Changes in Hardness of Stored Beans in Relation to Phytate and Dietary Fibre Fraction Contents

by

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

A storage study on beans (Phaseolus vulgaris) was done to determine changes in the total, soluble and insoluble dietary fibre, the ratio of the soluble /insoluble fractions (S/I), and phytate content in relation to hardening during time. Two bean varieties were used, a black variety (Tamazulapa), and a red variety (DOR-364). Beans with two different initial moisture contents (12% and 16%) were stored at 9°, 23° and 36° C, for 24 weeks. Samples were taken every three weeks and hardness was measured after cooking beans for 1 and 2 hours. Phytate, soluble and insoluble fibre contents were determined. Total dietary fibre (sum of soluble and insoluble fibre) and S/I were calculated.

Hardness of both varieties of beans increased when stored at 36° C, regardless of initial moisture content. Temperature was statistically the factor that affected hardness the most. Moisture content sometimes had an effect on hardness and a temperature*moisture interaction was found. Beans with 12% initial moisture content did not harden when stored at 9° C. Red beans with 12% initial moisture content when stored at 9° or 23° C were harder than the 16% initial moisture content, when measured after 1 hour cooking. When these same samples were cooked for 2 hours they showed equal levels of hardness. This was not so for Tamazulapa beans, showing that cooking rates are different for different varieties of beans.

Initial phytate content was higher for the red beans which were also harder than the black beans. Both varieties lowered their phytate content when stored at 36° C, and this effect was increased when the initial moisture content was higher. Moisture content by itself did not have a strong statistical effect on phytate content. Phytate had a negative relationship to hardness when analyzed for varieties, which was not as strong as the ones for fibre fractions. This relationship became a very weak one when analysis was done for all varieties pooled ($r=0.39$).

Total dietary fibre did not change during storage or relate to hardness. Insoluble fibre increased definitely when beans were stored at 36° C, regardless of variety or initial moisture content, whereas soluble fibre decreased for the same samples. Insoluble fibre showed a strong, positive relationship to hardness, regardless of variety, being the next best measurement in relation to hardness.

The S/I ratio, a calculation that has not yet been reported in the literature, was the most sensitive measurement when related to hardness, not only for each variety independently, but for varieties analyzed together.

SUMARIO

Un estudio sobre los efectos del almacenamiento en el frijol común (Phaseolus vulgaris) se llevó a cabo para determinar los cambios en las siguientes fracciones de fibra dietética: total, soluble, insoluble, la proporción soluble/insoluble, y en el contenido de fitatos, en relación con dureza a través del tiempo. Se utilizaron dos variedades de frijol: una variedad negra (Tamazulapa) y una variedad roja (DOR-364). Los frijoles se almacenaron con dos diferentes contenidos de humedad (12% y 16%) a 9°, 23° y 36° C, por un período total de 24 semanas, y se tomaron muestras cada tres semanas. Se determinó la dureza luego de cocinar los frijoles por una y por dos horas. También se determinaron los contenidos de fitato, y de fibra soluble e insoluble. Se calculó el contenido de fibra dietética total (sumatoria del contenido de fibra soluble y de fibra insoluble), y la proporción S/I.

La dureza de las muestras se incrementó cuando estuvieron almacenadas a 36° C, en las muestras con ambos contenidos iniciales de humedad. La temperatura de almacenamiento fue el factor que estadísticamente más afectó la dureza del frijol. El contenido inicial de humedad tuvo algunas veces efecto en la dureza y también se encontro una interacción de temperatura*contenido inicial de humedad. Los frijoles de ambas variedades con una humedad inicial del 12% cuando se

almacenaron a 9° C no presentaron ninguna dureza. Los frijoles rojos con 12% de contenido de humedad inicial que estuvieron almacenados a 9° o 23° C, presentaron una mayor dureza que los con 16% de contenido de humedad inicial, cuando la dureza se determinó después de una hora de cocción. Cuando se cocinaron por dos horas los frijoles presentaron niveles similares de dureza. Esto no fue así para los frijoles negros, demostrando que la velocidad de cocción es distinta para variedades diferentes de frijol.

El contenido inicial de fitato fue más elevado para los frijoles rojos, que a la vez presentaron mayor dureza que los frijoles negros. Ambas variedades disminuyeron su contenido de fitato cuando se almacenaron a 36° C. Este efecto se incrementó cuando el contenido de humedad inicial fue mayor. El contenido de humedad de las muestras no tuvo un efecto estadísticamente marcado por sí mismo en el contenido de fitato. El contenido de fitato presento una relación negativa con dureza cuando el analisis se hizo por variedad, efecto que no fue tan marcado como lo fue para las fracciones de fibra dietética. Sin embargo esta relación llego a ser muy débil cuando el análisis se hizo incluyendo ambas variedades en un solo grupo ($r=0.39$).

La fibra dietética total no presentó ninguna relación con dureza. La fibra insoluble aumento marcadamente cuando los frijoles se almacenaron a 36° C, para ambas variedades, con cualquier contenido inicial de humedad, mientras que la fibra

soluble disminuyó en el mismo grupo de muestras. La fibra insoluble presentó una marcada relación positiva hacia dureza, sin discriminar por variedad, relación que fue la segunda en importancia de las otras determinaciones evaluadas.

La proporción S/I, la cual no ha sido presentada en la literatura a la fecha, fue la determinación mas sensible en relación a dureza, no únicamente por variedad de frijol independientemente, sino también cuando se analizaron las variedades conjuntamente.

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I. INTRODUCTION

Although only about 10 to 12 species out of 13000 of the family **leguminosae** are of economic importance today, consumption of these species is high in some countries. Common beans (Phaseolus vulgaris) are a major source of protein in Latin America. Studies in the Central American subregion show that in Guatemala 95% of the population consumes beans (Rios-Sierra, 1988). In Guatemala the total bean production in 1986 was 121,909 metric tons (Garcia et al, 1988). Beans are essential to the nutritional well-being of large segments of the population because the bean protein complements the staple cereal proteins. Beans, however, are susceptible to the hardening phenomena which results in significant postharvest losses, increases energy expenditures for cooking (Hincks and Stanley, 1987), and results in undesirable flavour and texture, characteristics that deter acceptability (Uebersax and Bedford, 1980; Koehler et al, 1987). This leads to a decrease in crop utilization which causes economic losses and results in diminishing food supply for some segments of the population.

Common beans (Phaseolus vulgaris) are usually harvested with about 20% moisture content in the seeds. Then they are dried to about 10% before storage or consumption to prevent moulding or quality deterioration during storage (Sgarbieri and Whitaker, 1982). In Latin America beans are usually harvested twice a year, so that they have to be stored for at

least six months to maintain a constant supply. High temperatures and relative humidities which are the conditions normally encountered in the area contribute to the hardening of seeds.

Research has been done to find the possible causes for the hardening of beans, but no clear explanation exists. Possible explanations are related to the cell walls of bean cotyledons, but the approaches to solve this problem have not considered the study of dietary fibre and its components. It is logical to study this component of the bean as the dietary fibre in beans is largely composed of the cell wall components, and changes that occur during storage affect them. This approach could improve understanding of the hardening problem.

The general purpose of this research was to determine effects of hardening on the total, soluble and insoluble dietary fibre, the ratios of these components, and phytic acid content in two improved varieties of Guatemalan beans. These beans were stored under different temperatures for predetermined times to produce hardening.

The specific purposes were:

To determine the effect of various storage conditions and length of storage on the harding of different varieties of beans.

To determine the effect of various storage conditions on the total dietary fibre, on the soluble and insoluble

portions of this fibre, and on the proportion of these components, in two varieties of beans.

To determine the effect of three temperatures and two initial moisture contents on the phytic acid content of different varieties of beans during storage.

To correlate any changes in the dietary fibre and phytic acid content to hardening of bean.

To compare any changes in dietary fibre, phytic acid and hardening of the different varieties of beans.

II. LITERATURE REVIEW

2.1 HARDENING OF BEANS

A hard-to-cook defect has been observed in dry legumes when stored at elevated temperatures and humidity (Jones and Boulter, 1983; Varriano-Marston and Jackson, 1981; Sefa-Dedeh et al, 1979). It is different, and not necessarily related to the "hardshell" phenomenon, in which structural characteristics of seed coats are linked to the impermeability of the seed (Varriano-Marston and Jackson, 1981).

2.1.1 The Hard-to-Cook Defect

This defect is described as the inability of bean cotyledons to softening upon cooking (Hincks and Stanley, 1987). Not only do the beans harden, requiring large cooking time, but quality deterioration also occurs. After being subjected to adverse conditions, beans change their structure from the desirable soft, moist and pasty, to hard, lumpy and dry. Besides textural changes, quality deterioration is also related to changes in water absorption, cookability, pectin solubility, phytic acid content, calcium, magnesium and potassium contents, and migration of ions between seed coat and cotyledons occur.

Nutritional quality is affected when beans become hard. Antunes and Sgarbieri (1979), found a decrease in the Protein Efficiency Ratio (PER) of beans with relation to storage

conditions : the higher the storage temperature and relative humidity under which beans were stored, and the longer the storage period, the lower the PER values. More research needs to be done in this area. There also seems to be an effect in cooking time for legumes, above and below which, digestibility is impaired (Rockland and Radke, 1981).

2.1.2 Conditions That Favour the Hard-to-Cook Defect

Observations with light microscopy, and scanning electron microscopy, have shown noticeable changes in the cotyledon cells of hard-to-cook leguminous seeds (Varriano-Marston and Jackson, 1981; Sefa-Dedeh et al, 1979; Hincks and Stanley, 1987). Those changes are primarily failure of the cells to separate as result of cooking (Vindiola et al, 1986).

Cooking affects bean structure in the cotyledon by loosening the middle lamellae so that individual cells separate without the rupture of cell walls. The hard-to-cook legumes fracture or break across the intracellular materials (Stanley and Aguilera, 1985), showing little breakdown in the middle lamella (Sefa-Dedeh et al, 1979). The aging process affects the membrane permeability of beans, resulting in greater loss of solutes from the cotyledons (Jones and Boulter, 1983; Moscoso, 1981).

Length of storage and conditions affect the hard-to-cook defect. The conditions normally encountered in the tropics, that is high temperature and high relative humidities are

likely to cause the defect (Moscoso et al, 1984; Aguilera and Stanley, 1985)). Observations under controlled conditions show that at higher temperatures and humidities, beans harden faster (Hincks et al, 1987; Hohlberg and Stanley, 1987; Jones and Boulter, 1983). This is illustrated in the following figure where cooking times of intact black beans under different storage conditions for different length of time are shown against cooking rates. The samples compared are fresh beans, beans stored for 14 days at 41°C and 100% relative humidity, and beans stored for one year at room temperature:

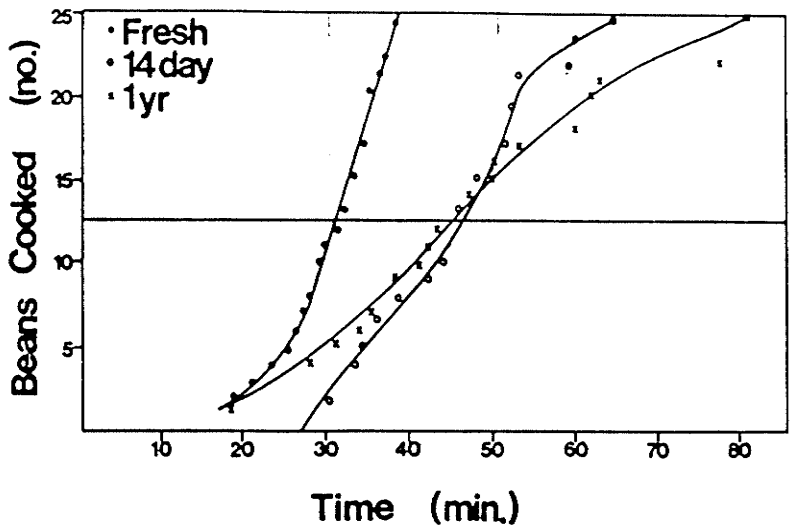

Figure 2.1: Cooking time of intact black beans as affected by storage (Jackson and Varriano-Marston, 1981).

Increase in moisture content due to high relative humidity during storage is one of the key factors in the initiation of hardening (Jones and Boulter, 1983).

2.2 APPROACHES TO UNDERSTAND THE PROBLEM

Based on the need to understand the hardening phenomena, attempts have been made to explain hardening mechanisms. Up to now, several mechanisms have been proposed but a definitive explanation does not exist (Srisuma et al, 1989; Garcia-Vela and Stanley, 1989; Holhberg and Stanley, 1987; Hincks and Stanley, 1987)). A factor that is generally accepted is that this mechanism is at least partially enzymatic (Stanley and Aguilera, 1985). The main proposed mechanisms are discussed in more detail below.

2.2.1 Proposed Mechanisms

2.2.1.1 A breakdown of phytic acid.

Phytic acid (phytate) occurs in plant seeds and grains, roots and tubers. Phytate may play a role in storage of phosphorus, storage of energy and initiation of dormancy. The accumulation site of phytate in dicotyledonous seeds, such as beans, is in the cotyledon (Oberleas, 1973). Phytate is located within the subcellular inclusions of aleurone grains which are one of the inclusions of the protein body. The protein bodies of legumes are surrounded by a lipoprotein membrane and contain crystalline inclusions, or globoids,

which are rich in phytin. The amount of phytate varies from 0.50 to 1.89% in cereals, from 0.40 to 2.06% in legumes, from 2.00 to 5.20% in oilseeds (except soybeans and peanuts which are included in the legumes). Phytate being a strong acid forms a variety of salts of both monovalent and divalent cations, and is primarily present in the seeds in this form. The solubility of most of phytate complexes is pH dependent and tend to be insoluble at physiological pH 6.0 (Reddy et al, 1982).

A good correlation between total phosphorus content and phytic acid ($r^2>0.9$) was observed for 50 varieties of dry beans (Phaseolus vulgaris L) (Lolas and Markakis, 1975). Based on these findings determination of total phosphorus may serve to indicate the amount of phytate. Nevertheless, not only phytate but also inorganic phosphorus, phosphatides and possibly nucleic acids are sources of phosphorus and values could be misleading (Cheryan, 1980).

Phytate has been considered of importance in bean hardening (Moscoso, 1984; Hincks and Stanley, 1986; Jones and Boulter, 1983; Kon, 1979). It has been reported that hard legumes contain less phytate than normal. Hard-to-cook legumes do not soften due to a lack of phytate (Kon, 1979), because a breakdown in phytic acid would inhibit chelation of divalent cations (Ca^{++} and Mg^{++}) allowing these to complex with the free carbonyl groups of pectates. Reduction of the methoxy content of pectates,through the action of pectin

esterase would permit increased bridging of pectin molecules by the divalent cations. Cross-linking of pectins of the middle lamella could prevent cell separation and result in harder beans (Moscoso et al 1984; Stanley and Aguilera, 1985).

2.2.1.2 Degradation of membranes.

The degradation of membranes as a result of phospholipid hydrolysing enzymes which lead to solute leakage has also been stated as a possible mechanism for hardening of beans (Jones and Boulter, 1983). A family of enzymes, the phospholipid hydrolases,are responsible for the membrane breakdown. Phytic acid is located at the lipoprotein membrane of the protein bodies of legumes. It is present as crystalline inclusions, or globoids, and deterioration of the membranes could make phytic acid accessible to phytase.

2.2.1.3 Lignification of the cell wall material

Hincks and Stanley (1987) reported heavy deposition of lignin in the middle lamella and secondary wall in cell wall material of hard-to-cook beans, with the heaviest depositions in the intercellular spaces. Cell walls from beans stored at low temperatures and low humidities exhibited only minimal depositions. These could be observed with transmission electron microscopy and scanning electron microscopy. Lignin is a product of the oxidation and polymerization of phenolic compounds mediated by cell wall bound peroxidase, and phenolic

substrates are present in the bean tissue. Adverse storage conditions cause a false germination reaction which makes phenolic substrates (aromatic amino acids) available as a result of protein degradation. It has been suggested that hardening occurs due to polymerization and complexing of these products (Hincks and Stanley, 1987).

2.2.1.4 Limited intra and intercellular water availability

Cooking of beans is a hydrothermal process which involves gelatinization and swelling of starch, denaturation of protein, solubilization of some polysaccharides and other physical and chemical changes that result in softening of structure and creation of flavour (Vindiola et al, 1986). Protein denaturation and starch gelatinization depend on the intra and intercellular water availability, so that any factor limiting water availability could interfere with the cookability of beans and cause hardening. Garcia-Vela and Stanley (1989) reported that gelatinization temperature of starch increased with storage time, and suggested that this could be explained by changes in the starch granule crystallinity or by the accumulation of low molecular weight compounds such as inorganic phosphate salts, peptides and amino acids. They also reported that protein denaturation was influenced by storage conditions and time, and suggested

that retardation in the thermal denaturation could be caused by a combination of protein hydrolysis and the unavailability of water.

2.2.1.5 Changes in neutral detergent residue

Cell wall material measured as Neutral Detergent Residue (NDR), and a fraction representing hemicelluloses increased in cotyledons and seedcoats after storage under harsh conditions (Rozo, 1982). Nitrogen in the residues also increased in the same samples and the extractability of tannins was reduced. These changes suggest that nitrogen compounds interacting with phenols may be contributing to the increase in cell wall content and hardness of beans.

2.2.2 Cell Wall Structure of Beans

A closer look at cell wall structures is needed, in order to understand the interrelationships among these constituents.

The shape of the cell and its texture is determined by the cell wall, Figure 2.2. Cell wall structures consist of cellulose microfibrils, hemicellulose and lignin. The primary walls of two cells are joined by the middle lamella. The middle lamella is mainly constituted of pectic substances which provide adhesion to hold the plant cells together and give physical strength. Pectic substances are associated with

calcium and magnesium ions. In mature cells a secondary cell and a primary cell wall lie outside of the plasma membrane. This is shown in Figure 2.3.

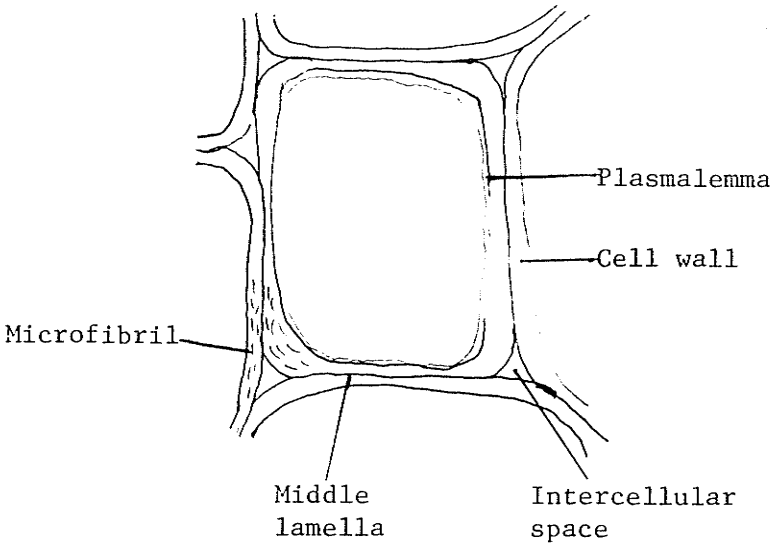

Figure 2.2: Diagram of the cell wall (John and Dew, 1986)

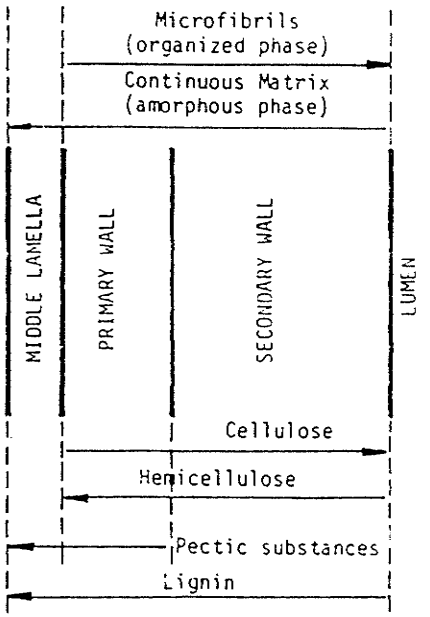

Figure 2.3: Schematic presentation of the cell wall (Rozo, 1982).

2.2.2.1 Components of cell wall

2.2.2.1.1 Cellulose

Cellulose is the major polysaccharide in the cell wall. Structurally it is a ß(1→4)-D-glucan of high molecular weight. It is present in the wall as fibrils. It is rather inert chemically unless the fibrillar structure is disrupted.

2.2.2.1.2 Non-cellulosic polysaccharides

These polysaccharides include water-soluble constituents such as pectins, arabinans, arabino-galactans, arabinoxylans, several gums and mucilages. Components insoluble in water such as xylans and mannans are also among the non-cellulosic polysaccharides of cell wall material. The pectic substances constitute a complex mixture of colloidal polysaccharides formed from pectin (the methyl ester), deesterified pectic acid and its salts (pectates) and certain neutral polysaccharides that form the galacturonan backbone (Stanley and Aguilera, 1985).

2.2.2.1.3 Lignin

Lignin is a component of the cell wall and contributes to structural rigidity. It gives the woody characteristics to fruits and vegetables. Lignins are not polysaccharides but high molecular weight aromatic polymers, which are formed by enzymatic dehydrogenation and subsequent polymerization of coniferyl, sinapyl and p-coumaryl alcohols. Lignification in

plants serves two functions: it cements and anchors the cellulose microfibrils and other matrix polysaccharides; and it prevents biochemical degradation and physical damage to cell walls. Lignin may increase in bean cotyledons as beans harden (Selvedran, 1983).

2.2.3 Dietary Fibre

Plant cell wall material and products derived from plant cell walls form what is termed dietary fibre. Dietary fibre can be defined as "the sum of lignin and polysaccharides that are not digested by the endogenous secretions of the human digestive tract" (Trowell et al, 1976). This material has also been considered as "unavailable carbohydrates" or "non-starch polysaccharides". This definition not only includes all polysaccharides and lignin from the plant cell walls, but also other indigestible polysaccharides such as gums, mucilages and algal polysaccharides. This definition of dietary fibre is not accepted by all scientists. The Expert Advisory Committee on Dietary Fibre of Health and Welfare, Canada (Health and Welfare, Canada,1988) has endorsed the following definition: Dietary fibre consists of "endogenous components of plant material in the diet which are resistant to digestion by enzymes produced by man. They are predominantly non-starch polysaccharides and lignin and may

include, in addition, associated substances". An interesting diagram of the dietary fibre components was presented by Asp and Johansson (1984):

TABLE 2.1: DIETARY FIBRE COMPONENTS

Total Dietary Fibre	Non-starch Polysaccharides (NSP)	Non-cellulosic Polysaccharides (NCP)	Other Polysaccharides	Soluble Fibre	Other Sugar Residues
					Uronic Acids
			Pectin		Rhamnose
					Arabinose
			Hemicellulose	Insoluble Fibre	Xylose
					Mannose
					Galactose
		Cellulose	Cellulose		Glucose
	Lignin	Lignin	Lignin		Lignin

For the purposes of this research, attention will be addressed to dietary fibre from the food point of view, not in relation to nutritional effects of food fibre. The cell wall changes during storage and hardening of beans will be looked at by using methodology developed for dietary fibre analysis, which quantitates soluble and insoluble fibre separately.

Common bean insoluble dietary fibre is mainly composed of celluloses and hemicelluloses. Insoluble dietary fibre consists primarily of cell wall remnants from which starch and protein storage bodies have been removed. Also there are partially digested fragments of the seedcoat palisade cell layer, and long, thin fibres from the nutrient transporting phloem (Hughes and Swanson, 1989).

Common bean soluble dietary fibre is composed primarily of pectic substances. Little is known about structural characteristics of soluble dietary fibre. Hughes and Swanson (1989) described it as "thin, irregularly shaped sheets attached to a framework of long thin rods". Legumes are a better source of soluble dietary fibre, which has a metabolic function in man.

2.2.3.1 Methods of determining dietary fibre

Dietary fibre is not a synonym for crude fibre, which has traditionally been determined to estimate food and feed digestibility. Crude fibre refers to the residue after a food has been sequentially extracted with ether, acid and alkali. This residue contains some of the cellulose and most of the lignin of the digested sample. Due to the variable relationship between dietary fibre and crude fibre in various materials, no standard conversion factor can be used (Asp and Johansson, 1984).

A number of procedures have been developed to determine dietary fibre. The development of the methodology began over 25 years ago but it is only in the past decade that reliable methods and their modifications have appeared. These have been developed because of the interest in dietary fibre and the need for reliable fibre values for foods and food products. These methods can be used to give information useful for understanding the hardening problem.

2.2.3.1.1 Total dietary fibre (TDF)

Analytical procedures for the measurement of TDF can range from a complete fractionation with measurement of all the various species present, to a simplified system involving grouping of different components (Southgate, 1978).

In the 1982 AOAC Spring Workshop it was decided to develop two types of methods to respond to the need for more information on food fibre. One method was developed that would give rapid and reliable information on the total dietary fibre content. A more comprehensive method was developed to determine the individual dietary fibre components. Southgate's method (Southgate et al, 1978), which determines the unavailable carbohydrates, represents a comprehensive approach. This method provides a measure of total dietary fibre as the sum of the components based the information on free sugars. This method does not quantitate the lignin.

The AOAC or Prosky TDF method (Prosky et al, 1984; Prosky

et al, 1985) is a rapid enzymatic gravimetric method to determine fibre. It is based on procedures developed by Asp et al (1983). Prosky's TDF is the sum of soluble and insoluble polysaccharides and lignin. These fractions can also be determined separately by a modification made by Asp et al (1983). To conform to first definition given, which describes dietary fibre as the sum of indigestible polysaccharides plus lignin, this method corrects for the undigested protein in fibre residues.

2.2.3.1.2 The neutral detergent fibre/soluble fibre (rapid gravimetric) method

An alternative method for determining TDF based on quantitation of neutral detergent residue and of soluble fibre has been developed by Mongeau and Brassard (1986). Like the AOAC method this is an enzymatic gravimetric procedure. Although the AACC method does not correct for undigested protein, interlaboratory collaborative studies show that the AACC method was in close agreement with values obtained with the AOAC (r^2=0.98), thus results are comparable, but the AACC method is more precise in terms of repeatability and reproducibility (Mongeau and Brassard, 1990). Health and Welfare Canada and the American Association of Cereal Chemists (AACC, 1988) have accepted this as an official method.

2.3 CARBOHYDRATE AND PROTEIN FRACTIONS AS FACTORS RELATED TO THE HARDENING PROBLEM

2.3.1 Protein and Starch Considerations

Hohlberg and Stanley in 1987 determined the fate of bean intracellular starch and protein during storage, and related starch and protein changes to the development of textural defects (or hardness in beans). The authors used black beans from Chile, with a moisture content of 8.3%. The beans were placed in storage for ten months within a month of harvest. Three different controlled environmental conditions were used for storage: high temperature and high humidity (HTHH) (30° C-85% RH); medium temperature and medium humidity (MTMH) (25°C - 65%); and, low temperature and low humidity (LTLH) (15°C - 35%). These conditions were chosen to resemble tropical, semitropical and temperate climatic conditions. Samples were taken from storage at 1, 2, 3, 4, 6, 8 and 10 months. Hardness of cooked beans was significantly different for the three treatments after the third month of storage. Conditions did not affect the melting temperature of starch, or the gelatinization energy, but both were affected by time, suggesting that a chemical or structural change occurs in bean starch which is independent of the environment. Other studies done in relation to starch gelatinization (Hincks et al, 1987; Garcia-Vela and Stanley, 1989) of beans stored at 30° C 85% RH; and 15° C 35% RH, showed hardening for the samples in high temperatures and high humidities. Water uptake was also

reduced for these samples and structural changes were observed, that revealed partially ungelatinized starch granules in hard-to-cook cooked beans (Hincks et al, 1987). Storage conditions and time had an effect on gelatinization enthalpies (Garcia-Vela and Stanley, 1989) increasing them. Differences between Hohlberg and Stanley (1987) and Garcia-Vela and Stanley (1989) can be explained by the way starch was studied. First mentioned researchers chemically isolated the starch, and the latter used whole raw or cooked ground beans. Since the method of determining these enthalpies was Differencial Scanning Calorimetry (DSC), isolated starch and whole beans could be expected to give different results.

The protein extracts by Hohlberg and Stanley (1987) showed no significant difference in enzyme activity with time or between environments, but a significant increase in the percent free aromatic amino acids was determined as a function of storage conditions. The authors suggested that there could be a relation between appearance of free aromatic amino acids and the hard-to-cook defect. It was theorized that these fractions might lead to polyphenol synthesis. Hydrolysis of storage proteins by proteases could be responsible for increased amounts of phenolic amino acids, but the authors did not find significant enzyme activity during storage.

2.3.2 Cell Wall Chemical Considerations

Rozo in 1982 studied the effect of extended storage under

simulated tropical conditions of high temperature and high relative humidity on cell wall constituents and polyphenolic compounds of red kidney beans, and related changes to the loss of cookability. Red kidney beans (Phaseolus vulgaris) of the Redkloud variety were used for this study. These were stored under three different regimes: 0°C in sealed containers, and 30°C and 40°C at 80% relative humidity. Three replicates of the beans were stored for eight months in the same chamber. Analyses were done at 0, 2, 4, 6 and 8 months. Moisture determinations of whole beans were done monthly. Cooking time was evaluated by puncture tests. Total nitrogen of seed coats and cotyledons, Neutral Detergent Residue (NDR), Acid Detergent Residue (ADR), condensed tannins and total phenol analyses were done.

Hardness increased with storage time. Storage of beans at high temperatures and relative humidities caused changes in hardness. Darkening of colour in seed coats was evident at 30° and 40°C. Neutral Detergent Residue, a measure of insoluble fibre, increased significantly from 5 to 8% of total cotyledon after eight months of storage at 40° C. No changes occurred in samples stored at 30° or 0°C, as can be seen in the following table:

TABLE 2.2: EFFECT OF STORAGE TIME CONDITIONS ON CELL WALL CONTENT (NDR) OF COTYLEDONS (g/100 g± 0.50 DWB)

Storage time (months)	Treatment		
	0°C-control	30°C - 90% RH	40°C - 80% RH
0	4.99	4.99	4.99
2	4.60	5.48	5.64
4	4.54	4.48	6.50
6	4.57	5.10	8.02
8	4.41	4.99	8.09

Source: Rozo, C. (1982)

The statistical analysis showed a high correlation of NDR with moisture content and hardness of whole beans. No significant changes occurred in cotyledons for lignin and cellulose content. The fraction NDR-ADR which measures hemicellulose increased significantly and correlated highly with hardness.

Lignin and cellulose in the seedcoat cell wall contents increased at 40°C. Nitrogen associated with the NDR increased significantly for beans stored at 30° and 40°C. The extractability of condensed tannins soluble in methanol and methanol-water decreased significantly during storage for samples at 30° and 40° C. The decrease in extractable tannins correlated with increases in moisture contents and hardness of whole beans.

The high correlation between NDR in cotyledons and

hardness of whole beans (r=0.86), and between NDR in seedcoats and hardness of whole beans (r=0.87) at 40°C suggest a synthesis of compounds that increase the NDR and that are related to hardness. Other investigators (Sada, 1980) have found decreases in total soluble carbohydrates, water soluble polysaccharides, starch, stachyose, raffinose and tryptophan in red kidney beans stored for eight months at 30° and 80% relative humidity.

Dietary fibre and specifically insoluble and soluble dietary fibre components are the aspects that need to be further studied in relation to hardness of beans.

Hardness correlated positively to NDF and negatively to soluble fibre in the black bean (Watts et al, 1990). No other comparisons of fibre fractions to hardness have been done. Change in soluble and insoluble fibre in beans due to cooking were reported by Hughes and Swanson, (1989).

A number of hypotheses have been suggested to explain the hard-to-cook phenomenon. Evidence for each of the hypotheses has been described but the actual series of reactions responsible for hardening has not been defined. Research on the possible factors affecting the hardening process is an ongoing one because still there is the need to identify the role of each of these factors in this process, and to clarify their mode of interaction.

III MATERIALS AND METHODS

3.1 MATERIALS

3.1.1. Beans

Two varieties of common beans (Phaseolus vulgaris), were obtained in Guatemala after the August 1988 harvest. Black beans, var. Tamazulapa, were bought from farmers in Atescatempa, Jutiapa. Red beans, Line DOR-364 were obtained from the CIAT-ICTA research station in Jutiapa. The area of Jutiapa, where the beans were grown, is approximately 900 meters above sea level and the temperature ranges from 15° to 29°C.

3.1.2 Storage Procedure

The fresh beans had an initial moisture content of approximately 20%. They had not been sun dried due to a heavy rainy season. The beans were cleaned by removing broken seeds and extraneous materials such as stalks, pods, soil, etc. They were air dried in a well ventilated area indoors until the moisture was lowered to 16% as determined with a Dole 400-B Moisture Tester[1]. Beans were turned and mixed twice each day. Half of each variety of beans was packaged at 16% moisture, while the remaining half was dried to 12% moisture before packaging. Beans were packaged in 100 gm portions in sealed pouches made of transparent polyethylene 8 X 10^{-3}

[1]James Dole Corporation, 1400-T Industrial Way, Redwood City, CA 94063

inches (0.2 mm) total thickness, in batches of approximately 100 g per pouch. The packaged beans were stored at 9°, 23° or 36° C for 24 weeks and were withdrawn from storage at three week intervals for testing. Beans of each variety at the two moisture levels, were tested at the beginning of the study ("0" time), and at three week intervals over a 24 week storage period. Immediately after the beans were removed from storage, moisture and hardness determinations were made. The beans were frozen until the chemical analyses were completed.

3.2 EXPERIMENTAL DESIGN

The following table (Table 3.1) shows the sampling design for the study:

TABLE 3.1: SAMPLING DESIGN FOR HARDNESS AND PHYTATE DETERMINATIONS

Variety	Temp °C	Moisture Content	Weeks of Storage 0	3	6	9	12	15	18	21	24
Tamazulapa	9	12 %	x	x	x	x	x	x	x	x	x
Tamazulapa	23	12 %		x	x	x	x	x	x	x	x
Tamazulapa	36	12 %		x	x	x	x	x	x	x	x
Tamazulapa	9	16 %	x	x	x	x	x	x	x	x	x
Tamazulapa	23	16 %		x	x	x	x	x	x	x	x
Tamazulapa	36	16 %		x	x	x	x	x	x	x	x
DOR364	9	12 %	x	x	x	x	x	x	x	x	x
DOR364	23	12 %		x	x	x	x	x	x	x	x
DOR364	36	12 %		x	x	x	x	x	x	x	x
DOR364	9	16 %	x	x	x	x	x	x	x	x	x
DOR364	23	16 %		x	x	x	x	x	x	x	x
DOR364	36	16 %		x	x	x	x	x	x	x	x

Moisture content, hardness values after one and two hour-cooking times, and phytate content were determined for both types of beans at all sampling periods.

The sampling design for the dietary fibre analyses is shown in Table 3.2. Tamazulapa beans for all treatments and all storage periods were analyzed for dietary fibre fractions. The DOR-364 samples for all treatments after storage for 0, 9, 18 and 24 weeks were analyzed for dietary fibre fractions.

TABLE 3.2: SAMPLING DESIGN FOR DIETARY FIBRE DETERMINATIONS

Variety	Temp	Moisture	Weeks of Storage								
	°C	Content	0	3	6	9	12	15	18	21	24
Tamazulapa	9	12 %	x	x	x	x	x	x	x	x	x
Tamazulapa	23	12 %		x	x	x	x	x	x	x	x
Tamazulapa	36	12 %		x	x	x	x	x	x	x	x
Tamazulapa	9	16 %	x	x	x	x	x	x	x	x	x
Tamazulapa	23	16 %		x	x	x	x	x	x	x	x
Tamazulapa	36	16 %		x	x	x	x	x	x	x	x
DOR364	9	12 %	x			x			x		x
DOR364	23	12 %				x			x		x
DOR364	36	12 %				x			x		x
DOR364	9	16 %	x			x			x		x
DOR364	23	16 %				x			x		x
DOR364	36	16 %				x			x		x

Weight of 100 seeds, seed coat percent, dry matter (moisture content), protein and ash content, seed size and seed size distribution were determined for the Tamazulapa and DOR-364 at the beginning of the storage study. The measurements, except for seed size and seed size distribution, were repeated on the samples stored 24 weeks, for each storage treatment.

3.3 METHODS

3.3.1. One Hundred Seed Weight

Three random samples of more than 100 seeds were removed from the whole batch of beans. Each sample was weighed in an analytical balance (precision 0.1 g), and beans were counted. The weight was divided by the number of beans and multiplied

by 100. The three values obtained were averaged to give the mean weight of 100 seeds in "as is" moisture basis.

3.3.2 Seed Size

The volume of 100 randomly selected unsoaked beans was measured by displacement of amaranth seeds as described by Elias et al (1986). The amaranth seeds (or any small type of seed such as Salvia hispanica) were placed in a graduated cylinder, which was tapped twice on the bottom to make sure there were no empty spaces, and the level recorded. Half the amount of the measured seeds was emptied and the beans were placed in the cylinder along with the other seeds. The cylinder was filled again with enough of the previously measured seeds to reach the recorded level. The remnant of displaced seed was then measured. The mean of the three volumes of displaced seeds (in mL) was divided by 100. This value was compared to the standards for beans.

3.3.3 Seed Size Distribution

This was determined by using a 1.5 kg sample of unsoaked beans and a stackable set of six sieves[2] with the following sizes of CEA Simon-Day Ltd meshes:

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[2]The Clipper Grain Seed and Bean Cleaners. Manufactured by A.T. Ferrel $ Co. Saginaw, MI.

TABLE 3.3: MESH SIZES USED FOR DETERMINING BEAN SIZE DISTRIBUTION

Number of Mesh	Size in Inches	Size of Grain
#1	14/64 X 3/4	large size grain
#2	13/64 X 3/4	large size grain
#3	3/16 X 3/4	large size grain
#4	11/64 X 3/4	medium size grain
#5	10/64 X 3/4	medium size grain
#6	9/64 X 3/4	medium size grain
#7	>9/64 X 3/4	small size grain

Beans were placed on the top sieve of the stack, which was shaken with an agitator, for 15 minutes. The weight of beans remaining on each sieve and in the bottom pan was measured to the nearest gram. Percentage was calculated from the original total weight and beans were classified according to size, as described in Table 3.3.

3.3.4 Percent Seedcoat

Percent seedcoat was measured as the weight of the dried seedcoats of 25 seeds in relation to the dried weight of cotyledons plus seedcoats and expressed as percent. The procedure followed is described by Elias et al (1986). Beans were soaked for 18 hours at room temperature in 50 mL distilled water, then were dried with a paper towel and the seedcoat removed manually. Both cotyledons and seedcoats were dried in a vacuum oven at 105°C and 25 mm Hg for 4 hours. The

dry weights of each of the parts was measured with a precision scale after cooling in a desiccator.

The value was calculated applying the following equation:

$$\% \text{ Seedcoat} = \frac{\text{Weight of dry seedcoat}}{\text{Wt dry cotyledon + Wt dry seedcoat}} \times 100$$

3.3.5 Moisture Content

3.3.5.1 For the chemical analyses

For the chemical analyses the moisture was measured using AOAC method 14.004 (AOAC, 1975).

3.3.5.2 For storage purposes

Moisture content was determined by utilizing a Dole 400-B Moisture Tester.

3.3.6 Protein Content

This was determined using a Kjeltec Analyzer from Tecator[3] (Model Auto 1030 Analyzer), by AOAC Method 47.021 (AOAC, 1975). The protein factor used was 6.25.

3.3.7 Hardening Tests

Sixty grams of beans were soaked in 300 mL distilled water at 23°C for 18 hours. Soaking water was discarded. Beans were cooked in boiling distilled water (temperature approximately 95°C) for 1-hour and duplicate weighed

[3]Tecator AB, P.O.Box 70, S-263 01 Höganäs, Sweden

subsamples of 30 grams drained beans were set aside. The remaining beans were left boiling to complete 2 hours of cooking, and duplicate weighed drained subsamples of 30 grams each were taken. Cooked beans were allowed to cool for one to two hours, and then hardness was measured with an Ottawa Texture Measuring System (OTMS)[4] using a 10 cm^2 extrusion cell[4] and a 454.5 Kg (1000 lb) loadcell. The OTMS was connected to an Apple II-E system.

The OTMS was calibrated to give an experimental maximum force of ±90.0 Kg. The experimental time was set at 30 seconds. The cross head speed at 6.60 cm/min and the plunger was calibrated to descend to 1 mm. Required calibrations were done every time before starting the compression/extrusion test in each sample. Hardness was measured as the peak force in newtons to extrude the 30 gram cooked sample, using the ESRI Texture Program (1986)[5].

The soaking, cooking and testing procedure was repeated on the following day to give replicate values.

3.3.8 Dietary Fibre

Soluble, insoluble and total dietary fibre were analyzed by AACC Method 32-06 (AACC, 1983; Mongeau and Brassard, 1986).

Using the method soluble dietary fibre was determined by

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[4]Canners Machinery Limited, P.O. Box 190, Simcoe, Ontario

[5]**Engineering and Statistical Research Institute (ESRI), Canada** Agriculture, Ottawa.

first autoclaving the sample, which was then treated sequentially with amylase (heat-stable amylase -A-0164, Sigma Chemical Co), amyloglucosidase (Fiberzym kit 7367503, Novo Biolabs) and protease (Fiberzym kit 7367503, Novo Biolabs) to remove starch and protein. Soluble fibre was then precipitated using 80% ethanol, the precipitate isolated by filtration and the filtrate dried and weighed. A shaking water bath (Model 1024) and a filtration module (Model 1023) from Tecator[2] were used. The dried residue was ashed in a muffle furnace at 500°C for 4 hours, and the weight of the ash deducted from the weight of the residue to give the weight of soluble fibre in the sample. The following equations were used to calculate soluble fibre:

$$\text{Wt of blank} = (\text{crucible} + \text{blank}) - (\text{crucible} + \text{ash})$$

$$\text{Wt of soluble fibre} = [(\text{crucible} + \text{fibre}) - (\text{crucible} + \text{ash})] - \text{wt blank}$$

$$\% \text{ soluble fibre} = \frac{\text{Wt soluble fibre}}{\text{Wt of sample}} \times 100$$

The insoluble dietary fibre portion was determined as a neutral-detergent residue supplemented with pancreatic alpha-amylase (Type VI-A, Sigma Chemical Co. A-6880; Type VI-B, A-3176 is equivalent), using a hot extractor (Model 1020) and a cold extractor (Model 1020) from Tecator[3].

$$\% \text{ NDF} = \frac{(\text{Wt crucible + residue}) - (\text{Wt crucible + ash})}{\text{Wt sample}} \times 100$$

All values obtained were corrected to percent dry weight. Tests were performed in duplicate, and where means had standard deviations higher than 0.6 (g/100g), the determinations were repeated.

Total Dietary Fibre was calculated as the sum of the soluble and insoluble fractions.

The ratio of the soluble to the insoluble fibre fractions was also calculated:

$$\text{SI Ratio} = \frac{\% \text{ Soluble Fibre}}{\%\text{NDF}}$$

3.3.9 Phytate

The phytate content of the samples was determined by the method of Latta and Eskin (1980). One gram samples were first extracted with 20 mL 0.65 N HCl and after shaking for one hour, samples were centrifuged. Five mL of supernatant were diluted to 25 mL and were passed through a AG1-X8 ion-exchange resin filled column and cleaned with 10 mL of 0.1 N NaCl. Phytate was eluted with 10 mL 0.7 N NaCl. Actual phytate content is measured in the by using Wade reagent (Wade and Morgan, 1955). After centrifugation the absorbance was measured at 500 nm.

3.4 STATISTICAL ANALYSIS

For the analysis of the data the Statistical Analysis System Version 5 (SAS, 1985), and the main frame computer of the University of Manitoba were used. The Statistical Advisory Service of the University of Manitoba was consulted on use of the appropriate statistical techniques.

Pearson's correlation coefficients were calculated using PROC CORR, between mean values of phytate, soluble, insoluble, total and Soluble/Insoluble ratio of dietary fibre and hardness measured as 1-hour and 2-hour cooking, for pooled data, and by bean variety. Probabilities for the test of the null hypothesis have been reported.

Split plot analysis of variance was used to obtain dietary fibre, phytate and hardness main and time effects for both the pooled data and the data sorted by bean variety. All repetition terms were pooled into a common error term under the assumption that there was no interaction between replicates and treatment or time.

IV RESULTS AND DISCUSSION

4.1 INTRODUCTION

Results presented here are organized as follows: a general description of the initial physical characteristics and proximate composition of fresh beans, and a final general physical and chemical determinations of beans done at the end of 24 weeks of storage. The storage study results are presented then: hardness of beans, phytate content changes, and dietary fibre fractions changes. The fibre fractions are total dietary fibre (the sum of the insoluble and soluble fractions), soluble fibre, insoluble fibre and soluble/insoluble ratio. These results are presented for Tamazulapa beans, then for the DOR-364 beans, and then for the samples pooled into one group. The samples were pooled in order to integrate, to interpret the results and compare them on the published data.

4.1.1. Initial Physical and Chemical Characteristics of Beans

At the beginning of the study, before any experimental activity was carried out, some physical determinations and proximate composition analyses were done in order to characterize the samples. The summary of the results from these determinations is presented in Table 4.1.

TABLE 4.1: INITIAL PHYSICAL AND CHEMICAL DATA

FOR TAMAZULAPA AND DOR-364 BEANS

Type of Data	Tamazulapa		DOR-364	
	Mean	SD[1]	Mean	SD
1. Physical:				
100 seed weight (g)[2]	18.42	0.19	18.51	1.16
Seed size distribution:				
% large[3]:	39.16		4.09	
mesh 1		1.36		0.03
mesh 2		6.81		0.38
mesh 3		30.99		3.68
% medium[3]:	60.22		94.40	
mesh 4		45.72		44.78
mesh 5		11.44		36.65
mesh 6		3.06		12.97
% small[3] (mesh 7):	0.62		1.51	
100 seed volume (mL)	18.33	1.15	18.33	0.58
% Seedcoat	9.53	0.18	10.72	0.28
2. Composition[4]:				
Protein	21.2	0.27	23.3	0.97
Fat	3.1	0.28	2.4	0.18
Ash	5.0	0.8	5.0	0.8
Carbohydrates (by dif.)	70.7		69.3	
Total Dietary Fibre	20.4	0.10	19.9	0.25

[1] SD = standard deviation
[2] "as is" moisture basis - approximately 12% for both varieties
[3] Total
[4] Dry matter basis

4.1.1.1 Physical characteristics

4.1.1.1.1 One hundred seed weight

The 100 seed weights were 18.42 grams for the Tamazulapa beans, and 18.51 grams for the DOR-364 beans. The means for the 100 seed weight were almost the same for the two varieties, but the DOR-364 sample had a much higher standard deviation. Standard deviation for the Tamazulapa beans was 0.19 grams but for the DOR-364 was 1.16 grams. It was observed during the weighing process that some of these seeds seemed heavier than others of the same size. This might have been responsible for the differences in the standard deviations.

4.1.1.1.2 Seed size distribution

Seed size distribution is shown in Table 4.1 Both varieties had their largest population in the medium range (60.22% for Tamazulapa, and 94.49% for the DOR-364). However, for Tamazulapa a large segment of the grains were in the large range (39.16%), whereas for the DOR-364 beans most of the seeds belonged on the medium range. Large seeds constituted only 4.09% of the bean population. The Tamazulapa had less seeds in the small region than the DOR-364 (0.62% for the Tamazulapa in comparison to 1.51% for the DOR-364).

4.1.1.1.3 One hundred seed volume

Seed size measured as displacement of amaranth seed was

the same for both varieties (18.33 mL) as shown in Table 4.1. The standard deviations differed, 1.15 mL for Tamazulapa and 0.58 mL for DOR-364. Using this method, any seed below 19.19 is considered small (Elias et al, 1986), so both varieties would be considered in the small seed group.

4.1.1.1.4 Percent seedcoat

The Tamazulapa beans had 9.53% and DOR-364 beans had 10.72% seedcoat. DOR-364 seedcoats were heavier than the ones observed for the Tamazulapa beans. This characteristic is usually described by Guatemalan consumers for red bean varieties. Red beans are generally considered to have "tougher" seedcoats. The characteristic consumers may be describing is heavier seedcoats. Standard deviations for percent seedcoat were not large for either variety, 0.18% for Tamazulapa, and 0.28% for the DOR-364.

4.1.1.2 Proximate composition

4.1.1.2.1 Moisture content

The freshly harvested Tamazulapa beans had 20% and DOR-364 beans had 17.4% moisture content. Beans had not been dried to the usual levels for marketed beans due to a heavy rainy season. Beans were dried in the laboratory to the desired moisture content levels by air-drying them in the shade.

4.1.1.2.2 Protein content

Both varieties had the protein content in the normal range for Phaseolus vulgaris. The DOR-364 had 23.3% protein on a dry matter basis. The Tamazulapa beans had 21.2% protein content on a dry matter basis.

4.1.1.2.3 Fat content

The Tamazulapa beans had a slightly higher content of fat (2.5%) than the DOR-364 (2.0%), but within the expected normal range.

4.1.1.2.4 Ash content

Ash content was 5.0 % grams for both varieties on a dry basis.

4.1.1.2.5 Carbohydrate

Carbohydrate was calculated by difference. There was a similar amount for both types of beans (70.7% for Tamazulapa and 69.3% for DOR-364). This was the result of the slight differences in protein and fat.

4.1.1.2.6 Total dietary fibre

Fresh samples of both bean varieties had about the same amount of total dietary fibre. Total dietary fibre content was 19.9% for the DOR-364 and 20.4% for the Tamazulapa on a dry basis.

4.1.2 Final Characterization

Physical determinations and compositional analyses done after samples had been stored for 24 weeks, are shown in Table 4.2.

TABLE 4.2: PHYSICAL AND CHEMICAL DATA FOR TAMAZULAPA AND DOR-364 BEANS STORED FOR 24 WEEKS

Storage Condition[1]		100 Seed Weight (g)		Seedcoat[2] %		Protein[2] g/100g		Ash[2] g/100g		Moisture Content %	
IMC	T	Mean	SD[3]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tamazulapa											
12	9	18.98	0.30	9.41	0.30	21.6	0.4	4.8	0.4	11.0	0.2
12	23	19.19	0.32	9.50	0.16	21.8	0.1	4.8	0.1	11.0	0.0
12	36	18.39	0.41	10.21	0.14	22.0	0.2	4.6	0.1	9.4	0.0
16	9	19.73	0.12	9.37	0.17	21.9	0.1	4.8	0.1	13.6	0.3
16	23	19.63	0.12	9.68	0.30	22.2	0.3	4.6	0.2	12.5	0.1
16	36	18.97	0.40	10.08	0.19	22.2	0.1	4.4	0.1	10.1	0.1
DOR-364											
12	9	18.43	0.23	11.83	0.77	22.3	0.1	4.6	0.4	11.2	0.0
12	23	18.58	0.14	10.55	0.13	22.0	0.0	4.4	0.2	10.7	0.1
12	36	18.17	0.17	10.64	0.05	22.0	0.0	4.2	0.4	9.1	0.0
16	9	19.26	0.24	10.73	0.14	22.1	0.1	4.6	0.3	13.2	0.0
16	23	19.31	0.11	10.90	0.16	22.3	0.1	4.5	0.1	12.0	0.1
16	36	18.53	0.19	10.90	0.11	23.0	0.1	4.7	0.1	10.2	0.0

[1] IMC = initial moisture content, %; T = temperature, ° C.
[2] Dry matter basis
[3] SD = standard deviation

4.1.2.1 Physical determinations

4.1.2.1.1 One hundred seed weight

Lower values were observed for the higher temperature treatments (36° C). Higher temperatures caused greater moisture losses because the packaging was not totally impermeable, and so the lower 100 seed weights reflected the lower moisture content of the samples after high temperature storage.

4.1.2.1.2 Percent seedcoat

At the end of 24 weeks Tamazulapa beans had an increase in the seedcoat percent for samples stored at the higher temperature (36° C), as compared to samples stored at lower temperatures (9° and 23° C). Seedcoat percent of the DOR-364 beans did not change with storage. The value for the DOR-364 samples stored at 9° C, 12 % initial moisture content was higher than the Tamazulapa beans. The DOR-364 beans had an initial high seedcoat percent and at the end of the study this difference was maintained.

An increase in percent seedcoat, as a result of harsh storage conditions for different black bean varieties, was reported by Rios-Sierra (1988). Samples stored at high temperatures for long periods had about 12% seedcoat and, recently harvested samples, kept under refrigeration had about 9%. Sereda (1989) also reported increases in seedcoats of black beans. Fresh samples had a mean of 9% seedcoat and

samples stored at high temperature-high humidity conditions for 6 weeks had a mean of 10% seadcoat. Higher percent seedcoat could be related to lower 100 seed weights after severe storage, and to a slight reduction in cotyledon weight.

4.1.2.2 Chemical determinations

4.1.2.2.1 Moisture content

At the end of 24 weeks both bean varieties, regardless of initial moisture content, showed a decrease in percent moisture as measured using the AOAC method 14.004 (AOAC, 1975). Decreases occurred at all three temperatures but were more marked for the beans stored at 36° C. For both varieties moisture content fell by approximately 5.8% for samples with an initial 16% moisture content, and fell approximately 2.8% for samples with an initial 12% moisture content. It was apparent that the plastic packaging allowed some moisture to escape, and the loss was greater at the higher temperatures.

Moisture content of the stored samples was monitored every three weeks with a Dole Moisture Tester when samples were taken for the experimental analyses (Appendix A). This tester does not register moisture contents below 11.18%, so the samples which started the study with 12% moisture content could not be read after reaching the 11.18% level. The Dole moisture tester is not an analytical piece of equipment, but

the monitored data of percent moisture of stored beans showed a drastic drop in the samples stored at 36° C, sometime between 9 to 12 weeks of storage.

4.1.2.2.2 Protein and ash content

The protein and ash content of the stored samples did not change significantly for either variety, regardless of the conditions under which samples were stored.

4.2 STORAGE STUDY OF BEANS

The storage results comprise all of the experimental data. Storage results are presented and discussed first in relation to hardness of each bean variety (Tamazulapa and DOR-364); then in relation to phytate content, and lastly all of the information for dietary fibre fractions is presented. In each section the information is presented for individual varieties first, and then analyses for the pooled data are shown and discussed.

4.2.1 Hardness of Beans

Hardness of the beans was tested after both a 1-hour and a 2-hour cooking period. Hardness was measured as the peak force in newtons recorded during compression and extrusion of 30 gram bean samples, using a 10 cm^2 testing cell of an Ottawa Texture Measuring System. Tests were conducted at the beginning of the storage study and then at three week

intervals over 24 weeks. Hardness results are presented first for Tamazulapa beans and then for the DOR-364 beans. Statistical analysis of the data for each variety and for the pooled data are also presented.

4.2.1.1 Tamazulapa

4.2.1.1.1 Hardening curves

Hardening across time of Tamazulapa beans is shown for each storage temperature (9°, 23° and 36° C) and each cooking time (1 and 2 hours). Figures 4.1 and 4.2 show results for samples with an initial moisture content of 12%. Figures 4.3 and 4.4 show hardening patterns for samples with and initial moisture content of 16%. The data supporting these figures are presented in Appendix B.

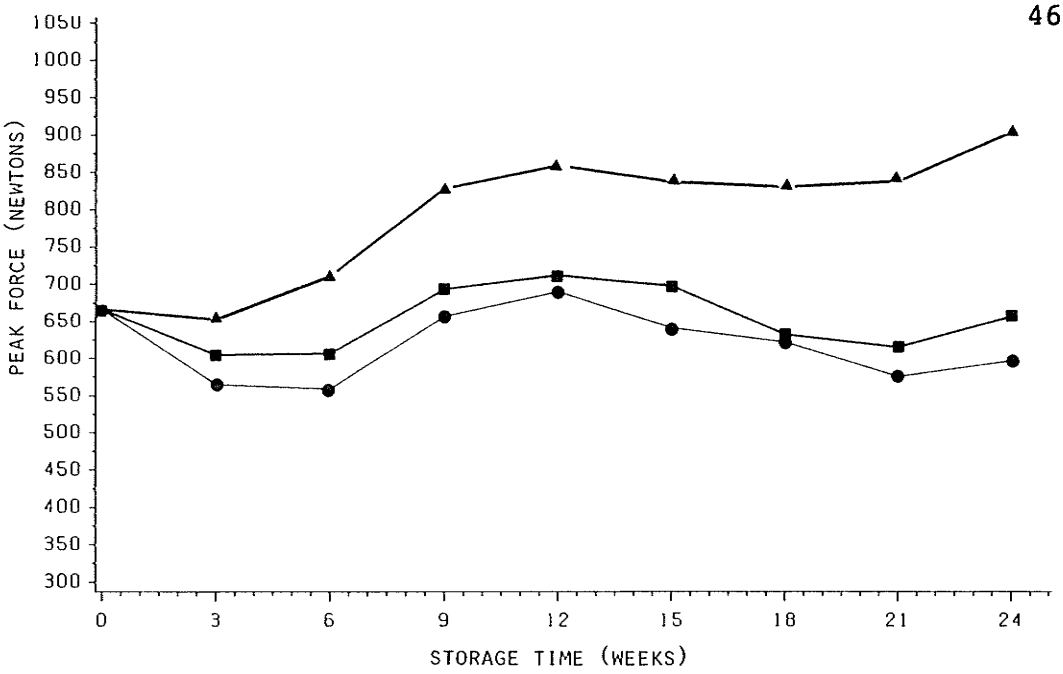

Figure 4.1: Hardness of Tamazulapa beans measured after one hour cooking time. Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

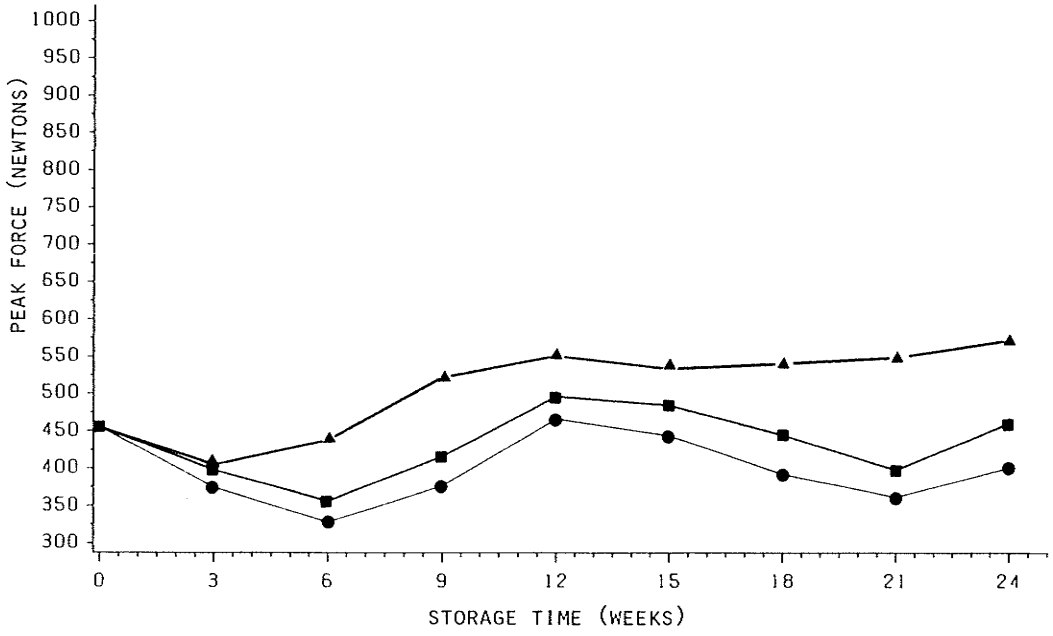

Figure 4.2: Hardness of Tamazulapa beans measured after two hour cooking time. Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

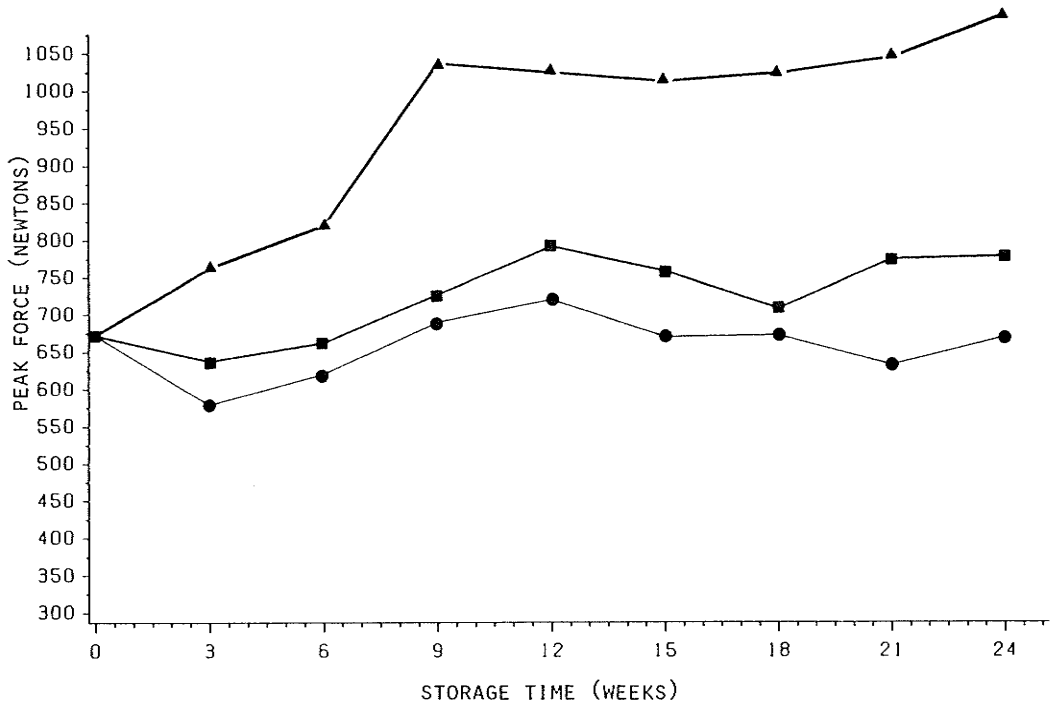

Figure 4.3: Hardness of Tamazulapa beans measured after one hour cooking time. Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

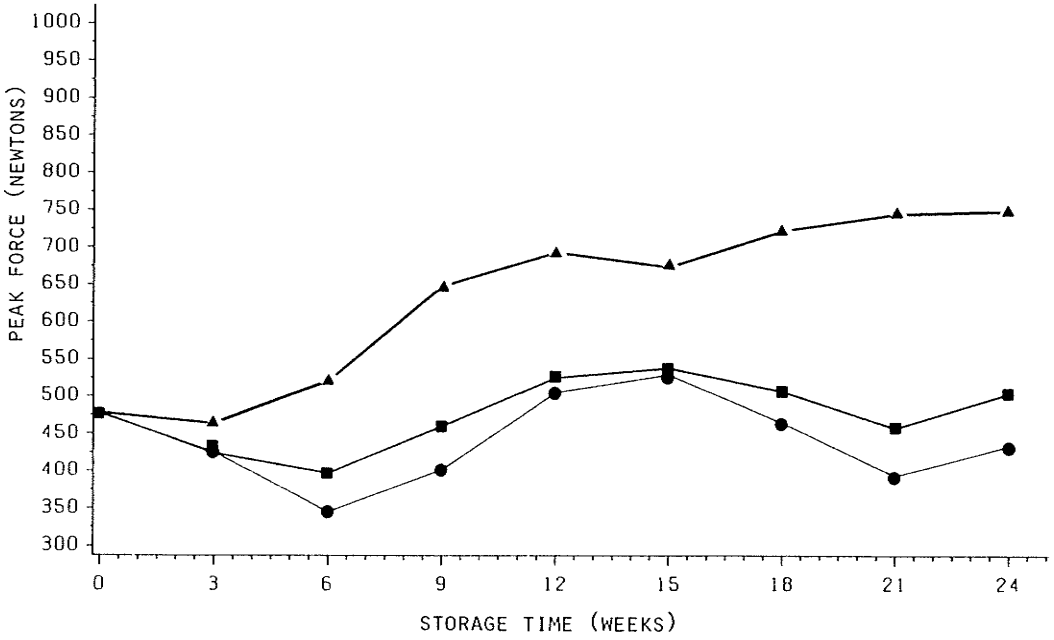

Figure 4.4: Hardness of Tamazulapa beans measured after two hour cooking time. Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

The actual values for hardness were different for samples cooked for 1 hour (Figures 4.1 and 4.3) than for samples cooked for two hours (Figures 4.2 and 4.4). Nevertheless, all figures show similar hardening patterns regardless of initial moisture contents. Beans with an initial moisture content of 16% had higher hardening curves, at all temperatures, than the ones with an initial moisture content of 12%. This was more apparent for samples cooked for 1 hour (Figures 4.1 and 4.3), than for samples cooked for 2 hours (Figures 4.2 and 4.4).

The four figures show a definite increase in hardness for beans stored at 36° C regardless of the initial moisture content and cooking time. Figures 4.3 and 4.4 show that at 23° C beans with the higher initial moisture content hardened to some extent, whereas the ones with an initially lower moisture content (Figures 4.1 and 4.2) did not appear to harden.

The beans seemed to harden more rapidly at the beginning of the study than at the end, as indicated by the changing slopes of the hardening curves. The monitoring of the changes in moisture content during the storage period showed a drop in the moisture of the samples with 16% initial moisture content when stored at 36° C. This set of samples is the one that showed this change in hardening patterns most clearly, and the change in hardening rate seemed to occur after 12 weeks of storage. It was at this point that the samples suffered the greatest drop in their moisture content (Appendix

A). Samples with an initially low moisture content could not be monitored as moisture fell below 11.18% and the Dole Moisture Tester could not measure it.

4.2.1.1.2 Statistical analysis

The data were analyzed for the effects of temperature, moisture content, storage time (as weeks), and for interactions of temperature*moisture content, temperature*week, moisture content*week, temperature*moisture content*week. When considering changes across storage time all repetition terms were pooled into a common error term under the assumption that there was no interaction between replicates and treatment or time. The results of this analysis is presented in Table 4.3.

TABLE 4.3: HARDNESS MAIN EFFECTS AND TIME EFFECTS FOR TAMAZULAPA BEANS[1]

	1-Hour Cooking		2-Hour Cooking	
Effects	F	p	F	p
Main effects				
Moist. Cont.	190.56	.0001	343.29	.0001
Temperature	485.16	.0001	574.13	.0001
MC/Temp	29.23	.0001	54.95	.0001
Time effects				
Week	45.23	.0001	96.91	.0001
Week/MC	4.05	.0002	5.78	.0001
Week/Temp	12.00	.0001	23.38	.0001
Wk/MC/Temp	0.89	.5839	2.75	.0008

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

The moisture content, temperature and moisture content*temperature were highly significant regardless of cooking time (p=.0001 in all cases) for Tamazulapa beans. As shown in the previous figures (4.1 - 4.4) at each temperature the curves were higher for the beans stored at 16% moisture content, than for beans stored at 12% moisture content. Moisture content*temperature interaction can be clearly observed from the greater hardening of the samples stored at 36° C with an initial moisture content of 16%.

All the variables included in storage time effects were highly significant at p<.0002, with the exception of the week*moisture content*temperature (F 0.89, p=.5839) when beans

were cooked for 1 hour. This confirmed that significant changes in hardness occurred over the storage period, related to the conditions of storage in this experiment.

4.2.1.2 DOR-364

4.2.1.2.1 Hardening curves

Hardening of DOR-364 beans across storage time is shown for each storage temperature (9°, 23° and 36° C) and each cooking time (1 and 2 hours). Figures 4.5 and 4.6 illustrate hardness values for samples with and initial moisture content of 12%. Figures 4.7 and 4.8 show hardening patterns for samples with and initial moisture content of 16%. The data supporting these figures are presented in Appendix B.

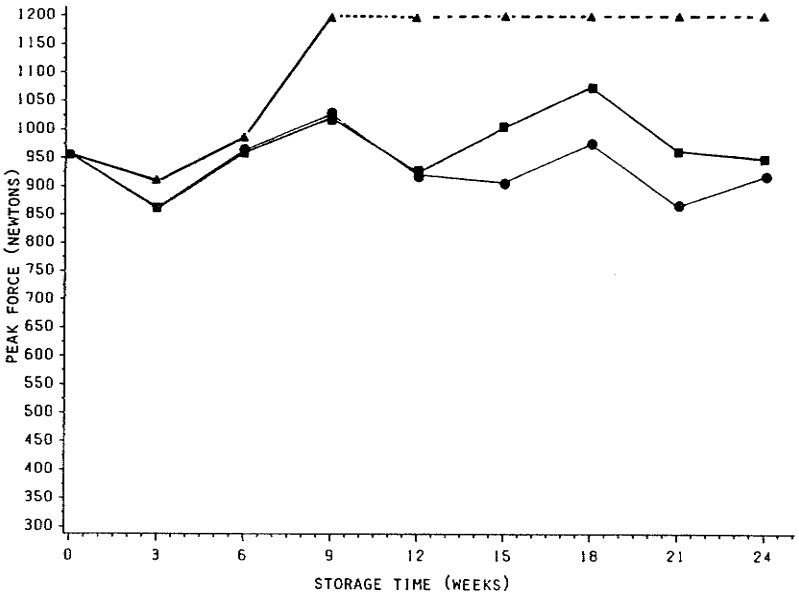

Figure 4.5: Hardness of DOR-364 beans measured after one hour cooking time. Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

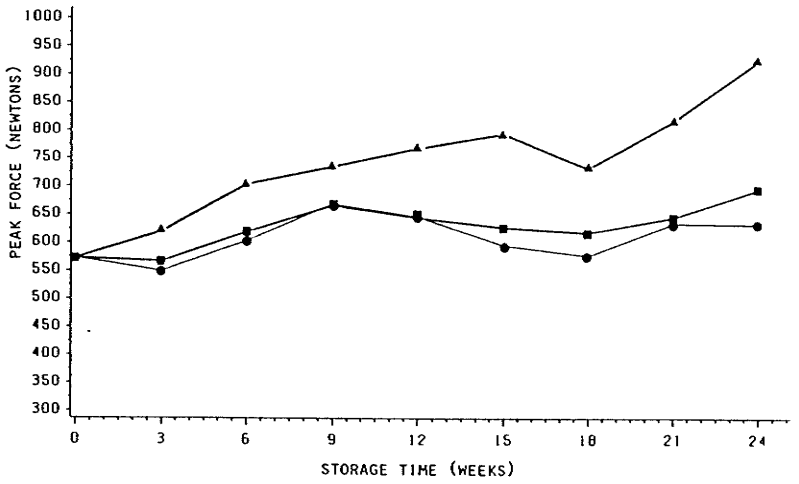

Figure 4.6: Hardness of DOR-364 beans measured after two hour cooking time. Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

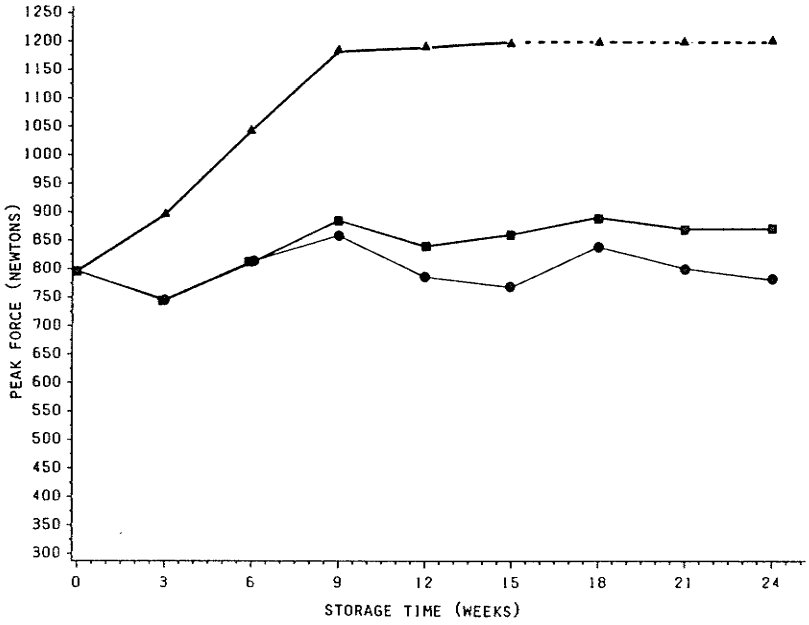

Figure 4.7: Hardness of DOR-364 beans measured after one hour cooking time. Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

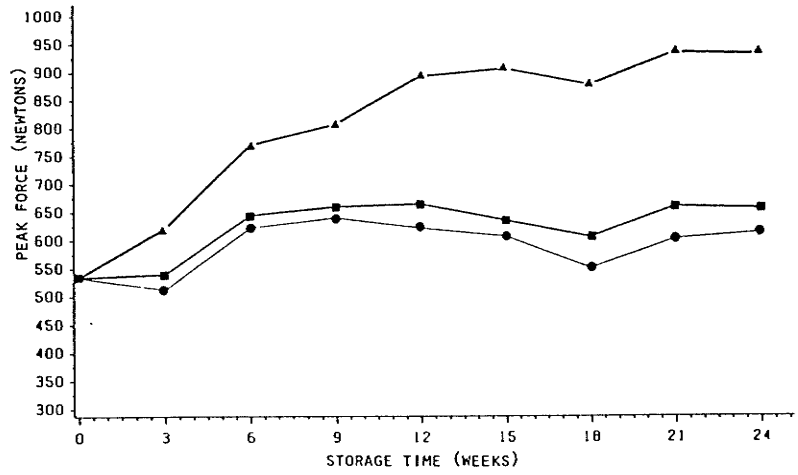

Figure 4.8: Hardness of DOR-364 beans measured after two hour cooking time. Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Hardness values of DOR-364 were initially higher than the values for Tamazulapa beans. These hardness values remained higher for the DOR-364 than for the corresponding Tamazulapa values, for the 24 weeks of storage regardless of cooking time, storage temperature, or initial moisture content.

For the samples cooked for 1 hour, some of the tests were not completed because the extreme hardness could have damaged the test cell. This happened for the samples stored at 36° C with either initial moisture content, after 15 weeks of storage. For beans with 12% initial moisture content also the tests were not completed for samples stored at 36° C, for 9 weeks and for some of the samples stored for 12 weeks.

The samples stored at 9° and 23° C with 12% initial moisture content when tested for hardness after one hour cooking time (figures 4.5 and 4.7) were harder than the ones stored with 16% initial moisture content. The same set of samples when hardness was measured after 2 hour cooking had the same hardening level for either moisture content.

Other previous research has shown that higher moisture content during storage usually results in more rapid hardening (Aguilera and Ballivian, 1987). However, in this study the DOR-364 samples 16% initial moisture content were softer at 1 hour cooking time.

The hardness of beans, measured after 2 hour cooking time, was the highest for the 36° C storage conditions at both

moisture levels. But samples with an initial 16% moisture content were generally harder than the samples with 12% initial moisture content.

4.2.1.2.2 Statistical analysis

The data were analyzed for effects of temperature, moisture content, storage time (week), and for interactions of temperature*moisture content, temperature*week, moisture content*week, temperature*moisture content*week. The same assumptions were made for this analysis as for the analysis of the Tamazulapa beans. The results of the analysis are presented in Table 4.4.

TABLE 4.4: HARDNESS MAIN EFFECTS AND TIME EFFECTS FOR DOR-364 BEANS[1]

Effects	1-Hour Cooking F	1-Hour Cooking p	2-Hour Cooking F	2-Hour Cooking p
Main effects				
Moist. Cont.	230.78	.0001	8.31	.0046
Temperature	615.83	.0001	541.05	.0001
MC/Temp	39.55	.0001	35.19	.0001
Time effects				
Week	57.03	.0001	91.67	.0001
Week/MC	2.38	.0195	4.62	.0001
Week/Temp	18.52	.0001	19.39	.0001
Wk/MC/Temp	1.17	.2974	2.07	.0132

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

When beans were tested after 1 hour of cooking, moisture content, temperature and moisture content*temperature were highly significant (p=.0001). This confirmed the changes observed in relation to greater apparent hardness of the bean for samples with initial 12% moisture content, at all storage temperatures.

When beans were tested after 2 hours of cooking, however, initial moisture content had less importance. This could be observed in the curves that did not vary in hardness level for the majority of the samples. Temperature and moisture content*temperature effect could be observed in the greater hardness of the 16% moisture content samples stored at 36° C, than the 12% moisture content samples stored at 36° C.

Week and temperature*week were highly significant (p=.0001) for DOR-364 beans. This confirmed the observation in the variability of the hardness patterns, in relation to temperature, of the samples for each week period.

Week*moisture content*temperature were not significant when hardness was measured for 1 hour, whereas for samples cooked for 2 hours there was some significance (p=.0132).

4.2.1.3 Pooled samples

When the pooled data were analyzed bean variety (type) was a factor in the analyses in addition to temperature, moisture content, and storage time (week). In this case besides the temperature*moisture content, temperature*week,

moisture content*week, temperature*moisture content*week interactions, there were interactions including type. To facilitate interpretation, the results were divided into main effects and time effects. These results are presented in Tables 4.5 and 4.6.

TABLE 4.5: HARDNESS MAIN EFFECTS OF POOLED SAMPLES AVERAGED ACROSS TIME[1]

Main	1-Hour Cooking		2-Hour Cooking	
Effects	F	p	F	p
Type	2699.11	.0001	3862.91	.0001
MC	0.34	.5618	194.05	.0001
Temp	1092.90	.0001	1105.20	.0001
Type/MC	419.34	.0001	88.85	.0001
Type/Temp	2.66	.0720	12.18	.0001
MC/Temp	67.33	.0001	86.46	.0001
Type/MC/Temp	1.01	.3656	0.23	.7910

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

TABLE 4.6: HARDNESS TIME EFFECTS OF POOLED SAMPLES[1]

Time Effects	1-Hour Cooking F	1-Hour Cooking p	2-Hour Cooking F	2-Hour Cooking p
Week	89.25	.0001	142.17	.0001
Type/Week	7.73	.0001	31.63	.0001
Temp/Week	28.13	.0001	37.28	.0001
MC/Week	5.39	.0001	8.19	.0001
Type/Temp/Week	2.28	.0038	4.52	.0001
Type/MC/Week	1.01	.4248	1.80	.0773
MC/Temp/Week	1.32	.1824	3.65	.0001
Type/MC/Temp/Wk	0.77	.7207	1.02	.4406

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

As shown in Table 4.5, bean variety had a definite, extremely significant effect (p=.0001) when the samples were pooled, regardless of cooking time. Temperature was a highly significant effect but moisture content for the 1 hour cooking time data did not show a significant effect. The high moisture content*type interaction (p<.0001) indicated that moisture effects could not be interpreted apart from variety. This confirmed the decision to interpret the data with the samples separately by each variety.

Table 4.6 shows that week had a strong significant effect for both cooking times (p=.0001). The table also shows that bean variety (type) had a strong, significant effect on time effects (p=.0001) when samples were pooled, as there were significant interactions for type with other variables.

Effect of storage period should therefore be considered for each bean type separately and not for the pooled data.

4.2.1.4 Comparisons between hardness obtained by the two cooking times

One hour and two hour cooking times were compared for each bean variety and also for the pooled data in order to determine if both cooking methods were measuring hardness in the same way.

Figure 4.9 shows the relationships of 1 hour and 2 hour cooking time for the pooled samples. The correlation coefficient obtained was 0.93 ($p < .0001$), which indicated a high degree of correlation between hardness values obtained by both methods.

Figure 4.10 shows the comparison of cooking time values for Tamazulapa beans. The linear relationship of the values obtained by the two cooking time methods can be observed. The correlation coefficient obtained was 0.94, ($p < .0001$).

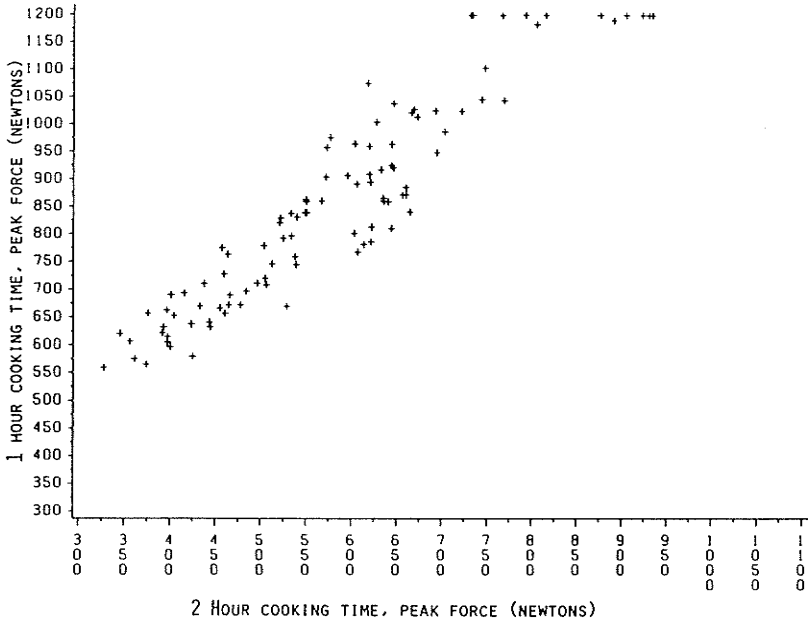

Figure 4.9: Comparison of one hour and two hour cooking times, for bean samples pooled into one group.

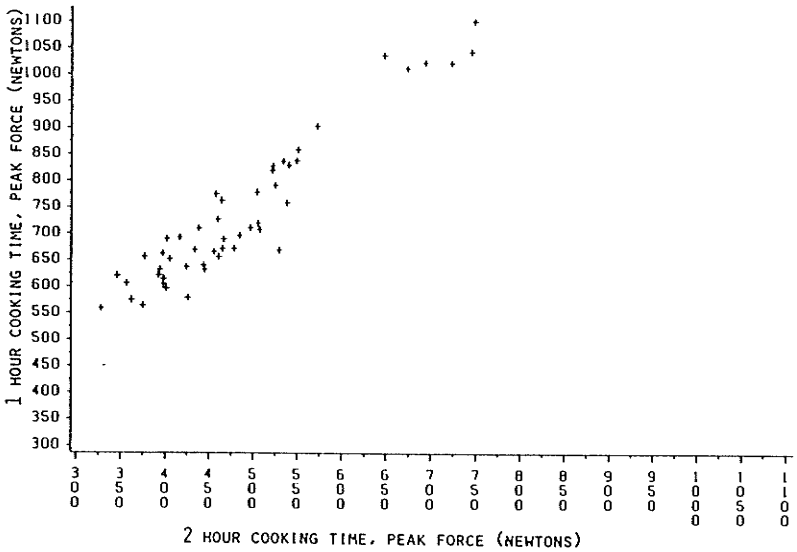

Figure 4.10: Comparison of one hour and two hour cooking times, for Tamazulapa beans.

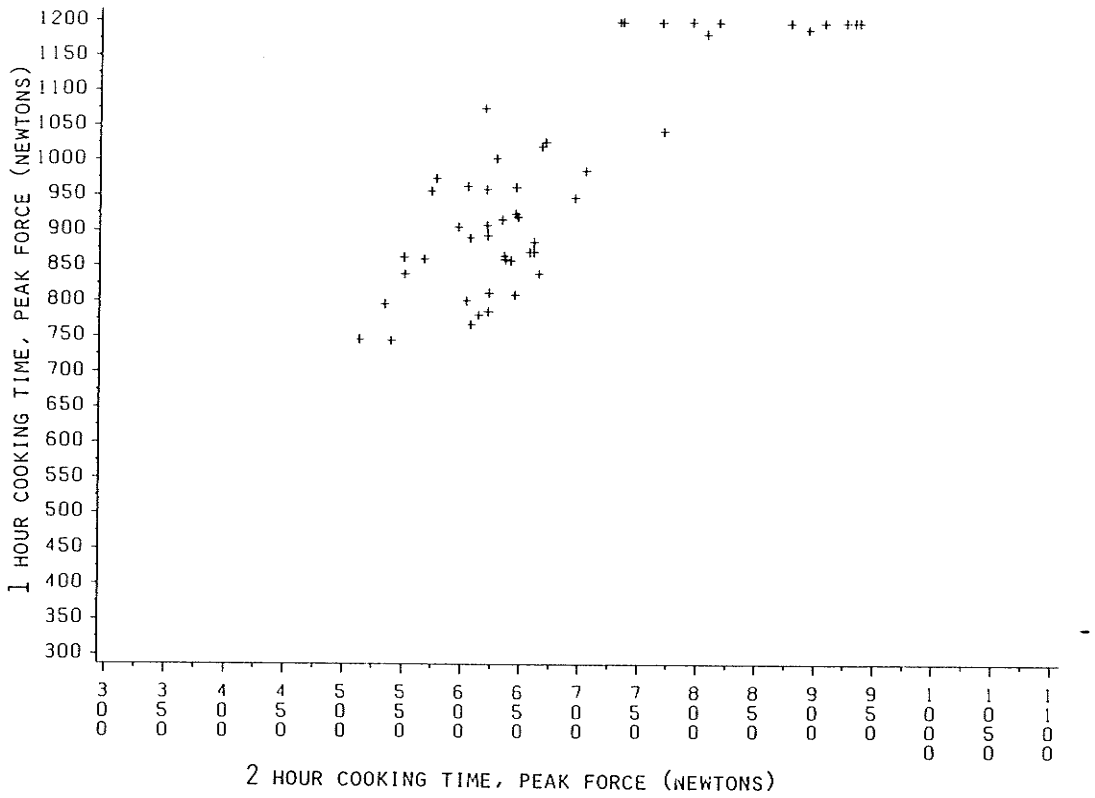

Figure 4.11: Comparison of one hour and two hour cooking times, for DOR-364 beans.

Figure 4.11 shows the correlation between the two cooking methods for DOR-364 beans. Although the figure indicates that the relationship was linear, points were widely scattered. The correlation coefficient obtained was 0.84 ($p<.0001$) when the data included all the uncertain values (>1200N). When these were omitted the correlation coefficient dropped to 0.70 ($p<.0001$).

4.2.1.5 Discussion

In this study there was a strong effect of temperature on hardness of beans but the effect of moisture content was

not as strong. Hardness of both varieties of beans increased when beans were stored at 36° C, regardless of initial moisture content. Bean hardening and storage studies reported in the literature have shown increases in hardening resulting from higher temperature and moisture content during storage (Hincks et al, 1987; Hincks and Stanley, 1986; Hincks and Stanley, 1987 Hohlberg and Stanley, 1987; Moscoso et al, 1984; Rozo, 1982; Sievwright and Shipe, 1986). Most of these studies have been restricted to one bean variety which has been stored under high temperature and high humidity and low temperature low humidity conditions. This study included three temperatures (high, medium and low) with high and low moisture contents for each temperature. An interaction of temperature*moisture content was found which showed an effect of moisture above and beyond the one found for temperature alone. This finding could be used as an explanation for the significant effect of moisture content and temperature on hardening reported in the literature. Another aspect to consider is that in this study beans were packed and sealed in polyethylene bags, and there was some permeability which allowed moisture to be reduced during the storage period.

Aguilera and Hohlberg (1988) summarized some findings on storage condition and the hard-to-cook phenomenon. They stated that beans stored at less than 8 - 9% moisture content regardless of temperature, or beans stored below 17° C regardless of moisture content, do not harden significantly

during prolonged storage. Both of the bean varieties used in this study, with an initial 12% moisture content, did not harden when stored at 9° C. This agrees with their statement. These authors attributed the moisture effect to water activity which is too low for mobilizing any reagent in a biological system, but no explanation was given for the temperature effect.

Relative humidities and moisture content values have been compared by Paredes-Lopez and coworkers (1989) who stated that beans held at 75% relative humidity (RH) reached an equilibrium moisture content of 15.8%. Holhberg and Stanley (1987) reported that samples stored at 85%, 65% and 35% RH, had average moisture contents of 13.5%, 10.8% and 9.6%, respectively and that the higher moisture levels resulted in greater hardening during storage. This study's samples with initial moisture content of 16% can be compared to the samples stored at 85% RH, and those with initial moisture content of 12% to the samples stored at 65% RH. Hincks and Stanley (1986) reported no hardening for their regular dried black bean samples (Phaseolus vulgaris var. Orfeo) stored for six months at 15° C, 35% RH (initial moisture content 11.7%) but the samples stored at 30° C, 85% RH were about 1.5 times harder than at the beginning of the study. Aguilera and Ballivian (1987) reported that black beans (Phaseolus vulgaris var. Orfeo) stored for six months in sealed polyethylene bags (0.13mm thickness), at 40° C, initial moisture content 12%,

hardened about 3.5 times during storage. Both studies reported hardening for beans stored at 6 months, but the level of hardening was not the same, in spite of beans being the same variety. Nevertheless, the higher the storage temperature the harder beans became. This agrees with the findings of this study. Black beans (Tamazulapa) with 12% initial moisture content, when stored at 36° C for 6 months, were about 1.25 times as hard as they were initially and samples with 16% initial moisture content stored at the same temperature and for the same time were about 1.56 times harder. Red beans (DOR-364) with 12% initial moisture content when stored at 36° C, for 6 months, were about 1.6 times harder at the end of 24 weeks and those with 16% initial moisture content stored under same conditions were 1.7 harder at the end of 24 weeks. These results agree with the date reported by Hincks and Stanley (1986). The beans hardened as expected, within the same range, but showed different hardening for the different varieties.

In the literature it has been reported that beans stored with a lower moisture content remain softer than ones with a higher moisture content (Aguilera and Ballivian, 1987; Jackson and Varriano-Marston, 1981). In this study it was found that the red beans stored at low moisture content (12%) and at 9° or 23° C, were harder after 1 hour of cooking that the beans stored with high moisture (16%). After 2 hours of cooking hardness values were similar for beans stored at either 12%

and 16% moisture and for both 9° and 23° storage temperatures. This showed that the initial moisture content had a different effect for different cooking times, for different varieties of beans. The cooking process of beans has been mentioned in the literature as one aspect that needs to be studied to provide a more accurate overall picture of the chemical, physical and biochemical changes affecting texture of beans (Hincks et al, 1987).

4.2.2 Phytate

The phytate content of the samples was determined by the method of Latta and Eskin (1980). Phytate content was measured after centrifugation by determining the absorbance at 500 nm. Values were calculated in g/100g on a dry basis.

4.2.2.1 Tamazulapa

4.2.2.1.1 Phytate content curves

Phytate changes across time of Tamazulapa beans are shown for each storage temperature (9°, 23° and 36° C). Figure 4.12 shows results for samples with an initial moisture content of 12%; and Figure 4.13 shows results for samples with an initial moisture content of 16%. The data supporting these figures are presented in Appendix C, Table C.1.

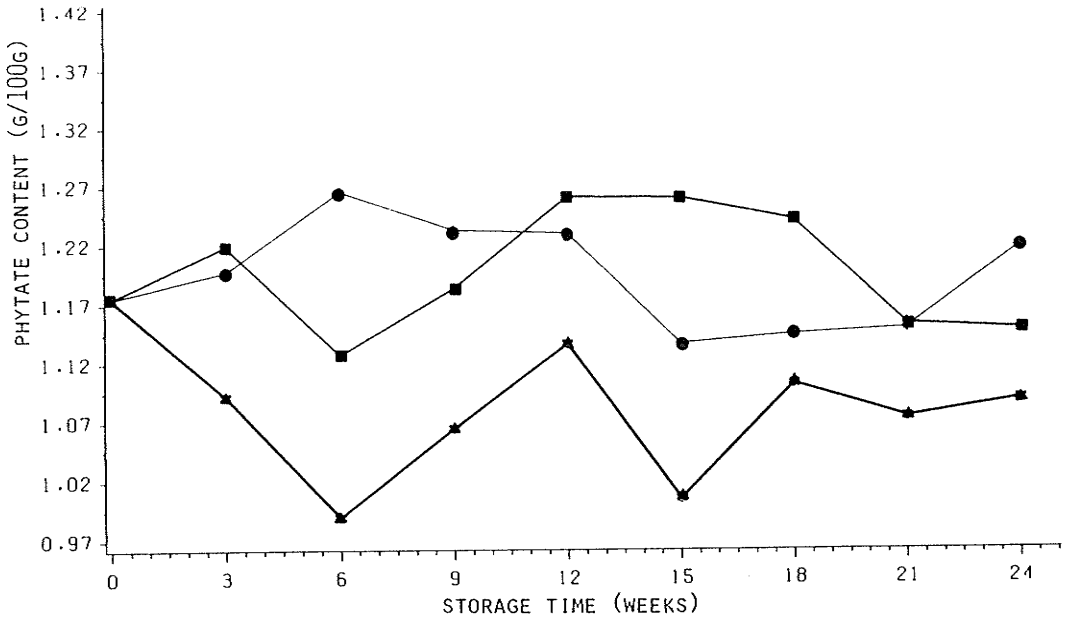

Figure 4.12: Phytate content of stored Tamazulapa beans Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

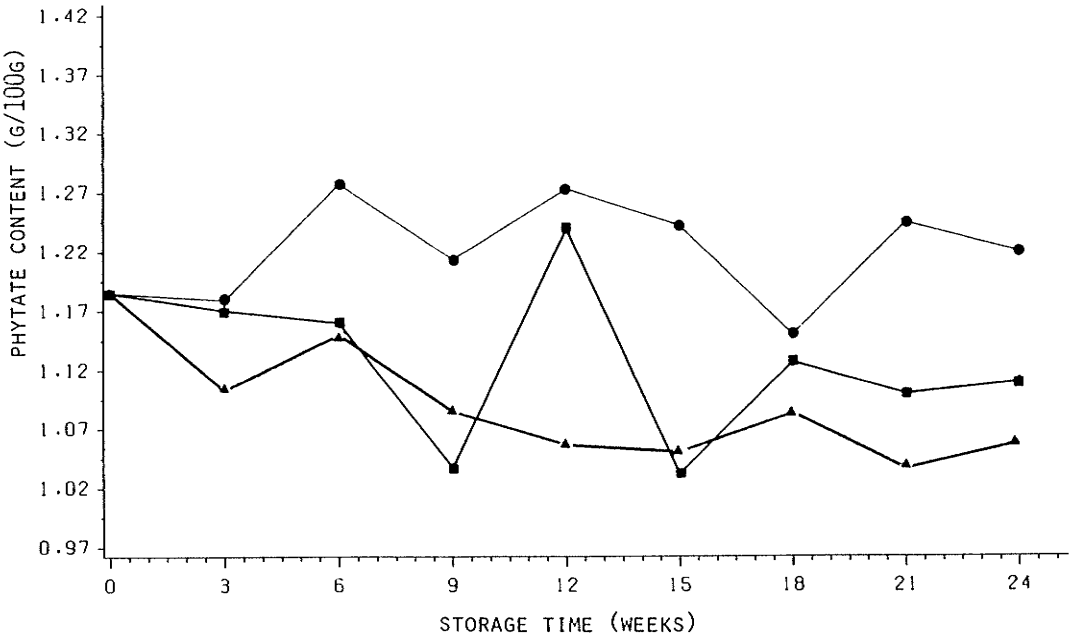

Figure 4.13: Phytate content of stored Tamazulapa beans Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

The samples with an initial 12% moisture content (Figure 4.12) showed a decrease in phytate when storage was at 36° C rather than at 9° or 23° C. This effect could also be seen in samples with initial moisture content of 16%. Samples stored at 9° C did not decrease in phytate as did the samples stored at 36° C Figure 4.13). Sixteen percent moisture content samples stored at 23° C had variable phytate content. (Analyses of the 23° C samples were repeated to confirm the values obtained). For the 12% moisture content samples stored at 23° C the phytate curve overlapped the curve for 9° storage; but for the 16% moisture content samples stored at 23° C the phytate curve overlapped the curve for 36° C storage. Initial phytate content was 1.18 grams for the 12% moisture content beans, and 1.19 grams for the 16% moisture content samples. Phytate was lower at the end of the study for the samples stored at 23° and 36° C: for samples stored with 12% initial moisture content phytate dropped to 1.15 and 1.09, respectively; samples stored with 16% initial moisture content reached 1.12 and 1.06, respectively (Appendix C, Table C.1).

4.2.2.1.2 Statistical analysis

These data were also analyzed for effects of temperature, moisture content, storage time (as weeks), and for interactions temperature*moisture content, temperature*week, moisture content*week, temperature*moisture content*week. When considering changes across time all repetition terms were

pooled into a common error term under the assumption that there was no interaction between replicates and treatment or time. The results of this analysis is presented in Table 4.7.

TABLE 4.7: PHYTATE MAIN EFFECTS AND TIME EFFECTS FOR TAMAZULAPA BEANS[1]

Effects	Phytate	
	F	p
Main effects		
Moisture Content	2.16	.1477
Temperature	93.80	.0001
MC/Temp	5.29	.0001
Time effects		
Week	5.37	.0001
Week/MC	2.48	.0229
Week/Temp	4.26	.0001
Wk/MC/Temp	3.32	.0005

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

Temperature had an effect (p=.0001) on phytate content. In the figures shown previously there was a marked decrease in phytate when samples were stored at 36° C but no decreases in phytate for samples stored at 9°, at both initial moisture contents, and only a small decrease for samples stored at 23° C with 12% initial moisture content (Figure 4.13). Moisture content did not have a significant effect on phytate content (p=.1471).

Storage time was a highly significant factor with phytate content dropping over the 24 weeks of storage.

The significant interaction of week*temperature and of moisture content*temperature*week can be understood from the figures shown (Figure 4.12 and 4.13). Week to week effects were not consistent and these were responsible for the interactions.

4.2.2.2. DOR-364

4.2.2.2.1 Phytate content curves

Phytate changes across time of DOR-364 beans are shown for each storage temperature (9°, 23° and 36° C). Figure 4.14 shows phytate levels of samples with an initial moisture content of 12%; and Figure 4.15 illustrates phytate levels of samples with an initial moisture content of 16%. The data supporting these figures are presented in Appendix C, Table C.2.

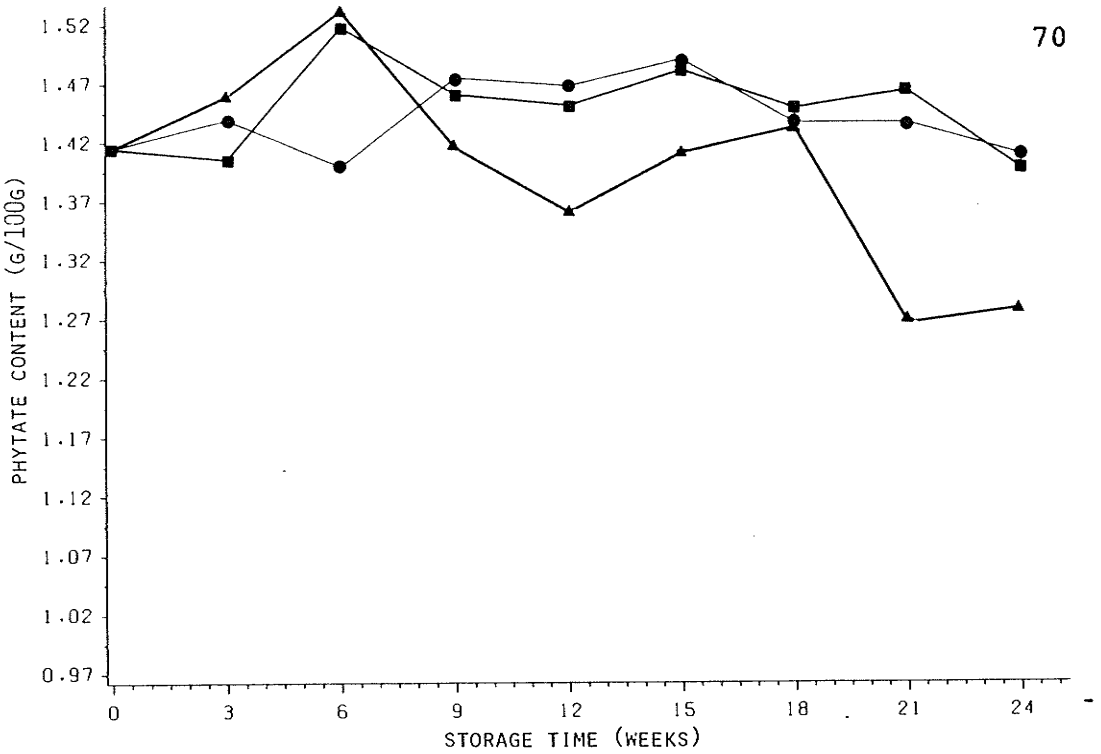

Figure 4.14: Phytate content of stored DOR-364 beans Initial moisture content 12%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

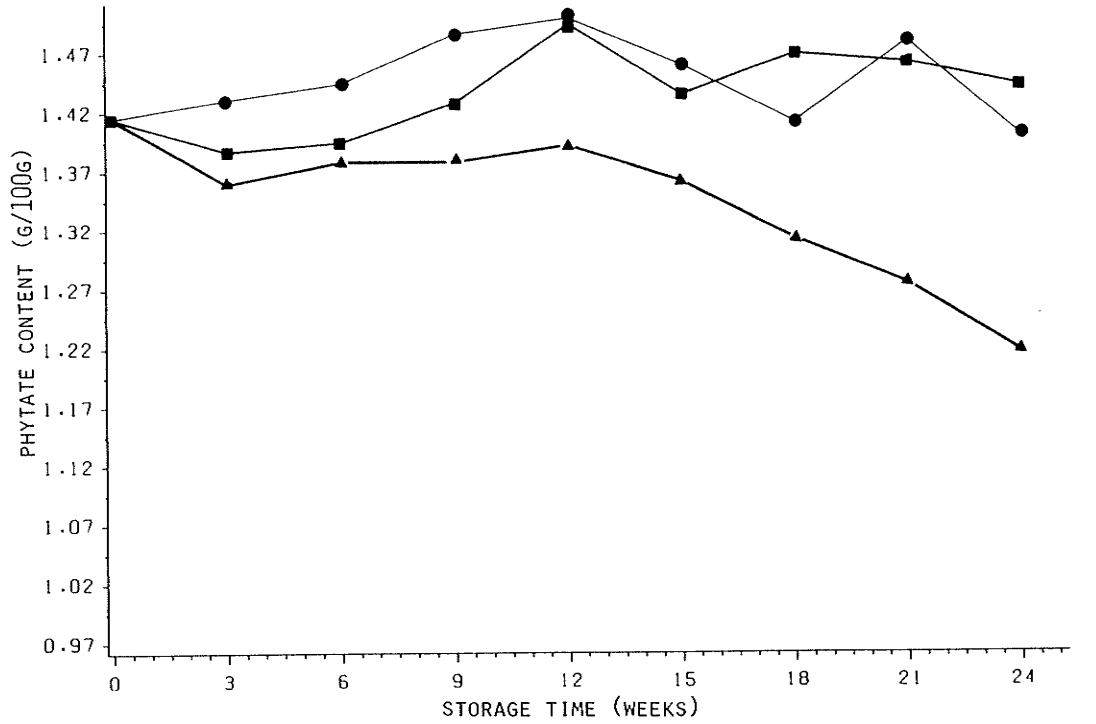

Figure 4.15: Phytate content of stored DOR-364 beans Initial moisture content 16%.
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Both figures show that there was a decrease in phytate content at the end of 24 weeks of storage for samples stored at 36° C. For samples with 16% initial moisture this decrease occurred throughout the storage period. The initial content of phytate was 1.42 g/100g for the DOR-364 beans, considerably higher than the mean initial value of 1.18 for the Tamazulapa beans. Even at the end of the study the phytate content of the DOR-364 remained higher than the initial content of the Tamazulapa beans. For the samples stored at 9° and 23° C phytate curves followed similar patterns for both initial moisture contents, and did not seem to vary during storage time, regardless of temperature.

4.2.2.1.2 Statistical analysis

The data was also analyzed as for the Tamazulapa beans, for effects of temperature, moisture content, time (as weeks), and for interactions temperature*moisture content, temperature*week, moisture content*week, temperature*moisture content*week. When considering changes across time the same assumptions were made. The results of this analysis is presented in Table 4.8.

TABLE 4.8: PHYTATE MAIN EFFECTS AND TIME EFFECTS FOR DOR-364 BEANS[1]

Effects	Phytate	
	F	p
Main effects		
Moisture Content	6.83	.0116
Temperature	43.63	.0001
MC/Temp	5.63	.0060
Time effects		
Week	6.44	.0001
Week/MC	2.44	.0250
Week/Temp	5.04	.0001
Wk/MC/Temp	1.24	.2969

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

Temperature, storage time (weeks) and week*temperature were statistically highly significant at p .0001. This can be clearly seen in Figures 4.14 and 4.15, where changes were observed over storage time, especially for samples stored at 36° C. The statistical analysis confirmed the temperature and time effects, and week*temperature interaction. There was a slightly significant moisture content effect (p=.0116), which can be explained by the marked fall in the curve that occurred for the samples stored at 36° C with 16% initial moisture content (Figure 4.15). The lack of significance for the interactions moisture content*week and the interaction of the

three factors could be explained by comparing both figures, as the pattern for the samples stored at lower temperatures were similar, regardless of storage time and moisture content.

4.2.2.3 Pooled samples

These data were analyzed with all samples pooled to form one data set. Bean variety (type) was also a factor included along with temperature, moisture content, time (week). In this case the interactions considering type were also determined. Results from this analysis were divided into main and time effects. These results are presented in Tables 4.9 and 4.10.

TABLE 4.9: PHYTATE MAIN EFFECTS OF POOLED SAMPLES AVERAGED ACROSS TIME[1]

Main Effects	Phytate F	Phytate p
Type	2596.77	.0001
MC	8.36	.0046
Temp	128.49	.0001
Type/MC	0.68	.4113
Type/Temp	8.42	.0004
MC/Temp	10.17	.0001
Type/MC/T	10.65	.0001

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

TABLE 4.10: PHYTATE TIME EFFECTS OF POOLED SAMPLES AVERAGED ACROSS TIME[1]

Time Effects	Phytate F	p
Week	7.46	.0001
Type/Week	4.36	.0001
Temp/Week	5.41	.0001
MC/Week	1.67	.1141
Type/Temp/Wk	3.90	.0001
Type/MC/Week	3.25	.0024
MC/Temp/Wk	1.71	.0545
Type/MC/Temp/Wk	2.83	.0007

[1]F = F ratio; P = probability. Based on Type III Sums of Squares.

Type and temperature effects were statistically highly significant (p=.0001) for phytate content of pooled beans. The type effect confirmed the differences observed in content and in patterns across storage time for each of the bean varieties, although both varieties had lower phytate values when they were stored at high temperature (36° C). Storage time (week), as expected was also highly significant. The interactions related to type, temperature and week were highly significant, whereas probability for the interactions that included moisture content were higher.

4.2.2.4 Discussion

Initial phytate content was higher for the red (DOR-364) beans than for the Tamazulapa but the red beans were generally

harder throughout the storage study. The literature states that legume seeds which have reduced levels of phytic acid take longer to cook (Bhatty and Slinkard, 1989), but this was not the case in this study. Kon (1979) stated that legumes that take longer to time to cook contain less phytate than legumes that take less time to cook. Phytate content differences in various bean varieties that were not related to cooking rate are shown in another study reported by Kon and Sanshuch (1981). This lack of relationship was overlooked by Kon and Sanshuch. They focused on reducing cooking time by adding phytic acid during cooking. These data confirm that the decreasing rate of phytate content rather than the absolute values should be considered in relation to the bean hardening process.

Both DOR-364 and Tamazulapa beans had much lower phytate contents after 6 months storage at high temperature (36° C). The amount of phytate was increased when the initial moisture content was high. Moscoso and coworkers (1984) reported that higher phytic acid phosphorus content in Redkloud beans favoured a more rapid rate of softening during cooking. They also reported alterations in the ratio of monovalent to divalent cations of soaked beans stored at high temperatures and high relative humidities (from 6.4 to 4.7). They concluded that decreased amounts of phytic acid and of monovalent cations would result in reduced solubilization of the pectic substances. More divalent cations would be

available to bind to the pectin. This would increase cooking time resulting in greater hardening. Elevated temperatures during storage have a significant effect on the loss of phytic acid of black beans after being stored for 6 months, as reported by Sievwright and Shipe (1986). Hincks and Stanley (1986) observed significant phytate degradation for beans stored at high temperature/humidity, but they found a lower correlation ($r=-0.716$) between phytate and hardening defect of beans. They concluded that phytate is a contributor but perhaps not the sole operating mechanism in the hardening defect. Hardness of beans has been explained by hydrolysis of phytic acid through the action of phytase, which would free divalent cations (Ca^{++}, Mg^{++}) allowing these to complex to the free carbonyl group of pectates, promoting cross-linking of pectins of the middle lamella. This cross-linking would prevent cell separation and thus hardness would occur (Moscoso et al, 1984; Stanley and Aguilera, 1985). The decrease in phytate content of stored beans has also been attributed to protein-phytate interactions which may make less phytic acid available for chemical assay (Sievwright and Shipe, 1986). Phytate would release calcium when bound to protein, and the available calcium could complex with pectins that would then become insoluble pectates. Prattley and Stanley (1982) suggested the existence of a protein-cation-phytate complex in situ. This would cause lower phytate without a corresponding increase in hardness. As will be shown later

in this study (4.3.3.4), phytate was not the most sensitive indicator of hardness. Hardening could be due to complex mechanisms which involve not only phytate but the soluble and insoluble fibre fractions.

4.2.3 Dietary Fibre

Dietary fibre of stored Tamazulapa and DOR-364 beans was measured by the fractions of insoluble and soluble fibre, and by the calculations of total dietary fibre and the ratio of soluble/insoluble fibre. Results will be presented in that order in the following section concluding with statistical analyses of the dietary fibre data. For dietary fibre one initial value for the samples prior storage (0 time) was used in the tables and calculations, using the means of both determinations, under the assumption that before any treatment was applied this value should be the same as the data is given in dry matter basis.

4.2.3.1 Tamazulapa

4.2.3.1.1 Dietary fibre fractions curves

4.2.3.1.1.1 Total dietary fibre

Total dietary fibre changes during time of Tamazulapa beans are shown for each storage temperature (9°, 23° and 36° C) in Figure 4.16 for samples with an initial moisture content

of 12%; and in Figure 4.17 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

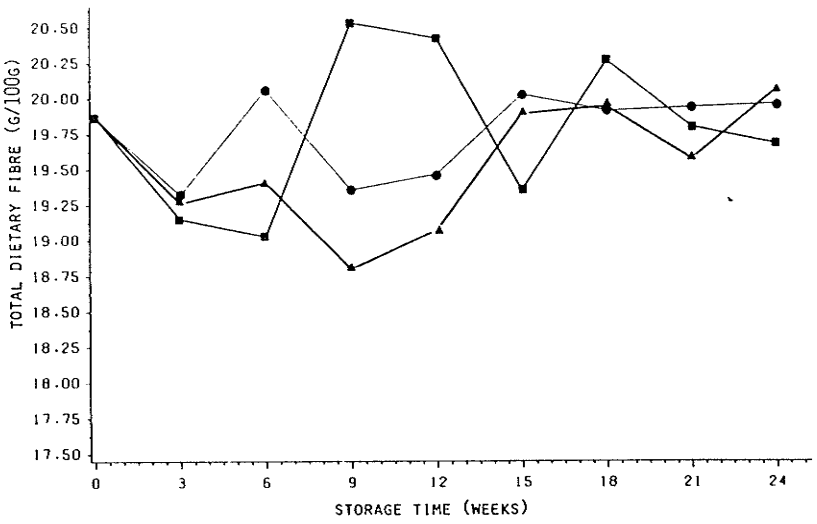

Figure 4.16: Total dietary fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 12% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

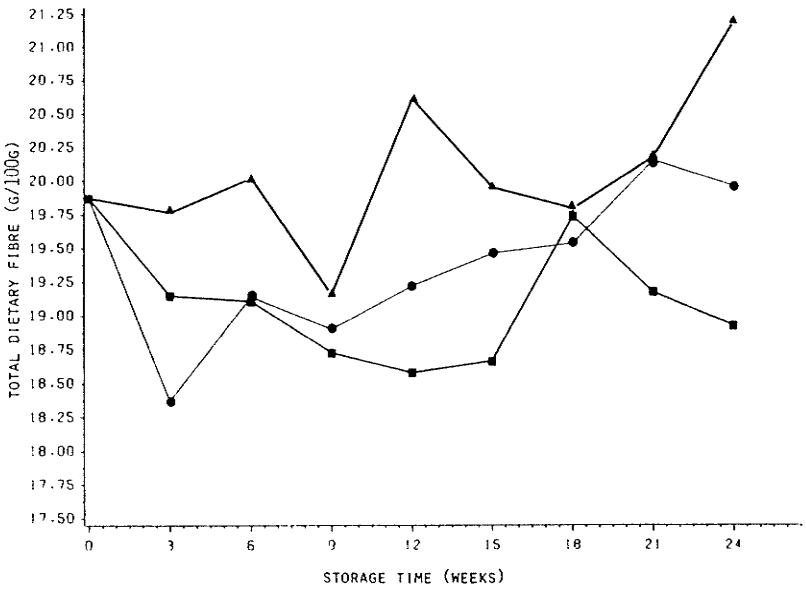

Figure 4.17: Total dietary fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 16% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Both of these figures show many variations over storage time. Changes in total dietary fibre seemed to followed no specific pattern. As shown in Figure 4.17 the only difference was for 16% moisture samples, where the 36° C storage appeared to result in higher total dietary fibre than with the 9° and 23° C storage.

4.2.3.1.1.2 Soluble fibre

Soluble fibre changes for Tamazulapa beans for the 24 weeks storage period are shown for each storage temperature (9°, 23° and 36° C). Figure 4.18 illustrates data for samples with an initial moisture content of 12%; and in Figure 4.19 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

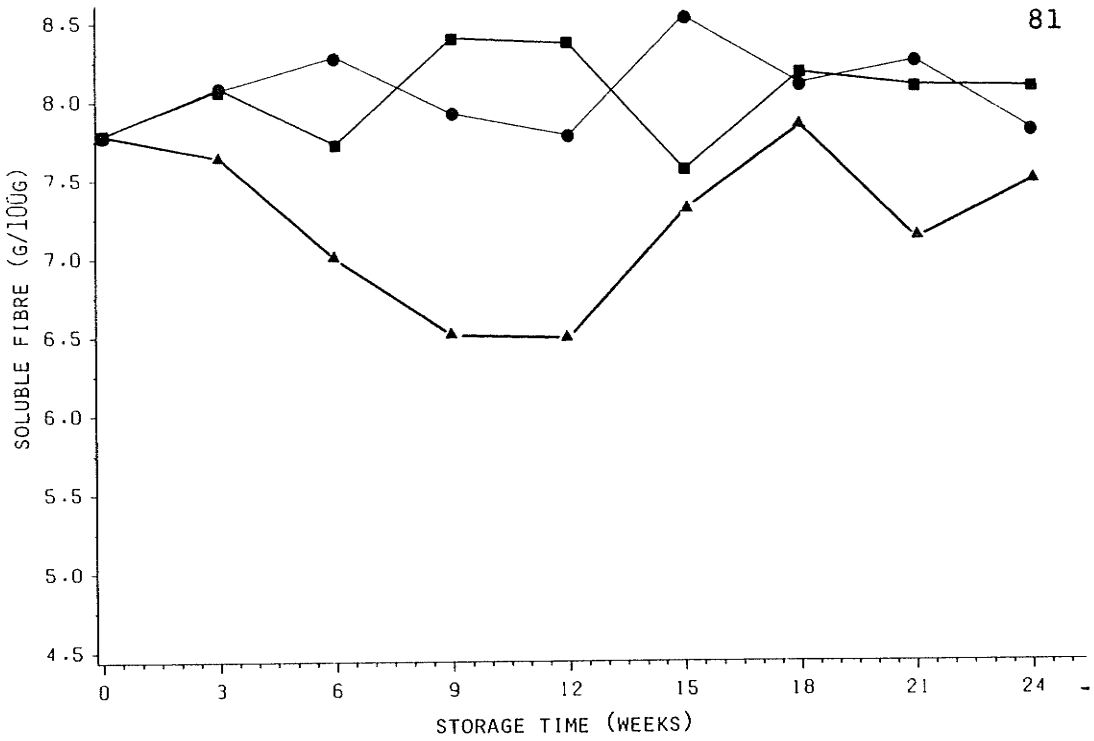

Figure 4.18: Soluble fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 12%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

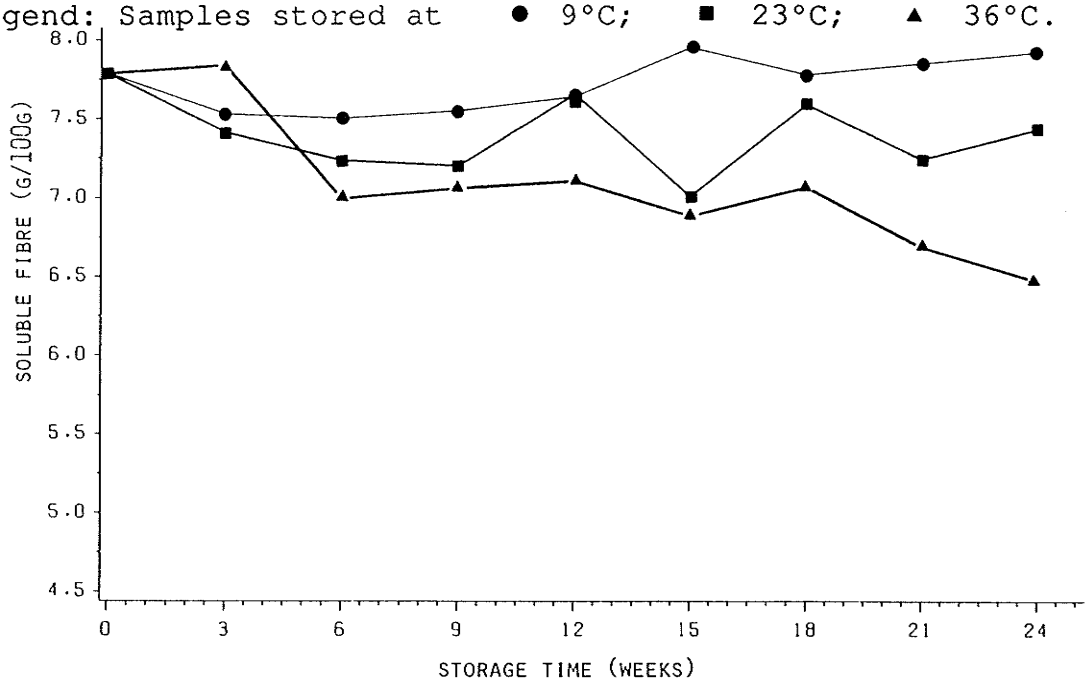

Figure 4.19: Soluble fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 16%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Both figures show that soluble fibre curves regardless of initial moisture content were lower for samples stored at 36° C than for samples stored at 9° and 23° C. Figure 4.19 also shows that for the 16% initial moisture content 36° C samples, not only were soluble fibre values lower during the whole period, but also there was a decrease over time. At the end of the study, the soluble fibre content of beans stored at 16% moisture content and 36° C was 6.5 g/100g, considerably lower than the initial value of 7.8 g/100g.

When beans were stored at 9° or 23° C, regardless of initial moisture content, the quantity of soluble fibre remained approximately the same from 0 to 24 weeks.

4.2.3.1.1.3 Insoluble fibre

Insoluble fibre changes during time of Tamazulapa beans are shown for each storage temperature (9°, 23° and 36° C). Figure 4.20 shows results for samples with an initial moisture content of 12%; and Figure 4.21 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

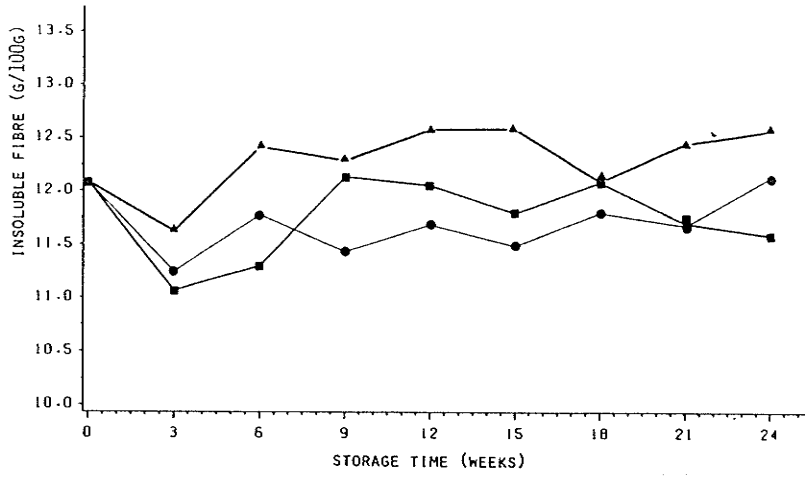

Figure 4.20: Insoluble fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 12% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

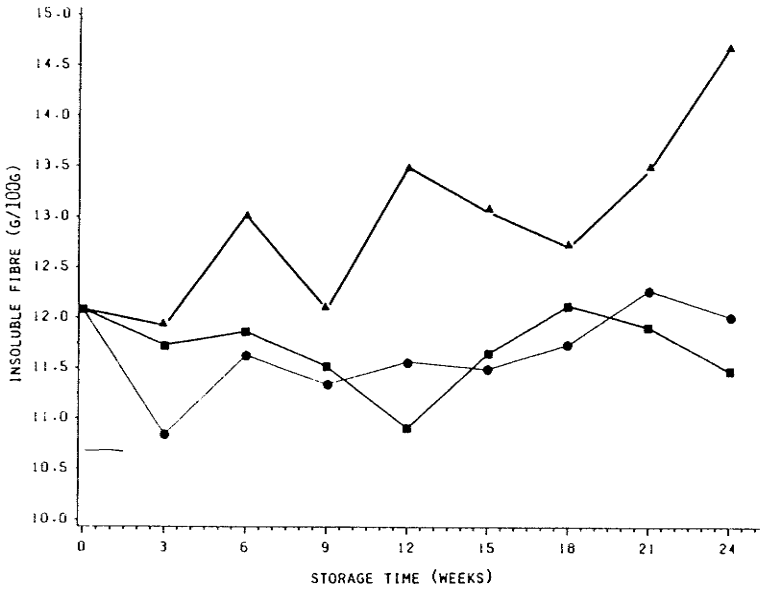

Figure 4.21: Insoluble fibre of Tamazulapa beans stored under various temperatures. Initial moisture content 16% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Both Figure 4.20 and Figure 4.21 show a higher content of insoluble fibre for samples stored at 36° C. The increase in insoluble fibre was greater for the sample with 16% initial moisture content (18% increase) than for the one with 12% initial moisture content (4% increase) at 24 weeks. Insoluble fibre values for samples varied from week to week at 23° and 9° C but did not show a consistent increase or decrease over the storage period.

4.2.3.1.1.4 Soluble/Insoluble (S/I) ratio

The S/I ratio changes during time for Tamazulapa beans are shown for each storage temperature (9°, 23° and 36° C). Figure 4.22 shows results for samples with an initial moisture content of 12%; and Figure 4.23 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

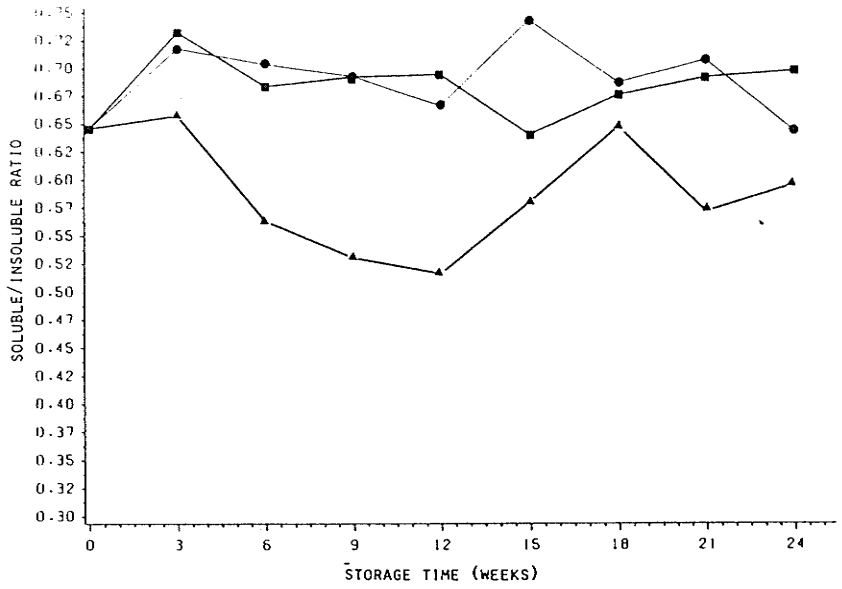

Figure 4.22: Soluble/insoluble ratio of Tamazulapa beans stored under various temperatures. Initial moisture content 12% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

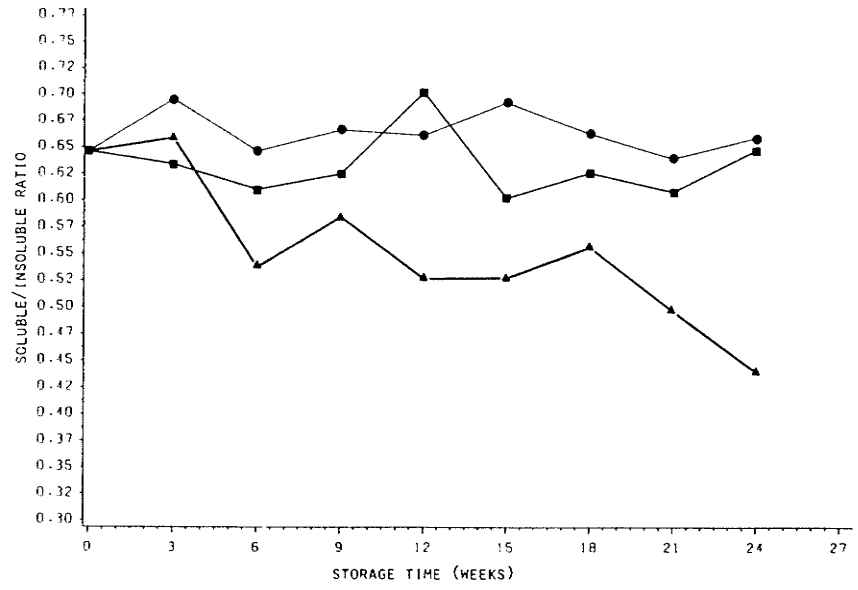

Figure 4.23: Soluble/insoluble ratio of Tamazulapa beans stored under various temperatures. Initial moisture content 16% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

The S/I ratio curves were lower for samples stored at 36° C, than for samples stored at 9° and 23° C regardless of initial moisture content, as shown in Figures 4.22 and 4.23. During storage at 36° C samples with 16% initial moisture content showed a pronounced decrease in the ratio. In general storage at 9° and 23° C did not seem to lower the S/I ratio.

4.2.3.1.2 Dietary fibre fractions statistical analysis

All of the dietary fibre data were analyzed for the main effects of temperature, moisture content, and the interaction temperature*moisture content; and also for time effects, for storage time (as weeks), and for interactions temperature*week, moisture content*week, temperature*moisture content*week. The results of this analysis is presented in Table 4.11.

TABLE 4.11: DIETARY FIBRE MAIN EFFECTS AND TIME EFFECTS FOR TAMAZULAPA BEANS[1]

	Total		Soluble		Insoluble		S/I	
Effects	F	p	F	p	F	p	F	p
Main effects								
MC	2.88	.0945	59.02	.0001	4.02	.0490	35.77	.0001
Temp	3.80	.0273	82.89	.0001	42.70	.0001	104.25	.0001
MC/Temp	11.61	.0001	8.92	.0004	6.19	.0034	1.17	.3155
Time effects								
Week	3.36	.0027	4.19	.0004	3.92	.0008	4.38	.0003
Week/MC	0.60	.7779	2.66	.0134	1.21	.3062	2.53	.0182
Week/Temp	1.46	.1444	6.21	.0001	2.63	.0031	5.58	.0001
Wk/MC/Temp	1.40	.1711	2.94	.0011	1.18	.3064	1.60	.0947

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

4.2.3.1.2.1 Total dietary fibre

The dietary fibre main and time effects analysis showed only one effect of significance, the moisture content*temperature. Total dietary fibre increased for the sample with initial 16% moisture content stored at 36° C but did not increase for the 12% initial moisture content, 36° C sample.

4.2.3.1.2.2 Soluble fibre

Results of the statistical analysis showed highly significant effects of both temperature and moisture content. These significant effects reflected the higher content of soluble fibre for the samples stored at lower temperatures.

There was also a highly significant effect of the interaction moisture content*temperature. Soluble fibre was lower for both samples stored at 36° C than for samples stored at lower temperatures, however the sample with 16% initial moisture content had a more marked decrease.

Time effects of the interaction week*moisture content*temperature was significant at p=.0011, and week*temperature was highly significant (p .0001). This could explain the drop in soluble fibre over storage time for samples stored at 36° C as compared to other samples. The interaction week*moisture content was just slightly significant (p=.0134). At certain storage periods there was a moisture content effect above those already observed. Week itself was also significant, and this effect can be shown by the variations at the different three week periods, and the observed decrease during storage time.

4.2.3.1.2.3 Insoluble dietary fibre

The analysis for insoluble dietary fibre showed a very significant effect of temperature (p .0001). Samples stored at 36° C were generally higher in content of insoluble dietary fibre, than samples stored at a lower temperature (Figures 4.20; 4.21). Moisture content had a slight effect (p=.0490). There was a significant moisture content*temperature interaction at p=.0034. This could be clearly observed in Figure 4.21, which showed that the marked increase in

insoluble dietary fibre for the samples stored at 36° C with an initial moisture content of 16%.

The week effect was highly significant (p=.0008) and week*temperature was also significant (p=.0031). Neither the interaction of moisture content*temperature*week nor the interaction week*moisture content were significant.

4.2.3.1.2.4 Soluble/Insoluble (S/I) ratio

The main effects of moisture content and temperature were statistically highly significant (p=.0001), but there seemed to be no combined effect, as the interaction moisture content*temperature was not significant. Figures 4.22 and 4.23 show lower ratios for samples stored at 36° C, regardless of moisture contents. The ratios were somewhat lower for the samples stored with an initial 16% moisture content throughout the study. This observation was confirmed by the lack of statistical significance for the interactions for time effects, when moisture was included. Week and week*temperature were highly significant, which is shown in the figures by the patterns of the samples stored at 36°C. Week effect was more obvious in the samples with initial 16% moisture content (Figure 4.23) where after 18 weeks the ratio dropped dramatically.

4.2.3.2 DOR-364

4.2.3.2.1 Dietary fibre fractions curves

4.2.3.2.1.1 Total dietary fibre

Total dietary fibre changes during time of DOR-364 beans are shown for each storage temperature (9°, 23° and 36° C) in Figure 4.24 for samples with an initial moisture content of 12%; and in Figure 4.25 for samples with an initial moisture content of 16%. The analyses for the DOR-364 beans were done only at 0, 9, 18 and 24 weeks. The data supporting these figures is presented in Appendix D.

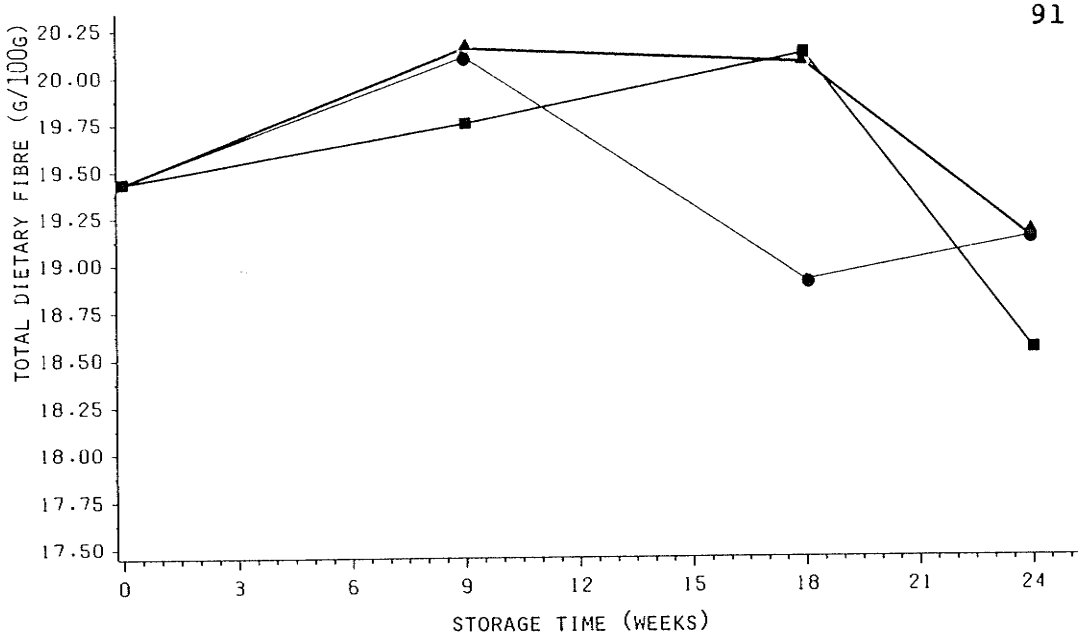

Figure 4.24: Total dietary fibre of DOR-364 beans stored under various temperatures. Initial moisture content 12% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

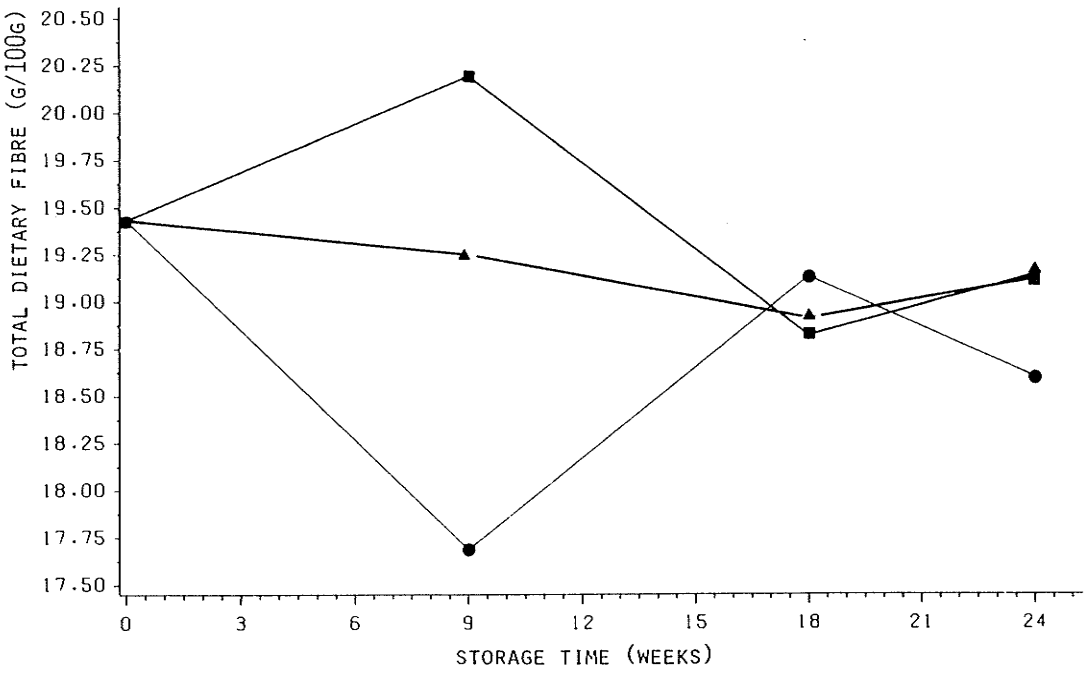

Figure 4.25: Total dietary fibre of DOR-364 beans stored under various temperatures. Initial moisture content 16% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Both figures showed that total dietary fibre content did not follow a specific pattern. None of the curves showed a consistently higher or lower amount of total dietary fibre than the other curves. Changes during time seemed to be towards maintaining the same amount of fibre, although total dietary fibre values at 24 weeks were slightly lower than at the beginning of storage.

4.2.3.2.1.2 Soluble fibre

Soluble fibre changes during time of DOR-364 beans are shown for each storage temperature (9°, 23° and 36° C) in Figure 4.26 for samples with an initial moisture content of 12%; and in Figure 4.27 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

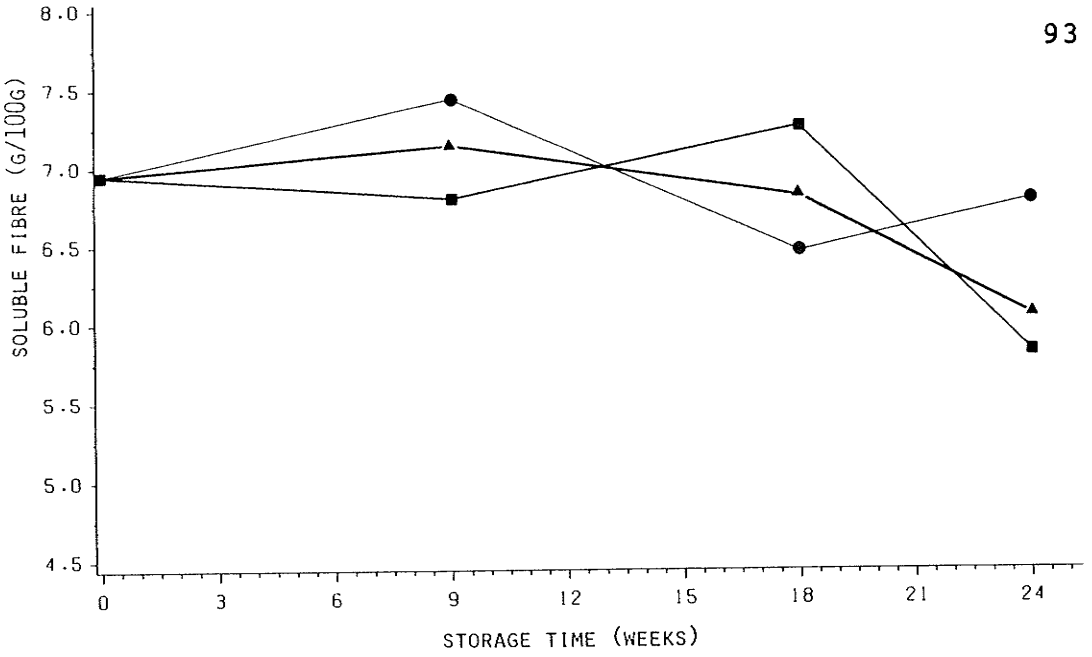

Figure 4.26: Soluble fibre of DOR-364 beans stored under various temperatures. Initial moisture content 12%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

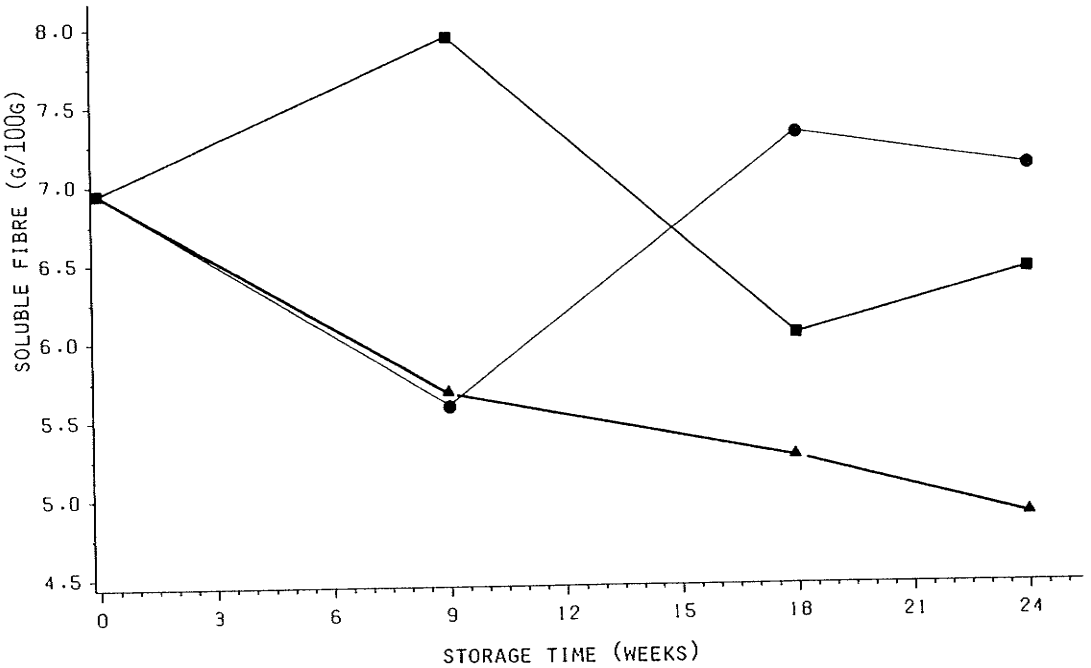

Figure 4.27: Soluble fibre of DOR-364 beans stored under various temperatures. Initial moisture content 16%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Figure 4.26 shows that there was generally more soluble fibre in samples with 12% initial moisture content. Figures 4.26 and 4.27 show that by the end of the 24 weeks soluble fibre decreased when samples had been stored at 23° and 36° C.

4.2.3.2.1.3 Insoluble fibre

Insoluble fibre changes during time of DOR-364 beans are shown for each storage temperature (9°, 23° and 36° C) in Figure 4.28 for samples with an initial moisture content of 12%; and in Figure 4.29 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

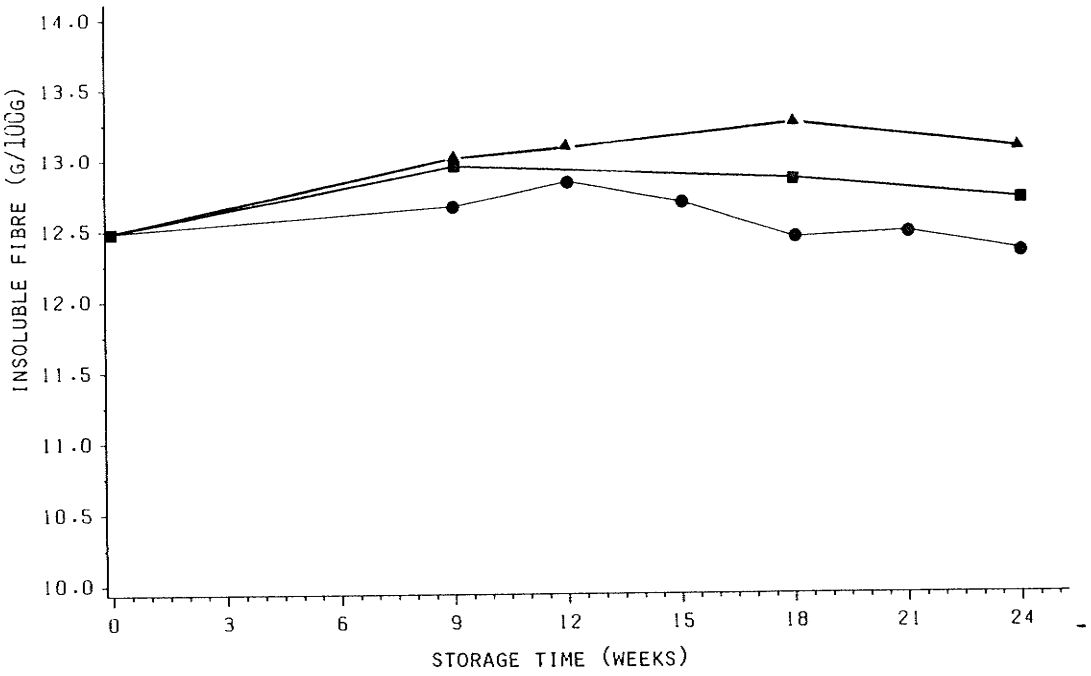

Figure 4.28: Insoluble fibre of DOR-364 beans stored under various temperatures. Initial moisture content 12%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

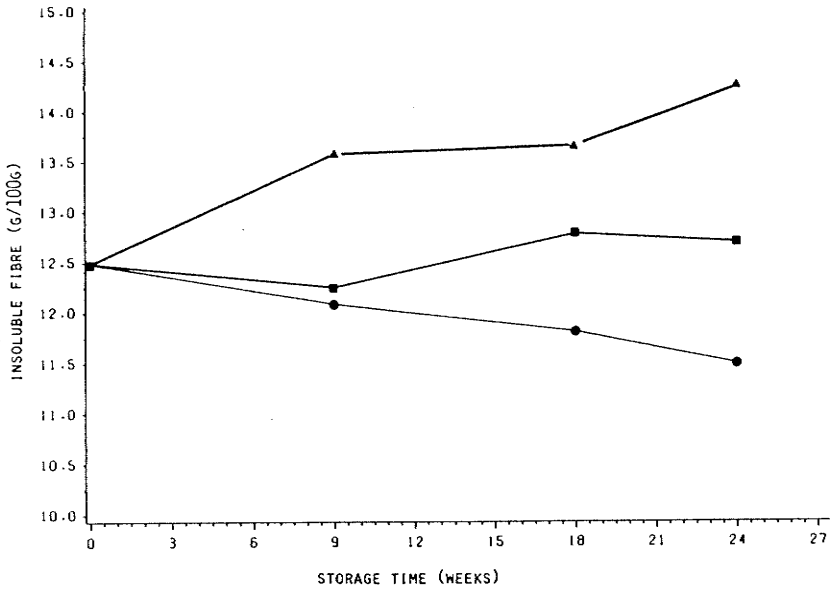

Figure 4.29: Insoluble fibre of DOR-364 beans stored under various temperatures. Initial moisture content 16%
Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

Figure 4.28 shows that samples with initial 12% moisture content stored at 36° C for the study period increased in insoluble fibre content while a small decrease could be observed for the 12% initial moisture content samples stored at 9° C. The increase in insoluble fibre could be observed even more clearly for samples with 16% initial moisture content(Figure 4.29). There was clearly a temperature effect as the samples stored at higher temperatures consistently had higher values of insoluble fibre than the ones stored at lower temperatures. For the 16% initial moisture content samples, the ones stored at 9° C had decreasing amounts of insoluble fibre over the 24 weeks.

4.2.3.2.1.4 Soluble/Insoluble (S/I) ratio

The S/I ratio changes during time for DOR-364 beans are shown for each storage temperature (9°, 23° and 36° C) in Figure 4.30 shows results for samples with an initial moisture content of 12%; and in Figure 4.31 for samples with an initial moisture content of 16%. The data supporting these figures is presented in Appendix D.

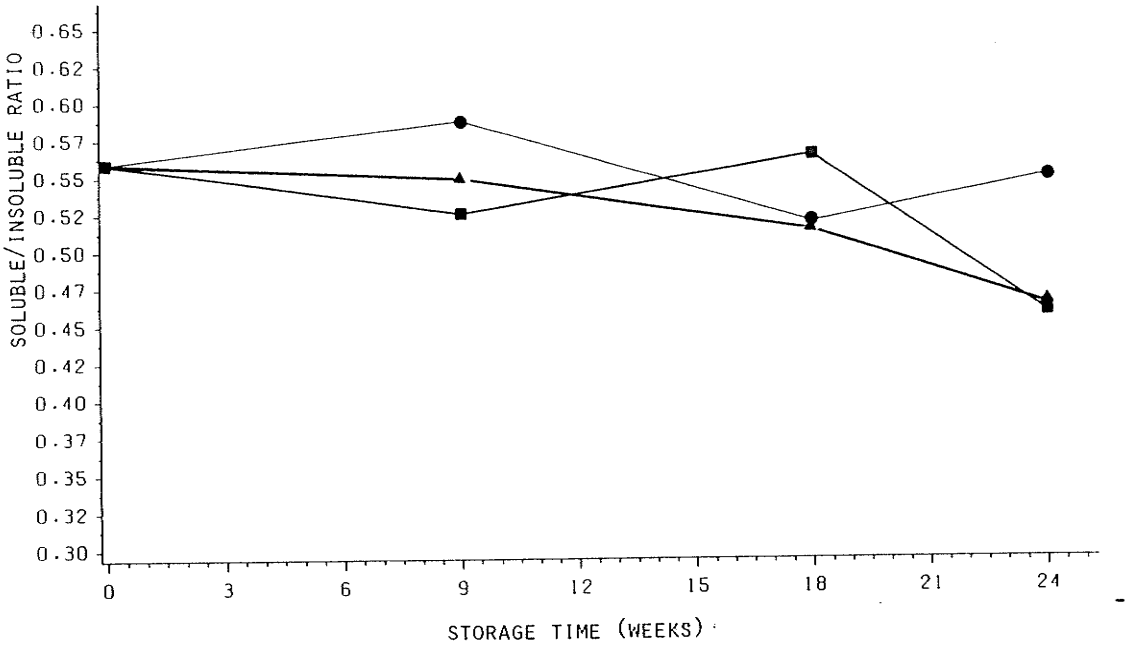

Figure 4.30: Soluble/insoluble ratio of DOR-364 beans stored under various temperatures. Initial moisture content 12% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

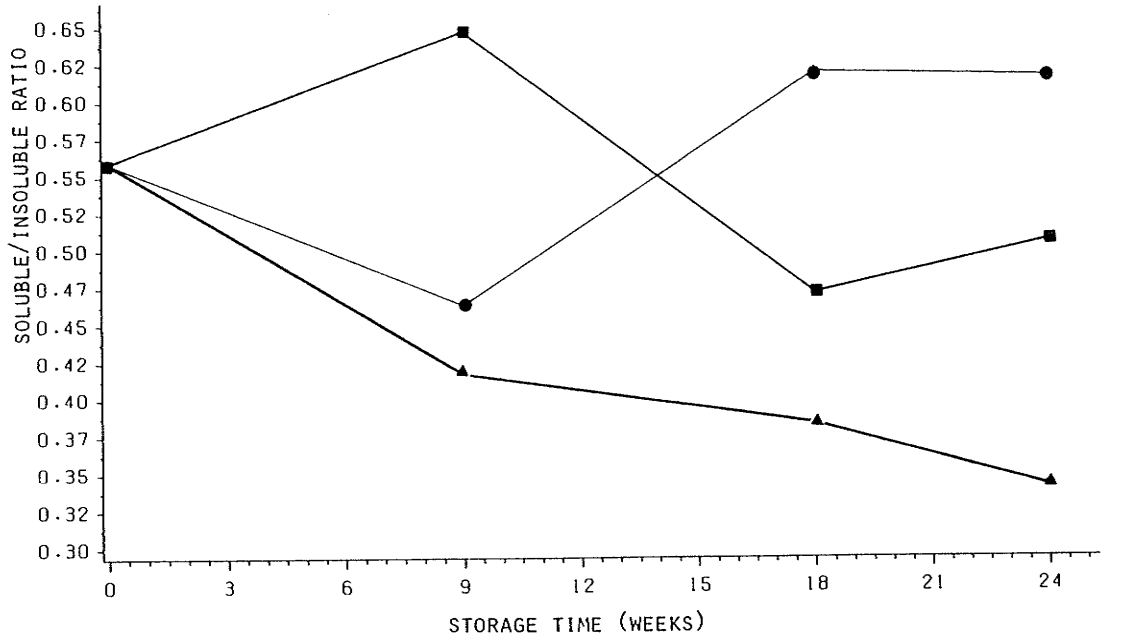

Figure 4.31: Soluble/insoluble ratio of DOR-364 beans stored under various temperatures. Initial moisture content 16% Legend: Samples stored at ● 9°C; ■ 23°C; ▲ 36°C.

There was a decrease in the ratio of soluble/insoluble fibre for the samples stored at 36° C, regardless of initial moisture content. This decrease was greater for the samples stored at 36° C with an 16% initial moisture content (Figure 4.31) than for the 12% moisture containing beans. For the samples stored at the other temperatures no consistent pattern was evident.

4.2.3.2.2 Dietary fibre fractions statistical analysis

The data was analyzed as the Tamazulapa data and the hardness and phytate content data, for the main effects temperature, moisture content, and the interaction temperature*moisture content; also for time effects, for time (as weeks), and for interactions temperature*week, moisture content*week, temperature*moisture content*week. The results of this analysis is presented in Table 4.12

TABLE 4.12: DIETARY FIBRE MAIN EFFECTS AND TIME EFFECTS OF DOR-364 BEANS[1]

	Total		Soluble		Insoluble		S/I	
Effects	F	p	F	p	F	p	F	p
Main effects								
MC	28.23	.0001	18.45	.0001	0.13	.7231	3.16	.0840
Temp	4.23	.0267	21.10	.0001	12.80	.0001	17.61	.0001
MC/Temp	2.28	.1241	17.27	.0001	3.91	.0281	9.10	.0006
Time effects								
Week	2.98	.0442	16.80	.0001	0.77	.5986	7.01	.0008
Week/MC	3.32	.0304	5.24	.0042	0.19	.9006	1.01	.3978
Week/Temp	1.24	.3078	7.66	.0001	2.11	.0650	5.32	.0005
Wk/MC/Temp	3.36	.0098	13.44	.0001	0.84	.5443	5.45	.0004

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

4.2.3.2.2.1 Total dietary fibre

The total dietary fibre main effects and time effects showed that there was a highly significant effect for moisture content (p=.0001), and a significant effect of temperature (p=.0267) and of week (p=.0442). Total dietary fibre increased for the samples with 12% initial moisture content when stored at 36° C. Total fibre generally decreased over time although initially there was an increase.

Time effects interactions of week*moisture content*temperature and week*moisture content were significant at p .0304, which can be seen in the many variations the different samples had during each week period. This determination is very difficult to interpret because being a sum of two fractions (soluble and insoluble fibre) it seems that the variations of each of them get more complex when added.

4.2.3.2.2.2 Soluble fibre

All the main effects and the week effect were highly significant for soluble fibre of DOR-364 beans. The figures generally show a decrease in soluble fibre content over time at all temperatures. This was more dramatic when samples were stored at high temperature (36° C) and had an initial high moisture content (16%).

Significant time effects (p .005) indicated that at each period the storage conditions affected samples differently.

4.2.3.2.2.3 Insoluble fibre

There was a highly significant effect of temperature (p .0001) on insoluble fibre. Insoluble fibre increased when samples were stored at higher temperatures, and decreased when storage temperatures were decreased. The moisture content*temperature effect was also significant (p=.0281). This effect is shown clearly in the figure of the samples with

16% initial moisture content where the effects of temperature are particularly evident.

Time effects were not significant for the insoluble fibre, meaning that at every period the samples seemed to follow the same variation, that changes in this type of fibre for DOR-364 beans are just related to temperature, with slight interaction of temperature*moisture content.

4.2.3.2.2.4 Soluble/Insoluble (S/I) ratio

Temperature had a highly significant (p .0001) effect on this ratio; with higher temperature storage resulting in a decrease. The interaction of temperature*moisture content (p=.0006) can be observed in Figure 4.31. For the samples that were stored with 16% initial moisture content, 36° C storage resulted in much lower S/I ratios than 9° and 23° C storage.

Time effects were very significant (p .0008), This can be explained by the general decrease in the ratio over storage periods. The interaction week*moisture content was not significant since at each period there was no effect that could be attributed specifically to moisture content. The interaction of week*temperature was significant. The interaction effects were illustrated in Figure 4.31.

4.2.3.3 Pooled samples

The fibre data were analyzed with all samples pooled to form one group. Bean variety (type) was a factor included with temperature, moisture content, time (week) in the analysis. In this case the interactions considering type were also included. Also results from this analysis were divided as main and time effects. These results are presented in Tables 4.13 and 4.14.

TABLE 4.13: DIETARY FIBRE MAIN EFFECTS OF POOLED SAMPLES, AVERAGED ACROSS TIME[1]

Main	Total		Soluble		Insoluble		S/I	
Effects	F	p	F	p	F	p	F	p
Type	16.73	.0001	376.00	.0001	38.15	.0001	227.49	.0001
MC	9.27	.0030	62.22	.0001	0.97	.3279	24.04	.0001
Temp	4.03	.0207	88.83	.0001	44.81	.0001	90.38	.0001
Type/MC	0.98	.3256	0.02	.8879	0.89	.3473	0.59	.4426
Type/Tem	0.91	.4048	0.03	.9710	0.78	.4601	0.49	.6141
MC/Temp	4.19	.0179	2.52	.0851	8.47	.0004	5.33	.0063
Type/MC/ Temp	5.00	.0084	16.13	.0001	0.41	.6670	4.59	.0123

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

TABLE 4.14: DIETARY FIBRE TIME EFFECTS OF POOLED SAMPLES[1]

Time	Total		Soluble		Insoluble		S/I	
Effects	F	p	F	p	F	p	F	p
Week	2.40	.0205	8.50	.0001	3.13	.0032	6.24	.0001
Type/Week	5.66	.0013	8.92	.0001	1.14	.3458	2.30	.0814
Temp/Week	1.57	.0900	8.61	.0001	2.81	.0008	6.10	.0001
MC/Week	1.66	.1160	3.96	.0004	0.97	.4626	1.92	.0651
Type/T/Wk	0.96	.4540	3.37	.0045	1.21	.3017	2.81	.0144
Type/MC/Wk	0.22	.8794	2.19	.0936	0.46	.7127	1.53	.2104
MC/Temp/Wk	1.48	.1228	4.36	.0001	1.29	.2171	2.42	.0040
Ty/MC/T/Wk	2.98	.0100	12.04	.0001	0.36	.9035	4.38	.0006

[1] F = F ratio; P = probability. Based on Type III Sums of Squares.

As shown in Table 4.13 when samples were analyzed as a pool, the main effect that was consistently highly significant (p .0001) for all the fibre fractions was type, that is bean variety. Temperature had a highly significant effect on all dependent variables, except total dietary fibre. This supports the decision to discriminate by variety for interpretation purposes.

Week had a significant effect (p=.0205) for all of the fractions, but the interpretation of changes during storage time is rather difficult due to the main interactions which occurred with type (bean variety) (Table 4.14).

4.2.3.4 Discussion

The relationship of total dietary fibre and of soluble and insoluble fibre to bean hardness has not as yet been

reported in the literature. Nevertheless there have been a few reports of soluble, insoluble and total fibre values for beans based on determinations made by recently developed dietary fibre methods. Results from these studies generally agree with the values found in this study. Hughes and Swanson (1989) reported 6.4 % for soluble fibre, 10.4 % for insoluble fibre, and 16.8 % for total dietary fibre contents of Tamazulapa beans. These authors used the AOAC method and it is assumed that data is presented on "as is" moisture basis. The values for the Tamazulapa beans in this study, on a 16% moisture content basis were 6.5 g/100g for soluble fibre, 10.2 g/100g for insoluble fibre and 16.7 g/100g for total dietary fibre. These values compare very closely, even though the methods used to determine the dietary fibre were not exactly the same, as Hughes and Swanson used the AOAC procedure. Mongeau and Brassard (1990) presented values for processed beans not identified by colour or variety, as part of a collaborative study to validate this method of analysis. These authors reported 7.25 % for soluble fibre, 10.94 % for insoluble fibre, and 18.19 % for total dietary fibre on a dry basis.

Total dietary fibre did not relate to hardness and this agrees with Watts et al (1990) who found no significant effect of storage method and length of storage on the total fibre content of black beans.

A definite increase in the insoluble fibre was observed

when beans were stored at high temperature (36° C), regardless of variety. Cotyledon cell walls of red beans were studied by Rozo (1982) and he reported large increases in the neutral detergent residue (NDR) when beans where stored at high temperature (40°C -80% RH). This increase was not evident when beans were stored at 30° C- 80% RH. The NDR is equivalent to insoluble fibre and includes cellulose, hemicellulose and lignin. Rozo (1982) did not find changes in the acid detergent residue (ADR) (comprised of celluloses and lignin) even with high temperature storage. The NDR-ADR value, which is an estimation of hemicellulose, increased almost two-fold over six months storage.

Watts and coworkers (1990) also reported an increase in neutral detergent fibre in stored beans. In the present study an increase in insoluble fibre was observed for beans stored at 36° C, 16% initial moisture content, regardless of variety, and some increase was observed for beans with 12% initial moisture content.

The soluble fibre also showed a definite decrease in this study, for beans stored at high temperature. There are no reports in the literature in relation to storage conditions, although Watts and coworkers (1990) did report a decreased soluble fibre in hardened beans.

The increase in insoluble fibre and the decrease in soluble fibre could account for the lack of change observed in total dietary fibre. Rozo (1982) did not observe changes

in lignin or cellulose content. Considering the fact that Rozo's study kept the moisture content constant and just temperature changed, his findings do agree with the findings reported here that temperature was the most important factor also in fibre changes. Since there was no absolute increase or decrease in total dietary fibre, a conversion from soluble to insoluble fibre could be accepted. Srisuma and coworkers (1989) reported that lignin content of navy bean seedcoats and cotyledons did not change in an 9 month storage study, even under extremely adverse conditions and development of the hard-to-cook defect. They concluded that enhanced lignification is not a major cause of bean hardening. They also found an increase in phenolic acid contents of stored beans, related to storage conditions. Although this was greater in the seedcoats than in the cotyledons it was sufficient in the cotyledon to account for an increase in phenolic compounds which would cross-link to pectin in the middle lamella. Their findings help interpret this study's: the lowering of the soluble fibre (pectin) by cross-links with phenolic compounds would result in greater insoluble fibre.

Soluble/Insoluble (S/I) ratio is a calculation that has not yet been reported in the literature. This ratio compares the fractions that did change during storage and it was the most sensitive measure of fibre changes in relation to hardness.

4.3 RELATIONSHIPS AMONG HARDNESS, PHYTATE AND DIETARY FIBRE FRACTION CONTENTS OF STORED BEANS.

Hardness of beans for each sampling period was measured as peak force after one and two hour cooking time, and related to phytate, S/I ratio, soluble, insoluble and total dietary fibre contents, to find the strength of these relationships.

4.3.1 Tamazulapa Beans

Comparisons of phytate content, dietary fibre fractions and hardness were done using Pearson's correlation coefficients. These coefficients, for Tamazulapa beans, are presented in Table 4.15.

TABLE 4.15: PEARSON'S CORRELATION COEFFICIENTS OF PHYTATE, DIETARY FIBRE FRACTIONS AND HARDNESS OF TAMAZULAPA BEANS

						Hardness	
	Phyt	S/I	Sol	Insol	TDF	1 h	2 h
Phytate	1.00	0.64^{c}	0.62^{c}	-0.48^{b}	-0.02	-0.61^{c}	-0.54^{c}
S/I		1.00	0.91^{c}	-0.85^{c}	-0.21	-0.82^{c}	-0.76^{c}
Soluble			1.00	-0.57^{c}	0.20	-0.73^{c}	-0.65^{c}
Insoluble				1.00	0.68^{c}	0.75c	0.73^{c}
TDF					1.00	0.25	0.29^{a}
Hard (1 h)						1.00	0.94^{c}
Hard (2 h)							1.00

^{a} $p \leq 0.05$
^{b} $p \leq 0.01$
^{c} $p \leq 0.0001$

Phytate correlated (p .01) with soluble fibre, soluble/insoluble (S/I) ratio, insoluble fibre and hardness regardless of cooking method. There was a positive relationship (r=.62) with soluble fibre and S/I ratio, and a negative correlation with hardness at one hour and 2 hour cooking time and with insoluble fibre. As beans hardened their phytate content decreased and insoluble fibre content increased. Phytate did not correlate with total dietary fibre.

S/I ratio correlated with its components, as expected, but also there was a correlation (p .0001) with hardness at both cooking times. This relationship was negative and strong (r=-.81) for one hour cooking, and negative and moderately strong (r=-.76) for 2 hour cooking. There was no significant relationship between S/I ratio and total dietary fibre.

Soluble fibre correlated (p .0001) negatively with insoluble fibre (r=-.57), and with hardness (r=-.73) for one hour cooking and (r=-.65) for two hour cooking. This indicated that as soluble fibre decreased, hardness increased and insoluble fibre also increased.

Insoluble fibre showed a moderately strong relationship with total dietary fibre (r=0.68). No relationship was found for total dietary fibre and phytate or hardness in Tamazulapa beans for samples cooked for 1 hour. There was a positive very weak relationship (r=.29) when samples were cooked for **2 hours.**

4.3.2 DOR-364 Beans

The same comparisons of phytate content, dietary fibre fractions and hardness were done using Pearson's correlation coefficients. The results of these comparisons for DOR-364 beans are presented in Table 4.16

TABLE 4.16: PEARSON'S CORRELATION COEFFICIENTS OF PHYTATE, DIETARY FIBRE AND HARDNESS OF DOR-364 BEANS

						Hardness	
	Phyt	S/I	Sol	Insol	TDF	1 h	2 h
Phytate	1.00	0.58[b]	0.56[b]	-0.57[b]	0.11	-0.54[c]	-0.63[c]
S/I		1.00	0.97[c]	-0.80[c]	0.42	-0.58[b]	-0.75[c]
Soluble			1.00	-0.63[b]	0.63[b]	-0.41[a]	-0.64[b]
Insoluble				1.00	0.20	0.81[c]	0.77[c]
TDF					1.00	0.30	-0.03
Hard (1 h)						1.00	0.70[c1]
Hard (2 h)							1.00

[a] $p \leq 0.05$
[b] $p \leq 0.01$
[c] $p \leq 0.0001$

[1]Values above 1200 newtons are excluded from this analysis.

Phytate content of DOR-364 beans correlated (p .0001) with hardness, regardless of cooking time. The relationships were negative and not strong (r -.54). Phytate was correlated positively with the soluble fibre and S/I ratio; and

negatively with insoluble fibre.

The S/I ratio correlated strongly with its components. The ratio had a moderately strong relationship with hardness when measured after 2 hour cooking (r=-.75), but not as strong a relationship (r=-.58) when measured after 1 hour cooking. These relationships were not as strong as were seen for the Tamazulapa beans.

Soluble fibre correlated negatively with insoluble fibre and hardness. This relationship was not strong, but it was stronger when hardness was measured after 2 hour cooking time. Soluble fibre had some relationship, for DOR-364 beans with total dietary fibre. This also differed from Tamazulapa beans.

Insoluble fibre correlated (p .0001) positively, with hardness regardless of cooking time method (r=0.81 for one hour cooking and r=.77 for two hour cooking). These relationships were stronger than were found for Tamazulapa beans. This suggests that as both types of beans harden their content of insoluble fibre increased.

Total dietary fibre correlated with soluble fibre (r=.63, p .01) but not with other components. No relationship was found for total dietary fibre and hardness in DOR-364 beans regardless of cooking time.

4.3.3 Pooled Samples

When samples were pooled some observations in the relationships of phytate and dietary fraction contents and

calculations, in relation to hardness were observed.

The Pearson's correlation coefficients for the pooled samples are presented in Table 4.17.

TABLE 4.17: PEARSON'S CORRELATION COEFFICIENTS OF PHYTATE, DIETARY FIBRE AND HARDNESS OF POOLED BEANS

						Hardness	
	Phyt	S/I	Sol	Insol	TDF	1 h	2 h
Phytate	1.00	-0.28[a]	-0.30[b]	0.18	-0.20	0.36[b]	0.39[c]
S/I		1.00	0.95[c]	-0.86[c]	0.11	-0.85[c]	-0.83[c]
Soluble			1.00	-0.67	0.40[c]	-0.76[c]	-0.78[c]
Insoluble				1.00	0.41[b]	0.81[c]	0.76[c]
TDF					1.00	0.06	-0.02
Hard (1 h)						1.00	0.92[c]*
Hard (2 h)							1.00

[a] $p \leq 0.05$
[b] $p \leq 0.01$
[c] $p \leq 0.0001$

* Values above 1200 newtons are excluded from this analysis.

When the data were pooled the relationships between phytate and hardness was not strong but was positive. For the varieties separately, the relationships between phytate and hardness were stronger and negative. This change in the direction of the relationship was also observed between phytate and soluble fibre, and phytate and the S/I ratio with

the pooled data.

The relationships observed for the S/I ratio for every bean variety were in the same direction and even got stronger when the data were pooled. The relationship for the pooled data can be seen in Figure 4.32.

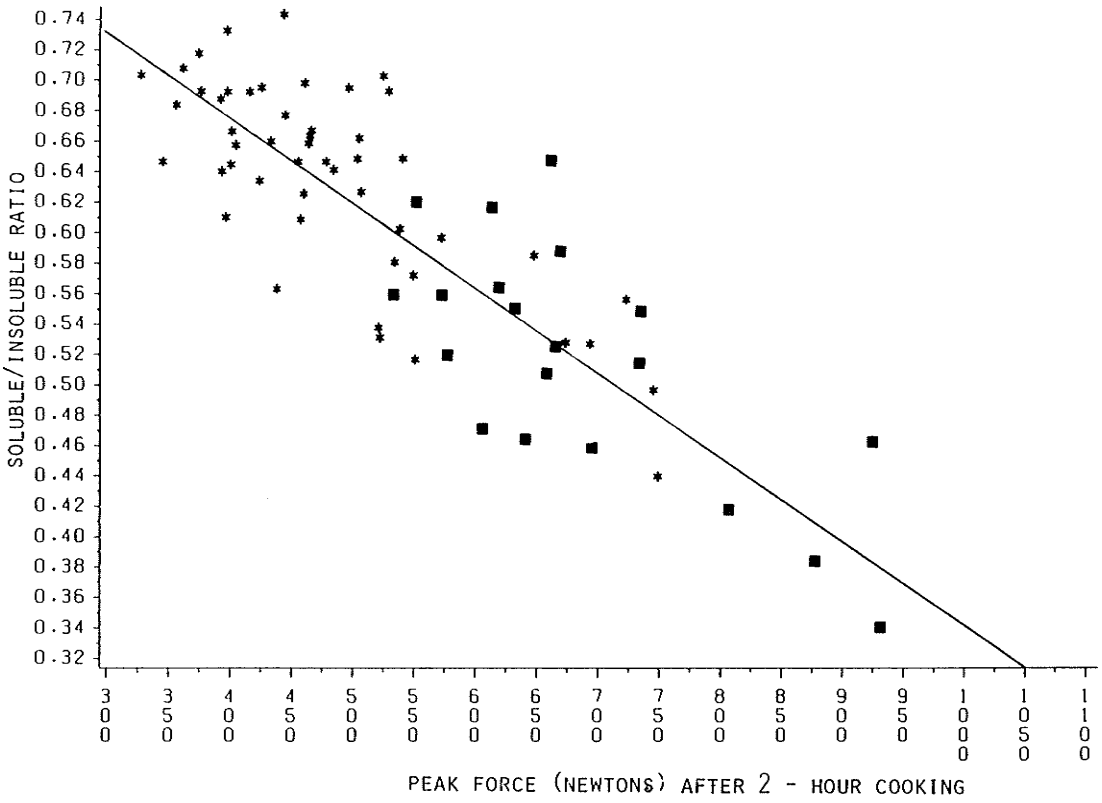

Figure 4.32: Relationship of Soluble/Insoluble ratio with hardness, measured for the samples pooled, after 2-hour cooking.
Legend: * Tamazulapa; ■ DOR-364.

The figure shows the line is extended when varieties are combined and this increases the r value. This relationship is stronger than for phytate/hardness for either bean variety, independently suggesting that the S/I ratio is a more useful measurement of hardness than phytate. The S/I ratio is not affected by bean variety.

4.3.3.4 Discussion

The soluble/insoluble (S/I) ratio, a calculation not yet reported in the literature, showed a better relationship to hardness than any other measurement for Tamazulapa beans, and the pooled samples. For the DOR-364 beans (using the 2-hour measurement for hardness as this data set is complete) the correlation coefficient for the S/I ratio was not the highest indicator of hardness, but was a very good indicator of hardness along with insoluble fibre (-0.75 for S/I and 0.77 for insoluble fibre). This relationship was a negative one, that is, the harder beans had a lower S/I ratio. The S/I ratio appeared to account for the changes occurring during storage, in relation to temperature and moisture content, more than the other measurements, and regardless of variety.

Insoluble fibre showed a strong, positive relationship to hardness, regardless of variety. This was the next most sensitive measurement in relation to hardness, and (when hardness was measured after 1-hour cooking time) this was the **best relationship (r=0.81). These results agree with the**

correlation of NDF to hardness (r=0.82) reported by Watts and coworkers (1990). The chemical determination of insoluble fibre used in this study, although gravimetric, has an acceptable precision as shown in a recent collaborative study by Mongeau and Brassard (1990). Insoluble fibre could give a good indication of stored beans hardness: as insoluble fibre increases, so does hardness.

Soluble fibre in relation to hardness varied in the strength of the relationship for each variety, although when varieties were pooled it improved the strength of the relationship. Watts and coworkers (1990) reported a correlation coefficient of -0.98 for soluble fibre and hardness, measured after 2-hour cooking. Their relationship was stronger because they did not discriminate storage conditions in their study, and their value included stored samples at low humidity, low temperature; high humidity and high temperature, and that were either initially field dried or heat treated to prevent hardness. Nevertheless, soluble fibre in relation to hardness did have a negative relationship for both studies, showing decreases in relation to storage method and/or hardness.

Phytate had a negative relationship to hardness (r from -0.54 to -0.63) but it was not as important as expected. It has been reported in the literature a relationship between phytic acid phosphorus content of soaked beans and softening rates of beans of r=0.96 by Moscoso et al (1984). And

Sievwright and Shipe (1986) reported a relationship between firmness and loss of phytic acid of r=0.88. Fibre fractions had a stronger relationship. When varieties were pooled there was no relationship between phytate content and hardness (r 0.39). The correlation coefficient reported by Hincks and Stanley (1986) between phytate loss and development of hardness in cooked beans was -0.76 ($p<0.01$). This was based on a study using one bean variety only. It can be concluded that for the individual varieties reduction in phytate is related to development of hardness, but absolute phytate levels do not correspond to hardness measurements when several varieties are compared.

Total dietary fibre did not have any relation to hardness, confirming results reported by Watts and coworkers (1990). Soluble fibre related to hardness but insoluble fibre gave a stronger relationship. This seems to indicate that hardness is not only related to pectic substances and phytate/cation exchange, but that hemicelluloses changes should be studied in more depth, to fully understand the bean hardening process.

V SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 SUMMARY

A storage study was conducted using two improved bush bean varieties (Phaseolus vulgaris), grown in Jutiapa, Guatemala from the crop of August 1988. One was a black variety (Tamazulapa) and the other a red variety (DOR-364). Beans with two different initial moisture contents (12% and 16%) were stored under three different temperatures (9°, 23° and 36° C) to determine changes in the hardening in relation to the total, soluble, insoluble dietary fibre, and soluble/insoluble ratio, and phytic acid content across time.

Initial hardness, tested after 2 hours of cooking, was higher for the red bean variety DOR-364 (mean 554 N) than for the black variety Tamazulapa (mean 467 N). Although changes occurred in relation to storage conditions, the higher relative hardness remained throughout the 24 weeks of storage. The difference in hardness was about 200 N, although it was higher (224 to 353 N) for samples with 12% initial moisture content than for samples with 16% initial moisture content (153 to 184 N). The phytate content of the DOR-364 was higher than that of the Tamazulapa at the beginning of the study (1.42 g/100g for red beans to 1.18 g/100g for black beans) and remained higher throughout the storage period. This indicated that there are considerable differences in phytate among varieties and that these differences do not relate to

hardness. For this reason, it can be assumed that hardness of beans cannot be predicted from the absolute value of phytate. It also shows that factors other than phytate content are predictive of hardness of beans.

Cooking samples for 1 and 2 hour gave similar results for each bean variety (r=0.93 for pooled data). It would not be advisable to use the data for one variety to extrapolate to another as cooking rate might vary. Cooking rate differences were observed in this study in relation to storage conditions for the two varieties.

Soluble fibre content and Soluble/Insoluble (S/I) ratio were initially high for both varieties of beans and both decreased with time as the beans hardened. Soluble fibre decreased from 7.8 to 6.5 g/100g in the Tamazulapa beans, and from 6.9 to 4.9 g/100g in the DOR-364, for samples with 16% initial moisture content stored at 36° C. There was a strong, negative relationship observed between hardness and soluble fibre (r=-0.78), and hardness and S/I ratio (r=-0.83). The relationship of soluble fibre to hardness although strong for the pooled data, was not maintained when varieties were analyzed separately (r=-0.65 for Tamazulapa and r=-0.64 for DOR-364).

The initial and final S/I ratios for the 16% initial moisture content, 36° C samples were: Tamazulapa 0.65 and 0.44, and DOR-364 from 0.56 and 0.34, respectively. The more the soluble fibre content, or the higher the S/I ratio of the

beans, the greater the degree of softness of the beans. The DOR-364 beans had initial increased hardness (about 90 N more) and also had a lower content of soluble fibre (0.9 g/100g less).

The S/I ratio is a more sensitive measure of hardness (r=-0.83 for 2-h cooking and r=-0.85 for 1-h cooking) than any of the fibre fractions (r=0.76 insoluble fibre, r=-0.78 soluble fibre), or phytate content (r=0.39). Relationships were similar for both varieties and for the pooled data. This was not the case for phytate. When varieties were analyzed separately the correlation of phytate with hardness was r=-0.63 for DOR-364 and r=-0.54 for Tamazulapa. For pooled samples r=0.39.

Initial insoluble fibre values were similar for both varieties (12.1 g/100g for Tamazulapa and 12.5 g/100g for DOR-364 beans). Insoluble fibre showed a strong positive relationship with hardness, r=0.76 for the pooled data, r=0.77 for DOR-364, and r=0.73 for Tamazulapa. When beans were stored at high temperature (36° C) and at 16% moisture content they increased their insoluble fibre content as they hardened by 2.6 g/100g for the Tamazulapa beans, and by 1.7 g/100g for the DOR-364 beans.

Total dietary fibre did not relate to hardness when data were pooled r=-0.02. When data were analyzed separately by variety a relationships of r=0.29 for Tamazulapa and r=-0.03 DOR-364 were found. The direction of these relationships was

not constant, sometimes it was positive and at other times negative. This suggested that total dietary fibre was not a reliable measure in relation to hardness.

In general, temperature was the variable which had the greatest effect on hardness, phytate and fibre fraction contents. Moisture content was also important but not a constant factor. It was not important for phytate content. Moisture content and temperature interactions were also important, giving an effect above and beyond the ones observed for temperature alone.

5.2 CONCLUSIONS

From the results in this study it can be concluded that

1. The soluble/insoluble (S/I) ratio is the best indicator for hardness of any of the fibre fractions and is better than phytate content, for beans stored under different temperature and initial moisture content conditions. This relationship is a negative one, so as beans harden the S/I ratio falls. This is a particularly useful indicator because the ratios are unitless, that is they reflect the proportion of the soluble to insoluble fibre but not the absolute amounts.

2. Phytate content is not a good indicator for hardness of stored beans when several varieties are compared. If one variety is studied then this measure could be a useful one,

but any of the fibre fractions measurements will give a better relationship to hardening. Phytate content decreases when beans are stored at high temperatures and therefore changes in the phytate content have a relation to other hardening changes.

3. Temperature is the most significant storage factor in relation to bean hardening. Temperature influenced all of the physical and chemical changes observed during storage. Moisture content affects some of the changes observed in relation to hardness. When high temperature and high moisture are combined there is a synergistic effect in almost all cases when changes are significant.

4. Insoluble fibre is the second most sensitive measure related to hardness. This is a simple reliable gravimetric technique, and as such, it could be used as an alternative to indicate hardness of beans.

5. Total dietary fibre is not related to hardness of stored beans.

6. The cooking time used in the hardness determinations can influence hardness comparisons.

7. Studies of hardening should include more than one variety if general inferences about beans are to be made.

5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

1. Cooking rate studies for various bean varieties should be done so that comparisons of hardness can be made among studies using different cooking times.

2. Studies including a number of varieties to determine differences in hardness in relation to initial phytate content are needed. These studies should consider changes in hardness in relation to phytate decreases in individual varieties.

3. Dietary fibre fraction determinations can be long and tedious, and it can be difficult to achieve low variability of results. Near Infrared Reflectance Spectroscopy (NIR) might be used to predict soluble and insoluble fibre. Research should be done to develop a standard equation for prediction.

4. Studies to determine the component sugars of the soluble fibres should be carried out, and changes in component sugars, in relation to storage should be investigated. This could give information about the pectin and hemicellulose components of the soluble fibre. Hardening might also be predicted on the basis of changes in these carbohydrate fraction.

5. Studies analyzing the changes in hemicellulose in relation to hardness of beans are needed to understand clearly the changes occurring during storage, specially at cell wall level.

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APPENDIX A

DOLE READINGS FOR MOISTURE PERCENT OF BEANS STORED UNDER VARIOUS TEMPERATURES MEASURED AT THREE-WEEK INTERVALS

Storage Condition[1]		Weeks							
IMC	T	3	6	9	12	15	18	21	24
Tamazulapa									
12	9	11.74	11.39	11.30	11.30	11.30	11.24	11.38	11.47
12	23	11.56	11.56	11.47	11.18	11.24	11.24	11.30	12.94
12	36	11.30	11.18	*	*	*	*	*	*
16	9	15.94	15.50	17.08	15.67	15.41	15.67	15.24	15.50
16	23	16.52	16.44	14.84	15.32	16.02	15.13	14.61	15.13
16	36	15.03	14.20	14.44	11.83	12.39	11.47	11.30	11.83
DOR-364									
12	9	11.47	11.75	11.47	11.47	11.56	11.83	11.83	11.38
12	23	11.83	11.47	11.66	11.66	11.30	11.47	11.38	11.47
12	36	12.00	11.30	*	*	*	*	*	*
16	9	16.02	15.86	14.93	16.02	15.67	16.12	16.21	16.21
16	23	16.11	15.50	16.02	15.86	15.41	15.77	14.84	15.41
16	36	16.06	14.12	14.20	12.94	12.56	11.83	11.66	11.66

[1] IMC = initial moisture content, %; T = temperature, °C.
*The Moisture Tester table used did not give values for moisture contents bellow 11.18%

APPENDIX B

HARDNESS OF STORED BEANS - RAW DATA

TABLE B.1: HARDNESS MEASURED AFTER 1-HOUR COOKING, OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(Newtons)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←------	666*±24	------→	←------	672*±14	------→
3	565± 6	605± 8	652±24	580± 8	638±13	764±27
6	559±25	606±30	711± 5	621±22	663±24	820±27
9[2]	657±25	694±20	828± 4	690±16	728± 4	1038±10
12	690±13	712±13	859±13	720±16	792±16	1025±24
15	641±14	697±10	837±27	670±18	759±11	1013±23
18	622±17	632±18	830±16	672±32	708±25	1024±14
21	576±18	615±29	838±33	632±25	775± 5	1045±24
24	605±26	657±21	904± 6	670±36	779±26	1103±40

[1] Measured with an OTMS. Means of 4 measurements (duplicates and replicates) and standard error of the mean.

[2] Means of 2 measurements (duplicates) and standard error of the mean.

APPENDIX B

HARDNESS OF STORED BEANS - RAW DATA

TABLE B.2: HARDNESS MEASURED AFTER 2-HOUR COOKING, OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(Newtons)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←------	456*± 2	------→	←------	478*±10	------→
3	374±11	398±10	405± 8	426±13	424±15	464± 2
6	328± 9	356±10	438± 9	346± 7	397± 5	521± 6
9[2]	376± 6	416± 8	522± 6	401± 4	460±20	647±18
12	466±18	496±16	551± 5	505±12	525±12	694± 8
15	444±23	484± 9	534±10	529± 9	538±12	674± 5
18	392±13	445±10	540±15	465±20	507± 9	723±12
21	362± 9	398± 5	549± 8	394±14	458±27	745±22
24	407±14	461±17	572±17	433±15	504± 5	748±11

[1] Measured with and OTMS. Means of 4 measurements (duplicates and replicates) and standard error of the mean.

[2] Means of 2 measurements (duplicates) and standard error of the mean.

APPENDIX B

HARDNESS OF STORED BEANS - RAW DATA

TABLE B.3: HARDNESS MEASURED AFTER 1-HOUR COOKING, OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(Newtons)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←-------	957±19	------→	←------	796±46	------→
3	863± 7	860±13	909±14	746± 5	745±13	894± 5
6[2]	964±11	960±36	986± 8	813±16	810±26	1044±42
9	1027±39	1021±27	*	859±17	885±20	1183±13
12	921±14	925±20	1199± 1	786±10	840±16	1190± 6
15	906±21	1004±30	*	768±15	860±19	*
18	975±39	1075±58	*	839±15	891±20	*
21	865±12	963±34	*	802±10	871±10	*
24	917±17	948±46	*	782±14	871±23	*

[1] Measured with and OTMS. Means of 4 measurements (duplicates and replicates) and standard error of the mean.
[2] Means of 2 measurements (duplicates) and standard error of the mean.
* Values above 1200 Newtons.

APPENDIX B

HARDNESS OF STORED BEANS - RAW DATA

TABLE B.4: HARDNESS MEASURED AFTER 2-HOUR COOKING, OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(Newtons)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←------	573*±7	------→	←------	534*±14	------→
3	550± 4	567±15	620± 9	513± 7	540± 4	621± 8
6[2]	604[3]	620±33	704[3]	622±10	644±16	770±56
9	670±16	667±11	735±11	641± 7	660± 7	807±12
12	646±25	644±27	769± 5	622±27	665±32	893±26
15	596±17	628±25	794±12	607±23	636±18	906± 5
18	577± 2	618±17	733±12	551± 8	606± 7	878±10
21	635±13	645± 7	817± 8	603± 8	660± 4	936± 4
24	633± 9	695±15	925±24	614±28	657±13	932±14

[1] Measured with an OTMS. Means of 4 measurements (duplicates and replicates) and standard error of the mean.
[2] Means of 2 measurements (duplicates) and standard error of the mean.
[3] Single determinations.

APPENDIX C

PHYTATE CONTENT OF STORED BEANS - RAW DATA

TABLE C.1: PHYTATE CONTENT OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←------	1.18±.00	------→	←------	1.19±.00	------→
3	1.20±.01	1.22±.01	1.10±.04	1.18±.07	1.18±.06	1.11±.02
6	1.27±.01	1.13±.00	1.00±.06	1.28±.02	1.16±.02	1.15±.03
9	1.24±.01	1.19±.03	1.07±.01	1.21±.02	1.04±.00	1.09±.01
12	1.24±.02	1.27±.00	1.14±.01	1.28±.01	1.24±.02	1.06±.02
15	1.14±.04	1.26±.02	1.01±.00	1.25±.02	1.04±.09	1.06±.01
18	1.15±.00	1.25±.01	1.11±.00	1.16±.03	1.13±.00	1.09±.01
21	1.15±.01	1.16±.06	1.08±.01	1.25±.01	1.10±.02	1.04±.00
24	1.22±.00	1.15±.00	1.09±.00	1.23±.04	1.12±.04	1.06±.00

[1] Means of duplicate measures and standard error of the mean.

APPENDIX C

PHYTATE CONTENT OF STORED BEANS - RAW DATA

TABLE C.2: PHYTATE CONTENT OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1] (g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	←-------	1.42±.00	-------→	←-------	1.42±.00	-------→
3	1.44±.01	1.41±.07	1.46±.02	1.43±.02	1.39±.04	1.36±.02
6	1.40±.01	1.52±.02	1.54±.05	1.45±.05	1.40±.05	1.38±.00
9	1.48±.01	1.46±.00	1.42±.01	1.49±.01	1.43±.08	1.38±.03
12	1.47±.01	1.46±.02	1.36±.03	1.50±.02	1.50±.03	1.40±.00
15	1.50±.02	1.49±.01	1.42±.00	1.46±.00	1.44±.02	1.37±.04
18	1.44±.00	1.45±.00	1.43±.01	1.42±.01	1.48±.01	1.32±.04
21	1.44±.00	1.47±.03	1.27±.01	1.49±.00	1.47±.02	1.28±.03
24	1.41±.06	1.40±.01	1.28±.01	1.41±.05	1.45±.04	1.22±.01

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.1: TOTAL DIETARY FIBRE OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(g/100g)

	12% moisture content			16% moisture content		
Week	9°	23°	36°	9°	23°	36°
0	----------19.9±0.4----------					
3	19.3±0.3	19.2±0.0	19.3±0.0	18.4±0.2	19.1±0.1	19.8±0.4
6	20.1±0.2	19.0±0.2	19.4±0.4	19.1±0.2	19.1±0.3	20.0±0.0
9	19.4±0.1	20.5±0.1	18.8±0.6	18.9±0.1	18.8±0.3	19.2±0.0
12	19.5±0.6	20.4±0.7	19.1±0.6	19.2±0.1	18.6±0.0	20.6±0.5
15	20.0±0.1	19.4±0.5	19.9±0.6	19.5±0.0	18.7±0.2	20.0±0.0
18	19.9±0.1	20.3±0.1	19.9±0.5	19.5±0.1	19.7±0.1	19.8±0.6
21	19.9±0.4	19.8±0.0	19.6±0.6	20.2±0.3	19.2±0.0	20.2±0.1
24	20.0±0.3	19.7±0.2	20.1±0.4	20.0±0.1	18.9±0.2	21.2±0.8

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.2: SOLUBLE FIBRE OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1] (g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- 7.8±0.1 ----------					
3	8.1±0.1	8.1±0.3	7.6±0.1	7.5±0.0	7.4±0.3	7.8±0.1
6	8.3±0.0	7.7±0.0	7.0±0.2	7.5±0.2	7.2±0.4	7.0±0.1
9	7.9±0.1	8.4±0.1	6.5±0.0	7.6±0.1	7.2±0.1	7.1±0.2
12	7.8±0.1	8.4±0.2	6.5±0.1	7.6±0.2	7.7±0.0	7.1±0.1
15	8.5±0.1	7.6±0.4	7.3±0.3	8.0±0.1	7.0±0.0	6.9±0.1
18	8.1±0.1	8.2±0.2	7.8±0.2	7.8±0.1	7.6±0.0	7.1±0.5
21	8.3±0.0	8.1±0.1	7.1±0.2	7.9±0.0	7.2±0.1	6.7±0.1
24	7.8±0.2	8.1±0.1	7.5±0.0	7.9±0.0	7.4±0.1	6.5±0.4

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.3: INSOLUBLE FIBRE OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- 12.1±0.4 ----------					
3	11.2±0.2	11.1±0.4	11.6±0.0	10.8±0.3	11.7±0.4	11.9±0.4
6	11.8±0.2	11.3±0.2	12.4±0.2	11.6±0.3	11.9±0.1	13.0±0.1
9	11.4±0.1	12.1±0.0	12.3±0.5	11.3±0.2	11.5±0.2	12.1±0.3
12	11.7±0.5	12.0±0.5	12.6±0.6	11.6±0.2	10.9±0.0	13.5±0.4
15	11.5±0.0	11.8±0.0	12.6±0.3	11.5±0.2	11.6±0.2	13.1±0.1
18	11.8±0.2	12.1±0.1	12.1±0.3	11.7±0.0	12.1±0.1	12.7±0.1
21	11.7±0.4	11.7±0.1	12.4±0.4	12.3±0.2	11.9±0.1	13.5±0.0
24	12.1±0.1	11.6±0.0	12.6±0.4	12.0±0.1	11.4±0.1	14.7±0.4

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.4: SOLUBLE/INSOLUBLE FIBRE RATIO OF TAMAZULAPA BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- .65±0.2 ----------					
3	.71±.00	.73±.06	.66±.01	.70±.02	.63±.05	.66±.03
6	.70±.01	.68±.01	.56±.00	.65±.03	.61±.04	.54±.01
9	.69±.02	.69±.00	.53±.02	.67±.02	.62±.00	.58±.03
12	.67±.02	.69±.01	.52±.01	.66±.02	.70±.01	.53±.01
15	.74±.01	.64±.03	.58±.05	.69±.02	.60±.01	.53±.01
18	.69±.02	.68±.02	.64±.00	.66±.01	.63±.00	.56±.04
21	.71±.02	.69±.02	.57±.00	.64±.01	.61±.01	.50±.01
24	.64±.01	.70±.01	.59±.02	.66±.00	.65±.00	.44±.01

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.5: TOTAL DIETARY FIBRE OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

(g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- 19.4±0.3 ----------					
9	20.1±0.1	19.9±0.2	20.0±0.7	17.7±0.0	20.2±0.1	19.2±0.4
18	18.9±0.4	20.1±0.2	20.0±0.4	19.1±0.1	18.8±0.4	18.9±0.1
24	19.1±0.2	18.6±0.1	19.1±0.0	18.6±0.1	19.1±0.5	19.1±0.5

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.6: SOLUBLE FIBRE OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1] (g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- 6.9±0.1 ----------					
9	7.4±0.2	6.8±0.2	7.1±0.4	5.6±0.2	7.9±0.2	5.7±0.1
18	6.5±0.4	7.3±0.2	6.8±0.4	7.3±0.4	6.0±0.2	5.2±0.0
24	6.8±0.1	5.8±0.0	6.0±0.1	7.1±0.1	6.4±0.1	4.9±0.3

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.7: INSOLUBLE FIBRE OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1] (g/100g)

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- 12.5±0.4 ----------					
9	12.7±0.1	13.0±0.0	13.0±0.3	12.1±0.3	12.2±0.1	13.6±0.3
18	12.4±0.0	12.9±0.0	13.2±0.0	11.8±0.3	12.8±0.1	13.7±0.1
24	12.4±0.3	12.7±0.1	13.1±0.0	11.5±0.2	12.7±0.4	14.2±0.2

[1] Means of duplicate measures and standard error of the mean.

APPENDIX D

FIBRE FRACTIONS OF STORED BEANS - RAW DATA

TABLE D.8: SOLUBLE/INSOLUBLE FIBRE RATIO OF DOR-364 BEANS STORED AT DIFFERENT TEMPERATURES AND MOISTURE CONTENTS[1]

	12% moisture content			16% moisture content		
Wk	9°	23°	36°	9°	23°	36°
0	---------- .56±.03 ----------					
9	.59±.02	.52±.02	.55±.02	.46±.03	.65±.02	.42±.00
18	.52±.04	.56±.01	.51±.03	.62±.05	.47±.00	.38±.00
24	.55±.02	.46±.02	.46±.01	.62±.01	.51±.01	.34±.02

[1] Means of duplicate measures and standard error of the mean.