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Improvement in the cooking and physico-chemical characteristics of hard-to-cook cowpeas by pre-conditinioning and micronization

BY

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DEDICATION

In the memory of my close and beloved relatives, this dissertation is dedicated to:

Salvador Mulhovo Ubisse, my dear father

Maria das Virtudes Alexandre Ingovene, my lovable mother

Armando Agostias Salvador Ubisse, my warm-hearted elder brother

May your souls rest in peace.

I love you all.

DECLARATION

I declare that the thesis which I hereby submit for the degree MSc (Agric) Food Science and Technology at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another university or institution of higher education.

Brasilino das Virtudes Salvador
July 2007

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ABSTRACT

Improvement in the cooking and physico-chemical characteristics of hard-to-cook cowpeas by pre-conditinioning and micronization

By

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Cowpeas (*Vigna unguiculata* L. Walp) are legumes widely consumed in developing countries. Because cowpeas are an important source of proteins, calories and vitamins, they have the potential to alleviate protein-energy malnutrition. However, the consumption of cowpeas is impaired by the hard-to-cook (HTC) defect, which develops when cowpeas are stored at high temperature and humidity conditions. HTC cowpeas require extended time to cook and have decreased protein, starch, vitamin availability and poor textural quality. The extended cooking time and poor textural quality reduce consumer preference and acceptability.

Soaking cowpeas in water or in a solution containing monovalent cations have been used by other researchers to reduce the cooking time of normal and HTC cowpeas, while micronization has been used to reduce the cooking time of normal cowpeas. Hence pre-conditioning in water or in a solution containing monovalent (Na^+) cations in combination with micronization, could have the potential to help in alleviating the HTC phenomenon in legume seeds, especially cowpeas.

This study was undertaken in two parts. The first part consisted of inducing the hard-to-cook (HTC) defect and determining its effect on cooking and physicochemical

characteristics of cowpeas. The second part consisted of attempting to alleviate the HTC defect in cowpeas by pre-conditioning cowpeas in water or in a solution with monovalent (Na^+) cations and its combination with micronization. The effect of these treatments on cooking and physicochemical characteristics of normal and HTC cowpeas were studied.

Storing cowpeas at high temperature and high relative humidity, increased the cooking time of cowpeas (*Mogwe-o-Kgotsheng*) from 89 to more than 270 min. The increase in the cooking time was associated with reduced pectin solubility, which was coincident with a decrease in phytic acid content and an increase in phytase activity. According to the "phytate-divalent cations-pectins" theory, at adverse storage conditions, phytase probably hydrolysed phytate to release divalent cations which migrated to the middle lamella to bind with pectins, reducing their solubility. Because of the reduced pectin solubility of HTC cowpeas, the hardness of cooked seeds increased, the degree of splitting reduced and water absorbed during cooking consequently reduced as compared with normal cowpeas. This research supports the "phytate-divalent cations-pectins" as an important mechanism to explain the HTC-defect in legume seeds.

From a practical standpoint, pre-conditioning cowpeas in water on its own was effective in reducing the cooking time of normal cowpeas from 89 to 44 min. This coincided with an improvement of pectin solubility, degree of splitting and decreased hardness. For HTC cowpeas, a combination of pre-conditioning in a solution containing monovalent (Na^+) cations and micronization was needed to optimally reduce the cooking time from more than 270 min to 59 min. This coincided with an improvement of pectin solubility, degree of splitting and decrease in the hardness of cooked cowpeas.

Pre-conditioning cowpeas in water induced the solubilization of pectins in the middle lamella of normal and HTC cowpeas. Pre-conditioning cowpeas in a solution with monovalent (Na^+) cations improved pectin solubility due to the solubilization effect of water as well as a conversion of insoluble pectins to soluble pectins by monovalent (Na^+) cations. Micronization improved pectin solubility further by breaking pectin molecules into lower and more soluble fractions, probably via the β-elimination reaction. Micronization also decreased the hardness of cooked seeds and increased the degree of splitting for both normal and HTC cowpeas. However, the reduction in hardness and increase in the degree of splitting were more pronounced in normal than in HTC cowpeas, probably because

more divalent cations were bound to the pectins of HTC cowpeas. For normal cowpeas, the improvement of pectin solubility, decrease in texture of cooked seeds and increase in the splitting as influenced by micronization was reflected in the increase of the amount of water absorbed during cooking, which could have contributed to the reduction in the cooking time. However, for HTC this was not the case as the water absorbed during cooking decreased. The reduction in the amount of water absorbed during cooking could be associated with protein denaturation during storage at adverse conditions and during micronization.

The improvement of pectin solubility was at higher levels when all the treatments were applied in HTC cowpeas than in normal cowpeas. However, this was not coincident with reduction in the cooking times. This suggests that factors (i.e. proteins, starch) other than pectin solubility could have contributed to the cooking time of HTC cowpeas.

Pre-conditioning cowpeas in a solution with monovalent (Na^+) cations in combination with micronization has a definite potential to help alleviate the HTC defect in cowpeas.

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1. INTRODUCTION AND PROBLEM STATEMENT

Cowpeas are widely consumed in different forms in most developing countries. In Mozambique, cowpeas are consumed mostly alone as a cooked whole grain, mixed with cassava, maize or vegetables to form a thick pasta called "*chiguinha*". In addition, cowpea seeds are also decorticated, ground into flour and mixed with chopped onions and spices and fried ("*akara*" or "*badgia*") or steamed ("*moin-moin*") (Taiwo, Akanbi & Ajibola, 1997a). According to Yousif, Deeth, Caffin & Lisle (2002), in some East Asian countries, legumes are consumed as a dessert or snack food, especially during celebrations and traditional festivals such as Chinese New Year. According to these authors, legumes in Japan are consumed as bean paste ("*ann*") or as filler for various sweet pastries, and as whole beans boiled and sweetened for snacks and confectionery items.

Cowpeas have a very rich nutritional profile, containing about 25% proteins and 60 to 69% soluble carbohydrates (Ajibola, Aviara & Ajetumobi, 2003). They are also an affordable source of minerals and B vitamins (Phillips, McWatters, Chinnan, Hung, Beuchat, Sefa-Dedeh, Sakyi-Dawson, Ngoddy, Nnanyelugo, Enwere, Komeny, Liu, Mensa-Wilmot, Nnanna, Okeke, Prinyawiwatkul & Saalia, 2003) and have high levels of folic acid and low levels of anti-nutritional and flatulence producing factors (Ehlers & Hall, 1997). However, cowpeas are produced in quantities that cannot be consumed within a short period of time leading to storage, most of the time, under high temperature and relative humidity conditions in developing communities or countries. This may induce a phenomenon known as hard-to-cook (HTC) defect.

According to Hentges, Weaver & Nielsen (1991), the most widely accepted reason for development of HTC phenomenon is attributed to the release of Ca^{2+} from phytate during storage, which migrates to the cell middle lamella where it binds to pectin. It contributes to pectin insolubilization, forming a barrier to water uptake and cotyledon softening during cooking. HTC cowpeas require longer cooking time because of the resistance of the seeds to softening during cooking and have low nutritional and textural quality (Liu, Phillips, Hung, Shewfelt & McWatters, 1992). The extended cooking time and poor textural quality reduce consumer preferences and acceptability (Phillips *et al.,* 2003).

Long cooking time is one of the most important parameters limiting the utilisation of HTC cowpeas. Reducing the cooking time of HTC cowpeas may improve their acceptability and result in higher nutrient retention during cooking by reducing the amount of solids leaching out during the process (Akinyele, Onigbinde, Hussain & Omololu, 1986). Various techniques have been introduced to reduce the cooking time of cowpeas. Micronization, an infrared (IR) heat treatment, is one of them and has been reported to reduce the cooking time by 36% for Bechuana white and 44% for Variety 462 cowpeas, when micronized to final surface temperatures of 153ºC (Mwangwela, Waniska & Minnaar, 2006) and by 30% for Bechuana White cowpeas, when micronized to final surface temperature of approximately 160ºC (Phadi, 2004).

The understanding of the mechanisms by which the HTC phenomenon takes place in legumes seeds is very essential to the process of designing techniques to alleviate the defect. This study aims to evaluate the potential of micronization and pre-conditioning in solution containing monovalent (Na^+) cations prior to micronization in reducing cooking time of HTC induced cowpeas.

2. LITERATURE REVIEW

2.1. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an annual, warm-season, herbaceous legume originating in Africa and widely grown in Africa, Latin America, Southeast Asia and in the Southern United States (Davis, Oelke, Oplinger, Doll, Hanson & Putnam, 1991). According to Ehlers & Hall (1997), cowpeas grow well under a wide variety of soil and soil conditions under both irrigated and non-irrigated regimes but responding more positively under irrigated conditions. The fact that cowpeas are more drought resistant than beans make them an important crop in many developing parts of the world where irrigation is still a problem.

The total worldwide production of cowpea is estimated at 3.3 million tonnes of dry grain, of which 64% is produced in Africa (FAO, 2001). Nigeria is the world's largest producer with 2.1 million tonnes, followed by Niger with 650 000 tonnes and Mali with 110 000 tonnes. The total production area of cowpeas is estimated at 9.8 million hectares; about 9.3 million hectares of these in West Africa (FAO, 2001). About ⅔ of the production and more than ¾ of the area of production is spread over the Sudan Savanna and Sahelian zones of sub-Saharan Africa from Senegal going east through Nigeria and Niger, from Angola across Botswana to Mozambique, Kenya and Tanzania (Ehlers & Hall, 1997).

2.2. IMPORTANCE AND UTILISATION OF COWPEAS

Cowpeas are an important source of energy and nutrients in developing countries of Africa, Latin America, and Asia. Cowpeas are good source of dietary protein, which complements cereals, starchy roots and tubers (Phillips *et al.,* 2003). They provide an alternative source of protein where meat and meat products are limited or expensive.

The dry grain of cowpea is the principal product used for human consumption. Leaves (mostly in eastern Africa), normal peas (the southern US and Senegal) and normal green pods (humid regions of Asia and the Caribbean) are also consumed and the crop can be used for green manure in south eastern US and Australia (Taiwo, Akanbi & Ajibola, 1997b).

2.3. STRUCTURE AND CHEMICAL COMPOSITION OF COWPEAS

The understanding of the structure and chemical composition of cowpeas seeds is essential for the explanation of physicochemical changes occurring during storage, soaking and cooking.

According to Sefa-Dedeh & Stanley (1979), a cowpea seed consists of a seed coat, cotyledons, hilum, micropyle and germ or embryo. The micropyle is situated just below the hilum. Some varieties have Y-shaped closed micropyle, while others have circular open micropyles (Sefa-Dedeh & Stanley, 1979). According to them, cowpea varieties have parenchyma cells (60 to 120 µm length) and these cells contain starch granules embedded in a proteinaceous matrix. Figure 2.3.1 shows the cowpea seed coat and different parts of the cotyledon.

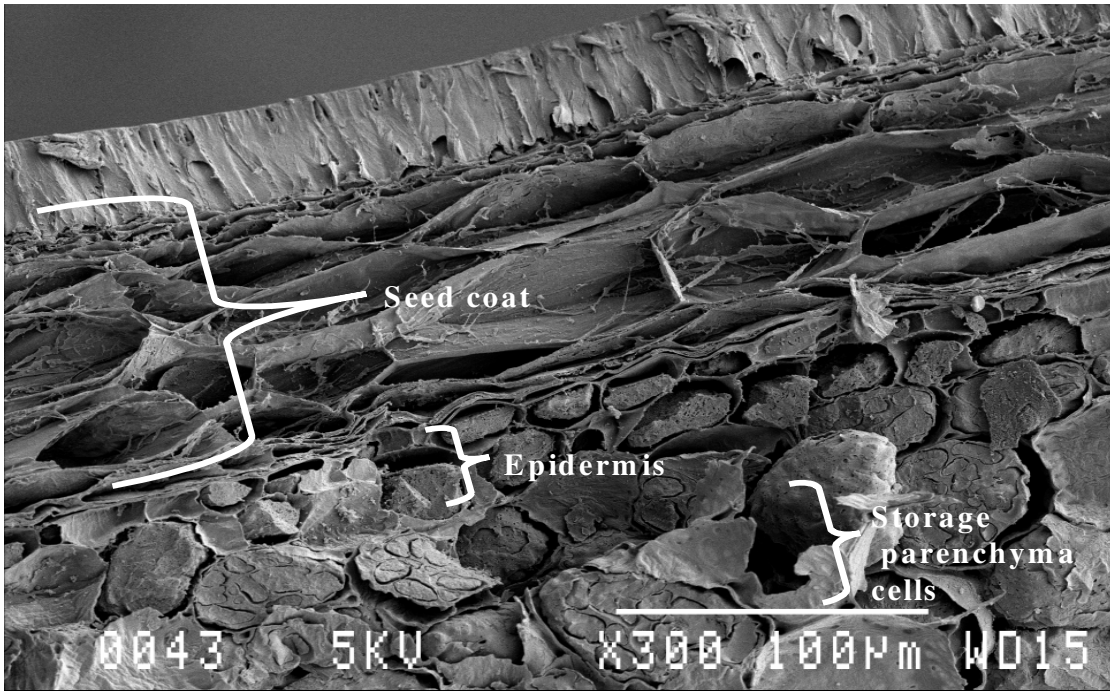

Figure 2.3.1. Micrograph of cowpea seed structure (Phadi, 2004)

The cowpea seed size (length, width and thickness), shape, testa texture, seed colour and seed weight vary considerable (Table 2.3.1).

Table 2.3.1. Seed size (length, width and thickness), weight, hilum length, and seed coat thickness of cowpea seeds

Seed Characteristics	Demooy & Demooy (1990)	Giami (2005)	Longe (1980)	Sefa-Dedeh & Stanley (1979)
Length (mm)	5.00 - 8.80	6.50 - 8.80	7.30 – 20.00	6.05 – 8.80
Width (mm)	5.30 - 6.80	4.80 - 5.80	3.70 – 6.30	5.20 – 7.30
Thickness (mm)	4.00 - 5.30	a	5.00 – 8.60	3.90 – 5.10
100-seed weight	7.52 - 19.16	12.50 – 18.60	a	a
Hilum (mm)	a	a	a	2.11 – 3.00
Seed coat (µm)	a	a	a	5.84 – 59.33

[a] No reported values

The testa texture can be rough, smooth or wrinkled (Demooy & Demooy, 1990). The seeds are oval, square, round, oblong or flat in shape. The seed colour may be red, black, white, brown, cream, maroon, dark brown or dark maroon (Longe, 1980; Giami, 2005). About 93% of proteins, 95% of the lipids, and 87% of the ash in cowpeas are found in the cotyledons and approximately 87% of the seed fibre is in the seed coat (Chavan, Kadam & Salunkhe, 1989). The percentage of seed coat in cowpeas varies from 9.3 to 33% (Ehlers & Hall, 1997; Olapade, Okafor, Ozumba & Olatunji, 2002). According to the values in Table 2.3.2, the proximate composition of cowpeas can differ considerable. Some differences in the proximate composition can be attributed to the effects of cultivar, soil, climate, and cultural practices (Hsieh, Pomeranz & Swanson, 1992).

Table 2.3.2. Proximate composition of cowpea seeds on wet basis

Parameter	Hsieh *et al.* (1992)	Phadi (2004)	Taiwo (1998)	Mwangwela (2006)
Moisture (%)	7.38 – 15.34	8.60	8.50 – 11.00	8.9-12.1
Ash (%)	3.68 – 4.36	3.50	a	a
Protein (%)	21.68 – 28.68	22.30	24.60 – 25.10	24.0-28.3
Lipid (%)	0.30 – 1.44	1.40	2.50 – 5.10	a
Carbohydrate (%)	65.92 – 73.17	64.20	54.20 – 58.60	a

[a] No reported values

Freitas, Teixeira & Ferreira (2004) found that globulins and albumins constitute the vast majority of the cowpea seed proteins, i.e. 51% and 45%, respectively. Glutelins (3%) and prolamins (1%) are the other fractions of proteins. Longe (1980) found starch the most abundant carbohydrate in cowpeas (37 to 42%). He also found other carbohydrates such as sugars (high levels of sucrose and traces of fructose, glucose, raffinose, stachyose and verbascose) and dietary fibre components in varying amounts. Proteins are the major water absorption components in cowpeas, although other components such as cellulose, starch and pectic substances also play an important role in water absorption (Sefa-Dedeh & Stanley, 1979).

Pectic substances are complex polysaccharides present in the primary cell wall and middle lamella where they play important roles such as hydrating agents and cementing materials for the cellulose network (Muralikrishna & Tharanathan, 1994). Figure 2.3.2 shows the structure of pectin, which is a linear polymer of α-D-galacturonic acid linked through the 1- and 4- positions, with a proportion of the carboxyl groups esterified with methanol (Coultate, 2002).

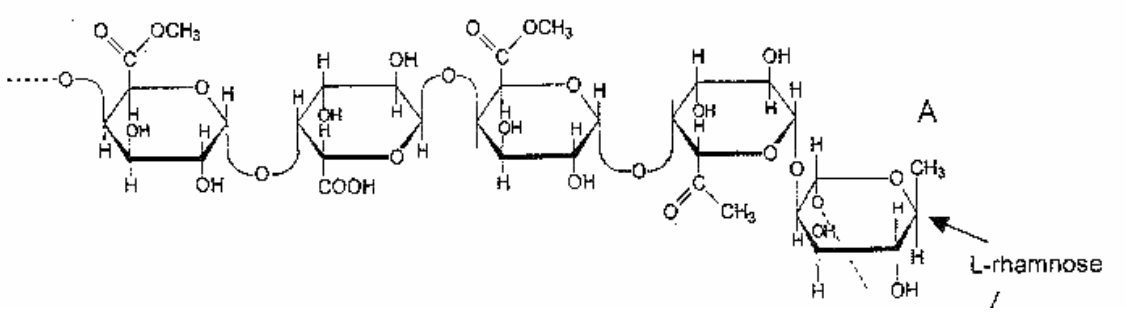

Figure 2.3.2. Pectin molecule (Coultate, 2002)

Cowpeas contain higher levels of folic acid and lower levels of antinutritional and flatulence producing factors compared to common beans (Ehlers & Hall, 1997). One of the most important antinutritional factors is trypsin inhibitor (TI), which is rapidly destroyed at temperatures above 100ºC and moisture content of about 20% (Phillips *et al.* 2003). Other antinutritional factors present are polyphenols (0.99 – 1.96 $mg.g^{-1}$) and phytic acid (1.15 – 2.10 $mg.g^{-1}$) (Giami, 2005). Polyphenols in legumes are mainly located in the seed coat (Giami, 2005). Brown and cream-coloured cowpeas contained higher amounts of polyphenols than white-coloured seeds. Davis *et al.* (1991) found that cowpeas are rich in lysine, and tryptophan, compared to cereal grains. However, they found that they are

deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seed is valued as good nutritional supplement to cereals and an extender of animal proteins.

2.4. MECHANISMS OF HARD-TO-COOK PHENOMENON IN LEGUMES

2.4.1. The development of HTC phenomenon during storage

Prolonged storage at high temperatures (30-35ºC) and high relative humidity (60-80%) has been related to development of HTC defect in legumes (Muneta, according to Taiwo, 1998). HTC phenomenon is a storage-induced textural failure of legumes stored at high temperatures and relative humidity to soften during cooking, resulting in longer cooking time and less palatability than softer seeds.

HTC cowpeas also undergo gradual loss of quality, such as, change in the seed coat colour and poor soaking imbibition, which causes the cowpeas not to soften sufficiently during cooking. Changes of the seed coat colour to brown were observed in lentil seeds stored under high temperatures and relative humidity (Nozzolillo & Bezada, 1984). The most dramatic changes in the seed coat occurred at 100% RH and between 20 and 30ºC after three weeks of storage. According to Nozzolillo & Bezada (1984), the browning of the seed coat resulted from the polymerisation of low molecular weight (MW) precursors (soluble tannins) to insoluble, unleachable brown-coloured high MW polymers (condensed tannins). The presence of condensed tannins in seed browning was supported by the deep colour (medium brown) of untreated seeds as compared to the yellow colour of pre-soaked seeds after four days of storage. Phenolics in these lentil samples were abundant and most originated from the seed coat.

Liu *et al.* (1992) studied the effect of storage-induced HTC defect in cowpeas and found that development of the phenomenon was a function of storage temperatures, relative humidity and time. Seeds stored at -18 ºC and 34% RH conditions did not show any development of HTC defect. However, when stored at 37 ºC and 75% RH conditions for 18 months, cowpeas showed HTC state seven times higher than that of the control. HTC cowpeas need more energy to cook and have lower nutritional and textural quality, which reduces consumer acceptability (Liu *et al*., 1992).

Jackson & Varriano-Marston (1981) and Liu & McWatters (1994) found that the HTC induced beans and cowpeas, respectively, lost more solids during soaking than their normal counterparts. According to the authors, these losses of solutes may be an indication of seed degradation. Due to membrane degradation, there is a greater loss of solutes in aged seeds than control seeds (Hentges *et al.,* 1991). Loss of seed viability was associated with seed browning and storage under high temperatures and relative humidity (Nozzolillo & Bezada, 1984). This was confirmed by impairment of germination proportional to the degree of browning during storage under aging conditions. Aged seeds were found to lose their ability to function and as a result there was liberation of large amounts of solutes into the imbibition medium.

Akinyele *et al.* (1986) observed that long cooking times are associated with seeds having high moisture contents, but could not find a direct association between moisture content and cooking time. Hentges *et al.* (1991) found beans with high moisture content (15.2%), stored at high relative humidity (65% RH) and at low temperatures (under 5ºC) maintained relatively low and stable cooking times. In addition, beans with low moisture content (12.9%) even stored at high temperatures (under 29ºC) and high relative humidity (65% RH) retained low and constant cooking times. It shows that the HTC defect appears to develop only in legumes seeds that have high moisture content and have been stored at high temperature and relative humidity.

Interactions among proteins and between proteins and starch or other components could occur during storage (Liu, McWatters & Phillips, 1992). These interactions may lead to changes in functional properties of seed protein and starch, which in turn could affect seed cookability and utilization.

Storing cowpeas at 30ºC and 64% RH led to decrease in pH of cowpeas from 6.64 to 5.57. The decrease in the pH of cowpeas was attributed to hydrolysis of lipids into fatty acids, oxidation of these acids into organic acids (Liu *et al.* 1992) and hydrolysis of phytates and storage proteins (Hohlberg & Stanley, 1987). This decrease in cowpea pH caused a decrease in protein extractability from 76.5 to 11.2% (Liu *et al.* 1992). A decrease in thermal stability of water-extractable proteins was also observed with time of adverse storage as the thermal transition temperature (Tm) decreased from above 100ºC to 56ºC. Protein coagulation or gelation is expected to occur before starch gelatinization (64 to

73ºC) since protein Tm had decreased below starch gelatinization temperature (Liu *et al.,* 1992). Insolubilized and thermal coagulated proteins in aged seeds would restrict starch from swelling during soaking or cooking, leading to HTC defect.

2.4.2. Mechanisms responsible for the development of hard-to-cook phenomenon in legumes

The development of HTC phenomenon in legumes has been related to multiple mechanisms that comprise the following theories:

2.4.2.1. Phytase-mineral and mineral-pectin interactions

The "phytase-mineral and mineral-pectin interactions" theory is the most widely accepted to explain the development of HTC defect in legume seeds. At high temperature, high relative humidity conditions, there would be increased metabolic activity, phytase activation and membrane degradation (Hentges *et al.,* 1991). Phytase hydrolyses phytate to release bound divalent cations. Since membranes of HTC legume seeds are degraded, these divalent cations could diffuse to the middle lamella where they bind to the pectic substances, forming insoluble pectates.

Aguilera & Rivera (1992) found a positive correlation between Ca^{2+} content and HTC defect. This is attributed to the fact that during storage Ca^{2+} is released from Ca^{2+} phytate complexes to the cell middle lamella. In the middle lamella, Ca^{2+} binds to the carboxyl groups of pectin, insolubilizing it, forming a barrier to water penetration and cell separation during cooking (Hentges *et al.,* 1991). A decrease in pectin solubility will increase the cooking time. The authors supported this theory with the fact that beans and cowpeas requiring long cooking times had decreased phytate and water-soluble pectin and increased water-insoluble pectin and leachable solids.

The HTC defect may also result from binding of many divalent cations other than Ca^{2+}. Liu *et al.* (1992) showed that Ca^{2+}- uptake by aged cowpeas seeds soaked in solutions containing Ca^{2+} is very close to that of control seeds. However, soaking in solution containing other cations (Mg^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Fe^{2+}, La^{3+} and Ce^{3+}) resulted in a much higher HTC level in aged samples than in control ones. HTC cowpeas have degraded membrane and high temperatures and humidities could induce activation of

pectin methylesterase causing demethylation of the cell wall pectin (Liu *et al.* 1992). These would create new binding sites through free carboxyl groups in the pectin, allowing more divalent and trivalent cations to be bound in HTC than normal cowpeas.

2.4.2.2. Involvement of phenolic compounds

Giami (2005) found the amount of poliphenols in cowpeas, expressed as mg phloroglucinol equivalents, varying from 0.99 to 1.96 $mg.g^{-1}$ seed flour. The amount of tannins found in 18 cultivars of Nigerian cowpeas ranged from 0.24 to 0.40% (Akinyele *et al.,* 1986).

Stanley (1992) found that storage of black beans under tropical conditions reduced the concentration of extractable tannins from black beans. This suggests that storage under high temperatures and relative humidity increases the water activity to a point allowing migration of condensed tannins to the cotyledons where they may bind with the macromolecular components of the cell wall (proteins) and middle lamella (pectins, cellulose). According to Stanley (1992), it strengthens the cell wall and it will reduce water penetration and swelling of the cotyledons, inhibiting cell separation and softening during cooking.

Garcia, Filisetti, Udaeta & Lajolo (1998) found the total amount of phenolics per gram of cotyledon to be five times higher for the control than for HTC beans. However, the distribution of different phenolic fractions was not the same. HTC beans had higher levels of free phenolic acid (four times) and phenolics bound to pectin (two times); control had about 45 times more phenolics as methanol-soluble esters and four times more ferulic acid associated with the water insoluble residue of the cell wall. It is believed that the presence of more ferulic acid bound to soluble pectin fraction, if involved in cross-links, may contribute to cell wall adherence, leading to cell defect during soaking and cooking. Two explanations were suggested: (1) possible tannin condensation during the development of HTC defect, which leads to decrease in phenolic compounds extractability and (2) feruloylated pectins may play a role in physiological and defence process, as well as, initiation of lignification.

2.4.2.3. HTC defect as a result of both theories

The phenomenon of HTC in legumes is still not fully understood. It should be theoretically easier to prevent the development of HTC defect in legumes by proper storage. However, it is not easy for the legume seeds producers/consumers to find the appropriate resources for proper storage in developing countries. Therefore, it is important to understand the different mechanisms that could play a role in the development of this phenomenon. Studies that have been done showed strong correlation between a role of different physicochemical characteristics and the cooking time of cowpeas (Hentges *et al.*, 1991; Liu, Phillips & McWatters, 1993b).

Hentges *et al.* (1991) found that during storage of legume seeds at high temperature and high RH, there is an overall decrease in the soluble protein fractions (albumins and globulins) and an increase in the insoluble fraction. The increase in insoluble protein was appointed as one for the reasons to the development of HTC defect in legume seeds.

According to Liu, Hung & Phillips (1993a), the HTC defect in cowpeas is caused directly by restricted starch gelatinization within the cell cytoplasm and lack of cell separation between cell walls during cooking. They attributed the restricted starch gelatinization to the decrease in protein solubility. In fact, proteins are the major water absorption components in legumes (Sefa-Dedeh & Stanley, 1979) and they play an important role in water uptake and could influence the amount of water absorbed by starch for its gelatinization during cooking. But the decrease in the soluble protein fractions (albumins and globulins) and an increase in the insoluble fraction can not be appointed as the main reason for the development of the HTC defect in legumes.

According to Stanley (1992), during storage of legume seeds at high temperatures and high RH, tannins can migrate from seed coat to the cotyledons where they can bind with the macromolecular components of the cell wall (proteins) and middle lamella (pectins, cellulose). Therefore, involvement of phenolic compounds is one of the main reasons for the increase in the insoluble proteins and consequently of the development of the HTC defect in legume seeds.

Phytase-mineral and mineral-pectin interactions theory can partly explain the HTC phenomenon in cowpeas as the insoluble Ca^{2+}-pectates in the middle lamella restrict or at

least reduce the rate of water penetration which is important in starch gelatinization. Then, the legume seeds will need more time to soften, resulting in longer cooking time and less palatability than softer seeds (Liu *et al*. 1992).

Although the phytate-mineral-pectin mechanism is the most widely accepted theory to explain the development of HTC defect in legumes, it is possible that the involvement of phenolic compounds may bring a synergistic effect for the development of HTC defect in legume seeds. Garcia *et al* (1998) found HTC phenomenon to be a complex process that affects different components of the cell (cell wall polymers, phenolics, starch, proteins). A combination of mechanisms taking place in the development of HTC defect in beans was suggested.

2.5. DIFFERENT TECHNIQUES FOR REDUCING COOKING TIME OF LEGUMES

2.5.1. Refrigeration

Preventing HTC cowpeas by proper storage is theoretical easier than understanding its mechanism. However, finding appropriate and sufficient resources for adequate storage is still a real challenge and an obstacle in developing countries. It has been proven that the HTC defect develops only in legume seeds stored at high temperatures and high relative humidities. Therefore, by storing legume seeds under low temperatures and humidities could be the answer to inhibit the development of the defect.

Liu *et al.* (1992b) and Liu *et al.* (1993a) demonstrated that cowpea seeds stored at -18ºC and ambient humidity had similar hardness to normal cowpeas and showed no HTC defect. Berrios, Swanson & Cheong (1999) demonstrated that beans stored under refrigerated hypobaric conditions (RHC) of 4.5ºC and 50 to 60% RH for 2 years exhibited quality factors characteristics of normal beans, such as shorter cooking time, smaller quantities of solids loss after soaking and faster initial water uptake during soaking. Beans stored at ambient conditions (AC) (23 to 25ºC and 30 to 50% RH) exhibited HTC defect. These differences could be attributed to microstructural differences of the stored beans. Berrios *et al.* (1999) observed large intercellular spaces and small adhesion areas between cotyledon cells in beans stored under RHC, small intercellular spaces and large adhesion areas between cotyledons cells in beans stored at AC.

Phytase under refrigerated conditions will not be activated and there will be no hydrolysis of phytate in the middle lamella. This implies that the insolubilization of the pectin in the middle lamella via binding with divalent cations released by phytate will not occur, avoiding the development of the HTC defect. Hentges, Weaver & Nielsen (1990) demonstrated that storing HTC beans and cowpeas under refrigerated conditions (6.5ºC and 71% RH) reversed the HTC defect.

Cooking times of the seeds became progressively shorter with additional storage. Mechanisms by which refrigerated conditions inhibit or reverse the HTC defect in legume seeds need to be investigated (Hentges *et al.*, 1990). Storing normal legume seeds under refrigeration conditions can prevent the development of the HTC defect, while when HTC legume seeds are stored under refrigerated conditions the HTC defect is reversed.

2.5.2. Soaking as a pre-conditioning treatment

Soaking legume seeds in water is the traditional and most widely known technique used by consumers to reduce the cooking time. Cooking involves both rehydration and heating. The two processes may take place simultaneously when cooking without pre-soaking or separately when rehydration is done before cooking (Mwangwela *et al.*, 2006). Soaking legume seeds reduces the time needed for the starch to absorb water needed for its gelatinisation during cooking. The texture of cooked cowpeas soaked at 25ºC softened with soaking time (Sefa-Dedeh, Stanley & Voisey, 1978). Similar results were obtained by Jackson & Varriano-Marston (1981) in HTC beans.

Water absorption by cowpeas is a complex process in which several anatomical and compositional factors become sequentially important – seed coat structure and thickness, seed size, hilum size and protein content (Sefa-Dedeh & Stanley, 1979). The seed coat thickness is an important factor during the first three hours of soaking since it is the first barrier the soaking water encounters during penetration. After this period, the hilum size accounts for most of the variation in water absorption. In the later stages of soaking, protein becomes increasingly important and the variations in the value for water uptake are mainly due to the amount of protein present and that binds with water (Sefa-Dedeh & Stanley, 1979). Both seed coat and hilum are external structures and that is why they are important in the initial stages of water absorption.

The water absorption patterns of beans stored under controlled and higher temperature and relative humidity conditions are characterized by rapid water uptake during the first 10 h of soaking (Berrios *et al.*, 1999). These researchers found that after 10 h of soaking, the rate of water uptake slowed and reached a saturation rate after 12 h. The initial water uptake was greater in HTC beans than normal beans. Moscoso, Bourne & Hood (1984), also found these results but the rapid water uptake was observed in the first 6 h, after which the rate slowed and reached a saturation point after 12 h. During imbibition of HTC beans (30ºC and 80% RH) the seed coat pulls away from the cotyledon at the palisade layer allowing a layer of bulk water to enter between the seed coat and cotyledons (Plhak, Caldwell & Stanley, 1989; Valle & Stanley, 1992). The large cavity between cotyledons in HTC beans can also have a different effect on water absorption after the initial resistance of the seed coat to water penetration has been overcome (Valle & Stanley, 1992). Therefore, HTC cowpeas were found to absorb more water than their normal counterparts. However, in terms of water holding capacity method, HTC beans were found to absorb approximately 25% less water during soaking than control beans.

Soaking cowpeas in distilled water at 45ºC for 1 h, showed greater amounts of water absorbed (90% of its maximum value) than those soaked at room temperature (Taiwo *et al.*, 1997a). They found that beyond that hour, there was no increase in the water absorbed. Cowpeas soaked at room temperatures continued absorbing water slowly after 1 h. The final amount of water absorbed was the same. Soaking at 45ºC for 1 h can be an alternative for reducing the time of soaking (≈18 h) and eliminate the possibility of excessive leaching of soluble solids.

HTC beans have been reported to undergo softening during cooking when first soaked in solutions containing monovalent cations, such as sodium and potassium (Valle, Cottrell, Jackman & Stanley, 1992). Aguilera & Rivera (1992) found a positive correlation between Ca^{2+} content and hardness of cooked black beans, while a high negative correlation was found between Na^{+} and K^{+} levels and hardness. The softening effect of soaking legume seeds in solutions containing monovalent cations is attributed to improvement of pectins solubilization due to exchange of divalent by monovalent cations in the pectin in the middle lamella. The data in Table 2.5.1 shows clearly that by increasing the ratio of

monovalent to divalent ions in cooking water there is a significant decrease of cooking time of HTC beans, either cooked in water or in the same soaking solutions.

These results confirm clearly the beneficial effect of monovalent cations in decreasing the cooking time of HTC legume seeds. Apart from ionic interchange, improved heat transfer properties, improved water absorption and water holding properties by saline solutions were reported to be responsible for the softening effect of monovalent cations (Léon, Elías & Bressani, 1992). Furthermore, CO_3^{2-} in the soaking solutions of dry beans can interact with biopolymers in the cotyledons cells to cause molecular unfolding and exposure to new sites for water binding (Valle & Stanley, 1992).

Table 2.5.1. Cooking time of HTC beans soaked at different ratios of monovalent to divalent ions (Léon *et al.*, 1992)

Ratio of monovalent to divalent ions	**Ions added in the solution**	**Cooking Time (min)**	
		Soaked and cooked with salt solutions	**Soaked with salt solutions and cooked with water**
0.30	Ca^{2+}, Mg^{2+}	> 450 a	>450 a
2.30	Ca^{2+}, Mg^{2+}	>450 a	>450 a
4.60	0	>360 c	360 c
6.30	Na^{+}, K^{+}	307 d	>420 b
8.30	Na^{+}, K^{+}	64.5 f	105 e
8.38	Na^{+}	35.5 h	43.5 g
9.80	Na^{+}	24.0 i	29.5 i

Means not followed by the same letter in cellare significantly different (*P<0.05)*

The use of salts (NaCl, $NaHCO_3$, Na_2CO_3 and $Na_5P_3O_{10}$) can elevate the gelatinization temperature of starch (Varriano-Marston & Omana, 1979) and raise the boiling point of solvents depending on their concentration (Garcia, Valle & Stanley, 1991). Valle *et al.* (1992) found that the presence of carbonate (CO_3^{2-}) ions decreased the temperature of protein denaturation.

The presence of the above mentioned salts in the soaking water caused some of the starch granules in the cooked beans to undergo initial loss of birefringence which proceeded from

the periphery of the granule to the hilum (Varriano-Marston & Omana, 1979). According to Varriano-Marston & Omana (1979), beans soaked in combined salts before cooking, followed by $Na_5P_3O_{10}$-soaked beans, showed high degree of cell separation and only thin cells remaining before cooking compared to those soaked in tap water. The latter authors associated these structural observations to the solubilization of pectic substances into soaking or cooking water. From these findings, it can be concluded that the exchange of divalent cations (Ca^{2+}, Mg^{2+}) by monovalent (Na^{+}) cations in the middle pectins can be associated with the dissolution of intercellular cement and subsequent cell separation, which leads to softer-to-cook legume seeds.

Soaking is not only important for reducing the cooking time but also for reducing the activity of trypsin inhibitors. However, cooking is the most effective method of reducing the activity of trypsin inhibitors (Wang, Daun & Malcolmson, 2003). Soaking of peas for 24 h reduced the activity of trypsin inhibitors between 4.5 and 8.8%, whereas 30 min cooking reduced the activity of trypsin inhibitors by about 79.9 to 86.9% (Wang *et al.,* 2003).

Egounlety & Aworh (2003) found that soaking of beans and cowpeas reduced the oligosaccharides and sucrose contents by 20 to 35% and 17 to 24%, respectively. Fructose, glucose and galactose are other components that were found in the soaking water in soybean, cowpea and groundbean. Melibiose was especially present in cowpeas and groundbeans. Soaking for 12 to 14 h reduced the tannin content by 54.6% in soybean, 72.2% in cowpeas and 30.9% in ground bean.

2.5.3. Decortication of legumes

Jackson & Varriato-Marston (1981), comparing cooking time of intact and decorticated beans, found the seed coat as the major barrier to bean softening during cooking. Decorticated samples showed significantly reduced cooking times. However, the contribution of cotyledons was found to be very important and became increasingly important over storage time. It can be attributed to the possible migration of condensed tannins to the seed cotyledons where they form insoluble complexes with the macromolecular components of the cell wall and middle lamella (Stanley, 1992).

Decortication reduced the cooking time of normal beans and 14-days aged beans from 62 min to 24 min and from 92 min to 40 min, respectively, compared to the cooking times of intact beans (Jackson & Varriato-Marston, 1981). The 55-days aged samples remained harder even after 6 h of cooking but after decortication, the cooking time reduced to about 240 min. Changes in the components of the cotyledons cannot be considered insignificant since about 40% of the total cooking time of intact normal and 7-days aged beans was associated with cotyledon softening. In the 14-days and 55-days aged beans, the cotyledons accounted for 45 to 60% of the cooking time. The results show that the seed coat contribution to cooking time exceeded that of cotyledons in the normal beans, but that the cotyledons contribution increased with storage time in aged beans.

Dehulling is an important pre-treatment in grain legumes to reduce the amount of tannins present in the hulls. Egounlety & Aworh (2003) found dehulling of legumes as the most effective practice to remove tannins. No tannins were detected in dehulled soybean, cowpeas and groundnuts. Nozzolillo & Bezada (1984) found that phenolics were abundant in lentil seeds and most originated from the seed coat.

2.5.4. Microwave cooking

Microwave cooking in sealed vessels has been reported to reduce the cooking time from 110 to 11 min for chickpeas and from 55 to 9 min for common beans (Marconi, Ruggeri, Cappelloni, Leonardi & Carnovale, 2000). The samples were pre-soaked for 18 h in distilled water before microwave cooking at 105ºC and 565 W. According to Marconi *et al.* (2000), the more extensive breakage of cell wall polysaccharides because of the higher temperatures and pressure may be responsible for the reduction in cooking time. Furthermore, they found that microwave cooking reduced the ash, proteins, and solids losses in the cooking water. This could be due to the fact that less cooking time was required and the seed: cooking water ratio was lower compared to the conventional cooking.

2.5.5. Micronization

Micronization is an infrared (IR) heat treatment of moisture-conditioned legumes that has been reported to reduce the cooking time of cowpeas (Mwangwela *et al.,* 2006; Phadi, 2005), lentils (Scanlon, Malcolmson, Arntfield, Watts, Ryland & Prokopowich, 1998; Arntfield, Scanlon, Malcolmson, Watts, Cenkowski, Ryland & Savoie, 1997).

According to Fasina, Tyler, Pickard, Zheng & Wang (2001), micronization involves short-time exposure of a material to electromagnetic radiation in the wavelength region of 1.8 to 3.4 µm (1800 to 3400 nm). According to them, for biological material, the penetration of infrared rays into the material causes the water molecules to vibrate at a frequency of 60 000 to 150 000 MHz. This causes rapid internal heating and a rise in water vapour pressure inside the material. This process offers the benefit of low costs associated with high efficiency of radiation heating compared to conventional technologies (Bellido, Arntfield, Scanlon & Cenkowski 2005).

Micronization technique has been used to increase digestibility and nutritional quality of cereals and grain legumes (Arntfield, Scanlon, Malcolmson, Watts, Ryland & Savoie, 1997). The trypsin inhibitors were reduced to approximately 35 to 50% in smaller seeds (green peas, black beans and lentils) and to less than 10% in larger seeds (kidney and pinto beans) (Fasina *et al.,* 2001).

When using micronization to reduce the cooking time, pre-conditioning pre-treatments to adjust the moisture content of the grains is very crucial. Scanlon *et al.* (1998) found cooking time reduced as moisture content increased and that at 40% moisture content the reduction in cooking time was more effective than at 20% moisture content. Mwangwela *et al.* (2006) found that micronization to final surface temperature of 153ºC, reduced the cooking time of cowpeas by 36% for Bechuana White and by 44% for variety 462, when pre-conditioned to approximately 41% moisture content. Phadi (2004) found a reduction of 30% on the cooking time of Bechuana white cowpeas pre-conditioned to 40% moisture content, when micronized to a final surface temperature of approximately 160ºC. However, Abdul-Kadir, Bargman & Rupnow (1990) found that the cooking time of pinto beans increased by 25% (IR-99ºC) for beans with 12.17% initial moisture and by 50% (IR-107ºC) for beans with 6.81% initial moisture, compared to unmicronized samples. Sarantinos & Black (1996) associated the increased cooking time of micronized chickpeas to the hardening of the texture and reduced water absorption capacity in micronized samples. The increased cooking time of legumes can also be attributed to the reduced hydration of the legumes seeds possibly caused by protein denaturation and starch damage.

The mechanisms by which micronization reduces the cooking time are not yet fully clear. However, several physicochemical changes have been measured to study the effect of this treatment on the properties of micronized materials. Some changes, which have been associated with the reduction of cooking time by micronization, are:

- Micronization produced some clear folds and fracture lines in lentils (Arntfield, Scanlon, Malcolmson, Watts, Cenkowski & Savoie, 2001) and in cowpeas seed coat and cotyledons, which improved water uptake, leading to a softer texture and increased splitting during cooking (Mwangwela *et al.,* 2006).

- Micronization pre-gelatinised starch, which can reduce the time and energy required to complete the starch gelatinisation during cooking. The percentage of starch that gelatinises increases with micronization of lentils (Cenkowski & Sosulski, 1997; Arntfield *et al.,* 1997; Arntfield *et al.,* 2001) and cowpeas (Mwangwela *et al.,* 2006).

- Micronization reduced protein solubility of lentils (Arntfield *et al.,* 1997; Arntfield *et al.,* 2001), navy beans (Bellido *et al.,* 2003) and cowpeas (Mwangwela *et al.,* 2006). Denaturation of proteins was considered responsible for the reduction in protein solubility. In the study done by Arntfield *et al.* (1997), the low protein solubility of lentils pre-conditioned at 25% and micronized to a final moisture content of approximately 7% did not produce softer lentils than those pre-conditioned and micronized to higher moisture levels. This may be because at lower moisture levels, denaturation of proteins is accompanied by polymerisation or cross-linking with other seed components, restricting water uptake during cooking. An other reason can be the low moisture content after pre-conditioning (25%), which can lead to a lower level of starch gelatinisation during micronization (Arntfield *et al.,* 1997).

2.5.5.1. The effect of micronization on phytate and pectin stability

Arntfield *et al.* (1997) found that the amount of pectic substances is reduced after micronization of lentils. Arntfield *et al.* (2001) found that micronization reduced the phytic acid but this was not correlated with the amount of soluble pectins in lentils.

There is a lack of literature dealing with the effect of micronization temperatures on pectin loss in legumes. Pectin is extremely sensitive to elevated temperatures in a near-neutral solution (Liu *et al.*, 1993; Sajjaanantakul, Buren & Downing, 2003). At these conditions, the pectin degrades to lower molecular weight products via breakage of glucosidic bonds adjacent to carboxymethyl groups (Liu *et al.,* 1993). This process is known as β-elimination reaction (Albersheim, 1959). During this reaction, there is breakage of glycosidic links between methylated galacturonate residues by an unusual β-elimination reaction where a *trans* double bond is inserted between C-4 and C-5 rather than the usual hydrolysis as shown is Fig. 2.5.1 (Coultate, 2002). Although this reaction has been reported in heat-related softening of many fruits and vegetables (Liu *et al.,* 1993), its effect on cowpeas and beans softening has not investigated yet. Liu *et al.* (1993) also found that heating had a greater increase in pectin solubility than soaking. This increase in pectin solubility can be associated with the involvement of β-elimination reaction in pectin degradation.

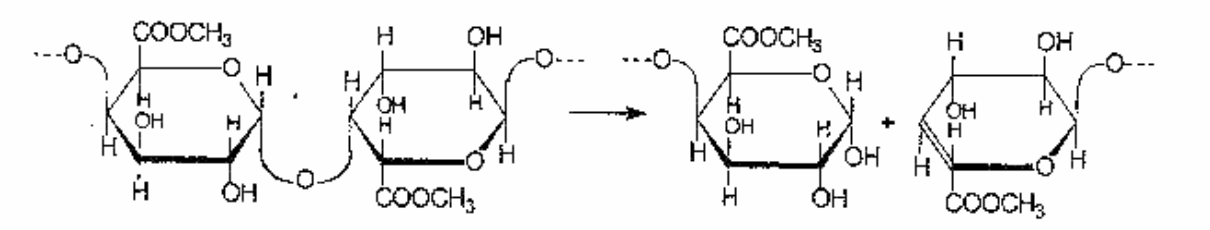

Figure 2.5.1. The β-elimination reaction (Coultate, 2002)

Phytase has proven to be highly heat stable between moisture contents of 10% to 35% (Affrifah, Chinnan & Phillips, 2005). About 63% of its initial activity was retained after subjected to the highest temperature (95ºC) and time (35 min) combination at the lowest moisture content (10 %). Moisture content, heating temperature and time were reported to have a significant effect on residual enzyme activity.

Moisture content is very important in enzyme inactivation. In dry or semi-moist food systems, phytase tends to be more stable (Affrifah *et al.*, 2005). According to these authors, this is because water is needed to promote the unfolding of proteins during thermal inactivation. Plots of phytase inactivation in cowpeas showed an initial drop in phytase activity during the first few minutes of heating at each temperature (i.e. 70, 75, 80,

90 and 95ºC). After that there is a gradual levelling in phytase activity. It shows that phytase inactivation is more dependent on heating temperature than heating time.

The effect of heating time is not as pronounced especially for samples treated at high temperatures (80 to 95ºC). Increasing moisture content facilitated the thermal inactivation of phytase. This is due to increased availability of solvent water which increases denaturation of the enzyme. It is noticeable that none of the treatments eliminated the phytase activity.

2.6. SUMMARY OF EXISTING KNOWLEDGE AND GAPS

It has been shown that when legume seeds are stored under high temperature and humidity conditions, they undergo physicochemical changes in the seed coat and cotyledons, which leads to the HTC phenomenon (Hentges *et al.* 1991; Liu *et al.* 1992; Moscoso *et al.,* 1984; Sefa-Dedeh *et al.*, 1978). According to the phytate-cations-pectins interactions theory, at these conditions phytate is degraded by phytase, releasing divalent cations (Ca^{2+}, Mg^{2+}), which bind with pectin in the middle lamella, resulting in less soluble pectin for water penetration during cooking or soaking (Hentges *et al.* 1991; Aguilera & Rivera, 1992).

Changes in the phytate content, phytase activity and pectin solubility during storage at adverse conditions could explain part of the mechanisms involved in legume seeds hardening. However, most of the literature available deals with one factor at time, i.e. phytate or pectin or phytase activity. There is a lack of integrated studies on how the three factors (phytate, pectin and phytase activity) relate to each other during adverse storage and on how this relationship affects physicochemical characteristics of cowpeas such as pH, moisture content, hydration properties, cooking time, degree of splitting, leachable solids and hardness of cooked seeds.

Soaking HTC legume seeds in water has been one of the most used methods to reduce the cooking time. Soaking beans (Jackson & Varriano, 1981; Berrios *et al.* 1999; Moscoso *et al.,* 1984) and cowpeas (Sefa-Dedeh *et al.*, 1978; Sefa-Dedeh & Stanley, 1979) in water at ambient temperature reduced the cooking time. Soaking in water at 45ºC for 1h has also been used to reduce the soaking time of cowpeas (Taiwo *et al.*, 1997a). Soaking in a solution containing monovalent cations (i.e. Na^{+}, K^{+}) have been found to reduce the

cooking time of HTC beans (Valle *et al.*, 1992; Aguilera & Rivera, 1992 and Léon *et al.*, 1992) and normal beans (Léon *et al.*, 1992) even further as compared to soaking in water alone. The reduction in cooking time when legume seeds are soaked in water is due to solubilization of pectins in the middle lamella (Clemente, Sánchez-Vioque, Vioque, Bautista & Millán, 1998). This solubilization effect is even greater when legume seeds are soaked in a solution containing monovalent (Na^+) cations due to conversion of insoluble to soluble pectins (Vidal-Valverde, Frías & Esteban, 1992)

Micronization of moisture-preconditioned legume has been shown to reduce phytate content in lentils (Arntfield *et al.,* 1997; Arntfield *et al.,* 2001), reduce phytase activity in cowpeas (Affrifah *et al.* 2005) and increase pectin solubility in the middle lamella of cowpeas (Liu *et al.,* 1993b). Micronization also improved water absorption properties during cooking and soaking, increased the degree of splitting, reduced the hardness of cooked seeds, thereby reducing cooking time of cowpeas (Mwangwela *et al.,* 2006; Phadi, 2004). At elevated temperatures, pectin was degraded to lower molecular weight products via breakage of glycosidic bonds adjacent to carboxymethyl groups (β-elimination reaction) (Liu *et al.,* 1993b).

However, the effects of micronization on the cooking/physicochemical characteristics as related to the cooking time of HTC legume seeds are not known. In addition to that, the effect of pre-conditioning legume seeds in a solution containing monovalent (Na^+) cations in combination with micronization on the cooking time and other physicochemical characteristics of HTC legume seeds still needs to be investigated.

2.7. HYPOTHESES

❶ Storing cowpeas under high temperature and relative humidity conditions will induce HTC defect in cowpeas, which are characterized by longer cooking times. This is because under these conditions, phytase will be activated and will hydrolyze phytate, releasing divalent cations such as Ca^{2+} and Mg^{2+}, which will bind to pectin in the middle lamella. Insolubilized pectins will form barrier to water penetration during soaking and cooking, reducing the degree of splitting during cooking and increasing the hardness of cooked seeds.

❷ Pre-conditioning of HTC cowpeas in a solution containing monovalent cations (Na^+) will reduce the cooking time because these cations will bind the free carboxyl groups and substitute calcium in pectins in the middle lamella, making the pectins more soluble. Subsequent micronization at moderate temperatures (153ºC) will increase pectin solubility in the middle lamella by breaking the pectins molecules into lower, more soluble molecular fractions via breakage of glycosidic bonds adjacent to carboxymethyl groups (β-elimination reaction). This will increase water absorption during cooking, the degree of splitting and reduce the hardness of cooked cowpea seeds.

2.8. OBJECTIVES

❶ To determine the effect of storing cowpeas at high temperatures and high relative humidity conditions on cooking characteristics and their relation to changes in other physicochemical characteristics of cowpeas.

❷ To determine the effect of pre-conditioning in either deionised water or solutions containing monovalent cations (Na^+) alone and in combination with micronization on cooking characteristics as they relate to changes in other physicochemical characteristics of normal and HTC induced cowpeas.

3. RESEARCH CHAPTER

3. 1. THE EFFECT OF HARD-TO-COOK PHENOMENON ON COOKING AND PHYSICOCHEMICAL CHARACTERISTICS OF COWPEAS

3.1.1 ABSTRACT

The effect of storing cowpeas at 42ºC and 67% RH for 21 days on cooking and physicochemical characteristics was evaluated. Storage of cowpeas under these conditions resulted in hard-to-cook (HTC) seeds with the cooking time increasing from 89 to more than 270 min. HTC cowpeas had decreased pH, pectin solubility and phytic acid content and had increased phytase activity, moisture content and a harder cooked texture. The decrease in pectin solubility in HTC cowpeas, which coincided with decreased phytic acid content and increased phytase activity, was significantly correlated with the reduced water absorption during 6 h of soaking and 3 h of cooking. The amount of water absorbed by HTC cowpeas after 18 h of soaking was significantly ($P \leq 0.05$) higher than that absorbed by control cowpeas. These results can be taken as further supporting evidence for the most widely accepted theory to explain HTC defect in legume seeds. The theory involves pectin insolubilization via binding to divalent cations (i.e. Ca^{2+} and Mg^{2+}) resulting from phytate hydrolyzes by phytase at relatively high temperatures and relative humidities.

Keywords: cowpeas, hard-to-cook phenomenon, cooking time, phytic acid, phytase activity, pectin solubility, texture, water absorption

3.1.2. INTRODUCTION

Cowpeas (*Vigna unguiculata* L. Walp) have been recognized as having a good nutritional profile, containing about 25% proteins and 60 to 69% soluble carbohydrates (Ajibola *et al.*, 2003). They are also an affordable source of minerals and B vitamins (Phillips *et al.,* 2003) and have high levels of folic acid and low levels of anti-nutritional and flatulence producing factors (Ehlers & Hall, 1997) as compared with beans. Therefore, cowpeas are a potential and a low cost solution for the malnutrition problem in most developing countries. However, one of the major constraints associated with consumption and utilisation of cowpeas is the storage induced hard-to-cook (HTC) defect. HTC cowpea seeds resist softening during cooking (Liu *et al.*, 1992b) and have decreased protein and starch availability and textural quality (Liu *et al.*, 1992a). The nutritional quality is impaired by a loss of vitamins and decreased protein availability (Garcia *et al.,* 1998). The extended cooking time and poor textural quality reduce consumer preferences and acceptability (Phillips *et al.,* 2003).

Many researchers have reported that extended storage under high temperature and high relative humidity conditions will promote HTC in legumes such as cowpeas. It is believed that hardening of legume seeds is due to structural changes in the cotyledons and middle lamella during storage at adverse conditions (Hohlberg & Stanley, 1987). Explanations for the development of the HTC phenomenon in legumes are based on two theories: (1) pectin insolubilization via binding with divalent cations resulting from phytate breakdown by phytase at relatively high temperatures and high relative humidities (Hentges *et al.*, 1991; Aguilera & Rivera, 1992) and cell wall lignification via cross-linking of phenolic compounds with cell wall proteins and middle lamella pectins and cellulose (Stanley, 1992). According to Hentges *et al.* (1991) the first theory is the most widely accepted to explain the development of HTC defect in legume seeds.

Although extensive research has been done on selected physico-chemical characteristics and their relation to cooked texture and cooking time of cowpea seeds, limited work has been done integrating all these parameters (e.g. water absorption during soaking and cooking, splitting, leachable solids, changes in the pectin content, phytase activity and phytic acid) in one study.

In order to get an integrated explanation for the involvement of different physicochemical characteristics on the mechanism of HTC defect in legume seeds and their changes during storage at high temperature and high relative humidity, a comparative study between normal and HTC cowpeas was conducted.

3.1.3. MATERIALS AND METHODS

3.1.3.1. Selection of raw materials

Three cultivars of cowpeas were cleaned to remove chaff, shrivelled and broken seeds. The increase in cooking time as influenced by aging conditions was used to select the cultivar most prone to the development of the HTC defect during storage. For this purpose, cooking times of normal and HTC-induced samples were determined using the Mattson Bean Cooker. The selection of these cultivars was based on their availability. Three cultivars of cowpeas (*Mogwe-o-Kgotsheng, Bechuana white and Mae-a-tsilwane)* obtained from Botswana in March 2005, were monitored for the development of the HTC phenomenon by storing them at 42ºC and 67% RH for 21 days. *Mogwe-o-Kgotsheng* was the variety most prone to the development of HTC defect as was evidenced by significantly higher increase in cooking time (i.e. 71.6%) than for the other varieties, i.e. *Bechuana white* (31.4%) *Mae-a-tsilwane* (30.5%). This experiment was done in duplicate. It was decided to use *Mogwe-o-Kgotsheng* for the experimental work.

3.1.3.2. Inducement of HTC defect

The HTC defect was induced according to procedure of Shomer, Paster, Lindner & Vasiliver (1990). *Mogwe-o-Kgotsheng* cowpeas seeds were incubated at 42ºC and 67% RH in a 57 cm x 46 cm x 33 cm container, over a saturated solution of KCl at the bottom of the container, for 21 days. The cowpea seeds were placed in two single layers over a net surface. The layers were switched once during the incubation period. The incubated cowpeas were kept overnight at ambient room temperature and humidity to reach a constant weight.

3.1.3.3. Cooking time

The Mattson Bean Cooker was used to determine the cooking time of normal and HTC cowpeas. For both samples, 25 cowpea seeds were positioned in the perforation zones of

the cooker, and then cooked in a heavy aluminium pan with 1500 ml of deionised water. The cooking time was recorded as the time required for 80% of the 90 g pins (20) to fall though the cooked seeds (Mwangwela *et al.*, 2006).

3.1.3.4. Moisture content

Moisture content was determined on the flours produced through milling representative samples of normal and HTC cowpeas using a Falling Number Mill 3100 (Falling Number, Huddinge, Sweden). Moisture was assayed according to the method described by American Association of Cereal Chemists – AACC (1983) with slight modifications. Moisture tins were dried in an oven at 103 ºC for 1 h and cooled in a dessicator for about 10 min. The cooled and dried tins were weighed and 5 g samples weighed into the tins. The samples were covered with foil and dried in a hot air oven for 4 h at ±103 ºC. The samples were cooled and weighed. The moisture content (%) was calculated as follows:
% moisture = (moisture loss (g)/original weigh of sample (g)) x100.

3.1.3.5. pH

The pH of the cowpeas was measured as described by Liu *et al.* (1992b). The sample was weighed (approximately 5 g of cowpea flour) and mixed with 100 ml of deionised water in a beaker and stirred for 45 min at ambient temperature. The pH of the mixture was recorded as cowpea tissue pH.

3.1.3.6. Water absorption during soaking and hydration capacity

Water absorption during soaking was determined according to the procedure of Agbo, Hosfield, Uebersax & Kimparens (1987) modified by Mwangwela *et al.* (2006). About 10 g of cowpea seeds were weighed and placed in Erlenmeyer flasks (100 ml) containing approximately 50 ml of deionised water. The flasks were placed in an incubator at 22ºC for 1, 2, 3, 4, 5 and 6 h. After soaking, the excess water was drained using a metal sieve (2.5 mm) and blotted dry with absorbent paper and weighed. The gain in weigh was expressed as g of water absorbed per kg of cowpeas. The amount of water (g) absorbed by 10 g of cowpeas after 18 h of soaking reflects the hydration capacity (Wang *et al.*, 2003). This was also expressed as g of water absorbed per kg of cowpeas.

3.1.3.7. Water absorption during cooking

The amount of water absorbed during cooking was performed according to a modified procedure of Cenkowski & Sosulski (1997). About 10 g of cowpea seeds were weighed and placed in Erlenmeyer flasks (100 ml) containing approximately 50 ml of deionised water. The flasks were placed in a heavy aluminium pan containing approximately 1500 ml of deionised water for the cooking process. The water in the pan was brought to the boiling point (95ºC), allowing 5 min for heating up. Two sample flasks were removed at 30 min intervals and excess water was drained using a metal sieve (2.5 mm) during the 180 min cooking. Then the cowpea seeds were cooled to room temperature (±22ºC) for 1 h, blotted dry with absorbent paper to remove excess water and weighed. The gain in weigh (g) was expressed as water uptake in g water per kg cowpeas. The boiled cowpeas samples were then used to determine splitting.

3.1.3.8. Splitting of cooked seeds

The splits were counted as the number of cowpeas with split seed coats and cotyledons and calculated as (no. split seeds)/ (no. whole seeds) x100 (Taiwo *et al.,* 1997a).

3.1.3.9. Leached solids

The concentration of solids in the cooking water were estimated according to the procedure of Valle *et al.* (1992) by drying duplicate aliquots of cooking water (2 ml weighed accurately) at 105 ºC in a convection oven to constant weight (18 h). Then, the solids lost were calculated by subtracting the weight of the dry pan from the weight of dry pan with dry broth.

3.1.3.10. Texture measurements during cooking

Texture was measured using a TA-XTPlus Texture Analyser (Stable Micro Systems, Godalming, UK) with an A/CKB craft knife adapter mounted with Hilite heavy-duty blades (Hilite Hardware, Pretoria, South Africa) (Mwangwela *et al.,* 2006). The work (area under the curve, N.mm) done by the texture analyser to cut each cowpea seed through a distance of 5 mm at a speed of 4 $mm.s^{-1}$ was measured as texture, according to the method described by Sefa-Dedeh *et al.* (1978). Each cowpea seed was positioned on its side and cut below the hilum along the cross section. Every blade was used to cut through five cowpea seeds consecutively, after which the blade was changed. For each sample

treatment, micronized or not, 10 cowpea seeds cooked for 30, 60, 90, 120, 150 and 180 min, were analyzed.

3.1.3.11. Phytic acid determination

The method described by Febles, Arias, Hardisson, Rodriguez-Álvarez & Sierra (2001) was used to determine the content of phytic acid in cowpea flour. The solutions used were: $2x10^{-2}$ M Fe (III) solution in 0.16 M HCl; 10^{-2} M EDTA standard solution; $4x10^{-1}$ M HCl solution with 5% of Na_2SO_4; and 20% sulfosalicylic acid solution.

In a 50 ml flask, 40 ml of HCl-Na_2SO_4 solution was added to a 2 g sample, and it was left for 90 min with intermittent agitation. In a neutral glass tube 20 ml of the floating liquid of the previous extraction (filtered if necessary), 20 ml of HCl-Na_2SO_4 solution, 20 ml of Fe (III) solution and 20 ml of 20% sulfosalicylic acid solution were placed. The mixture was then closed with a gum cup pierced by a narrow glass tube about 30 cm in length, and heated for 15 min in a boiling water bath. Then, it was cooled under tap water and left standing. From the clean floating material, 20 ml were separated and placed in a 250 ml precipitation beaker, filling it up to around 200 ml with deionised water and increasing the pH to 2.5 with 10^{-2} M EDTA solution. This was heated to about 70ºC and the Fe (III) titrated, while hot, with 0.010 M EDTA solution until bright yellow. The percentage of phytic acid was determined as follows:

Phytic acid (%) =1.32 x (10-V)/P; V-EDTA solution volume (ml); P-sample weight (g).

3.1.3.12. Phytase activity

The phytase activity was measured by direct incubation with sodium acetate buffer as described by Greiner & Egli (2003). Finely ground cowpea 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 45ºC. The reaction mixture was incubated at 45ºC for 30 min after which the amount of phosphorus released was calculated according to the following formulation (Eeckhout & Paepe, 1994):

Phytase units kg^{-1}= (Px1000)/ (Wx30), where P is micromoles of P liberated by phytase in 30 min, W is sample weight (g) and 30 is the incubation time taken into account.

A 2 ml portion of the incubate was transferred to a test tube containing 2 ml of 10% trichloro-acetic acid (TCA) to arrest the reaction and then centrifuged at 10000xg for 5 min. One ml of the supernatant was then added to 1 ml of a colour-forming reagent. The colour reagent was a mixture of four parts of solution A (15 g of ammonium heptamolybdate in 55 ml of 36 N H_2SO_4, made up to 1 l) and one part of solution B (27 g of $FeSO_4.7H_2O$, a few drops of 36N H_2SO_4, made up to 250 ml). A blue colour was formed and it was measured in a spectrophotometer (Lambda EZ150 Inc, Perkin Elmer, USA) at 700 nm after centrifugation at 10000xg for 3 min to remove any cloudiness formed. A calibration series containing 1 ml of 10% TCA per cuvette, 1 ml of P standard solution (0, 10, 20, 30 or 40 µmol P ml^{-1}) and 2 ml of colour reagent was produced to estimate the enzyme activity.

3.1.3.13. Soluble pectin

Pectin solubility was measured according to the modified method of Arntfield *et al.* (1997). The detailed procedure is as follows. A stock solution for the standard curve was prepared by adding 10 ml of 0.05 N NaOH to 120.5 mg of vacuum dried galacturonic acid and diluted to 1 l with water. Standards in the range of 10 to 70 µg anhydrogalacturonic acid per ml were used. To a 0.5 ml aliquot of each standard, 3 ml of 0.125 M sodium tetraborate in H_2SO_4 was added. It was boiled for 5 min and after cooling 50 µl of 0.15% m-hydroxybiphenyl (in 0.5% NaOH) was added and the mixture allowed to set for 20 min prior to reading absorbance at 520 nm.

Samples were prepared by first mixing 5 g ground sample in a 50 ml centrifuge tube with 40 ml of 95% ethanol. The mixture was left for 10 min with stirring and then centrifuged at 17300xg for 10 min. This extraction with 40 ml of 95% ethanol was repeated two more times using the residual pellet. The pellet was then vacuum dried prior to further analysis. To 1 g of dry pellet, 200 ml of 0.05% EDTA (pH 7) were added. This was boiled for 1 h replacing moisture lost through evaporation (except during the last 20 min). The mixture was then cooled and diluted to 250 ml with 0.05% EDTA (pH 7). A 50 ml aliquot was centrifuged at 15000xg for 5 min. To a 0.5 ml aliquot of supernatant 3 ml of 0.125 M tetraborate in H_2SO_4 were added. Then, it was boiled for 5 min and after cooling 50 µl of 0.15% m-hydroxybiphenyl (in 0.5% NaOH) was added and the mixture allowed to set for 20 min prior to reading absorbance at 520 nm. The blank was a sample treated in the same way except that the m-hydroxybiphenyl was replaced with 50 µl of 0.5% NaOH.

3.1.3.14. Statistical analysis

Experiments were conducted in duplicate. All the assays, with the exception of texture measurements, were done in triplicate. In the case of texture measurements, 10 seeds were analysed for each replicate per sample treatment. Analysis of Variance (ANOVA) and correlations were performed using the Statistica version 7.0 (StatSoft Inc, Tulsa, USA). The Least Significant Difference test (LSD-test) was utilized for comparison among means when the F-test was significant ($P \leq 0.05$).

3.1.4. RESULTS AND DISCUSSION

3.1.4.1. Effect of HTC defect on the seed coat colour

Figure 3.1.1 shows the effect of storing the cowpeas for three weeks at 42 ºC and 67% RH on the appearance of uncooked cowpeas. The colour of the seed coat of uncooked cowpeas changed from green to brown after these accelerated storage conditions (Fig. 3.1.1).

Figure 3.1.1. The effect of storing cowpeas for 21 days at 42 ºC and 67% RH on the cowpeas seed coat colour (normal cowpeas (a) and aged cowpeas (b))

Many other studies using accelerated storage conditions induced the HTC defect in cowpeas (Liu *et al.,* 1992b; Hentges *et al.,* 1991; Sefa-Dedeh *et al.,* 1978), beans (Hentges *et al.,* 1991) and red kidney beans (Moscoso *et al.*, 1984). HTC cowpeas were reported to undergo gradual loss in quality, such as, change in the seed coat colour (Liu *et al.*, 1992b). Nozzolillo & Bezada (1984) found that when lentils were stored for three weeks at 100% RH and between 20 and 30ºC, dramatic changes of the seed coat colour in terms of browning were observed. According to them, the seed coat colour changed from green to brown as a result of polymerisation of low molecular weight (MW) precursors (soluble

tannins) to insoluble, unleachable brown-coloured high MW polymers (condensed tannins). These findings were supported by the deep medium brown colour of unsoaked seeds as compared to the yellow colour of pre-soaked seeds after four days of storage. The presence of phenolics in general (Giami, 2005) and tannins in particular (Akinyele *et al.*, 1986) in cowpeas was previously reported. It is therefore possible that during storage of cowpeas for 21 days at 42 ºC and 67% RH, polymerization of soluble tannins to insoluble brown-coloured condensed tannins took place and it induced colour changes in the seed coats of cowpeas.

3.1.4.2. Effect of HTC defect on the cooking time, pH, moisture content, phytic acid, phytase activity and pectin solubility

Table 3.1.1 shows the effect of storing cowpeas at adverse conditions (42ºC and 67% RH for 21 days) on some physicochemical properties of cowpeas.

Table 3.1.1. The effect of HTC defect on physicochemical characteristics of cowpeas

Physicochemical characteristics	Normal cowpeas	HTC cowpeas
pH	6.5 ± 0.02 b	6.3 ± 0.03 a
Moisture content (%)	11.9 ± 0.09 a	13.8 ± 0.05 b
Cooking time (min)	89.1 ± 8.21a	> 270 b*
Texture of cooked cowpea seeds (N.mm)	3.5 ± 0.97 a	5.5 ± 1.54 b
Phytic acid content ($mg.g^{-1}$)		
as is	1.9 ± 0.13 b	1.5 ± 0.08 a
dry basis	2.50 ± 0.14 b	2.0 ± 0.02 a
Phytase activity (units. kg^{-1})		
as is	91.1 ± 4.78 a	104.2 ± 1.83 b
dry basis	103.4 ± 1.30 a	120.9 ± 0.80 b
Soluble pectin ($mg.g^{-1}$)		
as is	4.9 ± 0.36 b	3.2 ± 0.50 a
dry basis	5.6 ± 0.20 b	3.7 ± 0.30 a
Degree of splitting (%)	41.9 ± 2.85 b	5.5 ± 2.76 a
Leachable solids ($g.ml^{-1}$ cooking H_2O)	2.7 ± 0.04 a	3.0 ± 0.14 a

Means followed by the same letter within cell are not significantly different at level *P>0.05*

* The cowpeas remained uncooked after 4.5 h of cooking

HTC cowpeas had a significantly ($P \leq 0.05$) higher moisture content than their normal counterparts (Table 3.1.1). This phenomenon was previously reported by Sefa-Dedeh *et al.* (1978) who found cowpeas stored at high relative humidities (80 and 85%) showing an increase in the moisture content during 12 months of storage, regardless of the temperature of the storage environment.

The cooking time of normal cowpeas was drastically affected by the aging conditions. Cooking time of legume seeds is a function of storage conditions, time of storage, species and agronomic conditions and some of these factors may interact with each other during storage (Liu, 1995). The cooking times of normal *Mogwe-o-Kgotsheng* cowpeas are within the ranges reported by Akinyele *et al.* (1986). However, the aged samples remained uncooked even after more than 4.5 h of cooking; while the normal cowpeas were cooked after approximately 1.5 h. Liu *et al.* (1992b) found storing cowpeas seeds at 37ºC and 75% RH for 18 months showing the greatest hardening effect , followed by 25ºC and 75% RH and 30ºC and 64% RH.

Cooking time in this study is referred to as the time when 80% of the rods of the Mattson Bean Cooker (MBC) had fallen through the cooked cowpea seeds. The rods used in this study were 90 g. Proctor & Watts (1987) used rods of different weights to design MBC rods that would consistently reproduce cooking times correlated with that determined by sensory analysis. They found that lighter rods (49.75 g) provided the best indication of cooking time. It is possible that the rods used in this study underestimated the actual cooking time of cowpeas. But for the purpose of this study, it still provided a basis for comparison between the cooking time of normal and HTC cowpeas as well as for their correlations with some physicochemical characteristics such as pectin solubility, texture of cooked seeds, degree of splitting and water absorption during cooking and soaking.

There was no significant correlation (Table 3.1.2) between the cooking time and the moisture content of cowpeas. Akinyele *et al.* (1986) found cowpea seeds with long cooking times having high moisture contents, but could not find a direct association between moisture content and cooking time. Liu *et al.* (1992b) and Hentges *et al.* (1991) demonstrated that the interaction between both high temperature and high relative humidity conditions in atmosphere and final moisture content of stored seeds is very crucial for the development of the defect, not only one parameter. i.e. moisture content.

Table 3.1.2. Significant correlations coefficients between physicochemical characteristics of cowpeas at level P≤0.05

Physicochemical characteristic	pH	Cooking time	Moisture content	Texture of cooked seeds	Soluble pectin	Phytase activity	Phytic acid	Degree of splitting	Leached solids	W.A.C.[1]	W.A.S.[2]	H.C.[3]
pH		-0.97	-0.97									
Cooking time	-0.97			+0.97	-0.95	+0.70	-0.93	-0.91	+0.61			+0.89
Moisture content	-0.97										-0.95	
Texture of cooked seeds		+0.97			-0.99			-0.99		-0.73		
Soluble pectin		-0.95		-0.99			+0.90			+0.83		
Phytase activity		+0.70					-0.90					
Phytic acid		-0.93			+0.90	-0.90						
Degree of splitting		-0.91		-0.99						+0.76		
Leached solids		+0.61										
W.A.C.[1]				-0.73	+0.83			+0.76				
W.A.S.[2]			-0.95									
H.C.[3]		+0.89										

[1] Water absorption during cooking
[2] Water absorption during soaking
[3] Hydration capacity

Therefore, it is not expected to find a direct correlation between the cooking time and the final moisture content of cowpeas.

The cowpea pH is one of the parameters that was affected by the aging conditions (Table 3.1.1). There was a significant ($P \leq 0.05$) decrease in HTC cowpea pH as compared with the normal cowpeas. Liu, Phillips & McWatters (1993b) found that storing cowpeas at 30ºC and 64% RH for 18 months decreased pH of cowpeas from 6.64 to 5.57 and Liu *et al.* (1992a) found that the pH of cowpeas decreased linearly during 18 months of storage. During storage at high temperature and high relative conditions, the pH of cowpeas may decrease due to hydrolysis of lipids into fatty acids, oxidation of these acids into organic acids (Liu *et al.*, 1992a) and hydrolysis of phytates and storage proteins (Hohlberg & Stanley, 1987). There was a significant ($P \leq 0.05$) negative correlation (r = -0.97) between the pH of cowpeas and cooking time (Table 3.1.2). The increased level of HTC defect in cowpeas was partly attributed to the low pH of cowpeas possibly caused by hydrolysis of lipids into fatty acids, oxidation of these acids into organic acids (Liu *et al.*, 1992a) and hydrolysis of phytates and storage proteins (Hohlberg & Stanley, 1987). According to Liu *et al.* (1992a) the cowpea tissue pH can be a convenient and reliable indicator of seed HTC defect induced by adverse storage. It is possible that the decrease in the pH of cowpeas during storage caused by hydrolysis of phytates may have affected the cooking time. This is supported by the significant ($P \leq 0.05$) increase in the hardness of cooked cowpeas seeds after being subjected to adverse storage conditions for 21 days (Table 3.1.1).

A significant ($P \leq 0.05$) positive correlation (r = +0.97) was found between cooking time and texture of cooked cowpea seeds (Table 3.1.2). Texture has been used by several authors (Sefa-Dedeh *et al.* 1978; Sefa-Dedeh *et al.* 1979; Mwangwela *et al.*, 2006; Phadi, 2004) to indicate the level of softeness of cooked legume seeds and has been positively correlated with the cooking time. During the cooking process, the breakdown of the cowpeas middle lamella is an indication of the beginning of the softening process (Sefa-Dedeh *et al.* 1979). Cowpeas with HTC defect showed little middle lamella disintegration during cooking as compared with control cowpeas (Sefa-Dedeh *et al.* 1979).

The increase in the texture measurement of cooked cowpea seeds can be associated with the significantly ($P \leq 0.05$) decreased pectin solubility in HTC cowpeas (Table 3.1.1). A significant ($P \leq 0.05$) negative correlation (r = -0.95) was found between the soluble pectin and cooking time (Table 3.1.2). The amount of soluble pectins found in normal and HTC cowpeas was slightly higher than the values (0.07 to 3.25 $mg.g^{-1}$) reported by Hentges *et al.* (1991). These results from this study may suggest that adverse storage conditions significantly ($P \leq 0.05$) decreased the amount of soluble pectins in cowpeas. The same pectin solubility pattern was observed in cowpeas after 18 months of storage (Hentges *et al.* 1991). Decreased pectin solubility may contribute to development of HTC defect in legume seeds (Moscoso *et al.,* 1984). The decrease in pectin solubility can be attributed to pectin insolubilization via binding with divalent cations such as Ca^{2+} and Mg^{2+}, as a result of phytate breakdown by phytase at relatively high temperatures and humidities (Hentges *et al.,* 1991; Aguilera & Rivera, 1992).

The phytase activity values of normal cowpeas shown in Table 3.1.1 are within the value ranges reported in the literature, which are between 82.3 $units.kg^{-1}$ (Affrifah *et al.*, 2005) and 100.87 and 102.06 $units.kg^{-1}$ (Affrifah, Chinnan & Fang, 2006). Contrary to Hentges *et al.* (1991) who found the phytase activity of legume seeds stored at adverse conditions decreasing with time, the phytase activity of cowpeas in this study was found to increase after the storage at adverse conditions. This is not surprising since under high temperatures and relative humidity conditions phytase activation is expected to occur. To support this evidence, a significant ($P \leq 0.05$) positive correlation (r = +0.85) was found between phytase activity and the cooking time (Table 3.1.2). Affrifah & Chinnan. (2005) found phytase activity of cowpeas increasing from 115.3 to 133.9 $units.kg^{-1}$ when stored at 42ºC and 80% RH for five weeks.

Storing cowpeas at high temperatures and high relative humidity reduced the amount of phytic acid significantly (Table 3.1.1). This was coincident with the increase in the phytase activity (Table 3.1.1). A significant negative correlation (r= -0.93) was found between phytase activity and phytic acid in cowpeas (Table 3.1.2). These results suggest that during adverse storage conditions, phytase catalyzed hydrolysis of phytic acid in cowpeas. Phytic acid can readily form chelates with divalent cations such as Ca^{2+}, Zn^{2+}, Fe^{2+} and Mg^{2+} (Maenz, Engele-Schaan, Newkirk & Classen, 1999).

Once hydrolyzed, divalent cations, such as Ca^{2+} and Mg^{2+}, could migrate to the middle lamella, where they bind with pectins, contributing to reduced pectin solubilization. A significant ($P \leq 0.05$) positive correlation was found between pectin solubility and phytic acid (r = +0.90). Reyes-Moreno, Okamura-Esparza, Armienta-Rodelo, Gómez-Garza & Milán-Carrillo (2000) also found the phytic acid content of chickpeas decreasing significantly with storage at high temperatures (>25ºC) and high relative humidity (>65%). The values of phytic acid found in normal and HTC cowpeas are lower than the values (5.1 to 10.27 $mg.g^{-1}$) reported by Farinu & Ingrao (1991) and than those (8.18 to 9.46 $mg.g^{-1}$) reported by Preet & Punia (2000) but within the range (1.15 to 2.10 $mg.g^{-1}$) reported by (Giami, 2005), all in cowpeas. The wide variation in phytic acid content of cowpeas can be attributed to differences in storage conditions and time, cultivars and species (Hentges *et al.,* 1991). The first two parameters affect the phytase activity. Furthermore, a wide variation in phytic acid within different cultivars was reported by Farinu & Ingrao (1991). Long cooking times for legume seeds have been associated with low phytate content (Hentges *et al.,* 1991; Liu, 1995). A significant ($P \leq 0.05$) negative correlation (r = -0.89) was found between the cooking time and the phytic acid content of control and HTC cowpeas (Table 3.1.2). It supports the involvement of phytate in the development of the HTC phenomenon during storage at adverse conditions.

The "phytate-minerals-pectin interactions" is one of the earliest and most widely accepted reasons for the development of HTC defect in legume seeds (Liu, 1995). The increase of phytase activity, decrease of phytic acid content and the decrease in the pectin solubility after storage at adverse conditions clearly supports the insolubilization of pectins in the middle lamella due to their binding to divalent cations released by phytate, after its hydrolysis by phytase. It also justifies the harder texture and long cooking time found in HTC cowpeas. Phytate is an organic phosphate defined as the Ca^{2+}/Mg^{2+} salt of phytic acid (Garcia-Villanova, Garcia-Villanova & de Lope, 1982)). Phytase hydrolyzes the phosphate residues from phytic acid, thereby essentially destroying its affinity for minerals (Fennema, 1985). Inositol and phosphoric acid are finally formed through the action of this enzyme (Beal & Mehta, 1985 and Nayini & Markakis, 1983 according to Duodu, 1997).

The degree of splitting and leachable solids during cooking are other parameters that were significantly affected by storage at adverse conditions. The degree of splitting of normal cowpeas decreased with more than 80% after storage at adverse conditions (Table 3.1.1). HTC beans retain the cell middle lamella after cooking, while that of normal beans is disintegrated (Liu, 1995) allowing water penetration and swelling of starch granules. The pectins in the middle lamella of HTC cowpeas may have been bound to divalent cations such as Ca^{2+} and Mg^{2+}, forming an insoluble barrier that could have restricted water absorption during cooking as shown by the significant decrease in water absorbed after 3 h of cooking in Table 3.1.3. This could have contributed to harder texture and consequently, lower degree of splitting of HTC cowpeas as compared with normal cowpeas. There were significant negative correlations between the texture of cooked cowpea seeds and the degree of splitting (r =-0.99) and between texture of cooked seeds and pectin solubility (r = -0.99) (Table 3.1.2). This is not surprising since the rate of softening (texture) of cowpeas during cooking is mainly dependent upon the breakdown of the middle lamella (Sefa-Dedeh *et al.,* 1978). Many other studies using electron microscopy demonstrated that seed cookability is determined by the rate of cell separation during cooking (Sefa-Dedeh *et al.* 1978, Sefa-Dedeh *et al.* 1978; Shomer *et al.* 1990).

The amount of leachable solids in HTC cowpeas per ml of cooking water was not significantly ($P>0.05$) different from solids leached from normal cowpeas. There was however a significant but not very strong positive correlation (r =+0.61) between leached solids and cooking time of cowpeas (Table 3.1.2). Jackson & Varriano-Marston (1981) and Hentges *et al.* (1991) found that aged legume seeds lost more solids during soaking than their normal counterparts due to seed membrane degradation at aging conditions. Hentges *et al.* (1991) found very high positive correlations (r = +0.971, $p<0.01$) between cooking time and leachable solids in beans. The authors proposed that leakage is a result of cell membrane damage in aged beans. The low correlation between leached solids and cooking time found in this study is comprehensable since there were no significant differences in the amount of leached solids between normal and HTC cowpea samples. Leached solids during cooking are related to water taken up by the seeds during cooking (Phlak *et al.*, 1989). It is possible that *Mogwe-o-Kgotsheng* cowpeas have a strong adhesion between adjacent cells which became much stronger after storage at adverse conditions as shown by the harder texture in HTC cowpeas

(Table 3.1.1). This strong adhesion between adjacent cells could have limited water absorption during cooking (Phlak *et al.,* 1989) and partially blocked the channels by which solids leached in the cooking water. It is also possible that during storage at adverse conditions, seed membrane degradation was not enough to originate higher solid losses during cooking.

When cowpeas are cooked, the middle lamella is weakened resulting in cell separation (Mwangwela *et al*. 2006) which may result in splits in the seed coat and cotyledons and solid losses during cooking. Pectins are complex polysaccharides found in the primary cell wall and middle lamella where they play important roles such as hydrating agents and cementing materials for the cellulose network (Muralikrishna & Tharanathan, 1994). During cooking, the middle lamella is disintegrated allowing cell separation, texture softening, seed coat and cotyledon splitting and solid losses and consequently, shortening of the cooking time. But in HTC cowpeas, the extent at which these characteristics are affected during cooking might be less than in normal cowpeas because of the low pectin solubility in their middle lamella.

3.1.4.3. Effect of HTC defect on hydration capacity, water absorption during soaking and cooking in cowpeas

Table 3.1.3 shows the effect of storing cowpeas at adverse conditions on hydration properties of cowpeas after 6 and 18 h of soaking and after 3 h of cooking. The amount of water absorbed by HTC cowpeas after 18 h of soaking was significantly ($P \leq 0.05$) higher than that absorbed by normal cowpeas. To support these findings, a significant ($P \leq 0.05$) positive correlation (r =+0.89) was found between cooking time and water absorbed by cowpeas after 18 h of soaking (i.e. hydration capacity) (Table 3.1.2).

Table 3.1.3. The effect of HTC defect on hydration characteristics of cowpeas during soaking

Water absorption properties	Normal cowpeas	HTC cowpeas
Hydration capacity after 18 h of soaking (g H_2O. kg^{-1} cowpeas)	1121.5 ± 15.72 a	1334.7 ± 13.84 b
Water absorption after 6 h of soaking (g H_2O. kg^{-1} cowpeas)	1154.1 ± 18.08 b	1060.4 ± 17.91 a
Water absorption after 3 h of cooking (g H_2O. kg^{-1} cowpeas)	1286.5 ± 19.02 b	1216.5 ± 19.61 a

Means followed by the same letter within a row are not significantly different at level *P>0.05*

Hentges *et al.* (1991) and Jackson & Varriano-Marston (1981) demonstrated that water uptake after 18 h of soaking (corrected for solids loss), was higher in HTC beans than in control beans. During imbibition of HTC beans with water, the seed coat may have been pulled away from the cotyledon at the palisade layer allowing a layer of bulk water to enter between the seed coat and cotyledons (Plhak *et al.*, 1989; Valle & Stanley, 1992). The large cavity between the two cotyledons in cowpeas can also have a different effect on water absorption after the initial resistance of the seed coat to water penetration has been overcome (Valle & Stanley, 1992). It is possible that there was a large cavity between the cotyledons of HTC cowpeas and the fact that their seed coat might have been pulled away from the cotyledons could have contributed to larger amounts of water absorbed by HTC cowpeas after 18 h of soaking.

After 6 h of soaking, normal cowpeas had absorbed significantly ($P \leq 0.05$) more water than the HTC counterparts (Table 3.1.3). A significant ($P \leq 0.05$) positive correlation (r = +0.83) was found between the water absorbed after 6 h of soaking and soluble pectin. Less soluble pectin in soaking water can lead therefore to a slower rate of water uptake during soaking as pectin is known to play an important role in water absorption (Sefa-Dedeh & Stanley, 1979). Therefore, the decrease in the amount of water absorbed by HTC cowpeas during the first 6 h of soaking can be associated with their decreased pectin solubility (Table 3.1.1).

After 3 h of cooking, HTC cowpeas absorbed significantly ($P \leq 0.05$) less water than the control cowpeas. A significant ($P \leq 0.05$) positive correlation (r =+0.64) between water

absorbed after 3 h of cooking and pectin solubility was found. It is possible that solubilization of pectins in the middle lamella during cooking occurred at a slower rate in HTC cowpeas as they had a lower pectin solubility when compared with normal cowpeas. The low pectin solubility in the middle lamella of HTC cowpeas could therefore be associated with the lesser water absorbed after 3 h of cooking. A significant ($P \leq 0.05$) negative correlation (r = -0.73) was found between texture of cooked seeds and the amount of water absorbed after 3 h of cooking. Sefa-Dedeh *et al*. (1978) and Sefa-Dedeh & Stanley (1979) showed that the solubilization of the middle lamella of cowpeas during cooking results in cell separation and leads to a soft cooked texture. They postulated towards heat-catalyzed depolymerization of the middle lamella pectin polymers as being the mechanism underlying cell separation during cooking. Water absorbed after cooking and not water absorbed after soaking has been related to the hardness of cooked seeds (Jackson & Varriano-Marston, 1981). From these findings, it can be concluded that during cooking, the amount of water absorbed by cowpea seeds is affected by pectin solubility, which in turn affects the texture of the seeds during cooking.

Another important parameter that can be associated with the reduced water absorbed by HTC cowpeas as compared to normal cowpeas after 3 h of cooking is the decrease of pH induced by adverse storage from 6.5 to 6.3 (Table 3.1.1). Although this reduction is low, it is in agreement with Liu *et al.* (1993b) who found the cowpeas pH decreasing from 6.64 to 5.57 after 18 months of storage. Liu *et al.* (1992a) found that at pH 6.5 protein denaturation temperature was beyond 100 ºC and at pH 6.4 it reduced sharply to 78 ºC. According to these authors, under increased tissue acidity conditions, protein coagulation or gelation is expected to occur before starch gelatinization. Coagulated proteins could have formed a physical and water-restricting barrier and have been responsible for impeded starch gelatinization during cooking of HTC cowpea seeds, leading to less water absorbed during the cooking process.

3.1.4.4. Effect of HTC defect on the rate of water absorption during soaking and cooking and the degree of splitting

The amount of water absorbed per unit weight of seeds increased with soaking time in both normal and HTC cowpeas (Fig. 3.1.2). There was not a significant ($P \leq 0.05$) difference in the amount of water absorbed during the first hour of soaking but after that

period up to 6 h of soaking, normal cowpeas had significantly ($P \leq 0.05$) higher water uptake than the HTC cowpeas. It is possible that during the first 1½ h of soaking water absorption was not affected by pectin solubility. The seed coat structure and thickness may be the only factors involved in water absorption during that time (Sefa-Dedeh & Stanley, 1979).

After the water penetration has overcome these factors, pectin solubility would have played a crucial role in water absorption. It is clear from Fig. 3.1.2 that from the second to the sixth hour of soaking, HTC cowpeas absorbed less water than their normal counterparts probably due to lower pectin solubility observed in HTC cowpeas as compared with normal cowpeas (Table 3.1.1).

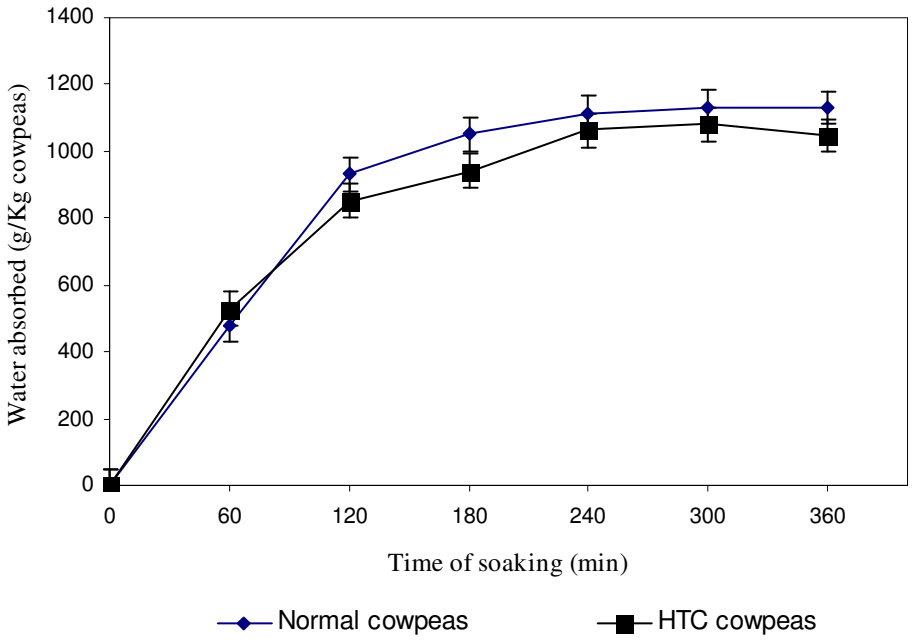

Figure 3.1.2. The effect of aging on the water absorption during 6 h of soaking (Vertical bars indicate the standard deviations of means; HTC: Hard-to-cook)

During the first hour of cooking, normal cowpeas absorbed as much water as the HTC cowpeas (Fig.3.1.3). After this, HTC cowpeas absorbed significantly less water as compared with control cowpeas during cooking. During cooking two different processes may occur in pectins of the middle lamella: solubilization in cooking water and disintegration via β-eliminative reaction.

The solubilization of pectins can be attributed to a conversion of insoluble pectinates to soluble pectins in the middle lamella (Vidal-Valverde *et al.*, 1992) and to the breakage of pectin molecules into lower, more soluble molecular fractions via β-eliminative reaction (Liu *et al.,* 1993b), thereby increasing pectin solubility. Pectin molecules of HTC cowpeas had showed lower solubility as compared with pectin molecules of normal cowpeas (Table 3.1.1). This can explain the slower rate of water absorption of HTC cowpeas as compared with normal cowpeas, from 1½ to 6 h of cooking.

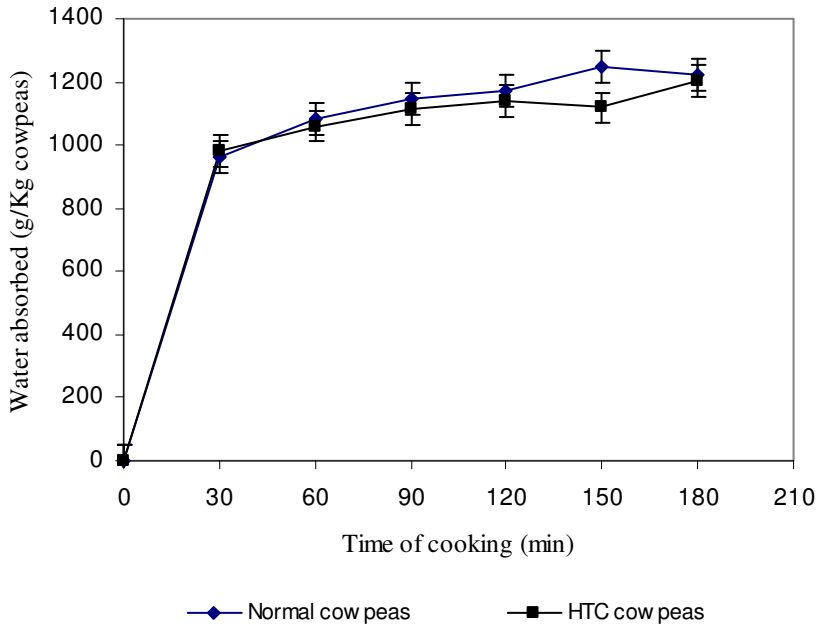

Figure 3.1.3. The effect of aging on the rate of water absorption during 3 hours of cooking (Vertical bars indicate the standard deviations of means; HTC: Hard-to-cook)

Another factor that can be linked to water absorption during cooking is the degree of splitting. A positive correlation was observed between splitting and water absorption during cooking (r =+0.76). Figure 3.1.4 shows the effect of HTC defect on the degree of splitting during 3 h of cooking. Both normal and HTC cowpea seeds were not prone to splitting during the first 30 min of cooking. After that period, splitting of normal cowpeas increased up to 41% while in HTC cowpeas it did not go further than 6% (Fig. 3.1.4).

There was a significant negative correlation (r = -0.99) between the degree of splitting and the texture of cowpeas and a significant ($P \leq 0.05$) negative correlation (r = -0.91) between splitting and cooking time. Taiwo, Akanbi & Ajibola (1998) reported that splitting of cooked cowpeas was positively correlated with drained weight and softness of cooked cowpea seeds. It is possible that the splits created in seeds and cotyledons of cowpeas contributed substantially to water penetration during cooking. Therefore, HTC cowpeas with low pectin solubility and harder texture had lower degree of splitting during cooking as compared to normal cowpeas and consequently, less water absorbed during the same period.

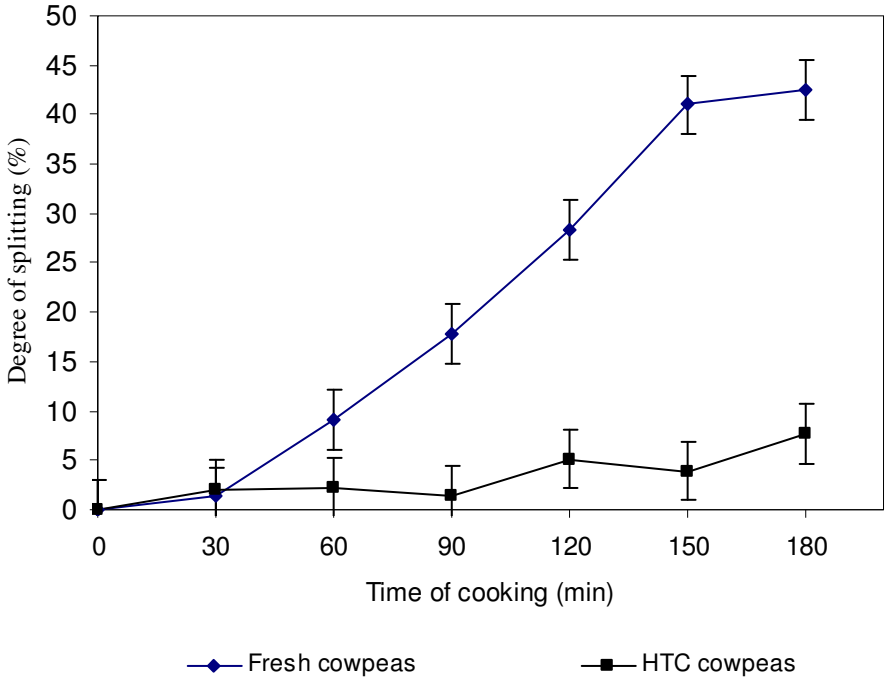

Figure 3.1.4. The effect of aging on the degree of splitting during 3 h of cooking. (Vertical bars indicate the standard deviations of means; HTC: hard-to-cook)

The very low rate of splitting in HTC cowpeas could be related to insolubilization of pectins in the middle lamella due to their binding with divalent cations released from hydrolysis of phytate by phytase at adverse conditions. To support this, an increase in phytase activity, decrease in phytic acid content and consequently the lower pectin solubility (Table 3.1.1) were observed after storing cowpeas at adverse conditions for 21 days. The low pectin solubility would restrict the starch granules from full swelling

during cooking (Liu *et al.*, 1992a). Hentges *et al.* (1991) and Aguilera & Rivera (1992) demonstrated that restricted water uptake during cooking was due to pectin insolubilization via binding with Ca^{2+} and Mg^{2+} resulting from phytate breakdown by phytase.

Cowpeas with harder texture will be less prone to splitting and will absorb less water during cooking. The texture measurements of both HTC and control cowpeas decreased with cooking time (Fig. 3.1.5). From 90 to 180 min of cooking, the texture of normal cowpeas did not differ significantly ($P \leq 0.05$), while for HTC cowpeas the texture did not change significantly from 120 min to 180 min of cooking.

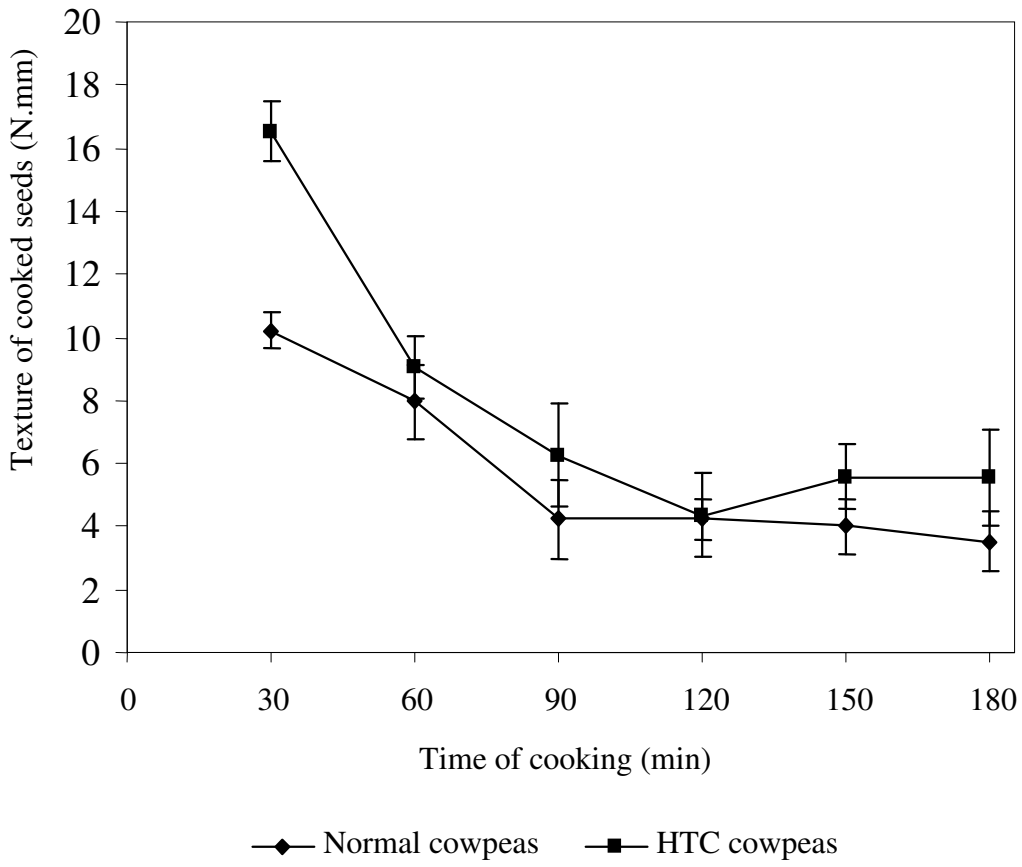

Figure 3.1.5. The effect of aging on the texture of cooked cowpeas during 3 h of cooking (Vertical bars indicate the standard deviations of means; HTC: hard-to-cook)

Overall, the texture of HTC cowpeas was significantly ($P \leq 0.05$) harder than that of normal cowpeas during the 3 h of cooking. The significant negative correlations found between texture and splitting (r =-0.99) and between texture and soluble pectin content (r =-0.99) (Table 3.1.1) in this study imply that during cooking there is a softening of the cowpea seeds which is accompanied by increased solubility of middle lamella and increased degree of splitting and consequently, increased water absorbed during cooking. In HTC cowpeas, because of low pectin solubility observed, the texture was harder and the rate of softening during cooking as well as the degree of splitting was lower as compared to normal cowpeas. It has been demonstrated that during cooking the rate of softening of cowpeas is mainly dependent on the breakdown of pectin molecules in the middle lamella (Sefa-Dedeh *et al.* 1978), which results in cell separation as shown by electron microscopy (Sefa-Dedeh *et al.* 1978, Sefa-Dedeh *et al.* 1978 & Shomer *et al.,* 1990).

3.1.5. CONCLUSIONS

Storing cowpeas at adverse conditions (42ºC and 67% RH for 21 days) significantly ($P \leq 0.05$) increases the cooking time from 89 min to more than 4½ h. The increase in cooking time of HTC cowpeas can be associated with decreased pectin solubility, which was coincident with increased phytase activity and reduced phytic acid content. At adverse storage conditions, phytase probably hydrolyses phytate to release divalent cations which bind to pectins in the middle lamella, reducing their solubility. Due to the reduced pectin solubility, HTC cowpeas have a harder texture, decreased degree of splitting and consequently, reduced water absorbed during cooking as compared with normal cowpeas. This research therefore supports the "Phytate-cations-Pectins interactions" mechanism to explain the HTC defect in cowpea seeds.

3.2. Improvement in the cooking and physico-chemical characteristics of hard-to-cook cowpeas by pre-conditinioning and micronization

3.2.1. ABSTRACT

The effects of pre-conditioning cowpeas in solutions with and without monovalent (Na^+) cations followed by micronization on physicochemical and cooking characteristics of normal and hard-to-cook cowpeas (*Mogwe-o-Kgotsheng*) were investigated. Pre-conditioning in water without further micronization is effective in reducing the cooking time of normal cowpeas. For HTC cowpeas, a combination of both pre-conditioning in a solution with monovalent (Na^+) cations and micronization is needed to optimally reduce the cooking time. These reductions in the cooking time when pre-conditioning on its own and in combination with micronization were respectively applied in normal and HTC cowpeas coincided with an improvement in pectin solubility, degree of splitting and decrease in the hardness of cooked cowpeas. Firstly, pre-conditioning in water improved the solubilization of pectins, while pre-conditioning in monovalent (Na^+) cations induced conversion of insoluble to soluble pectins. Secondly, micronization improved the pectin solubilization further by breaking pectin molecules in the middle lamella of cowpeas into lower and more soluble fractions, probably via the β-elimination reaction. Although all treatments improved pectin solubility of HTC cowpeas, this was not the case for reduction in cooking time. This suggests that factors other than pectin solubility influence the cooking time of HTC cowpeas. Storing cowpeas at adverse conditions increased the activity of phytase which coincided with a decrease in the amount of phytic acid. Micronization decreased the activity of phytase and the phytic acid content of both normal and HTC cowpeas. The reduction in phytic acid could have been a function of leaching Ca^{2+} and Mg^{2+} associated with phytic acid into water. Micronization decreased the amount of water absorbed by normal and HTC cowpeas during soaking probably because of denaturation of proteins.

Keywords: HTC cowpeas, micronization, cooking time, phytic acid, phytase activity, pectin solubility, texture, splitting, water absorption

3.2.2. INTRODUCTION

Legumes still play a significant role in dietary protein supply, especially in developing countries (Onigbinde & Ojeabulu, 1999). The limitation imposed on the consumption of legume seeds, such as cowpeas in tropical countries due the HTC defect has generated interest in the mechanisms of the phenomenon and especially in new and improved techniques to alleviate or eliminate the defect. The most widely agreed mechanisms for the development of the defect is related to pectin insolubilization due to binding with divalent cations such as Ca^{2+} and Mg^{2+} resulting from the release of divalent cations through action of phytase on phytate (Hentges *et al.*, 1991; Aguilera & Rivera, 1992). Another mechanism proposed is that of cell wall lignification via cross-linking of phenolic compounds with cell wall proteins and middle lamella pectins and cellulose (Stanley, 1992). Soaking in water and in solutions containing monovalent cations have been used to reduce the cooking time of cowpeas (Sefa-Dedeh *et al.*, 1978) and beans (Jackson & Varriano-Marston, 1981; Valle *et al.*, 1992; Léon *et al.*, 1992).

Micronization is an infrared heat treatment (1800 to 3400 nm) of moisture-conditioned grains and legumes that has been reported to reduce the cooking time of cowpeas (Mwangwela *et al.*, 2006; Phadi, 2004) and lentils (Scanlon *et al.,* 1998; Arntfield *et al.,*, 1997). However, the effect of pre-conditioning legume seeds in solutions containing monovalent cations prior to micronization on the cooking time, physicochemical changes and cooking characteristics of HTC legume seeds has not been investigated yet.

The objective of this study was to determine the effectiveness of pre-conditioning in either water or in a solution containing monovalent cations (Na^{+}) alone and in combination with micronization on cooking characteristics as they relate to changes in other physicochemical characteristics of normal and HTC induced cowpeas.

3.2.3. MATERIALS AND METHODS

3.2.3.1. Selection of raw materials

Cowpeas were selected as described previously (section 3.1.3.1).

3.2.3.2. Inducement of HTC Defect

The HTC defect was induced as described in section 3.1.3.2.

3.2.3.3. Pre-conditioning in deionised water and in a solution containing monovalent (Na^+) cations

The pre-conditioning process involved addition of deionised water in control samples (normal and HTC cowpeas) and of solutions containing monovalent cations (Na^+) in both normal and HTC cowpeas to reach approximately 40% moisture content. The solution used was a mixture of 2 g/l sodium bicarbonate and 1 g/l sodium carbonate, as recommended by Bellido *et al.* (2003). The amount of water required to achieve the desired moisture content was calculated as follows (Arntfield *et al.,* 1997):

Weigh of H_2O = weigh cowpeas x (%H_2O target - %H_2O original)/(100-%H_2O target)

After the pre-conditioning process, cowpeas were blotted dry on absorbent paper and held for a further 12 h at 22ºC to equilibrate the moisture throughout the seeds. Pre-conditioning legume seeds to 40% moisture content is a very crucial step before micronization and according to Scanlon *et al.* (1998), at this moisture, the reduction in cooking time is more effective than at lower pre-conditioning moisture contents.

3.2.3.4. Micronization

The micronization parameters (moisture content of conditioned seeds and micronizer settings) were selected according to Phadi (2004) and Mwangwela *et al.* (2006) in order to obtain relatively stable products in terms of final moisture content and without burning. A small scale micronizer was used, which consisted of three 2 KW Phillips IR lamps (Technilamp Pty, Johannesburg, South Africa) operating at 66.7% output. Before the micronization process, the micronizer was pre-heated for 20 min. Each time, a batch of 160 g cowpeas was micronized in a single layer (21 cm from the energy source) for 6 min, which according to Mwangwela *et al.* (2006) is believed to produce a final surface temperature of 153ºC on cowpeas. After the process of micronization, the cowpeas were spread on a tabletop and cooled to room temperature for 1 h before being packed in Zip-lock® bags (Plastilon Packaging, Pretoria, South Africa) and kept at 22ºC.

3.2.3.5. Cooking time

The Mattson Bean Cooker was used to determine the cooking time of normal and HTC cowpeas as described in section 3.1.3.3.

3.2.3.6. Moisture content

Moisture content was determined on the flours produced through milling representative samples of normal and HTC cowpeas using a Falling Number Mill 3100 (Falling Number, Huddinge, Sweden) as described previously (section 3.1.3.4).

3.1.3.7. pH

The pH of the cowpeas was measured as described by Liu *et al.* (1992b) (see section 3.1.3.5).

3.2.3.8. Water absorption during soaking and hydration capacity

Water absorption during soaking was determined according to the procedure of Agbo *et al.* (1987) modified by Mwangwela *et al.* (2006) as described in section 3.1.3.6.

3.2.3.9. Water absorption during cooking

The amount of water absorbed during cooking was performed according to a modified procedure of Cenkowski & Sosulski (1997) (see section 3.1.3.7).

3.2.3.10. Splitting of cooked seeds

The splits were counted as the number of cowpeas with split seed coats and cotyledons and calculated as (no. split seeds)/ (no. whole seeds) x100 (Taiwo *et al.*, 1997a).

3.2.3.11. Leached solids

The concentration of solids in the cooking water was estimated according to the procedure of Valle *et al.* (1992) described in section 3.1.3.9.

3.2.3.12. Texture measurements during cooking

Texture was measured using a TA-XTPlus Texture Analyser (Stable Micro Systems, Godalming, UK) with an A/CKB craft knife adapter mounted with Hilite heavy-duty blades (Hilite Hardware, Pretoria, South Africa) as described previously (section 3.1.3.10).

3.2.3.13. Phytic acid determination

The method described by Febles *et al.* (2001) was used to determine the content of phytic acid in cowpeas flour (see section 3.1.3.11).

3.2.3.14. Phytase activity

The phytase activity was measured by direct incubation with sodium acetate buffer as described by Greiner & Egli (2003) (see section 3.1.3.12).

3.2.3.15. Soluble pectin

Pectin solubility was measured according to the modified method of Arntfield *et al.* (1997) (see section 3.1.3.13).

3.2.3.17. Statistical analysis

Experiments were conducted in duplicate. All the assays, with the exception of texture measurement, were done in triplicate. In the case of texture measurements, 10 seeds were analysed for each replicate per sample treatment. Analysis of Variance (ANOVA) and correlation coefficients were performed using the Statistica version 7.0 (StatSoft Inc, Tulsa, USA). The Least Significant Difference test (LSD-test) was utilized for comparison between means when F-test was significant ($P \leq 0.05$). Main Effects ANOVA was used for overall effect of different treatments (storage conditions, pre-conditioning of seeds in water or in solution containing monovalent (Na^+) cations and micronization) and Factorial ANOVA was used to analyze the possible interactions between the three variables (storage, pre-conditioning in water or in a solution containing monovalent (Na^+) cations and micronization).

3.2.4. RESULTS AND DISCUSSION

3.2.4.1. Effect of pre-conditioning in a solution of monovalent (Na^+) cations and micronization on the cooking time, texture, moisture content and pectin solubility of cowpeas

The variables, i.e. state of cowpeas (normal or HTC), pre-conditioning process (in water or in a solution containing monovalent (Na^+) cations) and micronization interacted significantly (*P<0.001*) with each other in terms of the changes observed in the cooking time of cowpeas. Table 3.2.1 shows the effect of pre-conditioning cowpea seeds in a solution containing monovalent (Na^+) cations and subsequent micronization on the cooking time of normal and HTC cowpeas.

Table 3.2.1. The effect of pre-conditioning alone and in combination with micronization on the cooking time of normal and HTC cowpeas

Treatments	Cooking time (min)	
	Normal	**HTC**
Control	89 ± 3.2 e	270* g
Pre-conditioned in H_2O alone	44 ± 4.8 c	270* g
Pre-conditioned in monovalent (Na^+) cations alone	43 ± 5.3 bc	150 ± 9.6 f
Micronized after pre-conditioning in H_2O	37 ± 3.4 ab	148 ± 9.2f
Micronized after pre-conditioning in monovalent (Na^+) cations solution	30 ± 5.0 a	59 ± 4.0 d

Means followed by the same letter in cell are not significantly different at level *P>0.05*

* The cowpeas remained uncooked after 4.5 h of cooking

In control samples, HTC cowpeas had a cooking time that was more than three times higher than that of normal cowpeas (Table 3.2.1). Many other studies using accelerated storage conditions with various legume species confirmed that storing legume seeds at high temperature and high relative humidity lead to the development of HTC defect and hence longer cooking times (Hentges *et al.,* 1991; Liu *et al.,* 1992; Moscoso *et al.*, 1984; Sefa-Dedeh *et al.,* 1978).

Pre-conditioning cowpeas in water alone reduced the cooking time of normal cowpeas significantly but not that of HTC cowpeas. Pectic substances are found in the middle lamella of plant cell walls and contribute to the maintenance of structural integrity of the cell wall (Uzogara, Morton & Daniel, 1990). It is possible that an improvement in pectin solubility in the middle lamella when cowpeas were pre-conditioned in water (Table 3.2.2) resulted in a reduction of the cooking time of normal cowpea seeds (Clemente *et al.*, 1998). To support these findings a significant negative ($P \leq 0.05$) correlation (r = -0.75) (Table 3.2.3) was found between soluble pectin and cooking time for treated cowpeas. Despite the fact that pre-conditioning improved the pectin solubility of HTC cowpeas (Table 3.2.2), cooking time was not reduced. This implies that factors other than pectin solubility may have been involved in the long cooking time of HTC cowpeas. According to Sefa-Dedeh & Stanley (1979), not only pectins but also proteins, cellulose and starch can influence water absorption properties of cowpeas, thereby influencing the cooking time.

Pre-conditioning HTC cowpeas in a solution containing monovalent (Na^+) cations significantly reduced the cooking time of HTC cowpeas as compared with the control and HTC cowpeas pre-conditioned in water (Table 3.1.1). The effect of pre-conditioning normal cowpeas in a solution with monovalent (Na^+) cations on the cooking time did not differ significantly from that of pre-conditioning normal cowpeas in water alone (Table 3.1.1). From a practical point of view, pre-conditioning normal cowpeas in water was effective in reducing the cooking time of normal cowpeas.

Pre-conditioning in a solution containing monovalent (Na^+) cations improved soluble pectin by 65% and more than 100% (based on wet basis results) for normal and HTC cowpeas, respectively (Table 3.2.2). The solubilization of pectins as induced by this treatment, not only depends on the solubilization effect of water used during the pre-conditioning process (Clemente *et al.,* 1998), but also on the conversion of insoluble to soluble pectins in the middle lamella (Vidal-Valverde *et al.*, 1992).

Table 3.2.2. The effect of pre-conditioning alone and in combination with micronization on the soluble pectin of normal and HTC cowpeas

Treatments	Soluble pectin ($mg.g^{-1}$) (as is)		Soluble pectin ($mg.g^{-1}$) (dry basis)	
	Normal	**HTC**	**Normal**	**HTC**
Control	4.9 ± 0.4 b	3.2 ± 0.5 a	5.6 ± 0.2 b	3.7 ± 0.3 a
Pre-conditioned in H_2O alone	7.4 ± 0.5 c	5.3 ± 0.4 b	a	a
Pre-conditioned in monovalent (Na^+) cations alone	8.1 ± 0.7 d	6.9 ± 0.5 c	a	a
Micronized after pre-conditioning in H_2O	10.8 ± 0.5 f	9.9 ± 0.5 e	12.4 ± 0.6 c	12.3 ± 0.3 c
Micronized after pre-conditioning in monovalent (Na^+) cations solution	14.2 ± 0.9 h	11.8 ± 0.9 g	17.2 ± 0.7 e	15.0 ± 0.7 d

Means followed by the same letter in cell are not significantly different at level *P>0.05*

a Moisture content for these samples was not determined

Table 3.2.3. Significant correlation coefficients between physicochemical characteristics of pre-conditioned and micronized cowpeas at level P≤0.05

Physicochemical characteristic	Cooking time	Moisture content	Texture of cooked seeds	Soluble pectin	Phytase activity	Phytic acid content	Degree of splitting	Leached solids	W.A.C.[1]	W.A.S.[2]
Cooking time			+0.88	-0.75						
Moisture content										-0.85
Texture of cooked seeds	+0.88			-0.73			-0.98			
Soluble pectin	-0.91		-0.73			-0.51	+0.89			+0.82
Phytase activity						-0.48				
Phytic acid content				-0.51	-0.48					
Degree of splitting			-0.98	+0.89				+0.39	+0.61	
Leached solids							+0.39			
W.A.C.[1]							+0.61			
W.A.S.[2]		-0.85		+0.82						

Data from normal and HTC cowpea samples were pooled for the analyses, [1]water absorption during cooking, [2]water absorption during soaking

The latter is probably due to exchange of Ca^{2+}/Mg^{2+} in Ca^{2+}/Mg^{2+}-pectates by monovalent cations (i.e. Na^{+}) present in the pre-conditioning solution (Valle *et al.*, 1992; Léon *et al.*, 1992). Vidal-Vilaverde *et al.* (1992) found that the conversion of insoluble pectinates to soluble pectins in lentils proceeded in the order $H_2O<NaHCO_3$.

Pre-conditioning in water or in a solution containing monovalent (Na^{+}) cations improved pectin solubility for both normal and HTC cowpeas. The reduction in the cooking time of normal cowpeas as induced by these treatments coincided with the improvement in pectin solubilization. In HTC cowpeas, only pre-conditioning in a solution with monovalent (Na^{+}) cations reduced the cooking time as compared with control samples.

The combination of pre-conditioning in water and micronization reduced the cooking time of normal cowpeas by 58%, which was not significantly different from the 52% reduction imposed by pre-conditioning in a monovalent (Na^{+}) cations solution (Table 3.2.1). In addition to that, combining pre-conditioning in a solution containing monovalent (Na^{+}) cations with micronization showed no added advantage in terms of reducing the cooking time of normal cowpeas as compared to pre-conditioning in water and micronization.

In HTC cowpeas, pre-conditioning in a solution with monovalent (Na^{+}) cations alone as well as combining pre-conditioning in water with micronization had similar effects in terms of reducing the cooking time although the two treatments had different effects on solubility of pectins of HTC cowpeas. However, when HTC cowpeas were subjected to a combination of pre-conditioning in a solution with monovalent cations and micronization, the cooking time reduced by 80% (Table 3.2.1). The latter treatment was the most effective in reducing the cooking time of HTC cowpeas. However, the cooking time of HTC cowpeas subjected to this treatment was still higher than the cooking time of normal cowpeas subjected to any of the treatments.

Micronization of cowpeas could have induced breakage of pectin molecules into lower and more soluble molecular fractions in the middle lamella, probably via the β-elimination reaction (Liu *et al.*, 1993b). These findings are supported by the significant ($P \leq 0.05$) negative correlation between the cooking time and soluble pectin

(r = -0.75) (Table 3.2.3) in treated cowpeas. Other researchers also demonstrated that combining pre-conditioning in water and micronization reduced the cooking time of normal cowpeas (Mwangwela *et al.,* 2006; Phadi, 2004) and lentils (Scanlon *et al.,* 1998; Arntfield *et al.,* 1997).

Pectin is extremely sensitive to elevated temperatures in a near-neutral solution (Liu *et al.*, 1993b; Sajjaanantakul *et al.*, 2003). At these conditions, there is breakage of glycosidic links between methylated galacturonate residues by an unusual β-elimination reaction where a *trans* double bond is inserted between C-4 and C-5 rather than the usual hydrolysis (Coultate, 2002) to form lower molecular weight fractions (Liu *et al.,* 1993b). It is possible that the reduction in the cooking time of normal and HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized is a result of an additive effect of two different mechanisms in improving pectin solubility. The first mechanism is related to the possible exchange of divalent (Ca^{2+}, Mg^{2+}) by monovalent (Na^+) cations in the middle lamella of cowpeas (Valle *et al.*, 1992; Léon *et al.*, 1992) due to monovalent (Na^+) cations present in pre-conditioning solution, and the second is related to an improvement in pectin solubilization via β-elimination reaction (Coultate, 2002) when micronization was applied.

Table 3.2.4 shows the texture of micronized normal and HTC cowpeas after pre-conditioning in water or in a solution containing monovalent (Na^+) cations. There was no significant interaction between the variables, state of cowpeas (normal or HTC) and micronization in terms of texture of cowpeas after 180 min of cooking, therefore the overall effects of HTC defect and micronization are presented in Table 3.2.4.

The values for texture measurements of unmicronized and micronized normal and HTC cowpeas are within the range reported by Mwangwela *et al.* (2006), which ranged from 3.2 to 7.3 N.mm in unmicronized cowpeas and from 2.6 to 5.0 N.mm in micronized cowpeas. Overall, the HTC defect significantly increased and micronization significantly reduced hardness of cowpeas (Table 3.2.4). The increase in the hardness of cooked cowpeas as a result of the HTC defect could be related to insolubilization of pectins in the middle lamella as clearly indicated by a decrease in pectin solubility in HTC cowpeas (Table 3.2.2). Liu *et al.* (1993b) and Moscoso *et al.*

(1984) found aged cowpea seeds cooked for 30 and 45 min, respectively, having a harder texture than that of the normalcowpeas. They related the harder texture to poor pectin solubility during cooking of HTC cowpeas.

Table 3.2.4. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent (Na^+) cations on the texture of cooked seeds of normal and HTC cowpeas

Treatments	Texture of 180 min cooked cowpeas (N.mm)[1]		Overall Micronization Effect[2]
	Normal	HTC	
Control	4.5 ± 0.9 b	5.5 ± 1.5 d	5.0 ± 0.6 c
Micronized after pre-conditioning in H_2O	4.0 ± 1.1 a	4.9 ± 1.7 c	4.4 ± 0.6 b
Micronized after pre-conditioning in monovalent (Na^+) cations	3.8 ± 1.2 a	4.5 ± 1.0 b	4.1 ± 0.4 a
Overall HTC effect[3]	4.1 ± 0.4 a	4.96 ± 0.5 b	

[1]Means followed by the same letter in cell are not significantly different at level *P>0.05*

[2]Means followed by the same letter in the column are not significantly different at level *P>0.05*

[3]Means followed by the same letter in a row are not significantly different at level *P>0.05*

Overall, pre-conditioning in a solution with monovalent (Na^+) cations in combination with micronization was the most effective treatment to reduce the hardness of cooked seeds (Table 3.2.4). These findings are in accordance with cooking time results for HTC cowpeas in Table 3.2.1, where cowpeas subjected to this treatment had the lowest cooking times. There was a significant ($P \leq 0.05$) positive correlation between the cooking time and the texture of cooked cowpeas (r = +0.88) (Table 3.2.3). The overall reduction in the hardness of cooked cowpea seeds when pre-conditioned in a solution containing monovalent (Na^+) cations and micronized could be associated with the significant increase in pectin solubility (Table 3.2.2). A significant negative correlation was found between soluble pectin and cooked texture of treated cowpeas (r = -0.73) (Table 3.2.3).

In this study, both cooked texture and cooking time are associated with pectin solubility of cowpeas. Hentges *et al.* (1991) found that the cooking time of beans and cowpeas was positively correlated (r = +0.892) to insoluble pectin content. Texture

has been used by several authors (Sefa-Dedeh *et al.,* 1978; Sefa-Dedeh *et al.*, 1979; Mwangwela *et al.,* 2006; Phadi, 2004) to indicate the level of softness of cooked legume seeds and has been positively correlated with the cooking time. During the cooking process, the breakdown of the middle lamella of cowpeas is an indication of the beginning of the softening process (Sefa-Dedeh *et al*., 1979). Cowpeas with HTC defect showed little middle lamella disintegration during cooking as compared with control cowpeas (Sefa-Dedeh *et al*., 1979). It is possible that the reduction in the hardness of cooked cowpeas seeds as influenced by pre-conditioning in a solution with monovalent (Na^+) cations in combination with micronization in this research could have played a major role in the overall reduction in the cooking time of normal and HTC cowpeas.

Fig. 3.2.1 shows that with an increase in the cooking time, the texture measurements in terms of hardness of cooked cowpea seeds decreased. Overall, the cooked texture values of micronized cowpeas were lower than that of unmicronized cowpeas after the 3 h of cooking. This can be related to the fact that micronization improved the solubility of pectins in the middle lamella of both normal and HTC cowpeas (Table 3.2.2) probably via breakage of pectins molecules into lower and more soluble fractions (Liu *et al.* 1993b), allowing water penetration and cotyledons softening during cooking. The cooked texture of normal cowpeas pre-conditioned in water and micronized did not differ significantly ($P>0.05$) from that of normal cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized during the time of cooking.

However, the cooked texture of HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized was significantly lower than that of HTC subjected to pre-conditioning in water and micronized from 90 min of cooking.

A significant ($P\leq0.05$) negative correlation (r = -0.98) (Table 3.2.3) was found between the degree of splitting and cooked texture in both normal and HTC cowpeas. In terms of pectin solubility, it is possible that the same mechanism is involved in cowpea seeds softening and splitting during the cooking process. Pectin solubility could in part have influenced the texture and the degree of splitting of cowpea seeds during cooking.

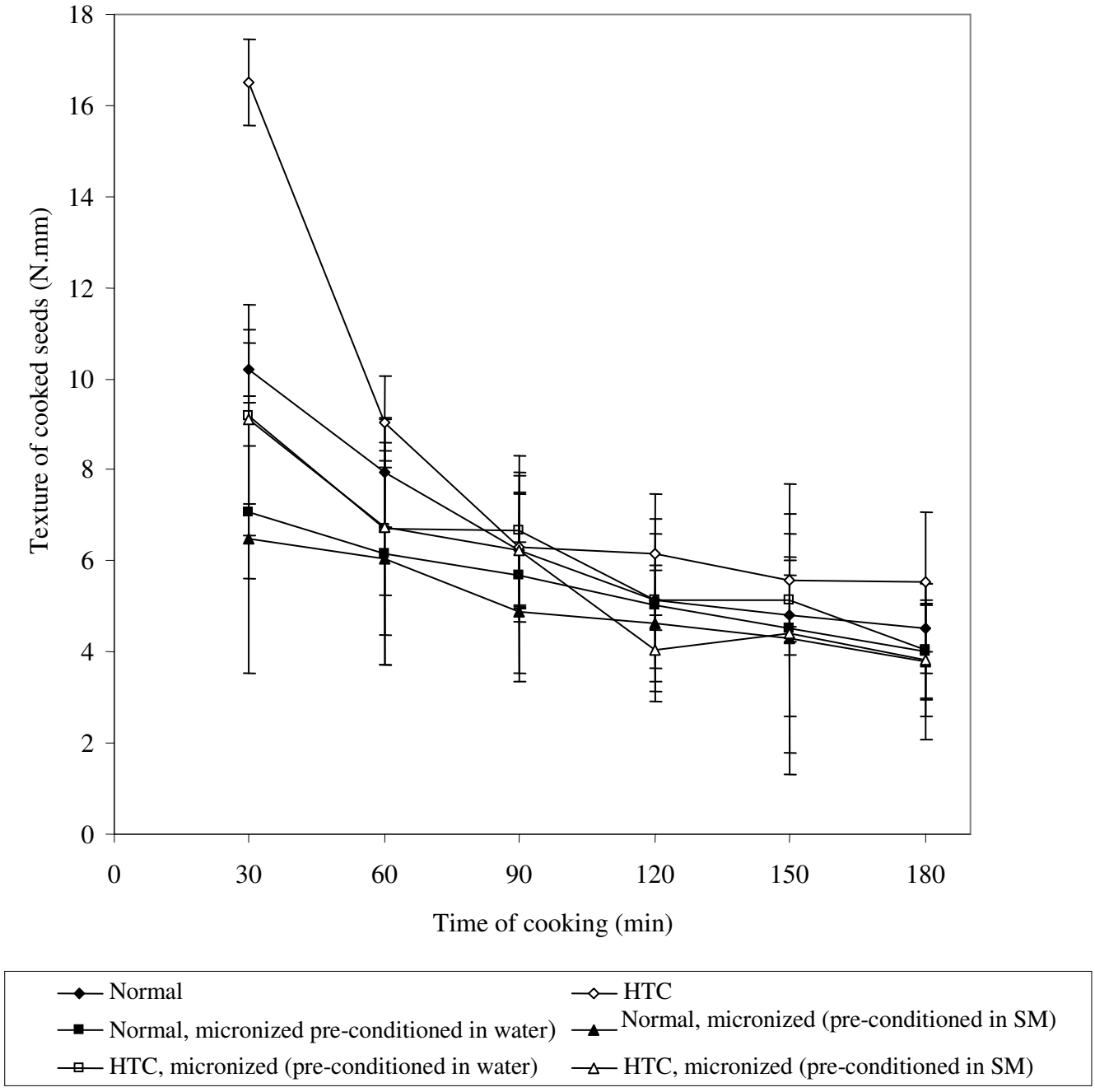

Figure 3.2.1. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on the texture of cooked cowpea seeds during 3 h of cooking (Vertical bars indicate the standard deviations of means; HTC: hard-to-cook)

The variables, state of cowpeas and micronization showed significant (*P<0.001*) interaction in terms of the degree of splitting of cowpeas as the effect of micronization on the degree of splitting depended not only on the pre-conditioning solution used but also on the state of cowpeas. Pre-conditioning cowpeas in either water or in a solution

containing monovalent (Na^+) cations in combination with micronization increased the degree of splitting of normal and HTC cowpeas when compared with control samples (Table 3.2.5).

Table 3.2.5. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent (Na^+) cations on the degree of splitting of normal and HTC cowpeas

Treatments	Degree of splitting (%)	
	Normal	HTC
Control	41.9 ± 2.9 b	5.5 ± 2.8 a
Micronized after pre-conditioning in H_2O	71.2 ± 2.6 e	56.1 ± 1.3 c
Micronized after pre-conditioning in monovalent (Na^+) cations	73.3 ± 2.9 e	61.4 ± 3.4 d

Means followed by the same letter in cell are not significantly different at level *P>0.05*

In normal cowpeas, pre-conditioning in water or in a solution with monovalent (Na^+) cations, both combined with micronization had the same effect in terms of reducing the cooking time and the texture and increasing the degree of splitting. It suggests that the degree of splitting, cooked texture and cooking time of normal cowpeas are affected by the same factors in normal cowpeas. The higher the pectin solubility in normal cowpeas, the higher the tendency to become softer, split and cook faster. These results can be taken as a further support for the involvement of the pectins in the insolubilization of the middle lamella of cowpeas. Pectin solubility may have affected the texture and degree of splitting of cooked normal cowpeas, which consequently affected the cooking time. These findings are clearly supported by the significant ($P \leq 0.05$) positive correlations between pectin solubility and degree of splitting (r = +0.89), soluble pectin and cooked texture (r = -0.73), pectin solubility and cooking time (r = -0.75) (Table 3.2.3). The breakdown of the middle lamella, leading to an easy separation of cells, has been reported to contribute to the softening of pulses during cooking (Sefa-Dedeh *et al.*, 1978).

However, in HTC cowpeas pre-conditioning in water followed by micronization had significantly lower effects in terms of reducing the cooking time (Table 3.2.1) and the

texture of cooked seeds (Table 3.2.4) and in terms of increasing the degree of splitting when compared with pre-conditioning in monovalent (Na^+) cations followed by micronization (Table 3.2.5). This phenomenon could be due to the fact that in the middle lamella of HTC cowpeas, more divalent cations (Ca^{2+}/Mg^{2+}), released from hydrolysis of phytate during storage at adverse conditions (Aguilera & Rivera, 1992; Hentges *et al.*, 1991) are available. The presence of more Ca^{2+} and Mg^{2+} released from phytate in the middle lamella could possibly have facilitated the action of monovalent (Na^+) cations in terms of exchanging with Ca^{2+} and Mg^{2+} associated with pectinates.

The development of cracks in the testa and cotyledon of cowpeas during micronization (Mwangwela *et al.,* 2006) probably increased the degree of splitting for both normal and HTC micronized cowpeas. The rapid temperature increase during micronization probably causes liquid water to turn into steam (gas). If the seed is able to hold the expanding gas, then pressure builds up and the cotyledon collapses under the pressure or explodes to release the pressure, resulting in modified cotyledon cells (Mwangwela *et al.,* 2006).

A significant positive correlation (r = +0.89) (Table 3.2.3) was found between the degree of splitting and soluble pectin in treated cowpeas. As pectins are known to contribute to the maintenance of structural integrity of the cell wall (Uzogara *et al.,* 1990), it is possible that the higher degree of pectin solubility as influenced by micronization could have contributed to the higher degree of splitting of these cowpeas during cooking.

The variables, state of cowpeas (normal or HTC), pre-conditioning in water or in a solution with monovalent (Na^+) cations and micronization interacted significantly in terms of phytase activity ($P<0.001$) (Table 3.2.6) and phytic acid ($P<0.05$) (Table 3.2.7) of cowpeas. Therefore, the effect of pre-conditioning alone on the phytase activity and phytic acid content depended on the state of cowpeas, while the effect of micronization depended on the state of cowpeas and on the pre-conditioning solution used.

Table 3.2.6. The effect of pre-conditioning alone and in combination with micronization on the phytase activity of normal and HTC cowpeas

Treatments	Phytase activity (units.kg^{-1}) as is		Phytase activity (units.kg^{-1}) (dry basis)	
	Normal	HTC	Normal	HTC
Control	91.1 ± 4.8 c	104.2 ± 1.8 d	103.4 ± 1.3 d	120.9 ± 0.8 e
Pre-conditioned in H_2O alone	93.6 ± 3.4 c	96.5 ± 4.9 c	a	a
Pre-conditioned in monovalent (Na^+) cations alone	90.1 ± 2.9 c	91.8 ± 2.6 c	a	a
Micronized after pre-conditioning in H_2O	47.0 ± 3.2 a	46.0 ± 2.9 a	54.3 ± 1.0 a	56.9 ± 1.7 b
Micronized after pre-conditioning in monovalent (Na^+) cations	46.0 ± 0.7 a	53.4 ± 16.7 ab	55.4 ± 1.0 a	67.8 ± 0.6 c

Means followed by the same letter in cell are not significantly different at level *P>0.05*

a Moisture content for these samples was not determined

Table 3.2.7. The effect of pre-conditioning alone and in combination with micronization on the phytic acid content of normal and HTC cowpeas

Treatments	Phytic acid ($mg.g^{-1}$) as it is		Phytic acid ($mg.g^{-1}$) on dry basis	
	Normal	**HTC**	**Normal**	**HTC**
Control	1.95 ± 0.13 e	1.49 ± 0.08 c	2.21 ± 0.14 c	1.73 ± 0.02 b
Pre-conditioned in H_2O alone	1.65 ± 0.13 d	1.38 ± 0.04 ab	a	a
Pre-conditioned in monovalent (Na^+) cations alone	1.44 ± 0.07 bc	1.41 ± 0.07 bc	a	a
Micronized after pre-conditioning in H_2O	1.40 ± 0.06 ab	1.38 ± 0.02 ab	1.62 ± 0.06 a	1.71 ± 0.10 b
Micronized after pre-conditioning in monovalent (Na^+) cations	1.29 ± 0.08 a	1.30 ± 0.02 a	1.56 ± 0.24 a	1.65 ± 0.06 ab

Means followed by the same letter in cell are not significantly different at level *P>0.05*

a Moisture content for these samples was not determined

The phytase activity found in normal cowpeas was within the range reported in the literature: 82.3 units.kg^{-1} (Affrifah *et al.,* 2005), 100.87 to 102.06 units.kg^{-1} (Affrifah *et al.*, 2006) and 115.3 units.kg^{-1} (Affrifah & Chinnan, 2005) for normal cowpeas. At adverse conditions (42ºC and 67% RH), phytase is activated as shown by the significantly increased activity of phytase in HTC cowpeas (Table 3.2.6). This could have hydrolyzed phytic acid as indicated by the decrease in phytic acid content in HTC cowpeas compared with normal cowpeas (Table 3.2.7). Phytase hydrolyzes the phosphate residues from phytic acid, thereby essentially destroying its affinity for minerals (Fennema, 1985), such as Ca^{2+} and Mg^{2+}. The 16% increase in the phytase activity (from 115.3 to 133.9 units.kg^{-1}) when cowpeas were stored at 42ºC and 80% RH for five weeks, in the study done by Affrifah & Chinnan (2005), was close to 17% (based on dry basis data) increase observed in this study. The values of phytic acid found in normal and HTC cowpeas (Table 3.2.8) are in accordance with those reported by Giami (2005) but only in normal cowpeas, which ranged from 1.15 to 2.10 mg.g^{-1}.

The decreased phytic acid content of HTC cowpeas was also reported by Hentges *et al.* (1991). This decrease can be attributed to the hydrolysis of phytic acid by phytase, which is activated under storage at adverse conditions (Liu, 1995). Hydrolysed phytic acid could have released divalent cations (Ca^{2+} and Mg^{2+}) which could have migrated to the middle lamella binding with pectins, forming an insoluble barrier to water penetration during soaking and cooking (Hentges *et al.,* 1991; Aguilera & Rivera, 1992). To support this, a significant ($P \leq 0.05$) negative correlation (r = -0.48) was found between phytic acid and phytase activity and a significant negative correlation between phytic acid and soluble pectin (r = -0.51) (Table 3.2.3).

Phytase activity of normal cowpeas was not affected by pre-conditioning (Table 3.2.6) probably because the enzyme phytase in cowpeas is thermostable and water only promoted the unfolding of proteins (Affrifah *et al.*, 2005) exposing the phytic acid. This suggests that phytase activity did not contribute to the significant reduction in phytic acid content (Table 3.2.7) observed in normal cowpeas when subjected to pre-conditioning. Similar results were found by Lestienne, Mouquet-Rivier, Icard-Verniére, Rochette & Tréche (2005) who found nearly no phytase degradation (1%) after 24 h of soaking.

In HTC cowpeas, pre-conditioning in water or in a solution with monovalent (Na^+) cations reduced the phytase activity significantly to the same extent as normal cowpeas (Table 3.2.6). Phytase activity during pre-conditioning probably plays a role in the reduction of phytic acid content (Arntfield *et al.,* 2001) of HTC cowpeas. Phytase is known to be responsible for catalyzing the hydrolysis of phytate (Affrifah *et al.* 2006; Hentges *et al.* 1991). Water promotes the unfolding of cowpea proteins (Affrifah *et al.*, 2005), thereby exposing the phytic acid. Because the phytic acid was probably exposed when cowpeas were subjected to pre-conditioning and the phytase in HTC cowpeas was activated (Table 3.2.6), pre-conditioning could have promoted hydrolyses of the phytic acid (Fennema, 1985), reducing the activity of the enzyme in HTC cowpeas.

Pre-conditioning in a solution with monovalent (Na^+) cations was more effective in reducing the phytic acid content in normal cowpeas as compared with pre-conditioning in water. This reduction in phytic acid content may have been a function of leaching Ca^{2+} and Mg^{2+} associated with phytate into water. The leaching of Ca^{2+} and Mg^{2+} into water is influenced by the concentration gradient, which governs the rate of diffusion (Sinha & Kawatra, 2003; Elmaki, Abdelrahaman, Idris, Hassan, Abiker & Tinay, 2006). But in HTC cowpeas, pre-conditioning in a solution with monovalent (Na^+) cations had a similar effect than pre-conditioning in water in terms of reducing the phytic acid. This is probably because the phytic acid in HTC cowpeas was strongly bound to divalent cations as compared to normal cowpeas. The effect of pre-conditioning in water on the phytic acid content of cowpeas is in agreement with the results obtained by Sinha & Kawatra (2003) and Elmaki *et al.* (2006), although their experiments consisted of soaking and not pre-conditioning cowpeas.

Pre-conditioning in water and pre-conditioning in monovalent (Na^+) cations did not differ in terms of reducing the phytase activity of cowpeas (Table 3.2.6). The same applies when the two treatments were combined with micronization based on wet basis data (Table 3.2.6). But the results on dry basis showed that pre-conditioning in a solution with monovalent (Na^+) cations followed by micronization was less effective in reducing the phytase activity than pre-conditioning in water followed by micronization for HTC cowpeas. These findings support the premises that the presence of monovalent (Na^+) cations in the pre-conditioning solution did not have a significant impact on the activity of phytase. Affrifah *et al.* (2005) found that the reduction in phytase activity of cowpeas when

heat-moisture treatments were applied ranged from 20 to 56% for samples with 25% and 35% moisture contents when heated at 95ºC. The thermal inactivation of phytase in cowpeas is highly dependent on heating temperature and moisture content (Affrifah *et al.,* 2005; Affrifah *et al.* 2006). The significant reduction in phytase activity of normal and HTC cowpeas when micronization was applied can probably be related to the higher final moisture contents of micronized cowpeas (Table 3.2.8) when compared with control cowpeas in combination with the high temperatures during micronization. Water is necessary to promote the unfolding of proteins during thermal inactivation of phytase (Affrifah *et al.,* 2005). The phytase activity of HTC cowpeas which had significantly higher final moisture content than that of normal cowpeas after micronization was about 47% after micronization, while in normal cowpeas it was about 52% after micronization compared with initial phytase activity.

Table 3.2.8. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent (Na^+) cations on the moisture content of normal and HTC cowpeas

Treatments	Moisture content (%)	
	Normal	HTC
Control	11.9 ± 0.1 a	13.8 ± 0.1 b
Micronized after pre-conditioning in H_2O)	13.5 ± 0.1 b	19.2 ± 0.6 d
Micronized after pre-conditioning in monovalent (Na^+) cations	17.1 ± 0.2 c	21.2 ± 0.2 e

Means followed by the same letter in cell are not significantly different at level *P>0.05*

There was a significant (*P<0.001*) interaction between the variables, state of cowpeas (normal or HTC) and micronization on the final moisture content of cowpeas. Generally HTC cowpeas have significantly higher moisture contents as compared with normal cowpeas (Table 3.2.8). This can be attributed to the higher relative humidity of the storage environment (Sefa-Dedeh *et al.,* 1978), which was 67% as compared with the 24% relative humidity of the environment. Micronized cowpeas, regardless of the type of the pre-conditioning solution employed, had significantly ($P \leq 0.05$) higher moisture contents than their unmicronized counterparts. Pre-conditioning in water was found to increase the moisture content of lentils (Arntfield *et al.,* 1997) and cowpeas (Mwangwela *et al.* 2006; Phadi, 2004) after micronization. Pre-conditioning in monovalent (Na^+) cations had a

greater effect than pre-conditioning in water in increasing the moisture content of micronized normal and HTC cowpeas. This can be attributed to increased water holding capacity provided by saline solutions (Garcia-Vela & Stanley, 1989).

Pre-conditioning in water or in a solution with monovalent (Na^+) cations combined with micronization had a similar effect in reducing the phytic acid content of normal and HTC cowpeas (Table 3.2.8). These results are in agreement with those obtained by Arntfiled *et al.* (2001) who found a significant reduction of the phytic acid content when normal lentils were micronized to final surface temperatures of 138ºC and 170ºC. A reduction of phytic acid content during cooking was reported in legumes (Vijayakumari, Siddhuraju & Janardhanan, 1997; Sinha & Kawatra, 2003) and it was attributed to formation of insoluble complexes between phytate and other components, to form phytate-protein and phytate-protein-mineral complexes. It is possible that micronization temperatures induced formation of insoluble complexes between phytate and proteins and with minerals and, accordingly, the amount of free phytic acid was reduced.

3.2.4.2. Effect of micronization after pre-conditioning cowpeas in a solution containing monovalent cations on the water absorbed during and after soaking and cooking, hydration capacity and leached solids

The effect of micronization on the water uptake was not only affected by the pre-conditioning process but also by the state of the cowpea seeds. This is reflected by the significant ($P<0.001$) state of cowpeas (normal or HTC) * micronization interaction that was found in terms of water absorbed after 6 h of soaking. HTC cowpeas absorbed significantly ($P\leq0.05$) less water than normal cowpeas (Table 3.2.9). This could be related to lower pectin solubility observed in HTC cowpeas (Table 3.2.2). This is supported by the significant positive correlation (r = +0.82) between soluble pectin and water absorption during soaking (Table 3.2.3). Less pectin soluble in soaking water or solution can lead to a slower rate of water uptake during soaking as pectin is known to play an important role in water absorption (Sefa-Dedeh & Stanley, 1979).

Micronized normal and HTC cowpeas regardless of the pre-conditioning solution used, absorbed less water than their control counterparts after 6 h of soaking (Table 3.2.9). This is in agreement with Mwangwela *et al.* (2006) who found unmicronized cowpeas absorbing much more water than micronized seeds.

Table 3.2.9. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on water uptake after 6 h of soaking of normal and HTC cowpeas

Treatments	Water uptake after 6 h of soaking (g H_2O. kg^{-1} seed)	
	Normal	HTC
Control	1154 ± 18 e	1060 ± 8 d
Micronized after pre-conditioning in H_2O	913 ± 25 c	741 ± 37 a
Micronized after pre-conditioning in monovalent (Na^+) cations	844 ± 13 b	723 ± 14 a

Means followed by the same letter in cell are not significantly different at level *P>0.05*

Cowpea protein contributes to water imbibition during soaking of cowpeas (Sefa-Dedeh & Stanley, 1979). Micronization probably reduced protein hydrophilicity of legume proteins owing to unfolding of protein molecules, thereby exposing hydrophobic sites (Zheng, Fasina, Sosulski & Tyler, 1998). This could have played a role in reducing the amount of water absorbed by micronized cowpeas after 6 h of soaking.

Normal cowpeas pre-conditioned in a solution containing monovalent (Na^+) cations and micronized absorbed significantly (*P≤0.05*) less water than those pre-conditioned in water and micronized (Table 3.2.9). A significant (*P≤0.05*) negative correlation was found between moisture content and water absorption after 6 h of soaking (r = -0.85) (Table 3.2.3). Normal cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized had higher final moisture content as compared to those pre-conditioned in water and micronized (Table 3.2.8). This could have contributed to less water absorbed by normal cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized during soaking. However, pre-conditioning HTC cowpeas in water or in a solution with monovalent (Na^+) cations in combination with micronization did not result in different amounts of water absorbed by cowpeas after 6 h of soaking. The seed coat and hilum size play a major role in water absorption during the first stages of soaking. At later stages protein becomes the most important component (Sefa-Dedeh & Stanley, 1979). The protein of HTC cowpeas can be strongly bound to phenolic compounds, e.g. tannins (Stanley, 1992) and denatured (Liu *et al.* 1992a) as a result of pH decrease during adverse

storage. Proteins are the major water absorption components in cowpeas (Sefa-Dedeh & Stanley, 1979). The effect of monovalent (Na^+) cations on water absorption as related to proteins of HTC cowpeas still need to be investigated. Figure 3.2.2 shows the effects of pre-conditioning cowpeas in water or in a solution with monovalent (Na^+) cations followed by micronization on the rate of water absorption during 6 h of soaking.

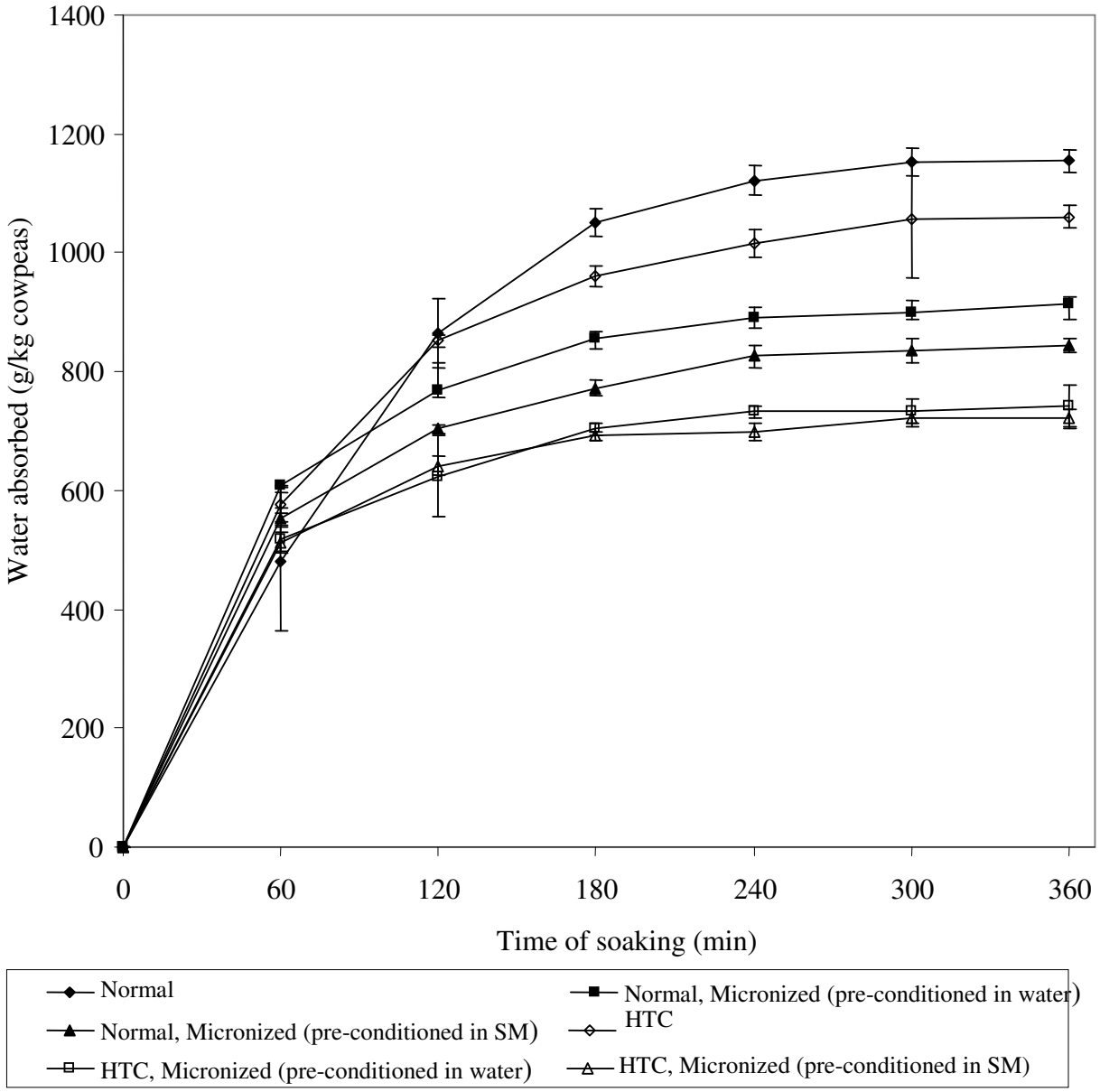

Figure 3.2.2. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on the rate of water absorption during 6 h of soaking (Vertical bars indicate the standard deviations of means; HTC: hard-to-cook)

During the first hour of soaking, unmicronized HTC cowpeas absorbed as much water as their normal counterparts. From 120 min of soaking, normal cowpeas absorbed significantly more water than the HTC cowpea seeds. Overall, micronized HTC cowpeas absorbed less water than micronized normal cowpeas during the 6 h of soaking (Fig. 3.2.1). These findings are in agreement with Garcia-Vela & Stanley (1989) who found beans stored at 80% RH and 30ºC having lower water-holding capacity as compared with normal beans. The low pH of HTC cowpeas (6.3) could have contributed to lower protein solubility (Garcia-Vela & Stanley, 1989), resulting in reduced water absorbed.

From 120 min of soaking, normal cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized absorbed less water than those pre-conditioned in water and micronized. It was reported earlier that cowpeas pre-conditioned in a solution with monovalent (Na^+) cations had significantly higher moisture contents than those pre-conditioned in water. It is possible that the less amount of water absorbed by cowpeas pre-conditioned in a solution with monovalent (Na^+) cations is related to their higher moisture content gained during the pre-conditioning process. Although the amount of water absorbed by HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized was slightly lower than that absorbed by HTC cowpeas pre-conditioned in water and micronized during the 6 h of soaking, there were no significant ($P>0.05$) differences. Another possible factor that could have contributed to lower water absorbed by cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized include the higher viscosities and lower water activities associated with relatively higher concentrations of monovalent (Na^+) cations as compared to water (Hsu *et al.*, 1983).

Table 3.2.10 shows the effect of pre-conditioning cowpeas in water or in a solution containing monovalent (Na^+) cations followed by micronization on the hydration capacity of cowpeas. The variables, state of cowpeas (normal or HTC) and micronization did not interact significantly ($P=0.418$) in terms of hydration capacity of cowpeas. It means that the action of pre-conditioning cowpeas in water or in a solution containing monovalent (Na^+) cations followed by micronization on hydration capacity was similar in both normal and HTC cowpeas.

Table 3.2.10. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on hydration capacity after 18 h of soaking of normal and HTC cowpeas

Treatments	Hydration capacity after 18 h of soaking (g H_2O. kg^{-1} seed)[1]		Micronization Effect[2]
	Normal	HTC	
Control	1121 ± 6 e	1334 ± 4 f	1227 ± 122 c
Micronized after pre-conditioning in H_2O	889 ± 14 b	1065 ± 16 d	977 ± 93 b
Micronized after pre-conditioning in monovalent (Na^+) cations	806 ± 26 c	1018 ± 9 c	912 ± 72 a
HTC effect[3]	939 ± 60 b	1139 ± 48 a	

[1]Means followed by the same letter in cell are not significantly different at level *P>0.05*

[2]Means followed by the same letter in the column are not significantly different at level *P>0.05*

[3]Means followed by the same letter in a row are not significantly different at level *P>0.05*

Hydration capacity in this study is defined as the amount of water absorbed by 10 g of cowpeas after 18 h of soaking (Wang *et al.,* 2003). Contrary to water absorption after 6 h of soaking, the hydration capacity significantly increased when the cowpeas were stored at adverse conditions (Table 3.2.10). Hentges *et al.* (1991) and Jackson & Varriano-Marston (1981) demonstrated that water absorption after 18 h of soaking (corrected for solids loss) was higher in HTC beans than in normal beans. It is possible that during the 18 h of the imbibition of cowpeas with water, the seed coat was pulled away from the cotyledon at the palisade layer allowing a layer of bulk water to enter between the seed coat and cotyledons (Plhak *et al.,* 1989).

As with the water absorbed after 6 h of soaking, hydration capacity of cowpeas was found to reduce when cowpeas were pre-conditioned in water and micronization (Table 3.2.10). The reduction was even further when cowpeas were pre-conditioned in a solution with monovalent (Na^+) cations and micronized. Reduced protein hydrophilicity of legume proteins owing to unfolding of protein molecules, thereby exposing hydrophobic sites (Zheng *et al.*, 1998) as a result of protein denaturation (Mwangwela, 2006) could have played a role in reducing the amount of water absorbed by micronized cowpeas. The higher moisture content of cowpeas pre-conditioned in water and micronized as compared to those pre-conditioned in a solution with monovalent (Na^+) cations and micronized

(Table 3.2.8) could also have influenced the reduction of water absorption after 18 h of soaking. This is supported by the significant negative correlation (r = -0.85) between moisture content and water absorbed after soaking.

The variables, state of cowpeas (normal or HTC) and micronization showed a significant (*P<0.001*) interaction in terms of water absorbed by cowpeas after 3 h of cooking. This means that the water absorbed by cowpeas after 3 h of cooking depended on the state and application of micronization (combined with pre-conditioning in water or in a solution with monovalent (Na^+) cations). HTC cowpeas absorbed less water during 3 h of cooking than normal cowpeas (Table 3.2.11). This can probably be related to the lower pectin solubility observed in HTC cowpeas as compared with normal cowpeas (Table 3.2.2).

Table 3.2.11. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on water uptake after 3 h of cooking of normal and HTC cowpeas

Treatments	Water uptake after 3 h of cooking (g H_2O. kg^{-1} seed)	
	Normal	HTC
Control	1287 ± 19 b	1217 ± 20 c
Micronized after pre-conditioning in H_2O	1396 ± 37 d	1132 ± 21 a
Micronized after pre-conditioning in monovalent (Na^+) cations	1307 ± 21 b	1122 ± 22 a

Means followed by the same letter in cell are not significantly different at level *P>0.05*

Another parameter that could have influenced water absorption after 3 h of cooking is the degree of splitting. A significant positive correlation (r = +0.61) (Table 3.2.3) was found between water absorption during cooking and the degree of splitting of treated cowpeas. Micronization was found to produce some clear folds and fracture lines in lentils (Arntfield *et al.,* 2001) and in cowpeas seed coat and cotyledons (Mwangwela *et al.,* 2006; Phadi, 2004), which probably improved the degree of splitting and consequently water uptake during cooking of cowpeas.

Normal cowpeas pre-conditioned in water and micronized absorbed much more water than control cowpeas (Table 3.2.11). However, normal cowpeas pre-conditioned in a solution

with monovalent (Na^+) cations and micronized absorbed as much water as unmicronized normal cowpeas after 3 h of cooking (Table 3.2.11). However, when HTC cowpeas were pre-conditioned in water and micronized, the water absorbed reduced significantly and similarly to when pre-conditioned in a solution with monovalent (Na^+) cations and micronized. The higher final moisture content of normal and HTC cowpeas subjected to the latter treatment (Table 3.2.6) could have limited water absorbed during 3 h of cooking. Other factors such as protein denaturation and solubility, cellulose and starch could have played a major role in the reduction in the amount of water absorbed by HTC cowpeas.

The improvement in pectin solubility of HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized, did not have a direct influence on the water absorbed by HTC cowpeas when compared with HTC cowpeas pre-conditioned in water and micronized. This supports the hypotheses that there may be other factors rather pectin solubility affecting the cooking process of HTC cowpeas. No significant correlation was observed between soluble pectin and water absorbed after 3 h of cooking.

A significant (*P<0.001*) state (normal and HTC) * micronization interaction was found in terms of solids leached into the cooking water. Micronization of cowpeas regardless of pre-conditioning solution used increased leached solids in normal and HTC cowpeas (Table 3.2.12).

Table 3.2.12. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on leached solids of normal and HTC cowpeas

Treatments	Leached solids ($g.ml^{-1}$ cooking water)	
	Normal	HTC
Control	2.71 ± 0.04 a	3.03 ± 0.14 b
Micronized after pre-conditioning in H_2O	3.16 ± 0.08 b	4.57 ± 0.21 c
Micronized after pre-conditioning in (Na^+) cations	3.03 ± 0.08 b	4.27 ± 0.79 c

Means followed by the same letter are not significantly different at level *P>0.05*

Increased leakage during cooking of legume seeds has been used as an indication of seed degradation. Hentges *et al.* (1991) and Jackson & Varriano-Marston (1981) found HTC legume seeds losing more solids into the soaking water than their normal counterparts. Valle *et al.* (1992) found that leached solids were generally higher for HTC beans than control beans during the early stages of cooking. This could be a result of membranes deterioration caused by lipid peroxidation, which leads to destruction of the lipids, proteins and other cellular components (Liu, 1995).

Leached solids of micronized legume seeds have been found to be higher than that of unmicronized legume seeds (Fasina *et al.*, 2001). This could be due to the cracks formed in micronized cowpeas, which could have facilitated the migration of solids out of the seed and leached into the cooking water (Fasina *et al.,* 2001). To support this, a very low but significant positive correlation (r = +0.39) (Table 3.2.3) was found between leached solids and the degree of splitting of treated cowpeas. Normal and HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized had lost as much solids in the cooking water as those pre-conditioned in water and micronized. When cowpeas were pre-conditioned in a solution with monovalent (Na^+) cations and micronized, the amount of water absorbed during cooking tended to decrease when compared to that of cowpeas pre-conditioned in water and micronized (Table 3.2.11). This decrease in the amount of water absorbed can be a result of the increased water holding capacity as a result of the presence of monovalent (Na^+) cations and could have contributed to less splitting during cooking and consequently, fewer solids leached into the cooking water.

Figure 3.2.3 shows the effects of pre-conditioning in water or in a solution with monovalent (Na^+) cations followed by micronization on the rate of water uptake during 3 h of cooking. During the first 30 min of cooking, there was a rapid increase in the amount of water absorbed by cowpeas. The rapid increase in the water absorption has been attributed to the action of pressurized gases trapped in the interior of the beans (Phlak *et al.*, 1989) and the filling of capillaries on the surface of the seed coat and the hilum (Hsu *et al.*, 1983). After 30 min of cooking, normal cowpeas pre-conditioned in water and micronized absorbed much water than those pre-conditioned in a solution with monovalent (Na^+) cations and micronized and than their control counterparts. But HTC cowpeas pre-conditioned in water absorbed as much water as those pre-conditioned in a solution with

monovalent (Na^+) cations and micronized, but less than that absorbed by control HTC cowpeas pre-conditioned in water and micronization. Generally, micronized cowpeas reduced the amount of water absorbed during cooking probably due protein denaturation (Mwangwela, 2006).

From 30 to 180 min of cooking, normal cowpeas pre-conditioned in water and micronized absorbed significantly ($P \leq 0.05$) more water than those pre-conditioned in a solution with monovalent (Na^+) cations and micronized and control normal cowpeas (Fig. 3.2.3). From 120 to 180 min of cooking, normal cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized absorbed as much water as control normal cowpeas.

From 120 min of cooking onwards, control normal cowpeas absorbed as much water as normal cowpeas pre-conditioned in water and micronized or pre-conditioned in a solution with monovalent (Na^+) cations and micronized. Generally, micronization improved the water absorption properties of normal cowpeas probably due to improvement of pectin solubility induced by micronization (Arntfield *et al.,* 1997) and the occurrence of fissures in the seed coat and cotyledons of micronized samples (Arntfield *et al.,* 2001; Mwangwela, 2006).

The amount of water absorbed by control HTC cowpeas tended to be constant between 90 and 150 min of cooking, but increased significantly after 180 min of cooking. From 90 to 180 min of cooking, the amount of water absorbed by micronized HTC cowpeas, independent of the pre-conditioning solution used, did not change significantly ($P > 0.05$). From 60 min of cooking, HTC pre-conditioned in a solution with monovalent (Na^+) cations and micronized absorbed significantly less water than unmicronized HTC cowpeas and HTC cowpeas pre-conditioned in water and micronized. The initial higher moisture content of HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized (Table 3.2.6) as compared to HTC pre-conditioned in water and micronized and control HTC cowpeas, could be associated with their lower rate of water absorption observed during cooking (Fig 3.2.3).

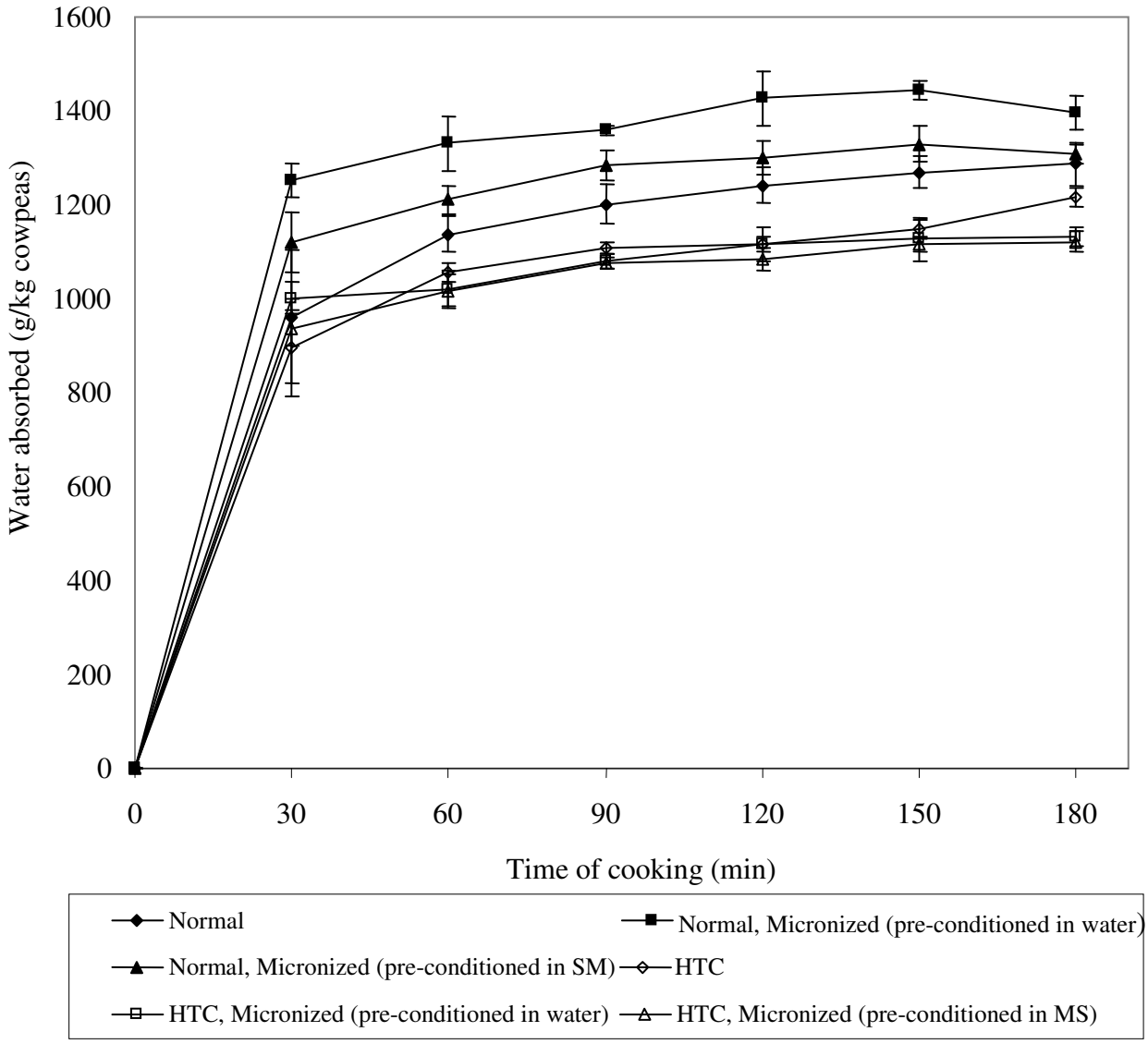

Figure 3.2.3. The effect of micronization after pre-conditioning cowpeas in solutions containing either water or monovalent cations (Na^+) on the rate of water absorption during 3 h of cooking (Vertical bars indicate the standard deviations of means; HTC: hard-to-cook; MS: solution containing monovalent (Na^+) cations)

3.2.4. CONCLUSIONS

From a practical point of view, pre-conditioning in water is effective in reducing the cooking time of normal cowpeas. For HTC cowpeas, a combination of micronization and pre-conditioning in a solution with monovalent (Na^+) cations is the most effective technique in reducing the cooking time. The reduction in cooking time for normal and HTC cowpeas is correlated with improvement in pectin solubilization. Pre-conditioning normal cowpeas in water induces pectin solubilization. Pre-conditioning in a solution with monovalent (Na^+) cations in combination with micronization reduces the cooking time of HTC cowpeas probably not only by the solubilization effect of water but also by a conversion of insoluble to soluble pectins due to the action of monovalent (Na^+) cations. Pre-conditioning does not change the phytase activity of normal cowpeas, but changed that of HTC cowpeas probably because the phytase was activated in HTC cowpeas and its action was further promoted by exposure of phytic acid by pre-conditioning. Generally, pre-conditioning reduces the phytic acid content probably due to its leaching into the pre-conditioning water. In addition to the action of monovalent (Na^+) cations, micronization could have improved the pectin solubilization further by inducing the breakage of pectins molecules into lower and more soluble fractions, probably via the β-elimination reaction. This coincides with an increase in the degree of splitting, decrease in the texture of cooked seeds and decrease in water absorbed during 6 and 18 h of soaking for both normal and HTC cowpeas. Micronization inactivates some phytase and reduces the phytic acid probably due to formation of insoluble complexes with proteins and minerals. Although all treatments improve the solubilization of pectins of HTC cowpeas, this is not the case for the reduction in cooking time. This suggests that there may be factors (e.g. proteins, starch, cellulose) other than pectin solubility involved in the reduction of cooking time of HTC.

4. GENERAL DISCUSSION

The chapter is divided into three sections. The first section deals with the appropriateness of the experimental design and methodologies used during the research. The second section proposes a model of the possible physiochemical changes taking place during the development of the HTC defect in legume seeds, which was induced by storing cowpeas at 42ºC and 67% RH for 21 days. The last section examines the changes in the physicochemical and cooking characteristics when cowpeas are pre-conditioned in a solution containing monovalent (Na^+) cations and then micronized (to a final surface temperature of 153ºC).

4.1. Critical review of experimental design and methodology

The main purpose of this study was to investigate the effect of pre-conditioning cowpeas in a solution containing monovalent (Na^+) cations followed by micronization on cooking characteristics of normal and HTC cowpeas as they relate to other physicochemical characteristics. The experiments were designed in such a way that assays were performed at three different steps during processing (Fig. 4.1.1).

This design helped to understand the physicochemical changes brought about pre-conditioning alone as a variable (step II), and by combining it with micronization (step III) as compared with untreated normal and HTC cowpeas (step I). All the assays were performed in steps I and III. At step II, only phytase activity, pectin solubility, phytate and cooking time assays were performed. As a result, the effect of pre-conditioning alone as a variable, on texture of cooked seeds, hydration properties, splitting, leached solids and moisture could not be explained. Therefore, it was difficult to explain whether the changes observed in some physicochemical characteristics of cowpeas, when pre-conditioning was combined with micronization, were due to micronization or pre-conditioning, or even a combination of the two variables. Although the main purpose of the study focused on the combined effect of pre-conditioning cowpeas in a solution containing monovalent (Na^+) cations and micronization, the fact that some determinations were not done at step II can be regarded as a weakness of this experimental design.

First, the HTC defect was induced in cowpeas by storing them at high temperature (42ºC) and high relative humidity (67%). The term “hard-to-cook” refers to a texture-related defect observed in legume seeds where extra cooking time is required to achieve the

proper tenderness. Legume cookability is a function of storage conditions, time, species, cultivars and agronomic conditions and some of these factors can interact with each other (Liu, 1995).

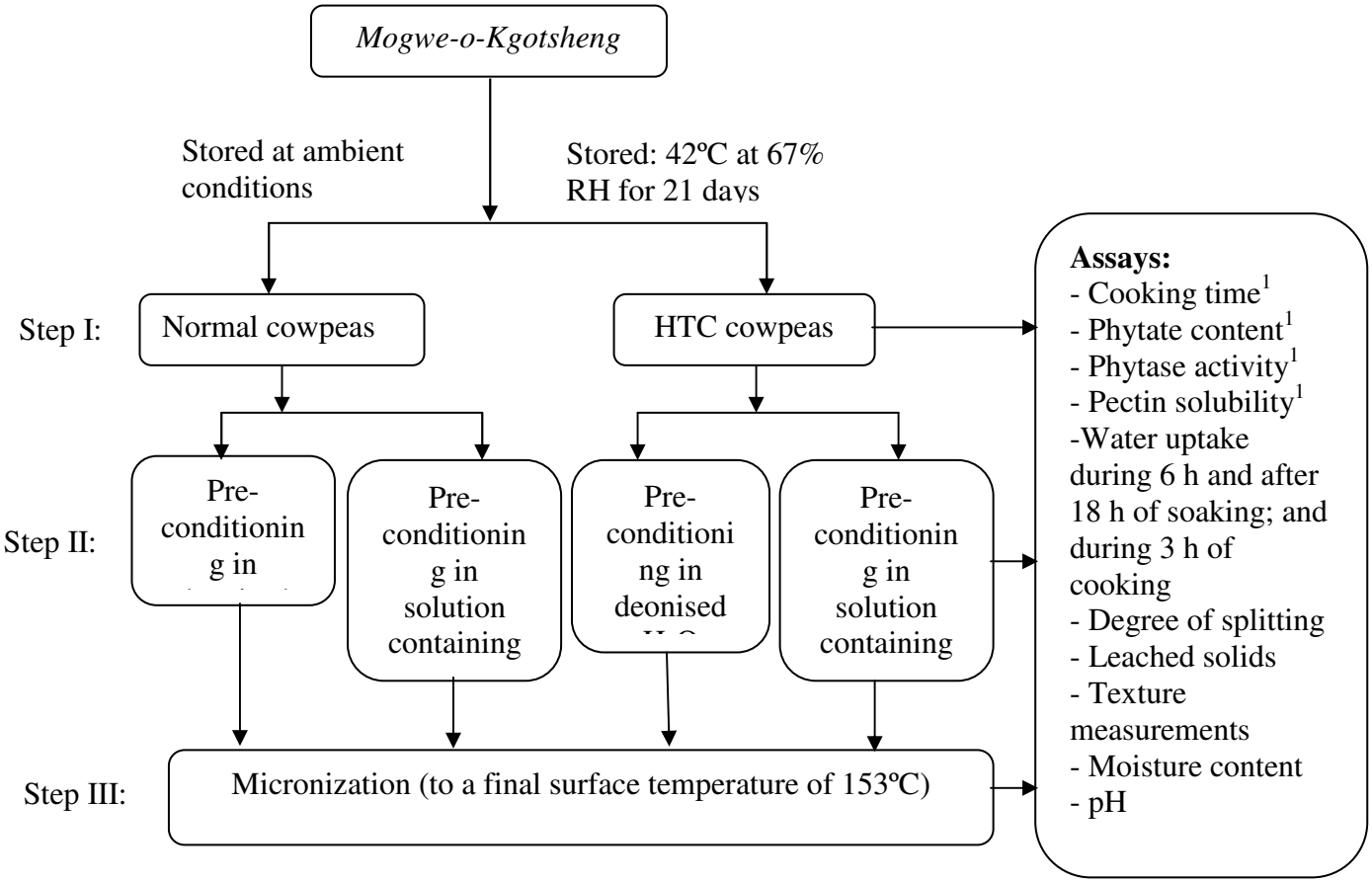

Figure 4.1.1. Experimental design for different assays at different steps

[1]Assays conducted after pre-conditioning

In order to obtain a variety that was susceptible to the HTC defect, three representatives cowpea varieties from Botswana, namely *Mogwe-o-kgotsheng, Bechuana white and Mae-a-tsilwane*, were monitored for HTC phenomenon by storing them at 42ºC and 67% RH for three weeks. *Mogwe-o-Kgotsheng* was the variety chosen for further experimentation as it showed a significantly higher increase in cooking time (≈72%) as compared with approximately 31% increase in cooking time shown by other two varieties.

Storage-induced or accelerated storage and chemical-induced methods have been used to induce the HTC defect in legume seeds. Storage-induced hardening consists of storing cowpeas at high temperature and high relative humidity and because it simulates the real

conditions in which cowpeas are stored in tropical conditions, it was chosen to induce the HTC defect in cowpeas. The two proposed mechanisms for hardening of legume seeds are: pectin insolubilization via its binding with Ca^{2+} and Mg^{2+} released from phytate breakdown by phytase (Hentges *et al.*, 1991; Aguilera & Rivera, 1992) and cell wall lignification via cross-linking of phenolic compounds with cell wall proteins and middle lamella pectins and cellulose (Stanley, 1992).

Chemical hardening consists of soaking legume seeds in a solution containing divalent (i.e. Ca^{2+}) cations such as $CaCl_2$ (Liu *et al.,* 1992b). Chemical hardening of legume seeds requires only 4 to 7 h in order to obtain the same or an even greater effect in terms of increasing the cooking time as compared with accelerated storage (Reyes-Moreno *et al.,* 1994). However, it is still not clear whether the mechanisms of chemical hardening are the same as those induced by storage at adverse conditions found in tropical countries. In addition to that, it is still not clear if the time for seeds hardening is enough to induce most of the mechanisms important during the development of the defect. These are some of the reasons that this procedure was not used to induce hardening of cowpeas in this study.

When very high relative humidity (100% RH, 41ºC for 14 days) were initially used as suggested by Jackson & Varrianto-Marston (1981), mould growth was observed on the cowpea seeds. To avoid this situation, saturated solutions of NaCl and KCl were successfully used to generate 75% RH (Shiga, Lajolo & Filisetti, 2004) and 80% RH (Downie, Georget, Smith & Waldron, 1997) in beans. In order to regulate the relative humidity of the storage environment, cowpea seeds in this research were stored over saturated solutions of KCl and 67% RH was generated.

A relatively prolonged storage as compared to three weeks used in this study, would probably be important to allow a completion of the mechanisms of the HTC defect during the inducement of the phenomenon. For instance, high temperature and humidity induced increase of phytase activity from 91.1 to 104.2 $units.kg^{-1}$ (14% increase) and reduced phytic acid content from 1.95 to 1.49 $mg.g^{-1}$ (24% decrease). There is a possibility that the increase in the activity of phytase and decrease in phytic acid content during storage could have been greater if the storage period was prolonged. This observation can be supported by the non-significant increase in the amount of leached solids after the storage of cowpeas at adverse conditions, which suggests that membrane degradation was only at the

initial stage. Leached solids have been used as an indicator of seed membrane degradation as a result of HTC defect in legume seeds subjected to aging conditions (Jackson & Varriano-Marston, 1981; Hentges *et al.* 1991).

The moisture content of cowpeas was performed at steps I and III in order to get the combined effect of pre-conditioning cowpeas in a solution containing monovalent (Na^+) cations and micronization on the water holding and hydration properties of the cowpea seeds. It was done according to accepted and approved procedures. However, because moisture content includes the water that is not directly involved in chemical reactions, water activity (a_w) is also recommended for future studies.

Legume seeds with HTC defect are not necessarily harder than normal seeds at the raw soaked stage (Liu, 1995). Thus, the presence of this defect can only be confirmed after cooking for a given period of time. Cooking time of cowpeas was used as a good and reliable predictor of the level of HTC defect in hardened cowpeas and was used to measure the effectiveness of pre-conditioning in a solution with monovalent (Na^+) cations and its combination with micronization in alleviating the HTC defect in cowpeas. Cooking time is defined as the time required for legume seeds to achieve a degree of tenderness during cooking acceptable to the consumers (Moscoso *et al.,* 1984).

A Mattson Bean Cooker (MBC) with 25 probes (of 90 g each) was used to measure the cooking time of cowpeas in this study. The use of this MBC is time consuming and it requires uninterrupted attention of an operator to observe the penetration of the probes into cooked seeds as cooking progresses. This drawback can be effectively eliminated by connecting an automatic recording system to the MBC device. Proctor & Watts (1987) found that lighter rods (49.75 g) provided the best prediction of the cooking time, based on its good correlation with the cooking time as determined by a sensory evaluation panel. In this study, the probes used in the MBC were 90 g each, which could have underestimated the actual cooking time required. The large variation in terms of the weight of probes used in the MBC, i.e. 90 g (Mwangwela *et al.,* 2006), 38 ± 1.2 g (Akinyele *et al.,* 1986), 82 g (Downie *et al.,* 1997) does not facilitate comparisons of cooking times from different studies and can lead to under or overestimation of the cooking time of legume seeds.

The cooking time in this study is referred to as the time when 80% of the rods (20/25 rods) of the MBC had fallen through the cooked seeds. There is a large variation in the literature in terms of the percentage of probes (in the MBC) fallen through cooked seeds that count for the measurement of the cooking time (cooking time point): $CT_{92\%}$ in beans (Proctor & Watts, 1987), $CT_{50\%}$ in cowpeas (Hentges *et al.,* 1991), $CT_{50\%}$ in cowpeas (Akinyele *et al.,* 1986), $CT_{50\%}$ in beans (Shiga *et al.,* 2004), $CT_{80\%}$ in cowpeas (Mwangwela *et al.,* 2006), $CT_{100\%}$ in black beans (Berrios *et al.,* 1999). Normally, the time required for the legume seeds to soften during cooking is taken as a basis for comparison. Although MBC provides a cooking time of legume seeds, which have been used as a basis for comparison by different authors, it is important that the probes used have the same weight and the CT need to be uniform. There is also a need for correlating different CT of legume seeds with sensory analysis in order to establish a uniform CT, which would be important in terms of establishing a basis for comparison.

Some authors soak cowpeas (Akinyele *et al.,* 1986; Hentges *et al.,* 1990) or beans (Downie *et al.,* 1997; Aguilera & Rivera, 1992) before cooking, while others cook cowpeas (Mwangwela *et al.,* 2006) or beans (Reyes-Moreno *et al.,* 1994) or chickpeas (Reyes-Moreno *et al.,* 2000) without soaking, depending on whether the seeds are HTC or not. Soaking can reduce the cooking time of legume seeds (Sefa-Dedeh *et al.*, 1978; Jackson & Varriano-Marston, 1981). It is also important to indicate whether the legume seeds were soaked or not before the determination of the cooking time using the MBC. In this study, instead of soaking, pre-conditioning was used in order to achieve specific moisture content (41%) necessary for micronization. The cooking time was measured before and after pre-conditioning cowpeas in water or in a solution containing monovalent (Na^+) cations in order to determine the effect of pre-conditioning, as a variable, on the cooking time of normal and HTC cowpeas. Pre-conditioning cowpeas in a solution with monovalent (Na^+) cations as well as its combination with micronization proved to be important steps as both reduced the cooking time of normal and HTC cowpeas.

The texture of cooked seeds as measured by the texture analyser is another important parameter that was used for the mechanical measurement of seed cookability and to differentiate HTC from normal cowpeas. Because the cooking quality varies within individual seeds, single seed measurements were performed using a blade to cut through ten consecutive seeds per sample treatment. It requires multiple performances during

different stages of cooking, and special attention to the positioning of the seeds on its side and this has proven to be very difficult and time consuming. To increase the efficiency, convenience and minimize variation between measurements, texture measurement for a group of seeds has been used by other researchers (Sefa-Dedeh *et al.,* 1978; Liu *et al.*, 1993a; Arntfield *et al.,* 2001) and it has become the most popular method for evaluating bean texture (Liu, 1995). This method consists of pouring a precise amount of seeds (i.e. 30 g) into a shear-compression cell or an extrusion cell and compressing them with a cross-head speed (Liu, 1995). Therefore, this method should be considered for future studies in texture of cooked seeds.

The texture of cooked seeds measured by using a texture analyser as well as by the MBC do not consider sensory attributes such as "cooked bean flavour" (Phadi, 2004) that has to be developed to an acceptable level during cooking (Proctor & Watts 1987). A descriptive sensory panel could have been used to determine the type and degree of attributes such as flavour (i.e. cooked bean flavour, raw bean flavour), texture (i.e. mushiness, lumpiness, graininess), and taste (i.e. beany taste, metallic taste) (Mwangwela, A –University of Malawi, Malawi, personal communication) of cooked cowpea seeds.

Before micronization (to a final surface temperature of 153ºC), cowpeas were first pre-conditioned in water or in a solution containing monovalent (Na^+) cations to a final moisture content of approximately 41%. It has been reported that the solubilization effect of water on pectins during soaking (Clemente *et al.,* 1998) and the conversion of insoluble pectinates to soluble pectins during soaking in a solution with monovalent (Na^+) cations (Valle *et al.*, 1992; Léon *et al.*, 1992) play a major role in the reduction of cooking time of HTC legume seeds. Pre-conditioning cowpeas in water was used to induce the solubilization effect of water on pectins. Pre-conditioning cowpeas in a solution containing monovalent (Na^+) cations was used to induce conversion of insoluble to soluble pectinates. To obtain 41% final moisture content in cowpea seeds, the following formulation (Arntfield *et al.,* 1997) was used:

Weigh of H_2O = weigh cowpeas x ($\%H_2O$ target - $\%H_2O$ original)/(100-$\%H_2O$ target)

It literally means that for every kg of HTC cowpeas, 461 g of water were needed to achieve a final moisture content of 41%. After the pre-conditioning process, cowpeas were blotted

dry on absorbent paper and held for a further 12 h at 22ºC to equilibrate the moisture throughout the seeds. Pre-conditioning of legume seeds prior to micronization using this method has been successfully used by several authors (Arntfield *et al.,* 1997; Scanlon *et al.*, 1998). However, it is important that the amount of cowpeas to be pre-conditioned have to be sealed in a plastic bag and mixed by shaking and rolling until all the water is absorbed and evenly distributed through the seeds.

Micronization was used due to its effect on reducing the cooking time of legume seeds (Arntfield *et al.,* 1997; Scanlon *et al.*, 1998; Phadi, 2004; Mwangwela *et al.*, 2006). When micronization is applied, there may be a breakage of pectin molecules into lower and more soluble molecular fractions in the middle lamella, via the β-elimination reaction (Liu *et al.,* 1993b). Pre-conditioning cowpeas in solution containing monovalent (Na^+) cations was combined with micronization in the expectation that the two treatments could have a greater effect on reducing the cooking time of HTC cowpeas than either one on its own. The reduction in the cooking time was expected to be due to the additive effect of the action of monovalent (Na^+) cations present in the pre-conditioning solution and due to high temperatures (153ºC) imposed by micronization.

The micronization parameters (moisture content and micronizer settings) were selected according to Phadi (2004) and Mwangwela *et al.* (2006) to obtain relatively stable products in terms of final moisture content and without burning. The micronizer was pre-heated for 20 min before micronizing the cowpeas in a single layer (21 cm from the energy source) for 6 min, which is believed to produce a final surface temperature of 153ºC, without excessive darkening and burning apart from reducing the cooking time (Phadi, 2004; Mwangwela, 2006). However it is possible that *Bechuana White* (Phadi, 2004; Mwangwela, 2006) and *Var.462* (Mwangwela, 2006) differed from that used in this study (*Mogwe-o-Kgotsheng*) in terms of thermal conductivity, which could have made the final surface temperature expected in this experiment (153ºC) to be different. Factors such as density (Taiwo *et al.,* 1998), size (Cenkowski & Sosulski, 1998), moisture content of the seeds (Arntfield *et al.,* 2001) are probably different in different varieties, and could influence the thermal conductivity of cowpea seeds differently.

The use of thermocouples attached to data-logging system could have been used to check the final surface temperature of cowpeas. While this experiment was conducted using a

small scale micronizer, the importance of controlling the temperature of the seeds is equally important when using a large-scale micronizer. Cenkowski & Sosulski (1998) showed that large and round peas are difficult to heat uniformly than small peas. Fasina *et al.* (2001) also showed that it was easier to uniformly micronize small seeded legumes (i.e. lentils and split peas) than larger seeded legumes (i.e. pinto peas). Vibrating troughs or belts have been used in large-scale micronizers to facilitate uniform exposure of the sample to the infrared radiation (Scanlon *et al.*, 1998). Although the small scale micronizer used in the experiment does not have vibrating belts, the treatment was still effective because the cowpeas used were small-seeded.

According to Liu *et al.* (1992b) the cowpea pH can be a convenient and reliable indicator of seed HTC defect induced by adverse storage at high temperature and high relative humidity. Therefore, the pH of cowpeas was measured in unmicronized, normal and HTC cowpeas. Liu *et al.* (1992b) performed pH measurements using flour slurry, 6 h soaking liquor and slurry of soaked seeds with normal water. Results showed that pH of the soaking liquor increased up to six months of storage and then decreased. The pH of the flour slurry and soaked seed slurry decreased linearly with storage time, but the slope of the flour slurry curve was steeper than that of the soaked seed slurry. The difference was apparently attributed to acid leakage during soaking. Therefore, flour slurry pH best represents cowpea tissue pH and it was used in this study.

The degree of splitting in legume seeds has been associated with water absorption, degree of cell separation during cooking and consequently, the texture of cooked seeds (Sefa-Dedeh *et al.*, 1978). Therefore, the degree of splitting helped to explain the role of pre-conditioning alone and when combined with micronization on cooking characteristics of normal and cowpeas as they relate to the cooking time. The degree of splitting was calculated as the number of cowpeas with split seed coats and cotyledons and calculated as (no. split seeds)/ (no. whole seeds) x100 as described by Taiwo *et al.* (1997a). The extent of seed splitting differs within different cowpea seeds, and depends on the cowpea treatments (normal or HTC, micronized or pre-conditioned in water or in a solution containing monovalent (Na^+) cations). One of the major problems with the degree of splitting assay is that there is no clear indication of what extent of splitting in cowpea seed counts for split seeds. In this study, only seed with a split longer than l/5 of the small circumference was counted as split seed (Taiwo *et al.*, 1997a).

Another important parameter that was measured in cooked cowpea seeds is the amount of leached solids. Excessive leakage in HTC legume seeds is associated with membrane breakdown and it is one of the important events in the initiating of the HTC defect (Liu, 1995). Leached solids of micronized legume seeds have been found to be higher than that of unmicronized legume seeds due to leakage of solids through the cracks created by micronization (Fasina *et al.*, 2001). In this study, leached solids were used to estimate the level of seed membrane degradation as induced by the HTC defect and to determine the effect of micronization on the solubilization of the middle lamella of cowpeas. Differently to this study that report solids that leak in the cooking water, some of the studies available in literature report leached solids in the water after 3, 6 and 18 h (Plhak *et al.*, 1989), 24 h (Jackson &Varriano-Marston, 1981), 18 h (Hentges *et al.,* 1991) of soaking. In this study, leached solids were quantified in the cooking water because the main objective of this study was to investigate the effect of micronization on cooking characteristics (i.e. cooking time, texture of cooked cowpeas, leached solids, etc) of HTC cowpeas. The results of leached solids need to be reported in terms of the amount that leached at a given time of cooking or soaking.

The importance of pectin solubility, phytic acid and phytase activity in this study, results from the most widely known theory for the development of the HTC defect, the "phytate-cations-pectin interactions" theory. According to this theory, at adverse storage conditions, the pectin in the middle lamella is insolubilized, via its binding with Ca^{2+} and Mg^{2+} released from phytate breakdown by phytase, resulting in HTC seed during cooking (Hentges *et al.*, 1991; Aguilera & Rivera, 1992). Therefore, the experimental design was designed in such a way that changes in pectin solubility were tested after every step of the experiment.

The soluble pectins in cowpea seeds were measured as the amount of galacturonic acid lost during pectin extraction as described by Arntfield *et al.* (1997). To estimate the amount of soluble pectin, a standard curve ranging from 10 to 70 µg anhydrogalacturonic acid per ml was used. For colour reaction, a 0.5 ml aliquot treated as described in the method was used. One of the major concerns with this method is the interference of neutral sugars in the final content of galacturonic acid (Liu *et al.,* 1993b). Liu *et al.* (1993b) found that the interference of neutral sugars in the final content of galacturonic acid was found to be minimal when amylase and glucose were added into soaking or cooking liquors. The

cowpea flour (5 g) was treated with 40 ml of 95% ethanol, which could have reduced the concentration of sugars to a level of negligible interference (Liu *et al.,* 1993b). Therefore, although the presence of glucose was reported in cowpeas by Longe (1980), it is not expected to have influenced the content of extracted pectin. This method not only reduced the interference of neutral sugars but was also more rapid when compared to traditional practice of measuring pectin solubility in terms of calcium pectate (Dietz & Rouse, 1952) and thereby eliminating large variations associated with the extractions. The traditional calcium pectate method is not sensitive to neutral sugars (Ibarz, Pagán, Tribaldo & Pagan, 2006) and measures the pectic acid content of a sample (Dietz & Rouse, 1952). The method applied in this study, report results in terms of galacturonic acid, since this is the basic structural unit of the pectin molecule.

The amount of phytic acid present in cowpeas was determined according to the procedure of Febles *et al.* (2001). The method consists of the titration of hot Fe (III) present in the floating liquid from the extraction with 0.010 M EDTA solution until bright yellow. This method is based on the formation of precipitate or insoluble complexes between phytic acid and Fe (III) (Garcia-Villanova *et al.*, 1982). The volume of EDTA used during the titration is the key for phytic acid estimation according to the following formulation:
Phytic acid (%) =1.32 x (10-V)/P; V-EDTA solution volume (ml); P-sample weight (g).

One of the disadvantages of this method is to establish a constant endpoint of the colour to bright yellow during titration as it keeps changing depending on the volume of EDTA used and the temperature of the solution to be titrated. During titration, depending on the time taken to reach the endpoint, different samples could have had different temperature variations. These temperature variations would have influenced the amount of EDTA necessary to reach the endpoint. Because large number of samples (10) was examined, the experiment became extremely tedious and required a lot of concentration in terms of controlling the temperature and endpoint. These problems (i.e. variations in the time to reach the endpoint and variations in the temperature among samples) could have been eliminated by using HPLC methods for phytic acid determination, although their use requires sophisticated equipment. The determination of phytate content could have given the best indication of the activity of phytase in hydrolysing the phytate to realease divalent cations.

Phytase hydrolyzes the phosphate residues from phytic acid, thereby essentially destroying its affinity for minerals (Fennema, 1985). The activity of phytase was measured based on the quantification of phosphorus liberated from phytate by phytase after 30 min incubation. From the method used, a phytase unit is defined as that amount of phytase activity which liberates inorganic phosphorus from a 100 µmol of Na-phytate solution, at a rate of 1 µmol.min^{-1} at pH 5.0 and at 45ºC (Eechout & Paepe, 1994).

About 60 to 90% of the total phosphate in plant seeds occurs as phytate-phosphate (Fennema, 1985). Because an excess phytate (100 µmol of Na-phytate as described in the method) was used in the assay, it could be assumed, however that the enzymatic hydrolysis of phosphorylated compounds other than phytate did not contribute, to a significant extent, to the phosphate liberated during phytate-degrading assay (Greiner & Egli, 2003). Insoluble phytate-mineral chelates tend to exist in a fine colloidal state that requires relatively high centrifugal forces to pellet out of solution (Maenz *et al.,* 1999). Incomplete pelleting may tend to overestimate the soluble and insoluble fractions of phytate-phosphorus complexes, thereby underestimating phytase activity. A relatively higher centrifugation force (10000 g for 3 min) was used to remove any cloudiness from the supernatant.

Other methods for determination of phytase activity are available, i.e. HPLC ion-pair chromatography. According to Greiner & Egli (2003), HPLC ion-pair chromatography separates and quantifies phytate and lower *myo*-inositol phosphates. The method consists of detecting the reduction in phytate during enzymatic hydrolysis. However, during the reduction of phytate significant amounts of lower *myo*-inositol phosphates could be generated in the assay mixtures reducing the accuracy of the method. Therefore, direct incubation of cowpeas flour in a buffered Na-phytate containing solution and quantification of liberated phosphate gives a better estimation of phytase activity and it is not as time-consuming as compared with HPLC ion-pair chromatography.

4.2. The effect of HTC defect on physicochemical and cooking properties of normal cowpeas

In this section the effect of HTC defect on physicochemical characteristics of cowpeas with a focus on the changes in the cooking characteristics will be discussed. The HTC defect was induced by storing cowpea seeds at adverse conditions (42ºC and 67% RH) for

three weeks. After that period, the HTC defect in cowpea seeds was confirmed by the significant increase in cooking time and cooked texture compared with normal cowpeas (Table 4.2.1).

Table 4.2.1. Summary of changes in cooking and physicochemical characteristics of cowpeas as induced by HTC defect in relation to that of normal cowpeas

Physicochemical characteristics	Percentage change (%) as induced by HTC defect in relation to normal cowpeas
pH	3 ↓
Moisture content	15 ↑
Cooking time	>203 ↑
Texture of cooked cowpea seeds (Hardness)	57 ↑
Phytic acid content	21↓
Phytase activity	14 ↑
Pectin solubility	35 ↓
Degree of splitting	87 ↓
Leached solids	↔
Hydration capacity (18 h of soaking)	19↑
Water absorption (6 h of soaking)	8↓
Water absorption (3 h of cooking)	5↓

↔ = No significant change, ↓= significant reduction and ↑ = significant increase

Most investigators did not concentrate their focus on integrated explanations of different physicochemical changes taking place during the development of the HTC defect in legume seeds. Rather they seem to focus on only one possible mechanism for the development of the HTC defect. However, it is apparent that the relationship between physicochemical changes during storage, soaking and/or cooking of legume seeds that lead to the HTC defect is very complex and proceeds via multiple channels (Figure 4.2.1).

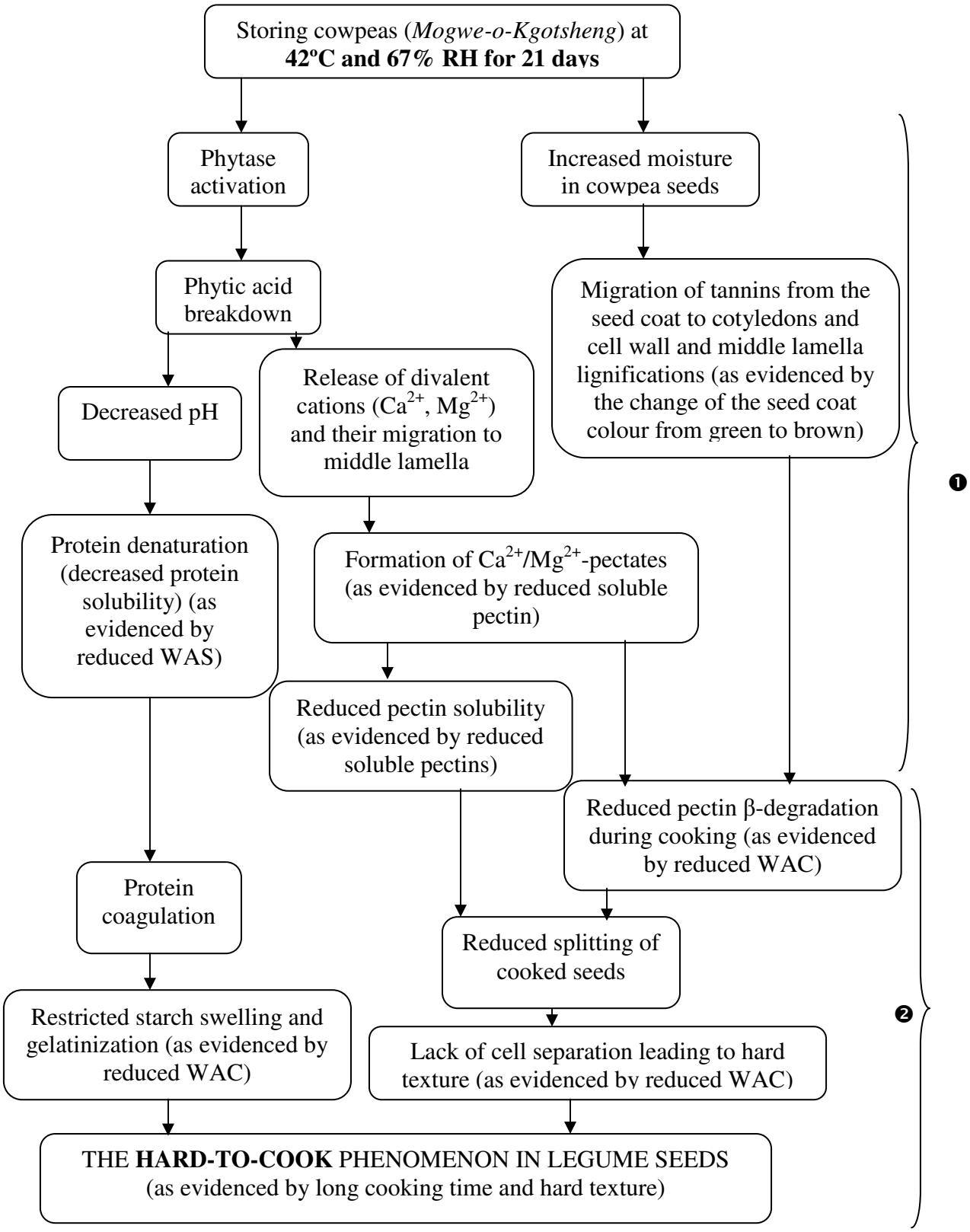

Figure 4.2.1. A model proposing multi-mechanisms with sequential events taking place during storage at high temperature and humidity, leading to HTC defect; WAS = water absorption during soaking, WAC = water absorption during cooking, ❶ = storage and ❷ = cooking

Therefore, a mechanistic model (Fig. 4.2.1) is proposed to explain the development of the HTC defect in legume seeds, based on the "Phytate-mineral-Pectin interactions" theory, which formed the basis of this study. For a complete understanding of the development of the HTC defect, "cell wall and pectin lignification" theory during improper storage also needs to be taken into consideration. The model proposes different physicochemical changes that take place during improper storage and the subsequent cooking process.

At high temperature and relative humidity conditions, phytase is activated and promotes phytic acid breakdown, as shown by significantly increased phytase activity and reduced phytic acid content in HTC cowpea seeds (Table 4.2.1). Phytase hydrolyzes the phosphate residues from phytic acid, thereby essentially destroying its affinity for minerals (Fennema, 1985). There was also a significant negative correlation (r = -0.90) between the two factors. Once the phytic acid is hydrolyzed, divalent cations (Ca^{2+}, Mg^{2+}) are released and they migrate to the middle lamella, where they bind with pectins, forming an insoluble barrier to water penetration during soaking and cooking (Hentges *et al.,* 1991; Aguilera & Rivera, 1992). However, in this study the amount of water absorbed by cowpea seeds during soaking and cooking has not proven to be a reliable indicator of seed cookability as there were no correlations found between the two parameters and the cooking time. Liu *et al.* (1993b) also could not find a direct association between the cooking time and water hydration during soaking, but Liu *et al.* (1992b) has found water absorption during cooking to be related to hardness of cooked cowpea seeds. The reduction in the amount of water absorbed by HTC cowpea seeds during soaking can also be associated with the increase in the moisture contents during adverse storage as shown by the significant negative correlation (r =-0.95) between moisture content and the moisture of cowpeas.

However, the water absorbed by HTC cowpeas after 18 h of soaking was significantly ($P \leq 0.05$) higher than that absorbed by normal cowpeas. The possible large cavity between the cotyledons of HTC cowpeas (Valle & Stanley, 1992) and the fact that their seed coat might have been pulled away from the cotyledons (Plhak *et al.*, 1989 and Valle & Stanley, 1992), could have contributed to large amounts of water absorbed by HTC cowpeas after 18 h of soaking.

Pectins contribute to the maintenance of structural integrity of the middle lamella of plant cell walls (Uzogara *et al.,* 1990). The reduced pectin solubility observed in this study as result of storage at adverse conditions can also be related to the involvement of phenolic compounds (Fig. 4.2.1). During improper storage, the moisture content of cowpeas increased (Table 4.2.1), which could have influenced the migration of tannins from the seed coat to seed cotyledons, where they could bind with macromolecular components, i.e. cell wall protein and middle lamella pectins and cellulose (Stanley, 1992; Garcia *et al.,* 1998). The formation of these complexes could have led to cell and middle lamella lignification (Stanley, 1992). However, in this research, no concrete evidence can be provided in this regard.

Pectin is extremely sensitive to elevated temperatures in a near neutral medium (Liu *et al.*, 1993b; Sajjaanantakul *et al.,* 2003). Under such conditions, pectin is degraded to lower and more soluble fractions, via β-eliminative reaction (Liu *et al.,* 1993b). Because the cowpea pH in this study was 6.5, it is believed that during cooking of normal cowpeas, the pectin was degraded into more soluble fractions, as shown by decreased pectin solubility (Table 4.2.1). However, since the pectic substances of HTC cowpeas are strongly bound to divalent cations (Ca^{2+}, Mg^{2+}) and possibly phenolic compounds, the extent of pectin degradation would have been less than what could have been expected.

The pectin solubility was positively correlated (r = +0.64) with water absorption during cooking. During the cooking process, there is a solubilization of the middle lamella of normal cowpeas resulting in cell separation leading to a soft cooked texture (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh & Stanley, 1979). Reduced pectin solubility and little β-degradation of pectins during cooking of HTC cowpeas could have contributed to less water absorbed during soaking and cooking leading to less splitting and thus a harder texture in cooked seeds. All these factors brought together, would have contributed to lack of cell separation during cooking of cowpeas and consequently, in HTC cowpeas.

The restricted water absorbed by HTC cowpeas can also be associated with the reduced protein solubility (Liu *et al.* 1992a) and restricted starch swelling of cooked HTC cowpea seeds as observed microscopically by Liu *et al.* (1993b). These authors showed that starch granules are ready to swell only at temperatures above 60ºC, and that the amount of water absorbed during cooking affected the final texture of cooked cowpeas. This observation

suggests that starch hydration and swelling are other important factors governing cooked texture and explain why water absorption during soaking is not a good indicator of seed cookability.

Another important event that happened during the storage of cowpea seeds under improper conditions is the decrease in cowpea seeds pH (Table 3.2.1). Although this decrease was very slight (from pH 6.5 to 6.3) it was significant, and should not be discarded in terms of its possible influence on the reduction in protein solubility. Liu *et al.* (1993b) found that a decrease in pH from 6.64 to 5.57 in cowpeas stored at 30ºC and 64% RH for 18 months was associated with increased hardness of the cowpea seeds. The decreased pH is probably due to hydrolysis of phytate as shown by the decrease in the phytic acid content (Table 4.2.1). Other factors that could have contributed to the decrease in the pH of cowpeas are: hydrolysis of lipids into fatty acids (Liu *et al.*, 1992a) and hydrolysis of storage proteins (Hohlberg & Stanley, 1987). The decrease in the pH of legume seeds stored at adverse conditions has been associated with the development of the HTC defect, via denaturation of proteins and reduction in protein solubility (Liu *et al.* 1992a).

At adverse conditions, the temperature of protein coagulation or gelation decreases from above 100 to 56ºC. This is below starch gelatinization temperatures (64 to 73ºC) (Liu *et al.* 1992a). Therefore, during cooking, protein gelation or coagulation may occur before starch gelatinization. Coagulated proteins may form a physical barrier to water penetration during cooking, preventing the starch granules from complete swelling, resulting in HTC seeds.

Based on the explanation above, it can be postulated that the HTC defect in cowpea seeds may in part be due to restricted swelling and incomplete gelatinization of starch during cooking because of physical barrier to water penetration formed by coagulated and less soluble proteins. In normal cowpeas, because of high protein solubility and high protein gelation point (above 100ºC) as compared with HTC cowpeas (Liu *et al.,* 1992a), starch gelatinization will take place before protein coagulation, leading to higher water absorption during cooking by starch granules, softer texture, higher degree of splitting, cell separation, and consequently, reduced cooking time.

Figure 4.2.2 shows a diagrammatic model focusing on the lignification of the cell wall and middle lamella macromolecular components, denaturated and less soluble proteins surrounding starch granules during storage at adverse conditions.

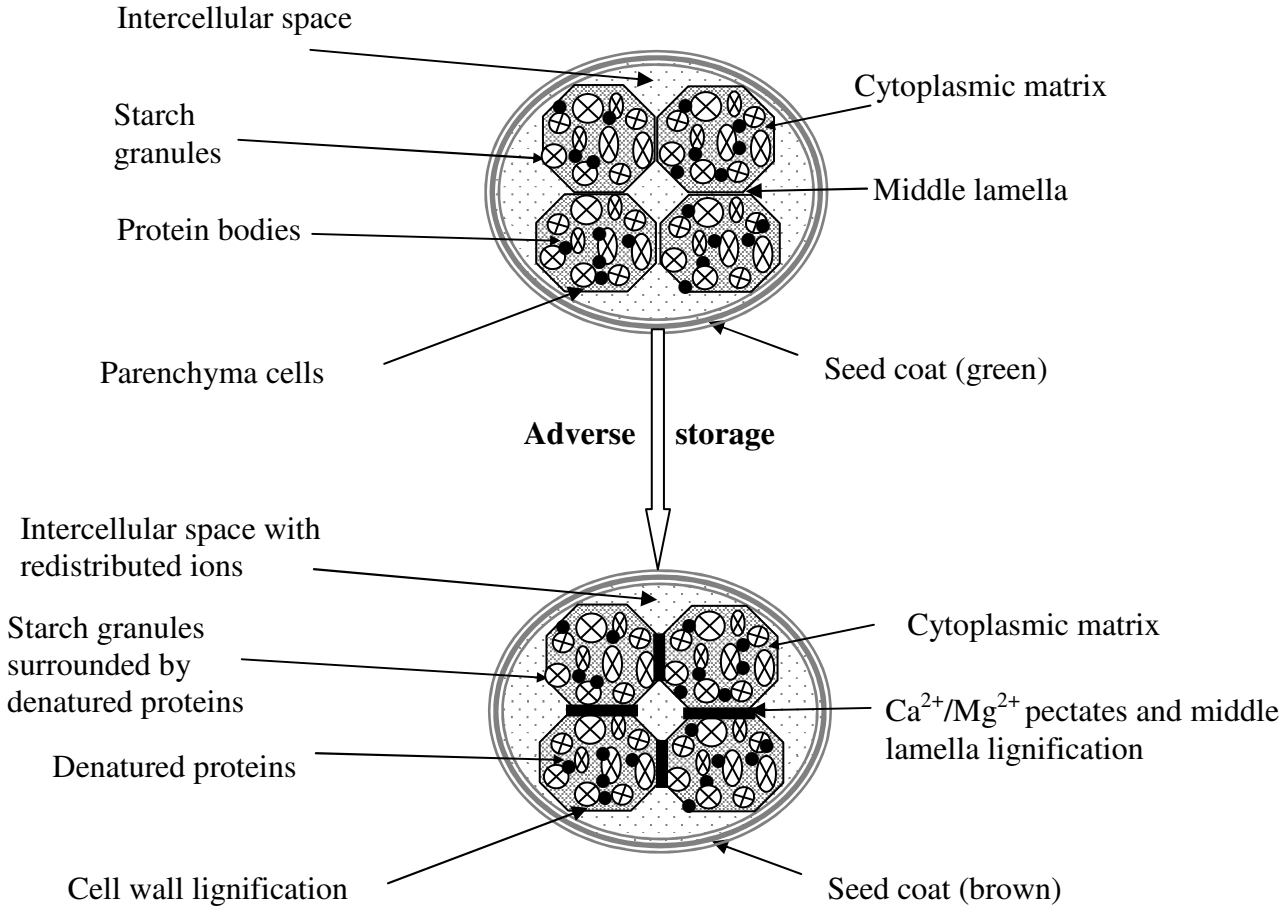

Figure 4.2.2. Diagram showing changes in the cell wall, starch granules, proteins and pectins of cowpeas during storage at adverse conditions (adapted from Mwangwela (2006) model)

After the storage period, the seed coat colour of cowpeas changed from green to light brown, which according to Nozzolillo & Bezada (1984) suggests polymeriaztion of tannins. The change of the colour of the seed coat is the only parameter that can be put forward in this research to support the involvement of phenolic compounds in the cell wall lignification, as it was not tested during the experiments.

From Figure 4.2.1, it is noticeable that there may be two direct causes of HTC defect in legume seeds, namely restricted starch swelling and gelatinization, and lack of cell separation. The mechanisms that affect both lack of cell separation and restricted starch

swelling and gelatinization, during cooking resulting in HTC cowpea seeds are summarized as follows:

(1) Little cell wall pectin solubilization and degradation due to cell wall lignification and binding of pectin with divalent cations (i.e. Ca^{2+}, Mg^{2+}) released during phytate degradation at adverse storage. The latter is a confirmation of the well-known theory for the development of HTC defect, "phytate-phytase-pectin interactions" theory.

(2) Protein coagulation caused by protein denaturation during adverse storage, restricts the starch granules from full swelling and gelatinization, as denatured proteins form a physical barrier preventing water to reach starch granules.

A more comprehensive study of the HTC defect in legume seeds, integrating phytate-phytase-pectins and cell wall lignification via binding with phenolic compounds is needed.

4.3. Improvement of cooking characteristics of normal and HTC cowpeas by pre-conditioning in water or in a solution containing monovalent (Na^+) cations and its combination with micronization

The effects of pre-conditioning (in water or in a solution containing monovalent (Na^+) cations) on its own and its combination with micronization on physicochemical characteristics of normal and HTC cowpeas (Table 4.3.1) will be discussed in this section. Diagrammatic models are proposed to explain how the pre-conditioning and its combination with micronization reduced the cooking time of normal (Fig. 4.3.1) and HTC cowpeas (Figure 4.3.2).

Pre-conditioning normal cowpeas in water or in a solution with monovalent (Na^+) cations improved pectin solubility (Table 4.3.1). The solubilization of pectins during pre-conditioning was probably due to the solubilization effect of water (Clemente *et al.,* 1998) and a possible conversion of insoluble to soluble pectins in the middle lamella by the action of monovalent (Na^+) cations (Vidal-Valverde *et al.*, 1992) (Fig. 3.2.1).

Table 4.3.1. Summary of changes in physicochemical and cooking characteristics of normal and HTC cowpeas as induced by pre-conditioning in water or in a solution containing (Na^+) cations and in combination with micronization in relation to control

Physicochemical characteristics	State of cowpeas	Percentage change (%) as induced by different treatments in relation to untreated (normal or HTC) cowpeas			
		Treatment I	Treatment II	Treatment III	Treatment IV
Moisture content	Normal	ND	ND	13↑	44↑
	HTC	ND	ND	39↑	54↑
Cooking time	Normal	51↓	52↓	58↓	66↓
	HTC	↔	44↓	45↓	78↓
Texture of cooked seeds	Normal	ND	ND	11↓	16↓
	HTC	ND	ND	11↓	18↓
Phytic acid content	Normal	a	a	26↓	29↓
	HTC	a	a	1↓	4↓
Phytase activity	Normal	a	a	47↓	46↓
	HTC	a	a	52↓	44↓
Pectin solubility	Normal	a	a	121↑	207↑
	HTC	a	a	232↑	305↑
Degree of splitting	Normal	ND	ND	70↑	75↑
	HTC	ND	ND	920↑	1016↑
Leached solids	Normal	ND	ND	17↑	12↑
	HTC	ND	ND	51↑	41↑
Hydration capacity	Normal	ND	ND	21↓	20↓
	HTC	ND	ND	28↓	24↓
WAS	Normal	ND	ND	21↓	30↓
	HTC	ND	ND	27↓	32↓
WAC	Normal	ND	ND	8↑	7↔
	HTC	ND	ND	2↓	8↓

↔ = No significant change, ↓= significant reduction, ↑ = significant increase, Treatment I= pre-conditioning in water, Treatment II= pre-conditioning in a solution containing monovalent cations, Treatment III= Treatment I + micronization, Treatment IV= Treatment II+ micronization, WAS= water absorbed after 6 h soaking, WAC= water absorbed after 6 h cooking, ND= not determined, a = no values on dry basis

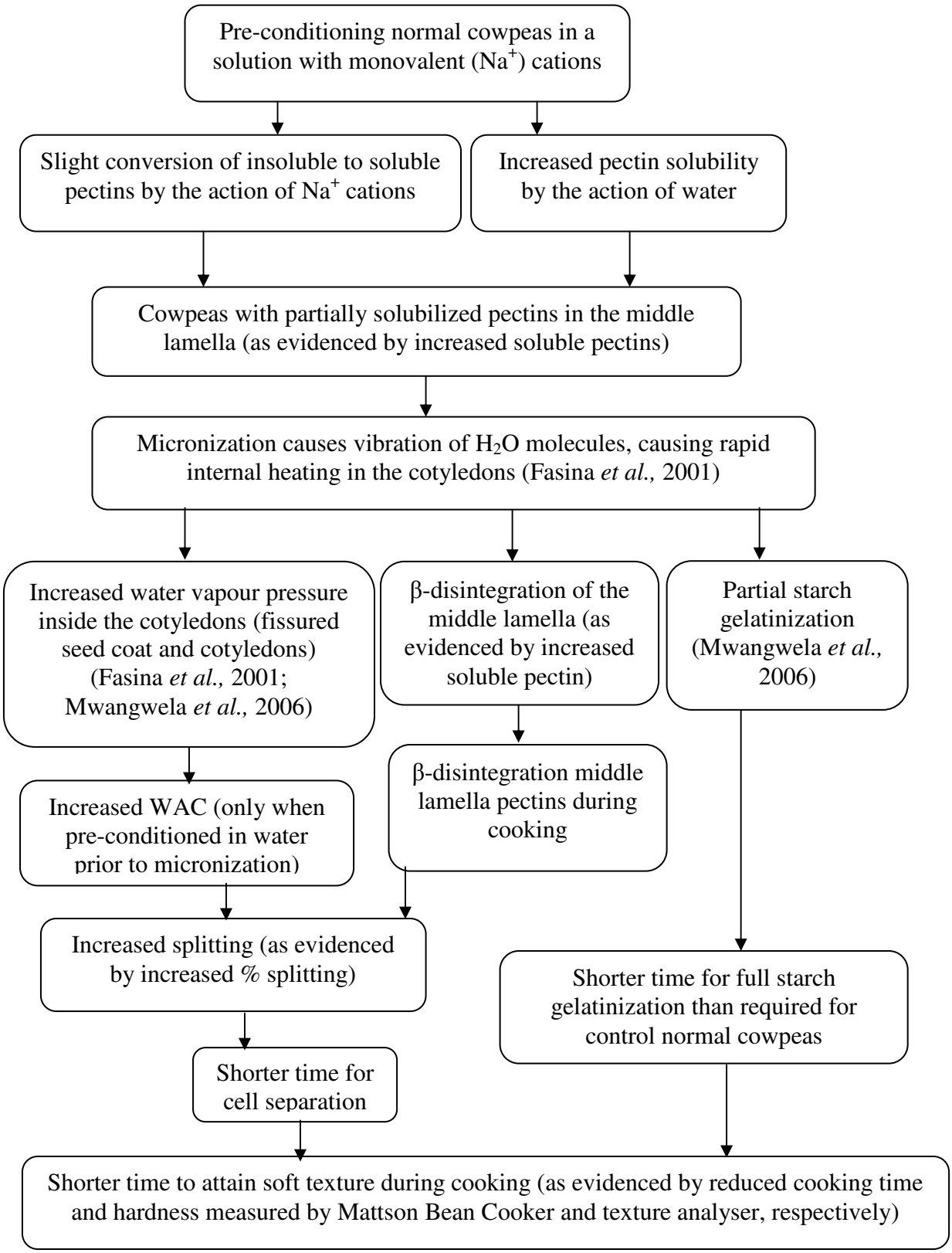

Figure 4.3.1. Proposed mechanisms of the combined effect of pre-conditioning in a solution containing monovalent (Na^+) cations and micronization on physicochemical properties in relation to cooking characteristics of normal cowpea seeds

After being pre-conditioned in water or in a solution with monovalent (Na^+) cations, the normal cowpea seeds were subsequently micronized. The penetration of infra-red rays induced by micronization probably caused the water molecules to vibrate, causing rapid internal heating and a rise in water vapour pressure inside the cotyledons (Fasina *et al.*, 2001), which could have caused the development of fissures in normal cowpeas (Mwangwela, 2006) (Fig. 4.3.1). The fissures created in normal cowpeas only when micronizd after pre-conditioned could have improved water uptake during cooking, leading to increased splitting and softer texture during cooking (Mwangwela, 2006). Micronization possibly induced degradation of the middle lamella of normal cowpeas through breakage of pectin molecules into lower and more soluble fractions, probably via β-degradation reaction (Liu *et al.*, 1993b) as shown by improved pectin solubility (Table 4.3.1). The β-degradation of pectins in the middle lamella is possibly further increased during cooking of normal cowpeas because of the elevated temperatures. Pectin degradation contributed to increased splitting leading to shorter time required for cell separation during cooking than required for control (untreated) normal cowpeas, leading to shorter time required for the seeds to attain a softer texture (Mwangwela, 2006). Pre-gelatinization of cowpea starch due to micronization is another factor that has been associated with the reduction in cooking time of normal legume seeds (Cenkowski & Sosulski, 1997; Arntfield *et al.,* 1997; Arntfield *et al.,* 2001; Mwangwela *et al.*, 2006). Because starch of normal cowpeas was probably partially gelatinized during micronization, the time required to complete starch gelatinization during cooking was shorter as compared with control normal cowpeas.

For HTC cowpeas, pre-conditioning in water or in a solution containing monovalent (Na^+) cations improved the pectin solubility within HTC cowpeas more extensively as compared with normal cowpeas (Table 4.3.1). This could be due to the fact that HTC cowpeas had increased amounts of divalent cations bound to pectins, which could have increased the conversion of insoluble to soluble pectins by Na^+ cations (Table 4.2.1).

This study attempted to link the improvement of pectin solubility in the middle lamella of cowpeas with the decrease in the cooking time. But for HTC cowpeas, although pre-conditioning in water significantly improved the pectin solubility in the middle lamella of cowpeas, it did not have an impact on the cooking time as compared with control samples.

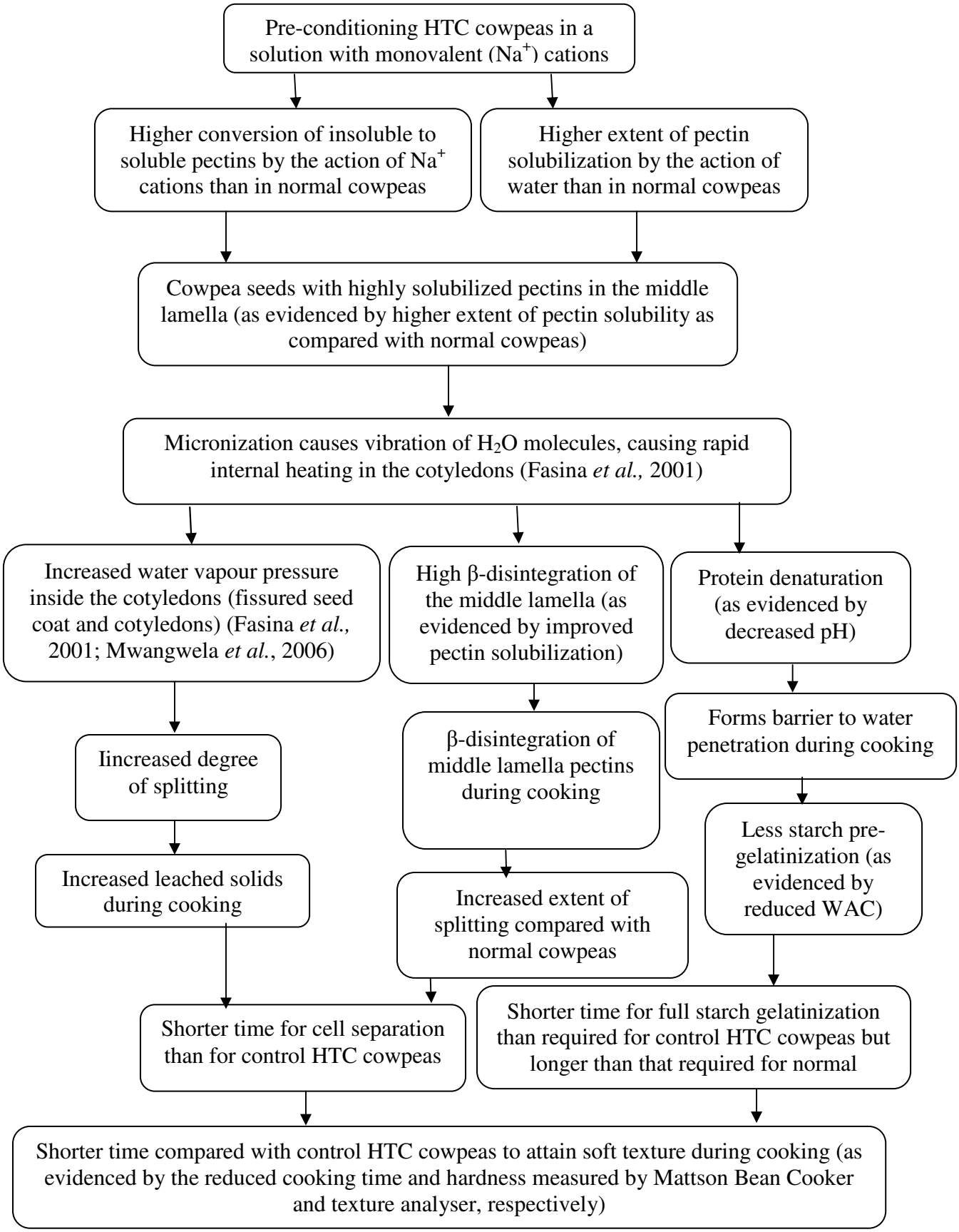

Figure 4.3.2. Proposed mechanisms of the combined effect of pre-conditioning in a solution containing monovalent (Na^+) cations and micronization on physicochemical properties in relation to cooking characteristics of HTC cowpea seeds

Another important observation is that although pre-conditioning in water or in a solution with monovalent (Na^+) cations had more extensive solubilization of pectins of HTC cowpeas when compared with normal, this was not the case in terms of the reduction in the cooking time. This suggests that factors other than pectin solubility influenced the cooking time of HTC cowpeas.

Micronization was the treatment that followed after cowpea seeds were subjected to pre-conditioning in water or in a solution with monovalent (Na^+) cations. Similarly to normal cowpeas, micronization possibly caused the development of fissures (Mwangwela, 2006) in HTC cowpeas (Fig. 4.3.2 and Fig. 4.3.3). Although micronization created fissures in HTC cowpeas, the water uptake during cooking decreased (Table 4.3.1). Reasons for reduced water absorbed by HTC cowpeas during cooking can be the reduced protein solubility induced by decreased pH (Liu *et al.,* 1992a) and by protein denaturation due to micronization (Zheng *et al.,* 1998; Mwangwela *et al.,* 2006). Increased splitting of micronized HTC cowpeas, which led to increased leached solids, could have contributed to shorter time required for cell separation and softer texture during cooking as compared with untreated HTC cowpeas (Fig. 4.3.2 and Fig. 4.3.3).

The β-degradation of pectins as induced by micronization (Liu *et al.*, 1993b) could have played a role in increasing the degree of splitting during the cooking of HTC cowpeas, leading to shorter time for cell separation as compared with untreated samples. The high temperature induced by cooking could also have increased the β-degradation of pectins in HTC cowpeas. As before, although the β-degradation of pectins in HTC cowpeas as induced by micronization was more extensive than in normal cowpeas (Table 4.3.1), this was not the case in terms of the reduction in the cooking time of HTC cowpeas. This suggests that factors other pectin solubility may have played an important role in the reduction of cooking time.

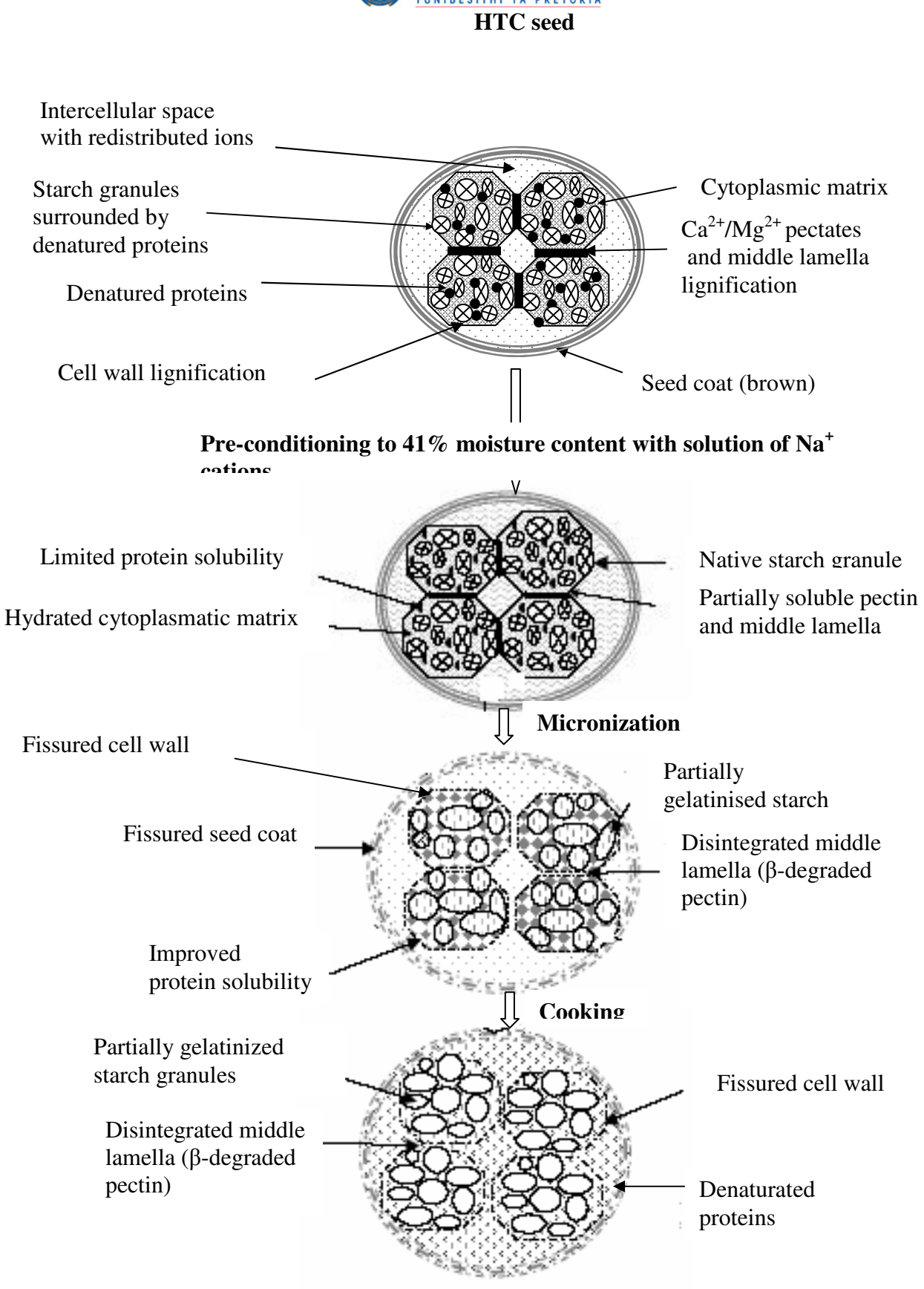

Figure 4.3.3. Diagrammatic view of changes taking place when HTC cowpea seeds are pre-conditioned in a solution containing monovalent (Na^{+}) cations, micronized and then cooked (adapted from Mwangwela (2006) model)

Proteins are the major water absorption components during cooking of cowpeas (Sefa-Dedeh & Stanley, 1979). At high temperature and humidity conditions, proteins in HTC cowpeas may be denaturated due to decreased pH (Liu *et al.,* 1992a) and due to micronization (Zheng *et al.,* 1998; Mwangwela *et al.,* 2006). At these conditions, the coagulation temperature of cowpeas could have decreased below that of starch gelatinization (Liu *et al.,* 1992a). Therefore, coagulation of proteins could have occurred before starch gelatinization during cooking. Coagulated and less soluble proteins could have formed a physical barrier to water penetration during cooking, leading to restricted starch gelatinization and consequently, harder texture than in case of normal cowpeas.

The effect of the presence of monovalent (Na^+) cations in the cooking water on proteins, starch, degree of cell separation and pectins in the middle lamella of HTC cowpeas in comparison with that of normal cowpeas still needs special attention in future studies. It has been reported that the presence of salts in the cooking medium elevates the temperature of starch gelatinization (Varriano-Marston & Omana, 1979) and raised the boiling point of water (Garcia *et al.,* 1991) in black beans. The presence of CO_3^{2-} ions has been associated with a decrease in the temperature of protein denaturation (Valle *et al.*, 1992).

A combination of mechanisms that induced softening of normal and HTC cowpeas, when micronization in combination with pre-conditioning in water or in a solution containing monovalent (Na^+) cations was used, can be summarized as follows:

(1) Pre-conditioning cowpeas in water improved pectin solubilization (Clemente *et al.,* 1998). Pre-conditioning cowpeas in a solution with monovalent (Na^+) cations induced a conversion of insoluble to soluble pectins (Vidal-Valverde *et al.,* 1992). The pectin solubilization occurred more extensively in HTC cowpeas than in normal cowpeas. However, this was not reflected in terms of reduction in the cooking time of HTC cowpeas.

(2) Pectin solubilization was further improved by β-degradation of pectins into lower and more soluble fractions during micronization (Liu *et al.*, 1993b) and cooking. Micronization induced the development of fissures caused by raise in vapour pressure within the cotyledons as induced by increased internal heating (Fasina *et al.*, 2001), which improved water absorbed by normal cowpeas, leading to splitting

and softer texture of legume seeds. Micronization induced the pre-gelatinization of starch (Cenkowski & Sosulski, 1997; Arntfield *et al.,* 1997; Arntfield *et al.,* 2001 and Mwangwela *et al.*, 2006), reducing the time required for full starch gelatinization during cooking. However, in HTC cowpeas insoluble and coagulated proteins could have formed a physical barrier to water penetration during cooking, leading to restricted starch gelatinization and consequently extended time to achieve a softer texture during cooking.

5. CONCLUSIONS AND RECOMMENDATIONS

Storing cowpeas at high temperature and humidity conditions (42ºC and 67% RH for 21 days) results in hard-to-cook (HTC) cowpea seeds, with the cooking time increasing from 89 min to more than 4½ h. This is attributed to insolubilization of pectins in the middle lamella, through binding with divalent cations (Ca^{2+} and Mg^{2+}) released from phytate breakdown by phytase. This is the most widely accepted theory to explain the HTC defect in legume seeds. The reduction in the cooking time of cowpeas as a result of the HTC defect is associated not only with reduced pectin solubility but also with increased hardness, reduced degree of splitting and consequently reduced water absorbed during cooking.

Storing legume seeds at low temperature and humidity conditions (i.e. refrigeration) could be the answer to inhibit the development of the HTC defect in harvested or normal legume seeds (Hentges *et al.,* 1990; Berrios *et al.,* 1999). However, finding appropriate resources for adequate storage is still a challenge in developing countries; therefore mechanisms to alleviate the HTC defect must still be investigated.

For normal cowpeas, pre-conditioning in water without further micronization is effective, from a practical standpoint, to reduce the cooking time (from 89 to 44 min). The reduction in the cooking time of normal cowpeas as a result of pre-conditioning in water is attributed in part to partial solubilization of pectins in the middle lamella.

Micronization of HTC cowpeas pre-conditioned in a solution with monovalent (Na^{+}) cations reduces the cooking time of HTC cowpeas (*Mogwe-o-Kgotsheng*) by approximately 80%. Pre-conditioning in monovalent (Na^{+}) cations improves the solubility of pectins due to the solubilization effect of water and a conversion of insoluble to soluble pectins by monovalent cations. Micronization improves pectin solubility further by breaking pectin molecules into lower and more soluble fractions, probably via the β-elimination reaction. However, although the combination of these two treatments had more extensive solubilization of pectins as compared with normal cowpeas, this is not the case with the reduction in cooking time. Factors such as protein denaturation and insolubilization, which develops during storage at adverse conditions due to low pH (Liu *et al.* 1992) and starch behaviour during adverse storage, should be considered in future studies in terms of how they affect water absorption properties as well as cell separation

during cooking. The effect of pre-conditioning in a solution with monovalent (Na^+) cations and its combination with micronization on HTC cowpeas protein and starch and its relation with water absorption properties should also be taken into account in future studies.

For future studies, it is recommened that a descriptive sensory panel be used to determine whether HTC cowpeas pre-conditioned in a solution with monovalent (Na^+) cations and micronized would be similar in sensory profiles to that of normal cowpeas. Consumer acceptance studies would also need to be conducted.

The cell wall lignification via binding with phenolic compounds and the pectin insolubilization via binding with divalent cations are believed to be the two direct causes of the HTC defect in legume seeds. It would be interesting to study how pre-conditioning in a solution with monovalent (Na^+) cations in combination with micronization would impact on the components (i.e. cell wall components, phenolic compounds, proteins) involved in the cell wall lignification theory in its entirety.

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