with the cooked hardness of the cowpeas following the steam treatment. However these indices have been previously reported to be related to the development of the hard-to-cook defect in untreated seeds. The observed variation in phytase activity was not a particularly good indicator of the textural characteristics of steamed cowpeas. Although the treatments resulted in better cooked texture, the improved storage stability of the treated cowpeas occurred through a pathway different from that typically associated with the hard-to-cook defect. This was evidenced by the lack of correlation between the cooked texture and the measured physiochemical indices.

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Table 5.2: Analysis of variance for moisture content (%), cooked texture (KN), water absorption (%), solids loss (g/10g), electrolyte leakage (mS/cm), phytic acid (%), phytase activity (units/kg), and pectin loss (mg galacturonic acid/g seed) of steam treated cowpeas for moisture content, steaming temperature, steaming time and storage condition

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<th>Cooked Texture (T)</th>
<th>Water Absorption (S)</th>
<th>Solids Loss (M x T)</th>
<th>Electrolyte Leakage (M x S)</th>
<th>Phytic Acid (T x T)</th>
<th>Phytase Activity (M x T x S)</th>
<th>Pectin (x 10^8)</th>
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<td>404**</td>
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<td>133**</td>
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<td>0.000</td>
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* - Significant at the 10% probability level. ** - Significant at the 5% probability level. *a – After 60 min cooking. *b – After 12 h soaking. *c – After 1 h soaking
Figure 5.1: Moisture content of steam-treated cowpeas under different storage conditions [INITIAL (□), MILD (○), SEVERE (△)]
Figure 5.2: Changes in cooked hardness of steamed cowpeas. Factors include:
A, storage conditions; INITIAL (□), MILD (○), SEVERE (△), for cowpeas steamed 4 min (open symbols) or control (closed symbols);
B, steaming temperature; 100°C (○), 121°C (△) and moisture content; 13% (closed symbols), 20% (open symbols);
C, steaming time; 2 min (■), 4 min (▲), 6 min (●), for cowpeas steamed at 100°C (Horizontal dashed line represents the peak force of optimally cooked control cowpeas.)
Figure 5.3: Changes in water absorption of steamed cowpeas. Factors include:
A, storage conditions; INITIAL (□), MILD (○), SEVERE (△), for cowpeas steamed 4
min (open symbols) or control (closed symbols);
B, steaming temperature; 100°C (○), 121°C (△) and moisture content; 13% (closed
symbols), 20% (open symbols);
C, steaming time; 2 min (■), 4 min (△), 6 min (○), for cowpeas steamed at 100°C
Figure 5.4: Solids lost during soaking of steamed cowpeas. Factors include:
A, storage conditions; INITIAL (□), MILD (○), SEVERE (▲), for cowpeas steamed 4 min (open symbols) or control (closed symbols);
B, steaming temperature; 100°C (○), 121°C (▲) and moisture content; 13% (closed symbols), 20% (open symbols);
C, steaming time; 2 min (■), 4 min (▲), 6 min (●), for cowpeas steamed at 100°C
Figure 5.5: Electrolyte leakage during soaking of steamed cowpeas. Factors include:
A, storage conditions; INITIAL (■), MILD (○), SEVERE (△), for cowpeas steamed 4 min (open symbols) or control (closed symbols);
B, steaming temperature; 100°C (○), 121°C (△) and moisture content; 13% (closed symbols), 20% (open symbols);
C, steaming time; 2 min (■), 4 min (△), 6 min (○), for cowpeas steamed at 100°C
Figure 5.6: Changes in phytate content of cowpeas following steam treatment and storage under different conditions [INITIAL (□), MILD (○), SEVERE (△)]
Figure 5.7: Changes in phytase activity in cowpeas following steam treatment and storage under different conditions [INITIAL (□), MILD (○), SEVERE (△)]
Figure 5.8: Changes in pectin loss in cowpeas following steam treatment and storage under different conditions [INITIAL (□), MILD (○), SEVERE (△)]. Solid lines represent loss during soaking for 6h and dashed lines represent loss during cooking at 100°C for 30min.
Figure 5.9: SEM images of the effect of different heat-moisture pretreatments on cowpea cotyledons. Cross sectional view showing cells from the outer (B, D, F, H) and inner (A, C, E, G) layers: control (A, B), 13%/100°C (C, D); 13%/121°C (E, F) 20%/121°C (G, H) [not subjected to storage conditions]
Figure 5.10: SEM images of the effect of different heat-moisture pretreatments on cowpea cotyledons after storage at 42°C/80% RH. Cross sectional view showing cells from the outer (B, D, F, H) and inner (A, C, E, G) layers: control (A, B), 13%/100°C (C, D); 13%/121°C (E, F) 20%/121°C (G, H)
CHAPTER 6

THE EFFECT OF DRYING CONDITIONS ON THE DEVELOPMENT OF THE HARD-TO-COOK DEFECT IN STEAM-TREATED COWPEA

Nicole S. Affrifah and Manjeet S. Chinnan
To be submitted to the Journal of Food Science
6.1 **ABSTRACT**

A three factor-three level Box-Behnken design was used to evaluate the effect of drying conditions (time, temperature and humidity) on changes in textural and physicochemical characteristics of steamed cowpeas in storage. The treatment combinations resulted in a significant lowering of the cooked hardness and were effective in preventing further hardening during storage under severe conditions (42°C/80%RH). Before storage, the cooked texture of treated samples as indicated by peak force was between 535-628N and 602N for the untreated control; and 516-649N and 1394N, respectively, after storage at 42°C/80%RH. The peak force of stored cowpeas significantly decreased as drying temperature increased. Steaming and drying reduced phytase activity to 59–64% of original activity and decreased phytate content from 0.133%to 0.074-0.105%. Storage however resulted in increased phytase activity and a corresponding decrease in phytic acid content. Drying time had a significant effect on phytase activity whereas all parameters significantly affected phytate concentration during storage. The treatments not only reduced the water absorption capacity but had a significant influence on the amount of water absorbed after a specific soaking time. The drying conditions showed both significant linear and quadratic effects on water absorption before storage. None of the drying parameters significantly affected pectin loss during soaking or cooking. The drying conditions applied after steaming significantly influences seed characteristics, therefore selection of optimum drying conditions may be used to control seed quality especially texture.

**Keywords**: Cowpeas, drying temperature, humidity, texture, hard-to-cook
6.2 INTRODUCTION

The acceptability of grain legumes is greatly affected by a variety of factors such as grain size, color, flavor, storage stability and cooking properties (Reyes-Moreno and Paredes-Lopez 1993). These physical and chemical properties of dry and cooked bean seeds influence consumer preference and processor standards. The hard-to-cook defect is one of the most important acceptability characteristic because the cooking time required for beans to reach an acceptable texture also greatly influences consumer perception of bean quality. The cooking quality of beans is defined as the cooking time required for beans to reach a cooked texture considered acceptable to consumers (Moscoso and others 1984).

The moisture content during storage has been related to the cookability of beans. Storage of beans at high moisture content generally results in longer cooking times compared with those stored at low moisture content (Burr and others 1968; Hughes and Sandsted 1975; Antunes and Sgarbieri 1979). The quality of beans can be preserved by maintaining the moisture content or water activity at a level where the undesirable reactions usually associated with the hard-to-cook defect are inhibited (Reyes-Moreno and Paredes-Lopez 1993). Morris and Wood (1956) reported that storing seeds with moisture content greater than 13% caused a significant deterioration in flavor and texture after 6 months at 25°C and became unpalatable after 12 months. Storing at the same temperature but at 10% or lower moisture content resulted in values comparable to the control after 2 years. According to Aguilera and Rivera (1990) the optimal moisture content for storage of black beans is 10-14% which is adequate for delaying hardening and minimizing mechanical damage during the handling of beans.

In a previous study, a steaming pretreatment was developed which was effective in preventing the hardening of cowpeas during storage under unfavorable conditions (Affrifah and
others 2004). However, steaming results in increased seed moisture content and in the light of the relationship between moisture content and hardening during storage, it is necessary to reduce the moisture content to acceptable levels. The steam treatment is therefore followed by a dehydration process. It is generally known that the rate of drying is a function of the air temperature and humidity. The drying conditions can be controlled by regulating the different combinations of operating variables such as temperature, relative humidity and air velocity. This is necessary in order to shorten the drying time and attain high quality (Inazu and others 2002). The rehydration characteristics of dried foods are influenced by the processing conditions, sample composition, sample preparation and the extent of structural and chemical disruptions induced by drying (Krokida and Maroulis 2000). The rate and extent of rehydration may be used as an indicator of food quality; foods that are dried under optimum conditions suffer less damage and rehydrate more rapidly and completely than poorly dried foods (Reyes and others 2002).

Data from another study in our lab showed some interactions between the effect of steaming time and the drying conditions employed after steaming on the cooking quality of cowpeas (Komey 1999). Increasing drying temperature generally decreased the cooked hardness of the treated seed. In addition, high temperature and intermediate humidity resulted in cooked bean hardness comparable to the untreated control. Results from her study indicated that the drying conditions could be selected to yield treated cowpeas with textural characteristics comparable to those of the control (Komey 1999). This study was therefore aimed at evaluating the effect of the drying conditions applied after steam treatment on the hardening of cowpeas during storage under unfavorable conditions.
6.3 MATERIALS AND METHODS

Freshly harvested cowpea seeds were obtained from SeedGrow, LLC (Meridian, Calif., U.S.A.) and stored at 4 °C until used. All chemicals used were of analytical grade and obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA).

Experimental design

The combination of seed moisture content and steam treatment (temperature and time) were selected based on their efficiency in reducing the cooking time and preventing hardening of stored cowpeas. From the results in our previous study (Affrifah and others 2004), the best treatment combination was found to be moisture content – 13%, steaming temperature – 121°C and steaming time – 4min. This treatment combination was used throughout this experiment. Three drying parameters (time, temperature and humidity) each at 3 levels were considered in the study. The factors and the corresponding levels are summarized in Table 6.1. The Box-Behnken (1960) experimental design was used to obtain 15 samples with the three center points being replicated (Table 6.2). To avoid bias, all the 15 runs were performed in a completely random order. An untreated control (C) was included for comparison.

Sample Preparation

Cowpea seeds were size-graded using a standard 16/64 circular sieve with seeds retained on the screen being used in the study. Fifteen samples of dry cowpeas each weighing 2 kg were placed in labeled low-density polyethylene Ziploc™ bags and randomly assigned to the treatment combinations. The entire content of a labeled bag was evenly spread out (1 layer thick) on a wire tray and steamed in a Dixie steam retort (Model RDSW-3, Dixie Canner Equipment Co.,
Athens, GA) at 121°C for 4 min, followed by drying in an environmental growth chamber (Model NQ2, Environmental Growth Chamber, Chagrin Falls, OH) set at the desired drying condition listed in Table 6.2. The dried seeds were sprayed with a solution of potassium sorbate in absolute methanol (1:8 w/v per kg) to control mold growth, and spread out in a single layer overnight to allow the methanol to evaporate before storage (Garruti and Bourne 1985). The treated dried samples were divided into 3 equal batches. One set was analyzed within a few days and were referred to as ‘initial’. The other 2 batches were placed into mason jars partially covered with cheese cloth. The jars were stored either in an environmental growth chamber (Model NQ2, Environmental Growth Chamber, Chagrin Falls, OH) set at 42°C and 80% RH (severe conditions) or in a walk-in cooler at 4°C (mild conditions). Samples were stored for a total of 5 weeks. Downie and others (1997) reported that storing runner beans at elevated temperature (42°C) and high relative humidity (80%) for 5 weeks was sufficient to induce the hard-to-cook defect. This was verified in an independent preliminary study using cowpeas.

For phytase activity and phytate content determinations, cowpea seeds were finely ground in a Wiley laboratory mill (Model 4, Arthur H. Thomas Co. Philadelphia, Penn., USA) to pass through a #40 mesh sieve.

**Moisture content**

Samples (3g) were dried at 130°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA) to a constant weight for the determination of moisture content (Rivera and others 1989).
Cooked bean hardness

A slight modification of the method described by Liu and others (1993a) was used. Cowpea seeds weighing about 30g were tied in cheese-cloth and cooked in deionized water for a total of 2 hours with a sample being removed after 30min intervals. The samples were allowed to cool to room temperature prior to measuring hardness (approx. 30min). An Instron Universal Testing Machine (Model 1122, Instron, Canton, MA) with a 500-kg load cell fitted with a Kramer Shear test cell was used to determine the hardness of the cooked beans. The test cell was filled with the entire content of the cheese-cloth and the test performed at a crosshead speed of 50 mm/min. The maximum force required to compress and shear the beans was determined from the force deformation curve. The peak force (N) was recorded as an average of duplicate measurements.

Phytase activity

The phytase activity was measured by direct incubation with sodium acetate buffer according to the method described by Greiner and Egli (2003) with slight modifications. About 1g of cowpea flour was suspended in 20ml of 0.1M sodium acetate buffer (pH 5.0) containing 100µmol of sodium phytate and pre-incubated at 45°C. The reaction mixture was incubated at 45°C for 30min after which the amount of phosphorus released was determined using the method described by Eeckhout and De Paepe (1994). A 2ml portion of the reaction mixture was added to a tube containing 2ml of 10% trichloroacetic acid to arrest the reaction and then centrifuged at 10,000g for 5min. One ml of the supernatant was then added to 1ml of a color forming reagent. The color reagent was a mixture of four parts of solution A (15g of ammonium heptamolybdate in 55ml of 36 N H₂SO₄, made up to 1L) and one part of solution B (27g of FeSO₄.7H₂O, a few drops of 36 N H₂SO₄, made up to 250ml). The blue color formed was measured at 700nm in a
diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, CA) after centrifuging to remove any cloudiness present. A calibration curve was produced over the range of 1 – 4 µmol of phosphate and used to estimate the enzyme activity. The analysis was performed in duplicate and reported as units/kg flour. One unit of phytase activity was defined as the amount of phytase which liberates inorganic phosphorus from a 0.001 M Na-phytate solution at a rate of 1 µmol/min at pH 5 and 45 °C.

**Phytate content**

The colorimetric method described by Vaintraub and Lapteva (1988) was used. About 1g of cowpea flour was extracted by stirring for 1h at room temperature with 20ml of 2.4% HCl. The slurry was centrifuged in a Beckman centrifuge (Model J2-21M, Beckman Instruments Inc., Palo Alto, CA) at 20,000 g for 15min at 15°C, decanted and 5ml of the supernatant diluted to 25ml. About 3 ml was pipetted into a centrifuge tube and 1ml of Wade reagent (0.03% FeCl₃·6H₂O and 0.3% sulfosalicylic acid in distilled water) added and vortexed for 5s. The mixture was then centrifuged for 5 min and the absorbance of the supernatant read at 500nm in a diode array spectrophotometer (Model 8451, Hewlett Packard, Palo Alto, CA). A standard curve was prepared using sodium phytate. The phytate concentration was calculated from the difference between the absorbance of the blank and that of the assayed sample and was reported as the average of duplicate determinations.

**Water absorption**

Ten grams of cowpea seeds were soaked in about 100ml of deionized water for 1, 3, 6, 12, 18 and 24 hours. After the soaking period, the seeds were removed, blotted to remove the surface
water and weighed. The amount of water absorbed was determined from the increase in weight. The soaking liquor was then partially dried at 90°C, then dried overnight at 40°C in a Lindberg Blue air oven (Model MO1440SC, Lindberg Blue, Asheville, NC, USA) and weighed to determine solids lost. The ‘corrected’ water absorption capacity (%) was calculated as described by Jackson and Varriano-Marston (1981):

\[ WA_c = \frac{Wt.\ after\ soaking - Initial\ Wt + Solids\ Loss}{Dry\ Wt} \times 100 \]

All the determinations were performed in duplicate and average results reported.

**Electrolyte leakage**

The amount of electrolyte leakage during soaking in deionized water was determined by measuring the conductivity of soaking liquors after removal of seeds (Berrios and others 1999). The volume of the soaking liquor was made up to the original volume with deionized water and the conductance measured using a digital conductivity meter (Fisher Scientific, Pittsburgh, PA). The conductivity was reported as mS/cm at 25°C.

**Pectin loss**

The method described by Liu et al. (1993b) was used. About 20g of seeds tied in cheese-cloth were soaked in 100ml deionized water for 6h, drained and the soaking liquor saved. Fresh deionized water (100ml) was added and seeds heated at 100°C for 30 min, drained and the cooking liquor saved. The soaking and cooking liquors were then separately made up to 100ml and centrifuged at 20000g for 15min. The supernatant (3 ml) was pipetted into 50ml centrifuge tube and 16ml of 95% ethyl alcohol added to obtain a final concentration of 80% alcohol. The tubes were stored at 4°C for 48h and then centrifuged at 20000g for 15min. The precipitate was
dissolved in 1ml water and 2 drops of 1N NaOH for 2 hours. A total of 9 ml of 78% sulfuric acid was added (4.5ml at a time) with each addition followed by vortexing and chilling in an ice bath. The tubes were sonicated for 3min, heated at 50°C for 10min and cooled in an ice bath. A 1ml aliquot was mixed with 2ml cold 6N NaOH, then 0.5ml of this mixture was added to a tube containing 0.5ml of 2% NaCl. This was followed by the addition of 4 ml of cold concentrated H₂SO₄, heating at 70°C in a water bath for 10min and then cooling in tap water for 10min. The color was developed by adding 0.1ml of 3, 5-dimethylphenol (0.1% in glacial acetic acid). The absorbance was read after 15min at 400 and 450nm. The difference in absorbance at the two wavelengths was used to calculate the galacturonic acid content. A standard curve was prepared using 0-125nmol galacturonic acid. The pectin loss was expressed as mg galacturonic acid/g dry seeds.

6.4 RESULTS AND DISCUSSION

For ease of discussion, the samples are grouped based on their storage condition and are referred to as follows: INITIAL – analyzed immediately following steam treatment; MILD – stored at 4°C; SEVERE – stored at 42°C/80% RH.

Moisture content

The moisture content of the cowpeas was reduced significantly as a result of the drying process. Before storage, the moisture content of the samples ranged between 4.61-10.71% (Table 6.3). In general, samples dried for a longer period and at a higher temperature had lower moisture content. The lowest moisture content was observed in samples dried using drying condition 15 (8 hours at 65°C and 15% RH). Other authors have reported that the hard-to-cook defect is
enhanced by storing seeds with moisture content of 13% or higher, whereas seeds with a moisture content of 10% or lower retain their textural quality (Hughes and Sandsted 1975; Antunes and Sgarbieri 1979). The low moisture content attained in this study should therefore contribute to maintaining the quality of cowpea seeds during storage. When the seeds were stored under severe conditions, the moisture content generally showed an increase, in some samples, it almost doubled. The moisture content after storage under severe conditions was found to be between 8.98-11.17%, whereas samples stored under mild conditions exhibited a moisture content of 5.36-10.58%. This was still generally lower than the moisture content of control cowpeas measured before and after storage.

**Cooked texture**

The treatment combinations investigated (Table 6.2) had a significant reducing effect on the peak force of cowpeas cooked at 100°C for different times. It was generally observed that initial samples showed peak forces comparable to or lower than that of the untreated control after 60 min cooking (Figure 6.1). In their study on roasted beans, Aguilera and Steinsapir (1985) reported that an initial softening occurred in the beans following heat treatment. They suggested that during the roasting process, a combination of escaping moisture and the temperature gradient present in the beans softened the intercellular material and permitted easier separation of the cells. Jones and Boulter (1983) considered beans to be cooked if the cotyledons yielded to only slight pressure when individual beans were squeezed between the forefinger and thumb. This method was used in this study to determine the optimum cooking time and corresponding peak force value for cowpeas. The peak force of a sample of optimally cooked control cowpeas was found to be approximately 602N after 60min cooking. The peak force values of the treated
seeds measured prior to storage (INITIAL samples) ranged from 535-628N after 60 min cooking. The lowest peak force was recorded for drying condition 7 where the seeds were dried for 6 h at 55°C and 24% RH. Only drying conditions 4, 13 and 15 resulted in samples which exhibited peak force values (612 – 628 N) higher than that of the control. This implies that in general, the treatments were effective in reducing the cooked hardness of cowpea seeds.

A majority of MILD samples showed a characteristic change in cooked texture. It was generally observed that the peak force values of these samples were lower than both INITIAL and SEVERE samples (Figure 6.1). A similar decrease in peak force was observed for the control samples stored under mild conditions. Thus overall, storage under mild conditions showed a slight improvement in texture. Other authors have also reported a shorter cooking time for beans and cowpeas following storage under low temperature conditions (Berrios and others 1999; Hentges and others 1991). It is generally known that the hard-to-cook defect is accelerated by storage under high temperature and humidity whereas low temperature storage maintains the texture.

When the treated cowpeas were stored under the severe conditions, it was observed after 60 min cooking that, in general, all the samples showed a decrease in peak force values (Figure 6.1). The only exceptions were for drying conditions 1, 5, 6 and 12 which showed slight increases in cooked hardness. On the other hand, the control sample stored under the same conditions exhibited an almost two-fold increase in cooked hardness. This is the typical hardening associated with legumes stored under high temperature and humidity conditions.

Aguilera and Stanley (1985) proposed an index known as the relative hardness (HR) which is the hardness (Ht) at any storage time, t divided by the hardness at the beginning of storage (Ho). This index was calculated to evaluate the effect of storage on cooked hardness.
Samples with $H_R > 1$ have increased in hardness whereas those with values $< 1$ show softening. The results are shown in Figure 6.2 for 30 and 60 min cooked samples. All MILD samples had $H_R$ values of approx. 1 or lower. This implies that storage under mild condition did not adversely affect the cooked texture of cowpeas. For SEVERE samples, a dramatic change occurred between 30 and 60 min cooking. After 30 min of cooking, only 5 drying conditions (4, 5, 11, 13 and 15) showed values less than 1, however cooking for an additional 30 min resulted in 11 samples with $H_R < 1$. Since in this study, the cooking time needed to achieve optimally cooked texture was judged to be 60 min, it can be concluded that on the whole the treated samples did not harden during storage, even under severe conditions. The treatments applied prior to storage were therefore effective in preventing hardening during storage.

The peak force values obtained before and after storage under the two conditions were compared using paired t-tests. Results from the statistical analyses indicated that the cooked texture of SEVERE samples was comparable to that of INITIAL samples. Conversely, peak force values of MILD samples were significantly lower than those of INITIAL or SEVERE samples, irrespective of the cooking time.

Regression analyses of the cooked texture for INITIAL samples showed that none of the drying parameters had a significant effect ($p > 0.05$) on the texture of cowpeas cooked for 30-120 min; however, all models had non significant lack of fit. The results of the statistical analysis for INITIAL samples are shown in Table 6.4 for samples cooked for 60 min and in Appendix 3 for 30, 90 and 120 min cooking. The results imply that the decrease in cooked hardness observed in the INITIAL samples was not influenced by the drying conditions applied after the steaming process. An analysis of the cooked texture (after 60 min cooking) of samples stored under mild conditions also showed that drying conditions did not significantly affect the
texture of the treated cowpeas stored at this temperature (Table 6.5). This suggests that the change observed (lower peak force values) in the texture of these samples was probably a result of the storage temperature and not the drying conditions. The statistical analysis for cooked texture of MILD samples cooked for 30, 90 and 120 min are summarized in Appendix 4; which also shows that none of the drying parameters significantly influenced the cooked texture under this storage condition.

However, samples stored for 5 weeks under severe conditions showed a different trend in that they were significantly affected by the drying conditions. Overall, the drying temperature had a stronger effect on the peak force values of samples cooked for 60 min. The other two factors (drying humidity and time) did not significantly influence the peak force of samples cooked for less than 90 min. The results of the statistical analysis for samples stored under severe conditions are shown for 60 min cooking in Table 6.6 and in Appendix 5 for 30, 90 and 120 min cooking. It therefore appears that the behavior of steamed seeds during storage under severe conditions is determined to some extent by the drying conditions. As such, steamed cowpeas meant for further storage should be dried under controlled conditions.

The regression models generated for cooked texture of cowpeas stored under severe conditions and cooked for longer than 30 min, showed non-significant lack of fit. This indicates the adequacy of the models to sufficiently predict cooked texture for any combination of the independent variables within the range studied. All the models were significant with high coefficient of determination values ranging between 86 and 98%. Only data for the results of the analysis for 60 min cooked samples are presented in Table 6.6. The predictive models obtained were used to draw response surface plots. However, since the models generated for INITIAL and MILD samples were not significant, only the data for SEVERE samples cooked for 60 min are
presented (Figure 6.3.). The shape of the resulting plots was dependent on the cooking time under consideration. Cooking for 60 min resulted in plots which showed a decrease in peak force with increasing drying temperature up to a point followed by a slight increase. This trend was observed at all levels of drying humidity. On the other hand, after cooking for 90 min, a general decrease in hardness with increasing temperature was observed. The effect of humidity was however dependent on the temperature, at low temperature the peak force increased sharply but peaked at intermediate humidity at high temperature values. The plots obtained for 120 min showed a gradual decrease in peak force with increasing temperature at all humidity levels. However there was a slight increase in peak force with increasing humidity up to a certain maximum value after which a decrease was observed.

Hung and others (1988) reported that the maximum force required to shear-compress cowpea seeds decreased with increasing pre-decortication drying temperature. Further studies on microstructural changes following the pre-decortication heat treatment revealed that the reduced shearing and fracturing forces observed with increasing temperature of cowpeas was possibly due to damages in the middle lamella which aids in maintaining the integrity of the cell (Hung and others 1990). The role of the middle lamella in bean softening during cooking has been reported by several authors (Sefa-Dedeh and others 1978; Hincks and Stanley 1986; Shomer and others 1990). The combination of steaming and subsequent drying could have resulted in an alteration of the middle lamella causing the cell to easily lose its integrity following cooking. This becomes more evident as the drying temperature increased and possibly more damage occurred in the middle lamella.
**Phytase activity**

The phytase activity of untreated cowpeas was found to be 115.3 units/kg. This was reduced by the combination of steam treatment and drying conditions to between 68.5-73.7 units/kg which represents 59–64% of the original activity (Figure 6.4). The lowest reduction in initial samples was observed in drying condition 10, referring to drying for 6h at 65°C and 13% RH, and the highest reduction was in drying condition 4 (4h of drying at 65°C and 15% RH). Storage of both the treated and untreated cowpeas resulted in an increase in phytase activity irrespective of the storage temperature, although the increase was more pronounced in samples stored under severe conditions (Figure 6.4). Enzyme activity increased to between 80-82.9 units/kg in severe samples and 74.4-80.3 units/kg in mild samples. Phytase activity in the control samples increased to 133.9 units/kg and 128.9 units/kg, respectively, after storage under severe and mild conditions. A similar increase in phytase activity during storage under high temperature and humidity was recorded by Hincks and Stanley (1986) and Mafuleka and others (1993). Paired t-tests analysis indicated that there were significant differences in the phytase activity measured in treated samples before and after storage at both temperatures. This implies the increase in activity observed was significantly affected by storage.

Regression analysis of data for phytase activity prior to storage generated a significant model (p<0.01) with non-significant lack of fit (Table 6.4). Drying time and its quadratic term as well as the quadratic term for temperature had significant influences on the measured phytase activity. The observed increase in phytase activity in the samples stored under both mild and severe conditions did not appear to be significantly affected by any of the drying parameters. From Tables 6.5 and 6.6, it is evident that no significant effects were observed in any of the factors under consideration. This means the phytase activity of all stored samples increased
irrespective of the drying conditions applied prior to storage. A response surface plot was drawn using the regression model generated for Initial samples (Figure 6.5). Activity decreased with drying temperature up to a certain level beyond which increasing temperature appeared to have a slight activating effect on activity. The drying humidity did not have a particularly characteristic effect on phytase activity (Figure 6.5). However at low temperature, phytase activity increased sharply with increasing humidity. Usually, increasing the temperature above the optimum for enzyme activity has an inactivating effect (Parkin 1993). This could account for the observed decrease in activity observed as drying temperature increased.

No correlation was recorded between the phytase activity and cooked texture of the treated cowpeas. This was true for Initial samples and also samples stored under either mild or severe conditions. A similar observation was made in our previous study where the changes in phytase activity were not significantly correlated with the cooked texture of steam-treated cowpeas (Affrifah and others 2004). It was suggested that the changes responsible for the reduction in cooked hardness after steam treatment were not directly related to changes in phytase activity.

**Phytate content**

Figure 6.6 shows the changes in phytate content of steamed and dried cowpeas measured before and after storage under different conditions. The phytate content of the cowpeas as determined before storage decreased from 0.133% in the untreated control to 0.074-0.105% in the steamed dried cowpeas. For Initial samples, the lowest phytate content was measured in drying condition 10 and the highest in drying condition 14. The reduction in phytate content was probably due to a combination of hydrolysis during steaming and phytase action. Drying
condition 10 had the highest residual phytase activity (73.7 units/kg) which could account for the higher reduction in phytate content. Although drying condition 14 did not have the overall lowest phytase activity (70.3 units/kg), it was on the lower end of the range. Storage resulted in further significant decreases in phytate content to between 0.071-0.098% under severe conditions and 0.065-0.095% under mild conditions (Figure 6.6).

The phytate content of INITIAL samples was significantly affected by drying humidity and its quadratic term as shown in Table 6.4. The predictive model had a non-significant lack of fit and explained approx 84% of the variation observed in phytate content. The response surface plot obtained using the predictive model showed that the phytate content increased sharply with increasing humidity to a peak after which it decreased (Figure 6.7A). Phytate content was highest in samples dried at intermediate temperature and humidity with lower values being observed on either side of the optimum. There was no significant correlation between phytate content and phytase activity prior to storage indicating that the most likely pathway for reduction in phytate concentration was through hydrolysis during the steaming process and subsequent drying.

From the analysis of the data on phytate content of MILD samples, it was observed that only drying time had a slightly significant effect (Table 6.5). The model generated was however not significant. The drying conditions applied following the steam treatment all had significant effects on the changes in phytate concentration during storage under severe conditions. The model explained 90% of the variation during storage and had non significant lack of fit (Table 6.6). All the factors had significant linear effects on the changes in phytate content of SEVERE samples although the effect of drying temperature was not as significant as drying humidity and time. The 2nd order quadratic terms were also significant and there was a significant interaction
between temperature and humidity. The response surface plot for the effect of drying conditions on phytate content is shown in Figure 6.7B. In general, the phytate content increased to a maximum with increasing drying humidity thereafter showing a decrease in concentration.

Paired t-test analysis showed significant differences in the phytate content of the samples before and after storage. Phytate content of severe samples was found to be significantly correlated ($r = -0.6423, p <0.05$) to the increase in phytase activity recorded during storage under the same conditions. Therefore part of the reduction in phytate content in stored samples may be attributed to the action of phytase.

Contrary to earlier reports (Kon and Sanshuck 1981; Longe 1983; Mafuleka and others 1993), no significant correlation was observed between the phytate content and cooked hardness at any cooking time. This was also true irrespective of the storage condition under consideration. This suggests that the textural changes noted following the treatments may be a result of changes in other constituents of either the seed coat and/or cotyledon and not so much as changes in the phytate content of the seed.

**Water absorption capacity**

The water absorption capacity was determined at different soaking times ranging from 1 to 24h. All the samples showed a similar trend where water absorption increased rapidly during the first 6 hours and then gradually slowed after 18 hours. In addition, the treated samples showed a decrease in water absorption capacity as compared to the untreated control. A representative graph showing the changes in samples dried under drying condition 2 is illustrated in Figure 6.8. Different observations have been made on the effect of heat treatment on the water absorption capacity in a variety of legumes. Some studies have recorded an increase in water absorption
following heat treatment of winged beans and cowpeas (Narayana and Narasinga-Rao 1982; Abbey and Ibeh 1988). However, Bellido and others (2003) recorded a decrease in hydration capacity of navy beans following a micronization treatment. Phillips and others (1988) reported that the water absorption capacity of cowpea meal exhibited a temperature dependency, increasing with temperature (up to 70°C) and then decreased as the treatment became more severe (90-130°C). One suggestion for the decrease in water absorption capacity could be the reduction in number of available water binding sites as a result of hydrophobic aggregation of the heated legume proteins (Zheng and others 1998). The water absorption capacity also decreased significantly with storage under both conditions. However, the decrease was greater in Severe samples as compared to Mild samples. This reducing effect of storage was also observed in our previous study (Affrifah and others 2004) and also by Liu and others (1992) and Reyes-Moreno and others (2000).

The results of the regression analysis of water absorption data of Initial samples indicated that the effect of the three factors was dependent on the soaking time. The effect of the drying conditions was significant only in samples soaked for 3, 6 and 18h. The drying conditions had significant linear and quadratic effects on the water absorbed at these soaking times as shown in Table 6.4 for 18 h soaking. The results of the statistical analysis for the other soaking times are summarized in Appendix 3. All the regression models had non significant lack of fit. The water absorption capacity of Mild samples was generally not significantly affected by any of the linear terms. However, the quadratic term for time was slightly significant (p < 0.1) for water absorption measured after 3 and 24 h of soaking (Appendix 4). Additionally, significant interactions, between drying time and temperature and between drying humidity and temperature, were observed for 18 h soaked cowpeas (Table 6.5).
A different trend was however exhibited for SEVERE samples where the drying conditions significantly influenced the water absorption capacity only after soaking for 24h. The regression coefficients are shown in Table 6.6 for 24 h soaked cowpeas. In addition, the quadratic terms for humidity and time were also significant in the 24h soaked seeds. The water absorption capacity for the other soaking periods was not significantly impacted by the drying conditions applied prior to storage (Appendix 5). The effect of drying conditions on the water absorption was further evident in the response surface plots generated; representative plots are shown in Figure 6.9. For INITIAL samples soaked for 18 hours, water absorption capacity was low in samples dried at the intermediate humidity and high in those dried at the low and high humidity conditions (Figure 6.9A). The water absorption capacity of the stored samples showed only a slight increase with increasing drying temperature especially at low humidity (Figure 6.9B). It was observed earlier that samples dried for a longer period generally had lower moisture content as compared to those dried for shorter periods. According to Smith and Nash (1961) although the seed coat is the principal factor controlling the rate of water absorption in soybeans, the initial moisture content also plays a role. The combination of increasing temperature and time has the effect of reducing the moisture content and therefore promoting water absorption capacity of these seeds.

Electrolyte leakage

The conductivity of the soaking liquor was measured as an indication of the concentration of electrolytes leached from the beans during soaking for 1-24h. Generally, conductivity increased as soaking time increased and also treated seeds leaked significantly more electrolytes as compared to the untreated control. A representative plot for the changes in electrolyte leakage is
shown in Figure 6.10 for drying condition 14. Additionally, storage resulted in a significant increase in electrolytes leached after all soaking periods especially for SEVERE samples. Several authors have indicated that stored beans generally leached more electrolytes as compared to fresh beans after any length of soaking time (Jackson and Varriano-Marston 1981; Plhak and others 1989; Berrios and others 1999). The increased electrolyte leakage in aged or stored beans is thought to be related to the loss of membrane integrity (Jones and Boulter 1983).

The effect of the drying conditions on electrolyte leakage was very dependent on the amount of soaking time and also the sample (initial or stored) under consideration. Overall, no general or consistent trend was observed in any of the samples. For INITIAL samples, the effect of drying temperature appeared to be the significant factor influencing the electrolyte leakage especially after 1 and 3 h soaking. The regression coefficients are shown in Table 6.4 for 3 h and Appendix 3 for the other soaking periods. For MILD samples, drying temperature and humidity had significant linear effects on electrolyte leakage after 3h, whereas drying time significantly affected the samples soaked for 18h. Additionally, there were some significant interactions between the drying conditions. These results are shown in Table 6.5 for 3h soaking and the rest of the data are presented in Appendix 4. Drying humidity and time were the significant factors for samples stored under severe conditions and soaked 6 hours (Table 6.6).

The predictive models all showed non-significant lack of fit and were used to draw response surface plots. Representative plots are shown in Figure 6.11 for 3 h soaking (INITIAL samples) and 6 h soaking (SEVERE samples). In general, increasing drying temperature resulted in an increase in electrolyte leakage at all soaking times and under both storage conditions. Liu and others (1992) studied the influence of pretreatment and cooking on electrolyte leakage in aged cowpeas. They observed that electrolyte leakage increased as the incubation temperature
increased. It has been previously reported that the integrity of plasma membranes is well maintained at temperatures below 45°C, however, exposure to temperatures above 55.5°C results in a loss of integrity (Thebud and Santarius 1982). This offers a possible explanation for the increased electrolyte leakage with increasing drying temperature.

**Pectin loss during soaking and cooking**

Pectin loss during soaking of treated cowpeas ranged from 0.218-0.31 mg galacturonic acid/g seed as compared to 0.072 mg galacturonic acid/g seed measured for the untreated control (Figure 6.12). For initial samples, cowpeas dried 8h at 45°C and 40% RH (drying condition 12) lost the least amount of pectin whereas those dried 8h at 65°C and 15% RH (drying condition 15) had the highest loss. The effect of storage under both mild and severe conditions on pectin loss during soaking was not very obvious. However, with a few exceptions it appears there was an overall decrease in pectin solubility.

From Figure 6.13, it can be seen that a different pattern was exhibited during cooking at 100°C for 30min. Pectin loss from treated samples before storage was between 0.706-1.01 mg galacturonic acid/g seed as compared to 1.03 mg galacturonic acid/g seed in the untreated control. With a few exceptions, storage generally resulted in a comparable or slight increase (0.744-1.04 mg galacturonic acid/g seed) in pectin loss in treated seeds suggesting an overall increase in pectin solubility. A decrease in pectin loss (0.603 mg galacturonic acid/g seed) was observed in the untreated control following storage under severe conditions. Hentges and others (1991) and Jones and Boulter (1983) all reported a decrease in pectin solubility in hard-to-cook seeds. The increased solubility recorded in this study could account for the reduction in cooked
hardness observed earlier. Results of a paired t-test for pectin loss during cooking showed no significant differences in the data obtained before and after storage.

The effect of drying conditions on the amount of pectin lost during 6h soaking in water and during cooking was determined. The overall regression model for both processes was not significant with non significant lack of fit indicating that a higher order model would probably be more suitable in describing the data (Table 6.4). Cowpeas stored under severe conditions showed a similar trend in that they were also not significantly affected by the drying conditions applied prior to storage (Table 6.6). However, storage under mild conditions resulted in slightly different results. Although, no significant effects were recorded for the loss of pectin during soaking, the drying humidity as well as the quadratic terms for time and humidity showed significant influences on the pectin loss during cooking (Table 6.5).

6.5 CONCLUSIONS

The treated cowpeas showed cooked texture which was comparable to or better than the control. The cooked texture following storage under mild conditions showed an overall improvement although the effect of drying conditions was not significant. The drying temperature made a significant contribution towards preventing hardening of steamed cowpeas during storage under severe or unfavorable conditions. The physicochemical properties monitored were more significantly affected by drying humidity and time as compared to temperature. The effect of drying condition on cooked texture and phytase activity was highly dependent on the storage condition of the cowpeas. Drying conditions significantly affected phytase activity before storage but not after; and only significantly affected cooked texture of stored cowpeas.
Acknowledgment: This study was supported by a grant from the Bean/Cowpea Collaborative Research Support Program, U.S. Agency for International Development (Grant # GDG-G-00-02-0012-00). We gratefully acknowledge the technical assistance of Mr. Glenn Farrell in the steaming process and Ms. Hannah Smith for her help with sample analysis.

6.6 References


Box GEP, Behnken DW. 1960. Some new three level designs for the study of quantitative variables. Technometrics 2(4):455-75.


Table 6.1: Process variables and levels

<table>
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<th>Independent Variable</th>
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<th>Uncoded</th>
<th>Levels</th>
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</tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Drying humidity</td>
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<td>(g water/kg air)</td>
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Table 6.2  Treatment combinations from the Box-Behnken experimental design

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<th>Drying humidity, g water/kg air (% RH)</th>
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1 Values in parentheses refer to equivalent relative humidity
Table 6.3: The effect of drying condition and storage on the moisture content of steamed cowpeas

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<tr>
<td>Coefficient</td>
<td>Peak Force[^d] (N)</td>
<td>Phytase Activity (units/kg)</td>
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<tr>
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<td>-------------------</td>
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<td>Lack of fit</td>
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[^a]: In models: X1, drying time; X2, drying temperature; X3, drying humidity. ^[b]: After 60min cooking. ^[c]: After 18h soaking. ^[d]: after 6h soaking

***Significant at level 0.01; **Significant at level 0.05; *Significant at level 0.10.
Table 6.5: Regression coefficients and analysis of variance of selected second-order models\(^a\) for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under mild conditions

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<th>Coefficient</th>
<th>Peak Force(^b) (N)</th>
<th>Phytase Activity (units/kg)</th>
<th>Phytate (%)</th>
<th>Water Absorption(^c) (%)</th>
<th>Electrolyte Leakage(^d) (mS/cm)</th>
<th>Pectin Loss (mg uronic acid/g seed)</th>
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<td></td>
<td>Soak</td>
<td>Cook</td>
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<tr>
<td>B(_1)</td>
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<td>B(_{11})</td>
<td>9.75(^*)</td>
<td>-0.249</td>
<td>0.002</td>
<td>0.287</td>
<td>-0.001</td>
<td>-0.003</td>
</tr>
<tr>
<td>B(_{22})</td>
<td>0.138</td>
<td>0.019(^**)</td>
<td>-0.000</td>
<td>-0.000</td>
<td>-0.000(^**)</td>
<td>0.003</td>
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<tr>
<td>B(_{33})</td>
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<td>0.045</td>
<td>-0.000</td>
<td>0.034</td>
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<td>-0.000</td>
</tr>
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<td>Interactions</td>
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<tr>
<td>B(_{12})</td>
<td>0.184</td>
<td>0.003</td>
<td>0.000</td>
<td>0.098(^*)</td>
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<td>0.000</td>
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<td>B(_{13})</td>
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<td>0.031</td>
<td>0.000</td>
<td>-0.073</td>
<td>-0.001</td>
<td>-0.000</td>
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<tr>
<td>B(_{23})</td>
<td>-0.393</td>
<td>0.006</td>
<td>0.000</td>
<td>-0.035(^*)</td>
<td>-0.000</td>
<td>-0.000</td>
</tr>
<tr>
<td>R(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F</td>
<td>2.44</td>
<td>2.11</td>
<td>1.42</td>
<td>3.28</td>
<td>25.94(^***)</td>
<td>0.47</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>2.17</td>
<td>34.2</td>
<td>0.16</td>
<td>3.59</td>
<td>0.39</td>
<td>0.32</td>
</tr>
</tbody>
</table>

\(^a\)In models: X\(_1\), drying time; X\(_2\), drying temperature; X\(_3\), drying humidity. \(^b\)After 60min cooking. \(^c\)After 18h soaking. \(^d\)After 3h soaking

\(^*\)Significant at level 0.01; \(^**\)Significant at level 0.05; \(^*\)Significant at level 0.10.
### Table 6.6: Regression coefficients and analysis of variance of selected second-order models for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under severe conditions

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Peak Force&lt;sup&gt;b&lt;/sup&gt; (N)</th>
<th>Phytase Activity (units/kg)</th>
<th>Phytate (%)</th>
<th>Water Absorption&lt;sup&gt;c&lt;/sup&gt; (%)</th>
<th>Electrolyte Leakage&lt;sup&gt;d&lt;/sup&gt; (mS/cm)</th>
<th>Pectin Loss (mg uronic acid/g seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Soak</td>
</tr>
<tr>
<td>Linear</td>
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<td></td>
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<td></td>
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<tr>
<td>B₁</td>
<td>-29.903</td>
<td>2.32</td>
<td>-0.032**</td>
<td>7.083**</td>
<td>-0.144*</td>
<td>-0.045</td>
</tr>
<tr>
<td>B₂</td>
<td>-64.420**</td>
<td>-0.36</td>
<td>0.005*</td>
<td>-0.002</td>
<td>0.009</td>
<td>-0.011</td>
</tr>
<tr>
<td>B₃</td>
<td>19.591</td>
<td>-0.535</td>
<td>0.016**</td>
<td>-1.564</td>
<td>-0.075*</td>
<td>-0.002</td>
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<tr>
<td>Quadratic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B₁₁</td>
<td>2.781</td>
<td>-0.111</td>
<td>0.001**</td>
<td>-0.358*</td>
<td>0.007</td>
<td>0.004*</td>
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<tr>
<td>B₂₂</td>
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<td>-0.000</td>
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<td>B₃₃</td>
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<td>0.019</td>
<td>-0.000**</td>
<td>0.057**</td>
<td>0.001</td>
<td>-0.000</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B₁₂</td>
<td>0.366</td>
<td>0.010</td>
<td>0.000</td>
<td>-0.021</td>
<td>0.002*</td>
<td>0.000</td>
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<td>B₁₃</td>
<td>-0.888</td>
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<td>0.000</td>
<td>-0.034</td>
<td>0.001</td>
<td>-0.001</td>
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<tr>
<td>B₂₃</td>
<td>-0.185</td>
<td>-0.003</td>
<td>-0.000*</td>
<td>-0.024*</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>R²</td>
<td>86</td>
<td>70</td>
<td>89</td>
<td>94</td>
<td>96</td>
<td>73</td>
</tr>
<tr>
<td>F</td>
<td>3.56*</td>
<td>1.33</td>
<td>4.64*</td>
<td>8.35**</td>
<td>12.59***</td>
<td>1.51</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>10.72</td>
<td>4.25</td>
<td>3.37</td>
<td>0.72</td>
<td>0.33</td>
<td>1.66</td>
</tr>
</tbody>
</table>

<sup>a</sup>In models: X₁, drying time; X₂, drying temperature; X₃, drying humidity. <sup>b</sup>After 60 min cooking. <sup>c</sup>After 24 h soaking. <sup>d</sup>After 6 h soaking

***Significant at level 0.01; **Significant at level 0.05; *Significant at level 0.10.
Figure 6.1: Effect of drying conditions on the texture of steam-treated cowpeas cooked for 60 min. Samples were analyzed before storage (INITIAL) and after storage under severe or mild conditions. ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
Figure 6.2: Hardness of steam-treated cowpeas cooked for 30 or 60 min, after storage under mild (A) or severe (B) conditions and expressed relative to hardness at time zero ($H_t/H_o$). ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
Figure 6.3: Response surface plot for texture of steam-treated cowpeas cooked for 60 min, after storage under severe conditions and as a function of drying temperature and humidity.
Figure 6.4: Effect of drying conditions on the phytase activity in steam-treated cowpeas, showing samples analyzed before storage (INITIAL) and after storage under severe or mild conditions. ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
Figure 6.5: Response surface plot showing effect of drying temperature and humidity on phytase activity in steam-treated cowpeas (INITIAL samples).
Figure 6.6: Effect of drying conditions on the phytate content of steam-treated cowpeas, showing samples analyzed before storage (INITIAL) and after storage under severe or mild conditions. ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
Figure 6.7: Response surface plot of the effect of drying temperature and humidity on phytate content in steam-treated cowpeas, showing initial samples (A) and after storage under severe conditions (B).
Figure 6.8: Changes in water absorption capacity of control (open symbol) and steam-treated cowpeas (closed symbol) with storage: INITIAL ( □ ); MILD (○); SEVERE (△) conditions. Steam-treated cowpeas dried using drying condition 2 (refer to Table 6.2).
Figure 6.9: Response surface plots of the effect of drying temperature and humidity on water absorption capacity of steam-treated cowpeas, showing INITIAL samples (A) and SEVERE samples (B). [INITIAL samples soaked for 18 h and SEVERE samples soaked for 24h].
Figure 6.10: Changes in electrolyte leakage of control (open symbol) and steam-treated cowpeas (closed symbol) with storage: INITIAL (□); MILD (○); SEVERE (△) conditions. Steam-treated cowpeas dried using drying condition 14 (refer to Table 6.2).
Figure 6.11: Response surface plots of the effect of drying temperature and humidity on electrolyte leakage in steam-treated cowpeas, showing INITIAL samples (A) and SEVERE samples (B). [INITIAL samples soaked for 3h and SEVERE samples soaked for 6h].
Figure 6.12: Effect of drying conditions on pectin loss from steam-treated cowpeas during soaking for 6 hours; showing samples analyzed before storage (INITIAL) and after storage under severe or mild conditions. ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
Figure 6.13: Effect of drying conditions on pectin loss from steam-treated cowpeas during cooking at 100°C for 30 min; showing samples analyzed before storage (INITIAL) and after storage under severe or mild conditions. ‘C’ refers to the control cowpeas. (Drying conditions are detailed in Table 6.2).
CHAPTER 7

SUMMARY AND CONCLUSIONS

The failure of stored legumes to soften even after prolonged cooking is an age-old problem which to-date has not been fully understood. The hard-to-cook defect has both economic and nutritional implications as a result of loss of income, increased fuel requirements and loss of nutritive value. Understanding the underlying mechanisms or at the very least developing preventive measures to control the defect is important in view of the fact that legumes are generally a comparatively cheaper source of proteins and other nutrients. This study was undertaken to explore the feasibility of designing a heating process capable of maintaining the textural and physicochemical attributes of cowpeas during storage under unfavorable conditions. The focus was to design a heating procedure to target one of the enzymes (phytase) thought to be involved in the mechanisms responsible for the hardening phenomenon.

This study has demonstrated that cowpea phytases are extremely stable to thermal inactivation between moisture contents of 10 and 35% (d.b.). It was not possible to completely inactivate the enzyme even after heating for 32 min at 35% moisture and 95°C. The inactivation was highly dependent on the heating temperature and moisture content of the medium. The kinetic data was not completely described by a simple first order reaction; however a modified model, using the fractional conversion technique, adequately explained and predicted the residual phytase activity in cowpea flour.

Phytase activity was not evenly reduced when cowpea seeds were steamed; the outer layers of the seed showed a higher reduction in activity as compared to the underlying layers.
The residual phytase activity was significantly lower when whole seeds were ground for analysis as compared to the activity measured in individual layers of the seed. Studying the changes in separate seed fractions may be a better approach to understanding the hardening process. A finite element model, relating the kinetics of phytase inactivation and the heat/mass transfer process occurring during steaming, was used as a predictive tool for the inactivation of phytase in cowpea seeds. Overall, the predicted values were generally slightly lower than the experimentally measured activity for any treatment combination. The predictions for residual activity in the outer layers were better as compared to the predictions in the inner and middle layers, and this was particularly obvious as steaming time increased.

Steaming treatments were generally effective in reducing the cooked texture of cowpeas prior to storage. A heating regime was subsequently developed which was successful in preventing the characteristic storage-induced hardening by steaming cowpeas with an initial moisture content of 13% at 121°C for 4 or 6 min. Steaming caused a significant reduction in physicochemical properties specifically water absorption capacity, phytate content and phytase activity whereas solids loss, electrolyte leakage and pectin loss were improved. A lack of significant correlation between the measured physicochemical indices and the cooked texture suggested that the reduction in cooked hardness observed occurred through a pathway different from that typically associated with the development of the hard-to-cook defect in cowpeas. Although the steam treatment was effective in improving or maintaining the textural characteristics, the changes in phytase activity did not adequately correspond to the observed changes in textural properties of cowpea seeds. This is indicative of the complexity of the hard-to-cook defect and also suggests that other factors may be involved in the development of the defect as well as in the improved texture recorded.
Drying conditions, especially temperature, had a significant role in preventing further hardening of cowpeas during storage under unfavorable conditions. Steamed and dried cowpeas stored under mild conditions showed an overall improvement in cooked texture after 5 weeks. Physicochemical attributes, particularly water absorption, phytase activity and phytate content, were more significantly affected by drying humidity and time as compared to the effect of drying temperature. The drying conditions applied after steaming has a significant effect on the changes that occur in cowpea seeds during storage and should therefore be selected carefully.

It is our recommendation that in order to prevent hardening during storage, cowpeas should be steamed at 121°C for 4 min followed by drying at 60°C to moisture content of about 10%.

A more comprehensive study on the changes directly associated with steaming of cowpeas and their relationship with the observed reduction in texture would need to be conducted. This is necessary in order to be able to extend the developed steam treatment to other legumes and it might also be a step closer to finally understanding the underlying mechanism of the hard-to-cook defect.
APPENDICES
Appendix 1: Analysis of variance of cooked texture (KN) and water absorption capacity (%) of steam treated cowpeas for moisture content, steaming temperature, steaming time and storage condition

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Cooked Texture</th>
<th></th>
<th>Mean Square</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90 min</td>
<td>120 min</td>
<td>1 h&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture (M)</td>
<td>1</td>
<td>0.001</td>
<td>0.004</td>
<td>0.002</td>
<td>9.621**</td>
</tr>
<tr>
<td>Steaming Temperature (T)</td>
<td>1</td>
<td>2.56**</td>
<td>0.101**</td>
<td>0.054**</td>
<td>318.4**</td>
</tr>
<tr>
<td>M x T</td>
<td>1</td>
<td>0.004</td>
<td>0.012**</td>
<td>0.001</td>
<td>65.51**</td>
</tr>
<tr>
<td>Storage (S)</td>
<td>2</td>
<td>2.39**</td>
<td>0.187**</td>
<td>0.108**</td>
<td>716.2**</td>
</tr>
<tr>
<td>M x S</td>
<td>2</td>
<td>0.055**</td>
<td>0.001</td>
<td>0.003**</td>
<td>0.091</td>
</tr>
<tr>
<td>T x S</td>
<td>2</td>
<td>0.265**</td>
<td>0.034**</td>
<td>0.004**</td>
<td>4.682</td>
</tr>
<tr>
<td>M x T x S</td>
<td>2</td>
<td>0.013</td>
<td>0.001</td>
<td>0.002</td>
<td>1.509</td>
</tr>
<tr>
<td>Steaming Time (t)</td>
<td>2</td>
<td>0.094**</td>
<td>0.004</td>
<td>0.003**</td>
<td>68.3**</td>
</tr>
<tr>
<td>M x t</td>
<td>2</td>
<td>0.039*</td>
<td>0.001</td>
<td>0.000</td>
<td>18.00**</td>
</tr>
<tr>
<td>T x t</td>
<td>2</td>
<td>0.062**</td>
<td>0.005</td>
<td>0.000</td>
<td>13.9**</td>
</tr>
<tr>
<td>M x T x t</td>
<td>2</td>
<td>0.004</td>
<td>0.000</td>
<td>0.001</td>
<td>27.9**</td>
</tr>
<tr>
<td>S x t</td>
<td>4</td>
<td>0.110**</td>
<td>0.014**</td>
<td>0.006**</td>
<td>2.89</td>
</tr>
<tr>
<td>M x S x t</td>
<td>4</td>
<td>0.022</td>
<td>0.004</td>
<td>0.001</td>
<td>9.3**</td>
</tr>
<tr>
<td>T x S x t</td>
<td>4</td>
<td>0.004</td>
<td>0.002</td>
<td>0.000</td>
<td>2.45</td>
</tr>
<tr>
<td>M x T x S x t</td>
<td>4</td>
<td>0.018</td>
<td>0.003</td>
<td>0.002</td>
<td>2.141**</td>
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<tr>
<td>Error</td>
<td>36</td>
<td>0.013</td>
<td>0.003</td>
<td>0.001</td>
<td>3.22</td>
</tr>
</tbody>
</table>

*+, ** - Significant at the 10% and 5% probability levels respectively. <sup>a</sup> – Cooking time. <sup>b</sup> – Soaking time.
Appendix 2: Analysis of variance of solids loss and electrolyte leakage from steam treated cowpeas for moisture content, steaming temperature, steaming time and storage condition

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean Square</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Solids Loss (g/10 g)</td>
<td>3 h&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 h</td>
</tr>
<tr>
<td>Moisture (M)</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>Steaming Temperature (T)</td>
<td>1</td>
<td>0.000</td>
<td>0.027&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.063&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>M x T</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
<td>0.005</td>
</tr>
<tr>
<td>Storage (S)</td>
<td>2</td>
<td>0.001</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>M x S</td>
<td>2</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>T x S</td>
<td>2</td>
<td>0.002</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>M x T x S</td>
<td>2</td>
<td>0.001</td>
<td>0.000</td>
<td>0.006</td>
</tr>
<tr>
<td>Steaming Time (t)</td>
<td>2</td>
<td>0.004&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.003</td>
<td>0.012</td>
</tr>
<tr>
<td>M x t</td>
<td>2</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>T x t</td>
<td>2</td>
<td>0.004&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>M x T x S x t</td>
<td>4</td>
<td>0.002</td>
<td>0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>*</sup>, <sup>**</sup> – Significant at the 10% and 5% probability levels respectively. <sup>a</sup> – Soaking time.
Appendix 3: Regression coefficients and analysis of variance of selected second-order models for textural and physicochemical characteristics of steamed cowpeas before storage

<table>
<thead>
<tr>
<th></th>
<th>Peak Force (N)</th>
<th>Water Absorption Capacity (%)</th>
<th>Electrolyte Leakage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min (^b)</td>
<td>90 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_1)</td>
<td>71.8</td>
<td>49.2</td>
<td>-48.1</td>
</tr>
<tr>
<td>(B_2)</td>
<td>-62.4 (*)</td>
<td>-34.8</td>
<td>-7.52</td>
</tr>
<tr>
<td>(B_3)</td>
<td>-29.9</td>
<td>-19.1</td>
<td>30.7</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_{11})</td>
<td>-0.126</td>
<td>3.94</td>
<td>8.82 (*)</td>
</tr>
<tr>
<td>(B_{22})</td>
<td>0.648 (**)</td>
<td>0.326</td>
<td>0.099</td>
</tr>
<tr>
<td>(B_{33})</td>
<td>1.44</td>
<td>0.871</td>
<td>-0.337</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_{12})</td>
<td>0.287</td>
<td>0.023</td>
<td>-0.233</td>
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<tr>
<td>(B_{13})</td>
<td>-3.48</td>
<td>-3.95 (**)</td>
<td>-1574</td>
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<tr>
<td>(B_{23})</td>
<td>-0.375</td>
<td>-0.024</td>
<td>-0.101</td>
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<tr>
<td>(R^2)</td>
<td>71</td>
<td>78</td>
<td>69</td>
</tr>
<tr>
<td>(F)</td>
<td>1.35</td>
<td>1.95</td>
<td>1.24</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.66</td>
<td>0.41</td>
<td>1.11</td>
</tr>
</tbody>
</table>

\(^a\) In models: \(X_1\), drying time; \(X_2\), drying temperature; \(X_3\), drying humidity. \(^b\) Cooking time. \(^c\) Soaking time. \(*\) Significant at level 0.05; \(**\) Significant at level 0.10.
### Appendix 4: Regression coefficients and analysis of variance of selected second-order models\(^a\) for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under mild conditions

<table>
<thead>
<tr>
<th></th>
<th>Peak Force (N)</th>
<th>Water Absorption Capacity (%)</th>
<th>Electrolyte Leakage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min(^b)</td>
<td>90 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_1)</td>
<td>-95.6</td>
<td>-54.8</td>
<td>-24.2</td>
</tr>
<tr>
<td>(B_2)</td>
<td>15.1</td>
<td>19.9</td>
<td>-10.2</td>
</tr>
<tr>
<td>(B_3)</td>
<td>91.8</td>
<td>21.8</td>
<td>42.4</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_{11})</td>
<td>14.1</td>
<td>4.48</td>
<td>8.97**</td>
</tr>
<tr>
<td>(B_{22})</td>
<td>-0.106</td>
<td>-0.144</td>
<td>0.185</td>
</tr>
<tr>
<td>(B_{33})</td>
<td>-1.68</td>
<td>-0.303</td>
<td>-0.289</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_{12})</td>
<td>-1.18</td>
<td>-0.248</td>
<td>-0.812</td>
</tr>
<tr>
<td>(B_{13})</td>
<td>-1.14</td>
<td>0.386</td>
<td>-1.41</td>
</tr>
<tr>
<td>(B_{23})</td>
<td>0.072</td>
<td>-0.164</td>
<td>-0.268</td>
</tr>
<tr>
<td>(R^2)</td>
<td>68</td>
<td>70</td>
<td>84</td>
</tr>
<tr>
<td>(F)</td>
<td>1.19</td>
<td>1.3</td>
<td>2.89</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>1.89</td>
<td>0.27</td>
<td>2.67</td>
</tr>
</tbody>
</table>

\(a\) In models: \(X_1\), drying time; \(X_2\), drying temperature; \(X_3\), drying humidity. \(^b\) Cooking time. \(^c\) Soaking time.

**Significant at level 0.05; *Significant at level 0.10.
Appendix 5:  Regression coefficients and analysis of variance of selected second-order models\(^a\) for textural and physicochemical characteristics of steamed cowpeas after 5 weeks storage under severe conditions

<table>
<thead>
<tr>
<th></th>
<th>Peak Force (N)</th>
<th>Water Absorption Capacity (%)</th>
<th>Electrolyte Leakage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min(^b)</td>
<td>90 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(_1)</td>
<td>-247.3</td>
<td>21.7</td>
<td>77.9(***)</td>
</tr>
<tr>
<td>B(_2)</td>
<td>42.0</td>
<td>2.47</td>
<td>-35.1(***)</td>
</tr>
<tr>
<td>B(_3)</td>
<td>3.88</td>
<td>49.8(^*)</td>
<td>41.3(***)</td>
</tr>
<tr>
<td>Quadratic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(_{11})</td>
<td>0.366</td>
<td>-1.07</td>
<td>-1.45</td>
</tr>
<tr>
<td>B(_{22})</td>
<td>-0.108</td>
<td>0.111</td>
<td>0.359(***)</td>
</tr>
<tr>
<td>B(_{33})</td>
<td>0.650</td>
<td>-0.401</td>
<td>-0.530(**)</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B(_{12})</td>
<td>0.417</td>
<td>-0.534</td>
<td>-0.745(**)</td>
</tr>
<tr>
<td>B(_{13})</td>
<td>7.25</td>
<td>0.406</td>
<td>-0.917(**)</td>
</tr>
<tr>
<td>B(_{23})</td>
<td>-1.53</td>
<td>-0.609(**)</td>
<td>-0.119</td>
</tr>
<tr>
<td>R(^2)</td>
<td>83</td>
<td>92</td>
<td>99</td>
</tr>
<tr>
<td>F</td>
<td>2.64</td>
<td>6.17(**)</td>
<td>40.61(***)</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>5.76</td>
<td>0.75</td>
<td>0.84</td>
</tr>
</tbody>
</table>

\(^a\)In models: X\(_1\), drying time; X\(_2\), drying temperature; X\(_3\), drying humidity. \(^b\)Cooking time. \(^c\)Soaking time.  
\(***\) Significant at level 0.01; \(**\) Significant at level 0.05; \(^*\) Significant at level 0.1