Yield and Quality of Soymilk and Tofu Made from Manitoba-grown Food Grade Soybeans

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

Effects of variety and environment on seed characteristics and processing potential of Manitoba-grown food-grade soybeans were determined and compared to Harovinton, a well-established Canadian food-grade soybean variety. Seed size and seed protein and sucrose content were measured. A laboratory-scale procedure for soymilk and tofu production was adapted to evaluate soymilk yield and colour and tofu yield, colour, and hardness. Eleven soybean varieties at three sites (Carman, Morris, Rosebank) were evaluated in 2005 and ten soybean varieties at three sites (St. Adolphe, Morris, Rosebank) were evaluated in 2006. Both site-year and variety main effects were found to significantly affect soybean seed characteristics (protein), soymilk colour, and tofu hardness ($P < 0.0001$). The site by year interaction was significant for seed protein, soymilk colour, and tofu hardness. Seed size for Manitoba-grown soybean varieties were generally smaller than the commercial Harovinton sample; exceptions were the varieties OT05-21 and OT05-20, which were only grown in 2006. Protein content of the varieties CL987704, Kaminchis and Lotus, grown in 2005 only, were similar to Harovinton; however, all other Manitoba-grown soybeans were lower in protein content. The varieties OAC Prudence, Jim, OAC 01-12, OAC Erin and OT05-20 grown in 2006 had higher seed sucrose content than Harovinton. For soybeans with lower protein content, soymilk and tofu yield and tofu color were lower than for Harovinton. Soymilk colour and tofu hardness for all Manitoba-grown soybeans compared well to Harovinton. While it is possible to make acceptable soymilk and tofu from Manitoba-grown soybeans, the cultivars that meet acceptable agronomic criteria are deficient in terms of yield and/or seed size.

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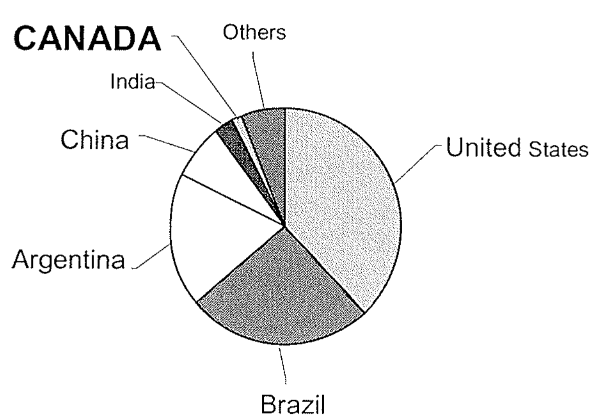
CHAPTER 1

1 Introduction

Although Canada accounts for only 1.4% of the world's soybean production (Figure 1.1) it is recognized as a leader in soybean quality. Through its Identity Preservation [IP] program Canada can guarantee buyers that the soybeans they are purchasing have the safety and quality they require. IP soybeans are desired for food products because food safety and quality are critical to both processors and consumers. Approximately 75% of all Canadian soybeans sold in Asian markets are IP certified and are primarily used for the production of soymilk and tofu (Canadian Soybean Exporters Association, 2006).

Figure 1.1. The estimated world soybean production in 2006.



Source: (http://www.pecad.fas.usda.gov/wap.cfm)

Most soybean production in Canada takes place in Ontario (77.1%) followed by Quebec (15.3%) and Manitoba (7.3%) (Canadian Grain Commission, 2006). Soybean production in Manitoba is quite recent, since until the past five years there were few soybean varieties that could be grown in locations as far north as Manitoba. When soybean production was first attempted in Manitoba, the varieties grown had been developed for the southern Ontario growing environment. Varieties bred for Southern Ontario had difficulty growing in Manitoba because of differences in day length between the two growing areas. This was a problem because soybean plants are photoperiod sensitive and therefore grow and develop according to the day length (hours of sunshine). An example of the importance of selecting a soybean variety that is suitable for growing in a specific growing region with a specific day length is given by Scott and Aldrich (1970):

> "Flowering of a southern-grown variety is initiated by a shorter day than that of varieties adapted to the northern region. At New Orleans Louisiana, on July 3 there are about 14 hours of sunlight between sunrise and sunset; at Winnipeg, in Canada, there are about 16 hours. A variety adapted to Louisiana is selected to start flowering when the day length is 14 hours or less. In the Winnipeg area this variety would flower in the middle of August when the day length at that latitude finally shortens to the necessary 14 hours. Conversely, if a variety adapted to the northern area is moved south, it will begin flowering much earlier than if kept in its own area of adaptation." (p. 16)

Soybeans are categorized into 10 maturity groups based on the growing location for which they have been adapted. These categories range from the most northern 00 group (i.e. Southern Canada) to the most southern group X (i.e. South America). Because these variety groupings did not originally encompass Manitoba, the 00 group has been further divided into three growing seasons: short-, mid-, and long-season areas, with varieties in the long season area typically being better suited to Southern Ontario. The short and mid-season area varieties are better suited for growing in Manitoba. However, short season varieties will also typically have lower soybean yields than varieties grown in long season areas. In recent years the development of improved short-season soybean varieties has resulted in a steady increase in soybean production in Manitoba. In 2006 there were 360,000 acres of soybeans sown, which was a two-fold increase from the 2005 crop year (Manitoba Agriculture Services Corporation, 2006).

One of the major challenges facing Manitoba soybean producers is determining which soybean varieties are best suited for their growing environment and which food-grade varieties have the desired characteristics suitable for soymilk and tofu production. Evaluating the quality of food-grade soybeans cannot be adequately measured by determining the macronutrients and physical characteristics of the seeds alone (Mullin *et al.,* 2001). A more thorough evaluation of the quality of food grade soybeans requires the small scale production method of soymilk and tofu to assess yield, colour, and texture. At this time there is no standardized method for testing the quality of soybeans for the production of soymilk and tofu. Thus there is a need to develop a laboratory-scale method to evaluate the suitability of Manitoba-grown food-grade soybeans for soymilk and tofu production.

The development of laboratory-scale method to evaluate the suitability of Manitoba-grown food-grade soybeans for soymilk and tofu production would aid breeders in selecting suitable soybean varieties for the Manitoba growing area. The development of more suitable Manitoba soybean varieties would allow Manitoba to become more prominent in supplying high quality soybeans to soymilk and tofu processors. Therefore, the objectives of this study were as follows:

(1) To establish a laboratory-scale method for preparing soymilk and tofu.

(2) To examine the effects of soybean variety, growing location, and crop year on soymilk and tofu yield and quality.

(3) To compare the soymilk and tofu quality of Manitoba-grown food grade soybeans with an established high quality, food grade soybean variety.

CHAPTER 2

2 Review of Literature

Historical evidence suggests that the first soybean crops were grown in Northern China during the 11^{th} century B.C. (Liu, 1999). Soybean production remained limited to Asia until its introduction into Europe in the 1700's. It was upon their arrival in Europe that the soybean was given its botanical name *Glycine Max* (L) Merr. Translated, *Glycine* means "sweet", which is a term used to describe all groundnut species of legume, and *Max* means "large", which makes reference to the characteristic large nodules of the soybean plant (Liu, 1999).

The introduction of soybeans into North America is believed to have occurred around 1764, but large-scale production of the crop did not come about until the 1900s. The demand for oil after the onset of World War II bolstered the importance of soybeans as a commodity and drove an increase in production and processing (Liu, 1999). Soybeans were used solely for oil during this time and it became important to find a use for the soybean meal, a by-product from oil processing and a nuisance to discard. Eventually, the meal was used as a high protein source in animal feed and became as important as the oil.

The introduction of soybeans in Canada was advanced by the creation of a soybean research program in 1923 by Dr. F. Dimmock at Harrow, Ontario. (Ontario Soybean Growers, no date given). Today, Agriculture and Agri-Food Canada's Greenhouse and Processing Crops Research Centre (GPCRC), located at Harrow, Ontario continues to be the leader in soybean variety testing and development. Research performed at Harrow has lead to the development of soybean varieties that are suited to the Canadian climate. The GPCRC has also focused on the development of food-grade soybeans with specific attributes such as high protein to meet the requirements of soybean food processors.

2.1 Soybean Seed Structural Parts

Soybean seeds are primarily comprised of three principal components: the seed coat, the cotyledons, and the embryo (Liu, 1999).

2.1.1 Seed Coat

The seed coat's function is to protect the embryo before and after planting. A crack in the seed coat makes the seed very susceptible to bacteria and fungus and makes further plant development very unlikely. The seed coat is marked with a hilum, which is either oval or round and ranges in colour from black, brown, yellow or grey.

2.1.2 Cotyledons

The two cotyledons are part of the embryo and account for most of the seeds mass. The cotyledons serve as the primary storage structure for protein and oil and provide nourishment to the developing plant (Liu, 1999).

2.1.3 Embryo

The embryo is made up of the radicle, the hypocotyls, and the epicotyl. The epicotyl is very small and acts as the main stem and growing point. The radicle eventually becomes the primary root and the hypocotyl's function is to lift the cotyledons above the soil surface. Both the radicle and hypocotyl are located at one end of the Hilum (Liu, 1999).

2.2 Soybean Seed Composition

The typical proximate composition of soybeans is shown in Table 2.1. Among all other legumes and cereals, soybeans have the highest protein content with an average of 40% (Liu, 1999). The soybean's oil content of approximately 20% is the second highest of all food legumes. Isoflavones, which are a relatively minor component in soybeans, have received more attention recently because of their association with several health benefits (McCue and Shetty, 2004). Other important components in soybeans include: vitamins, fiber and minerals. The exact quantity of each component will vary with variety and environmental conditions (Liu, 1999).

Table 2.1. Typical proximate composition of soybeans and their structural parts.

	% in Whole Seeds	Chemical Composition (% Dry Matter)			
		Protein	Lipid	CHO	Ash
Hull	8	9	1	86	4.3
Hypocotyl	2	41	11	43	4.4
Cotyledons	90	43	23	29	5.0
Whole Seeds	100	40	20	35	5.0

Adapted from Liu, 1999 (p.26).

2.2.1 Soybean Protein

Soy protein accounts for the largest amount of dry matter in whole soybeans. Since the protein content is so high in soybeans some researchers believe that soybeans should be considered a protein seed instead of an oilseed. Most soy protein is used for animal feed and has traditionally been underutilized in human diets particularly in North America.

Soy protein contains all nine essential amino acids, but like proteins from other legumes it is low in sulfur-containing amino acids (Liu, 1999). Of the sulfur-containing amino acids, methionine is found in a lower amount in soy protein. Most soy protein is considered to be globulin (soluble in a salt solution) with the 11S and 7S globulins being the most prominent (Liu, 1999).

2.2.2 Soybean Lipids

Nutritionally, soybean oil compares well with other highly unsaturated oils (Liu, 1999) (Table 2.2). Soybean oil contains relatively low levels of saturated fatty acids (~15%) and high levels of polyunsaturated fatty acids [PUFAs] (~61%), most of which is linoleic acid (~53%). The high PUFA content of soybean oil makes it less stable to oxidation than other oils high in saturated fats.

Table 2.2. Typical composition of selected fatty acids for common edible oils.

Oil	Palmitic	Oleic	Linoleic	Linolenic
Soybean	11.0	23.4	53.2	7.8
Canola	3.9	64.1	18.7	9.2
Corn	12.2	27.5	57.0	0.9
Sunflower	6.8	18.6	68.2	0.5
Olive	13.7	71.1	10.0	0.6
Peanut	11.6	46.5	31.4	0.0

Adapted from Liu, 1999 (p.26).

2.2.3 Soybean Carbohydrates

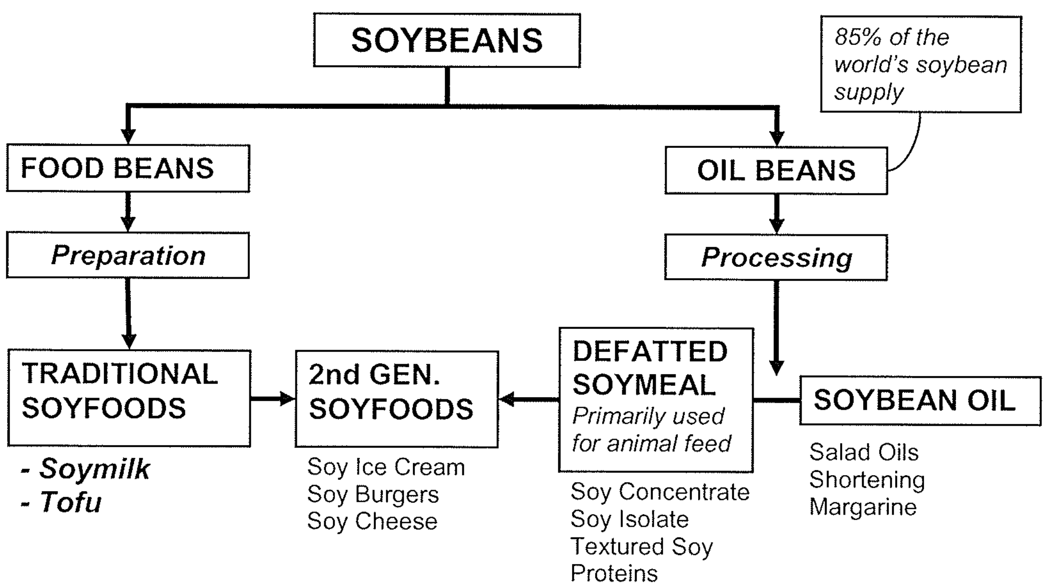
Although carbohydrates are the second most abundant component in soybeans, they are considered to be of little economical value. The carbohydrate component of soybeans is not considered to be as important as soybean protein and oil and therefore has not been given much attention by researchers. The carbohydrate component in soybeans is only viewed as an extra source of energy in animal feed.

Only negligible amounts of monosaccharides such as glucose are found in soybeans. Disacharrides, primarily sucrose, and oligosaccharides, such as raffinose and stachyose are found in measurable amounts. Sucrose, which is usually in the range of 2.5 – 8.2%, is important to soymilk and tofu processors because it adds a desirable sweetness to their products (Dr. L. Woodrow, personal communication, May 28, 2007). Raffinose and stachyose are also important to food manufacturers because they are believed to cause flatulence and abdominal discomfort, which is associated with bean consumption (Liu, 1999).

2.3 Soybean Seed Classification

Soybeans are either crushed into edible oil and defatted meal for animal feed or made into a number of food products for human consumption (Liu, 1999). The differences in applications have led to the formation of two distinct categories of soybeans: (1) food beans and (2) oil beans (Figure 2.1).

Figure 2.1. Summary of uses for soybeans.



(Adapted from Liu, 1999)

2.3.1 Food Beans

Although any soybean can be used for food consumption, the term "food beans" or "food grade soybeans" specifically refers to soybeans that have been selected and bred for direct food consumption (Liu, 1999). In general, food grade soybeans have a high protein content (>44%), large seed size (>22g/100 seeds), and lighter coloured seed coat and hilum than oil beans. Food grade beans are also required to meet higher grading standards and may have to meet other specifications set by processors (Liu, 1999). Typically, most soybean food processors will use only conventional or non-genetically modified organisms (non-GMO) soybean varieties due to public concerns over the safety of GMO foods (Dr. L. Woodrow, personal communication, May 28, 2007).

The quality of food grade soybeans encompasses both functional properties and nutritional value. However, from a processors perspective, food grade soybean quality primarily refers to the functional qualities of the soybeans that affect the soybean food product. Nutritional value is looked at as more of a value added feature.

2.3.2 Oil Beans

The bulk of the soybeans grown in the world are considered to be "oil beans" or "commodity beans", which includes GMO varieties (Liu, 1999). Typically, an oil bean will have a smaller seed size, darker hilum and higher oil content than a food grade soybean. The popularity of soybean oil and the demand for soybean meal for animal feed has resulted in the dominance of soybeans in oilseed world markets.

2.4 The Nutritional Value and Health Benefits of Soybeans

The popularity of soybean based foods has grown because of the health benefits associated with soybeans. Sales figures have reflected the rise in popularity of soybean foods worldwide with an increase from $300 million to $3.9 billion between 1992 and 2004 (Soyfoods Association of North America, , 2006).

2.4.1 Soybean Protein

One of the main reasons for the interest in soy protein has been the approval of a health claim in the United States which allows manufacturers to state: "Diets low in saturated fat and cholesterol that includes 25 grams of soy protein a day may reduce the risk of heart disease." (Food and Drug Administration, U.S. Department of Health and Human Services, 1999). Along with heart disease prevention, soy protein has been found to slow progression of renal disease (Fair et al., 2004).

Soybeans are considered to be a good source of dietary protein. According to the Protein Digestibility Corrected Amino Acid Score [PDCAAS], soy protein in a purified form (soy protein concentrate) is equal in quality to animal protein (Liu, 1999). Soy protein quality varies among soy foods due to processing treatments, which alter digestibility, absorption, and utilization. Processing can increase soy protein digestibility and absorption by: (1) removing biologically active substances such as saponins, phytic acid, and phenolics (2) changing the chemical form of the protein and/or (3) inactivating proteinase inhibitors (Liu, 1999).

2.4.2 Soybean Isoflavones

The health benefits associated with soybean isoflavones make them of particular interest to soy food processors because of the health benefits they may add to their products. The health benefits of isoflavones are linked to their ability to mimic estrogen. Isoflavones have a chemical structure that is very similar to that of mammalian estrogen and are therefore considered to be phytoestrogens. The capacity to regulate hormonal responses suggests that isoflavones may be particularly effective at protecting against hormone-dependent condition including breast and prostate cancer and osteoporosis (Setchell and Cassidy, 1999).

Soybeans and soy foods are the primary source of dietary isoflavones, but the exact amount in which they are present varies depending on factors such as growing conditions and processing (Liu, 1999). Genotype and environment have been demonstrated to have a significant effect on isoflavone levels (Kim *et al.,* 2007; Kim Wicker, 2005; Seguin *et al.,* 2004). Of particular interest to Manitoba producers are the findings that temperature has a significant effect on isoflavone levels (Caldwell *et al.,* 2005; Lee *et al.,* 2003; Tsukamoto *et al.*, 1995). Research has indicated that under cooler growing temperatures, soybeans may develop higher amounts of isoflavones. From a value added perspective, soybeans grown in the cooler Manitoba temperatures may have a nutritional advantage over soybeans grown in warmer climates.

2.5 The Potential Health Risks of Soybean Consumption

2.5.1 Soybean Protein

The amino acid requirements to support growth and development are not well understood, which makes it difficult to evaluate the significance of soy protein's lack of methionine. (Tome and Bos, 2000). Soy protein appears to have an adequate amount of sulfur-containing amino acids to support adult growth, but there is debate as to whether it can support infant growth. For this reason, methionine is often added to soy infant formula to increase its nutritional quality (Friedman and Brandon, 2001) and ensure that infant amino acid requirements are met.

2.5.2 Soybean Isoflavones

The consumption of traditional soy foods is considered safe because of their long history of use. There are concerns however, regarding the safety of soy when it is consumed in a purified extract or supplement form (Branca and Lorenzetti, 2005). Under particular scrutiny is the consumption of large doses of purified isoflavones. Many health benefits of soy are attributed to the estrogenic action of isoflavones, however consuming isoflavones in mega doses may cause undesirable hormonal effects. The major concern regarding the intake of soy isoflavone is centered on the consumption of soy-based formula by infants.

Several animal studies have suggested that isoflavones may alter development and behavior. One such study indicated that perinatal exposure to genistein resulted in reduced body mass, persistent aggressive behaviour and demasculinization of genitalia in male mice (Wisniewski et al., 2005). Although data from animal studies suggests isoflavones may alter reproductive status (Fitzpatrick, 2003), there is no significant clinical evidence that reports any adverse effects from feeding infants soy formula (Branca and Lorenzetti, 2005).

Other health concerns involving soy include infertility, the promotion of breast cancer and increased length of menstrual cycle. These concerns have been shown to have no clinical relevance and are based on anecdotal evidence, however this does not necessarily guarantee that soy is safe (Fitzpatrick, 2003). For centuries traditional soy foods have been consumed safely, however in recent times it has become common practice to consume large doses of concentrated soy compounds. Research will be needed to determine optimal safe doses (Branca and Lorenzetti, 2005).

2.6 Soybean Food Products

Soybean food product or "soyfood" can be classified as traditional soyfoods or second generation soyfoods.

2.6.1 Traditional Soyfoods

Traditional soyfoods are those which have been produced and consumed in Asian countries for centuries. These foods can be divided into two categories: fermented and non-fermented (Golbitz, 1995). A description of each food in each category and the way it is used can be found in Table 2.2.

Table 2.3. Description and use of commonly consumed traditional soyfoods.

Category	Traditional Soyfood	Description	Use
Non-Fermented	Soymilk	-an aqueous extraction of whole soybeans	-dairy milk alternative -base for tofu
	Tofu	-a curd made from salt or acid coagulated soymilk -resembles a soft white cheese	-its bland flavour and porous texture allows it to be used with foods such as soup or stir fry -meat or cheese substitute
	Okara	-soybean pulp that is a by-product of soymilk production -consists primarily of insoluble fibre, but is also a good source of protein and minerals	-can be incorporated as an ingredient into foods such as salads, soups, baked goods and desserts
Fermented	Tempeh	-a cake of cooked and fermented soybeans	-meat alternative
	Miso	-white, brown or reddish-brown soybean paste -salty flavour	-soup base and flavouring ingredient
	Soy Sauce	-dark brown liquid extracted from a fermented mixture of soybeans and wheat -salty and sharp flavour	-all purpose seasoning -most widely accepted of all fermented soyfoods
	Natto	-prepared by soaking and steaming soybeans, followed by a short fermentation with *Bacillus natto.*	-typically consumed as a breakfast food - highly prized for its health promoting and nutritional qualities

Source: Golbitz, 1995.

2.6.2 Second Generation Soyfoods

Second generation soyfoods arose from the creation of soyfoods designed to match the food preferences of the Western population (Golbitz, 1995). They include such products as soy ice cream, soy yogurt, soy cheese, and soy burgers. Second generation food products are targeted towards vegetarians and people looking for an alternative to meat. A large reason for the exponential growth of the soyfood industry is attributed to the immense popularity of second generation soyfoods.

Soy protein ingredients are used in the formulation of second generation food product and are usually made from defatted soybean meal (Soya & Oilseed Bluebook, 2007). Products in this category include soy flour, soy concentrate, soy protein isolate and texturized soy protein. Soy protein ingredients are added to a variety of food systems such as meat, dairy, and bakery products, breakfast cereal, infant foods, and beverages either as a functional additive or as an inexpensive alternative to animal protein.

2.7 Soymilk and Tofu Production

Soymilk and tofu are two traditional soyfoods that have gained popularity in North America. Soymilk is an aqueous extraction of soybeans, while tofu is a protein gel that is made by heating soymilk and then adding a coagulant, which forms a curd. Although most of the soymilk and tofu manufactured today is made in modern processing facilities, the basic steps in production have remained unchanged for centuries (Liu, 1999).

2.7.1 Soymilk Production

In most traditional and commercial methods, the first step in soymilk processing involves soaking the whole soybeans in order to incorporate water for the extraction of water-soluble components. By soaking, the soybeans processors benefit by reducing power input required for grinding, removing some oligosachharides, decreasing cooking time, and increasing yields. (Liu, 1999).

The next step involves grinding the soybeans which can be done using a variety of devices including large blenders, mills and commercial grinders. The grinding step is required to disrupt soybean tissues and release protein, lipids and other solids into the water-soybean slurry (Liu, 1999). The coarseness of the grind needs to be considered carefully because both too fine and too coarse of a grind will lead to reduced soy milk yield. Ideally, the soybeans should be ground fine enough to maximize proteins that are solubilized in the filtered soy-water extraction, but coarse enough to allow for easy filtration (Liu, 1999).

Following grinding, an extraction of the soymilk from the soybean slurry takes place. Depending on the preference of the processor, extraction can occur before or after heating. Extraction occurs when the slurry is filtered through a screen or cloth under vacuum or pressure or through centrifugation. The extraction process will remove all fibrous material (the okara) and maximize soymilk yield by extracting water soluble components in the soybean. Some modern processing methods will by-pass the extraction step and instead homogenize the soybean slurry to maximize the retention of solids (Liu, 1999).

During soymilk processing there is always at least one heat treatment. Heat treatment serves the purpose of denaturing soybean proteins to increase digestibility, inactivating anti-nutritional factors (i.e. trypsin inhibitors), inactivating enzymes (lipoxygenases) that cause off-flavours and improving shelf-life by destroying microorganisms. Two common types of heat treatment are the traditional process and the hot water grind process. During the traditional process, heating occurs after the soybean slurry has been filtered. This is the only heating step during the process and usually involves heating at 93-100°C for 30 minutes. The hot water grind process allows for easier grinding and is designed to assist in the deactivation of lipoxygenase, which contributes to the undesirable beany flavour associated with soymilk. During grinding, boiling water is added to the soaked soybeans resulting in a slurry with a temperature around 80°C. The slurry is then held at this temperature for 10 minutes before filtering.

The heat treatment process is often considered one of the most important processing steps because of the major influence it has on the final soybean food product. Heat treatment influences nutritive properties of the end-product by reducing vitamin and isoflavone contents. It also destroys spoilage microorganisms and affects the colour and flavour of the end product (Kwok and Niranjan, 1995). Soymilk is a good medium for bacterial growth and therefore needs to be heat treated to reduce the number of microorganisms, Pasteurization, sterilization, and ultra-high temperature processes are three common heat treatments used to extend shelf life (Liu, 1999). However, heat treatment of soymilk can result in browning and unwanted flavour development.

The final step in soymilk production often involves the addition of sweeteners and flavours as well as fortification with vitamins and minerals. Sweeteners and flavours help mask any beany flavours which are undesirable to most Westerners and also allows processors to cater to the tastes of their target market. The fortification of soymilk usually involves the addition of calcium, vitamin B_{12}, and methionine (Liu, 1999). The typical nutritional value of soymilk is summarized in Table 2.4.

Table 2.4 Typical nutritional composition per 100 grams (g) of soybean, soymilk and tofu.

Soybean Product	Moist (g)	Energy (Kcal)	Prot (g)	Lipid (g)	CHO (g)	Fibre (g)	Ash (g)
Soybeans, Raw	8.5	416	36.5	19.9	30.2	9.3	4.9
Soymilk fluid (Raw, no additives)	93.3	33	2.8	1.9	1.8	1.3	0.3
Tofu (calcium sulfate)	84.6	76	8.1	4.8	1.9	0.3	0.7

Source: 2007 Soya and Oilseed Bluebook

2.7.2 Tofu Production

The process of making tofu involves the complex interaction of many factors such as soybean chemical composition and processing conditions. Cai and Chang (1999) studied the quality characteristics and yield of tofu produced using a commercial method, a pilot-scale method and bench method and found that processing steps such as heating, grinding, extraction and coagulation have a significant effect on tofu texture and yield.

Tofu is produced from the extracted soymilk. The process involves heating the soymilk and then adding a coagulant either during or immediately following the heating process. The most commonly used coagulant is calcium sulfate, which is inexpensive, gives high yields, and can be used to make tofu with a variety of textures (Liu, 1999).

It is necessary to heat the soymilk to denature the protein, which in conjunction with the coagulant, allows for the formation of a gel (Liu *et al.,* 2004). When the protein denatures, the disulfide bonds, and hydrophobic amino acid side chains are exposed (Liu *et al.,* 2004). If an acid coagulant such as glucono-delta-lactone (GDL) is added, the negative charges on the protein molecules and the protons from the GDL promote coagulation (Liu, 1999). If a salt coagulant such as calcium sulfate is added the calcium ions will promote coagulation.

Four important factors need to be considered during the coagulation process: (1) type of coagulant (2) concentration of coagulants (3) temperature of soymilk when coagulant(s) are added and (4) the mode of adding and mixing the coagulant(s) (Liu, 1999).

(1) Type of Coagulant

The type of coagulant used will change the microstructure and texture of the tofu (Kao *et al.,* 2003). The two most common types of coagulants used in tofu manufacturing are salt or acid. The most commonly used salt coagulants are calcium sulfate and magnesium chloride. Calcium sulfate is ideal because it can be used to make both soft-textured tofu and firm tofu. However, tofu made from calcium sulfate often has a flavour that is slightly inferior to tofu made from other salt coagulants such as magnesium chloride (Liu, 1999).

Acid coagulants have only recently become popular in tofu production. Glucono-delta-lactone (GDL) is the most popular acid coagulant used in modern tofu production because it can produce a tofu with a smooth, creamy, soft texture. GDL is slower acting than calcium sulfate and therefore has a longer coagulation time resulting in tofu with smaller particle sizes, which results in a smoother texture. One disadvantage of GDL tofu is that it has been found to have a less desirable flavour than calcium sulfate tofu (Dr. L. Woodrow, personal communication, May 28, 2007).

(2) Concentration of Coagulants

Adding the correct amount of coagulant to the soymilk is a critical factor in tofu manufacturing (Liu *et al,* 2004). The amount of coagulant added will impact the texture, colour, and flavour of the tofu (Kao *et al.,* 2003). Too much coagulant results in a bitter taste, yellowish curd, and coarse texture; too little coagulant will result in a cloudy appearance and the presence of small amounts of uncoagulated soymilk (Liu, 1999).

(3) Temperature of Soymilk when Coagulants are Added

The temperature of the soymilk when the coagulant is added is an important factor in determining the rate of coagulation. Hard textures and low yields occur when the soymilk is too hot and the opposite is true when the temperature is too low. A temperature range of 70-80°C has been demonstrated to be optimal with regards to texture and yield (Liu, 1999).

(4) Mode of Adding Coagulants

One of the most difficult and crucial steps in tofu production is the mode by which coagulants are added to the soymilk. Traditionally, the coagulant is added to the soymilk without stirring in order to produce a high yield and good texture. The method of coagulant addition usually involves pouring the soymilk and coagulant solution simultaneously to ensure mixing without having to stir.

After the soymilk has coagulated for about 30 minutes, the formed soy curd is stirred, broken and poured into a mold. The mold is typically lined with fine mesh or cloth which will allow water and whey to be removed from the curd when pressed. The amount of pressure applied and the duration of pressing influence the hardness of the tofu. Pressing the curd only slightly will result in soft tofu, while increased pressing will result in firm or hard tofu. For silken tofu production, the curd is neither stirred nor pressed, which results in a tofu with a consistent texture that is very smooth and soft.

The type of tofu being produced will ultimately decide what variations to the tofu method need to be made in order to get the desired texture. Tofu is classified by its texture and includes: dry tofu, soft tofu, firm tofu, silken tofu, and filled (packed) tofu (Liu *et al.,* 2004). Dry tofu is the firmest variety of tofu and is usually boiled in a mixture of soy sauce and seasoning to make a savory dish (Tsai *et al.,* 1981). Soft tofu is lightly pressed, but typically too soft to be cut and is generally topped with soy sauce and eaten with a spoon (Tsai *et al.,* 1981). Hard tofu is made firmer than soft tofu by pressing more water out, making it more suitable for use in stir-frying as well as deep-frying. Silken tofu has the highest moisture content of all tofu. It is very soft and creamy and is typically eaten as a dessert. Filled tofu involves a unique process in which the coagulant is added to cooled soymilk then heated, but not mixed (Liu *et al.,* 2004). Filled tofu is also made inside the package so it does not require any further cutting or packaging. Table 2.4 summarizes the nutritional value of tofu.

2.8 Factors Affecting Soymilk and Tofu Yield and Quality

2.8.1 Laboratory-Scale Procedures for Preparing Soymilk and Tofu

Lim *et al.* (1990) developed a laboratory-scale procedure for preparing and assessing soymilk and tofu that followed traditional commercial practices which became the model method used and adapted by other researchers. Table 2.5 summarizes the methods that have been used by researchers since the method by Lim *et al.* (1990) was published. At present there is no standard method used by researchers; however, the method developed by Mullin *et al.* (2001) is considered to be the most reliable procedure by Canadian soybean breeders and exporters.

The lack of a standard method makes it difficult to compare results from one study to another since processing has a major effect on the end quality of soymilk and tofu (Cai and Chang (1999). In addition, the conditions under which soybeans are stored have also been found to influence quality and yield of soymilk and tofu (Hou and Chang, 2004; Cai and Chang, 1999).

2.8.2 Soybean Protein

Protein is the largest component in soybeans, comprising approximately 40-45% of the total dry matter (Liu, 1999). It is generally believed that high protein soybeans (> 44% dry matter) will increase the yield and quality of soymilk and tofu (Bhardwaj *et al.*, 1999). Soybean protein content is highly dependant on environmental conditions (Poysa and Woodrow, 2002; Aziadekey *et al.*, 2001; Vollman *et al.*, 2000; Rao *et al.*, 1998) with protein levels typically increasing with increased air temperatures and dry conditions (Dornbos and Mullen, 1992). Cooler growing temperatures may be one reason why Manitoba-grown soybeans typically have lower protein content than soybeans grown in warmer climates.

Although protein quantity is used as the major criteria for selecting food-grade soybeans, recent research has indicated that protein quality may be a more important factor in determining food-grade soybean quality. The main focus of soybean protein research has been directed at the two key salt soluble storage proteins glycinin (11S globulin) and β-conglycinin (7S globulin), which make up 40 and 30% of the total soybean seed proteins, respectively (Prak *et al.,* 2005). Differences in physiochemical properties between glycinin and β-conglycinin give them each unique functional properties that affect soy protein quality (Rickert *et al.,* 2004).

In most soybean varieties glycinin will account for over 40% of the seed protein making it the largest protein fraction (Poysa *et al.,* 2006). From a nutritional standpoint, glycinin is more valuable than β-conglycinin because it typically contains 3-4 times more sulfur containing amino acids. (Liu, 1999). Glycinin has five subunits, which are further categorized into two groups based on amino acid sequence (Zarkadas *et al.,* 2007). Group I contains the subunits $A_{1a}B_{1b}$, A_2B_{1a}, and $A_{1b}B_2$ and Group II contains the subunits A_3B_4 and $A_5A_4B_3$. All of the subunits are comprised of an acidic (A) polypeptide and a basic (B) polypeptide that are linked by a disulfide bond (Zarkadas *et al.,* 2007).

β-conglycinin is composed of three subunits that are given the designations α, α', and β, respectively. Unlike glycinin, the β-conglycinin subunits contain no disulfide bonds and are all considered to be glycoproteins. The α and α' subunits have similar amino acid compositions and both contain no cysteine and very little methionine (Liu, 1999). The β subunit represents the largest proportion of β-conglycinin and is devoid of methionine (Zarkadas, *et al.,* 2007).

Differences in the water holding capacity of 11S and 7S proteins is suggested to impact soymilk and tofu yield. Khatib *et al.* (2005) observed that soybean varieties with higher 11S/7S protein ratios had greater water holding capacities than varieties with lower 11S/7S protein ratios. Greater water holding capacity translates into higher soymilk and tofu yields. In contrast, Ji *et al.* (1999) found that an increase in the 11S/7S protein ratio did not increase tofu yield and hypothesized that there were other components, which they did not expound on, that are important to yield. It is possible that differences in the methods used to prepare soymilk and tofu in these two studies would account for discrepancies in results.

Texture is the most important parameter of tofu quality since tofu colour and flavour are considered to be neutral (Liu, 1999). The ideal tofu texture is described as being smooth, firm and coherent (Poysa and Woodrow, 2002). Because tofu is a protein gel it is believed that its texture is primarily determined by the gelation properties of the two major storage proteins 11S and 7S. Several researchers have investigated the role each protein plays and how the ratio of 11S/7S affects tofu texture.

Poysa *et al.* (2006) studied the effect of soy protein composition on tofu quality by making tofu from selectively bred soybean lines which were deficient in either all of the 11S subunits, some of the 11S subunits or the 7S subunit α'. The protein subunit composition of the selected soybean lines was determined using electrophoresis and tofu was made with a laboratory-scale method. The tofu production method used a constant water to protein ratio of 18:1 in order to differentiate the quality of tofu on the basis of protein quality rather than protein quantity. Soybean lines lacking the 11S subunits were found to significantly reduce the protein gel forming ability to the extent that a tofu cake could not be sufficiently produced. These findings are consistent with the results of studies by Tezuka *et al.* (2000) and Ji *et al.* (1999). The ability of 11S to form a stronger gel than 7S is assumed to be a result of the higher sulfur-containing amino acid content of 11S (Ji *et al.*, 1999). It is suggested that because 11S has more sulfur-containing amino acids than 7S it will produce firmer gels due to the formation of covalent bonds through disulfide bonding (Saio *et al.*, 1975).

The capacity of 11S to form a strong gel is largely dependent on temperature. Both 11S and 7S will form heat induced gels, but will do so at different denaturation temperatures (Braga *et al.*, 2006). Several studies have determined the denaturation temperature of the 11S and 7S globulins to be ~90°C and ~80°C, respectively (Rickert *et al.*, 2004; Ji *et al.*, 1999; Nagano *et al.*, 1994). The higher denaturation temperature of 11S suggests that although it forms firmer gels, it must be heated to a higher temperature in order to unfold and form disulfide bonds. This is an important factor when considering processing conditions because failure to heat the soymilk to an adequate temperature will likely result in insufficient gelation.

The mean protein ratio of 11S:7S varies from 1.6 to 2.5 among different soybean varieties (Zarkadas *et al.*, 2007). The effect of the 11S/7S protein ratio on tofu quality has been examined by a number of researchers and has become a controversial topic because of conflicting results.

Kang *et al.* (1991) reported that the 11S/7S protein ratio had a significant effect on tofu texture. Similar results were observed by Kim and Wicker (2005) and Tezuka *et al.*, 2000. However, the methods used in these three studies did not necessarily reflect actual tofu processing conditions. Kang *et al.* (1991) prepared gels using only purified soy proteins and a heat treatment. The other two studies used a laboratory-scale method for tofu preparation, but their methods diverged from traditional commercial tofu making methods. When Ji *et al.* (1999) used traditional tofu preparation methods to test the effect of the ratio of 11S/7S proteins they found that the ratio had no effect on tofu texture.

2.8.3 Effect of Variety and Location on the Yield and Quality of Soymilk and Tofu

There is limited information on the effect of variety and growing location on soymilk and tofu yield and quality. There have been no studies of this nature conducted on Manitoba-grown soybeans; however, the literature that does exist is helpful in understanding the factors that might possibly affect the yield and quality of soymilk and tofu made from Manitoba-grown soybeans.

Aziadekey *et al.* (2002) examined the contribution of variety, environment, and year and their interactions on soymilk and tofu quality. They found that variety was primarily responsible for variation in the quality characteristics of soymilk and tofu with year having very little effect. They also noted that for tofu texture there was a large variety by environment interaction which needs to be considered when developing soybean varieties for tofu production.

Bhardwaj *et al.* (1999) found that variety had a significant effect on soymilk colour ($p < 0.01$), tofu colour ($p < 0.01$) and tofu yield ($p < 0.01$). Growing location was only found to significantly effect tofu texture ($p < 0.01$). Difference in seed size caused by genetic variations among the soybean varieties was attributed to variety having a greater impact on soymilk and tofu characteristics than growing location.

Poysa and Woodrow (2002) found that varietal effects were more substantial than growing location effects for soymilk and tofu yield, tofu colour and tofu texture. In addition, they also found year effects to be greater than growing location effects in contrast to the findings of Aziadekey *et al.* (2002). A more recent study by Min *et al.* (2005) found variety and growing location had a significant effect on soymilk colour and tofu yield supporting the findings by Poysa and Woodrow (2002), Aziadekey *et al.* (2002), and Bhardwaj *et al.* (1999).

Table 2.5. Summary of laboratory–scale methods for soymilk and tofu production.

RESEARCHER	SOAKING	GRINDING	EXTRACTION	HEATING	COAGULANT	PRESSING
• Lim *et al.*, 1990 • Aziadekey *et al.*, 2002	16-h @ 20°C, rinsed and drained	Water added with beans and blended 4 min in commercial blender	Commercial juice extractor line with filter cloth	Soymilk brought to a boil	2.7-g of Calcium sulfate in 7.5 mL distilled water set for 15 min	Covered with cheesecloth, pressed with a weight for 15 min
Bhardwaj *et al.*, 1999	No soaking – ground soybean was used	Ground soybean blended with water	Previously heated slurry was run through a commercial juice extractor lined with cheesecloth	Prior to extraction - Slurry was steam cooked and held @ 98°C for 4 min then cooled	10% GDL solution added to soymilk and then held at 85°C for 45 min. – left to cool overnight	n/a
• Mullin *et al.*, 2001 • Poysa and Woodrow, 2002	22-h @ 13°C, rinsed and drained	Water added with beans and blended for 4 min using a commercial blender	Commercial juice extractor lined with two layers of polyester mesh	Two 500-ml portions were heated separately at (98°C for 4 min.)	1 soymilk portion was coagulated with 1.5-g GDL in 20 mL water, the other with 2-g calcium sulfate in 20-ml water-left to cool 1.5-h	n/a
Kim and Wicker, 2005	16-h @ room temp. , rinsed and drained	Food processor used to chop beans - Water added with beans and ground using a homogenizer	Previously heated slurry squeezed through two layers of cheesecloth	Prior to extraction - Slurry heated to and held @ 90°C for 15 min.	Soymilk heated again to 95°C and 1.2-mL magnesium chloride was added and left for 2 min	Mixture placed in mold lined with cheesecloth and pressed
Min *et al.*, 2005	16-h @ room temp. , rinsed and drained	Boiling water added with beans and blended 3 min in commercial blender	Filtered through four layers of cheesecloth	Soymilk boiled for 10 min.	Soymilk cooled to 75°C and 0.02M calcium sulfate solution was added	Mixture transferred to mold and weight placed on top

Table 2.6. Key findings from studies using laboratory-scale methods to assess soymilk and tofu yield and quality.

RESEARCHER	KEY FINDINGS
Lim *et al.*, 1990	• Tofu yield was not associated with seed size • Proposed a model for predicting tofu yield that suggests high protein and ash levels in soybeans, coupled with low phosphorous levels, will produce higher tofu yields.
Bhardwaj *et al.*, 1999	• Soybean variety had a significant effect on seed protein, seed size, soymilk colour, tofu colour, and tofu yield. • Location was found to have a significant effect on seed protein, seed size, and tofu strength. • Tofu yield was positively correlated to seed size while seed protein was negatively correlated to tofu yield and positively correlated to tofu strength.
Mullin *et al.*, 2001	• Reproducibility of results was very good within labs; however, there was a lack of reproducibility across labs. • The differences across labs were attributed to possible human error during the addition of coagulants and differences in equipment calibrations. • It was concluded that this method was best suited for use in comparative studies performed in a single laboratory.
Poysa and Woodrow, 2002	• The effects of variety and year were greater than the effects of location on protein content and seed composition, soymilk and tofu yield, and tofu colour, and texture. • Soymilk and tofu yield and tofu texture were positively correlated with seed protein content.
Aziadekey *et al.*, 2002	• Environmental conditions were shown to have a significant effect on seed characteristics. • Soybean variety was shown to have a significant effect on tofu yield and colour. • In experiment 1: seed protein was not significantly correlated to tofu yield or and texture ; however, seed protein was significantly correlated to these properties • In experiment 2: Seed size was correlated significantly with tofu yield in experiment 1, but not in experiment 2.
Kim and Wicker, 2005	• The difference in soybean protein subunit composition between the two varieties was attributed to differences in the physical properties of soymilk and tofu. • The variety with higher protein content and higher ratio of 11S protein produced tofu with a firmer texture.
Min *et al.*, 2005	• Soybean variety and growing location had a significant affect on soybean seed protein content, soymilk colour, and tofu yield and hardness. • The most important factor in determining soymilk and tofu was found to be soybean seed protein content.

CHAPTER 3

3 Materials and Methods

3.1 Selection of Soybean Samples

The soybeans used in this study were grown as a part of the Manitoba Crop Variety Evaluation Trials [MCVET]. MCVET is managed by members representing seed growers, the Manitoba Pulse Growers Association [MPGA], the University of Manitoba and the federal and provincial governments. Soybean varieties grown in the trials are either registered or experimental lines submitted by seed companies. Data collected from MCVET provides Manitoba producers with growth and yield information on new cultivars to assist them in the selection of varieties most suited to their growing conditions. It also provides seed companies with data to support the registration of experimental lines (Manitoba Agriculture, Food and Rural Initiatives, 2006).

The sample set selected for study was comprised of 13 conventional (non-genetically modified organisms [GMO]) food-grade soybean varieties (Table 3.1). The soybeans were collected from six different site-years. The term site-year refers to a specific growing site during a given crop year (i.e. one site for one year). The site-years in this study included: Carman-2005 (C-05), Morris-2005 (M-05), Rosebank-2005 (R-05), St. Adolphe-2006 (S-06), Morris-2006 (M-06), and Rosebank-2006(R-06). Table 3.2 summarizes the varieties examined in each site-year. There were no soybeans grown at the Carman site in 2006 because of excess water accumulation; samples from the St. Adolphe site were used as an alternative. The varieties CL987704, Kaminchis, and Lotus were dropped from the trials after 2005 because they were not well suited for Manitoba's growing conditions. The varieties OT05-20 and OT05-21 were included in the trials in 2006 and were developed specifically for tofu production.

Table 3.1. Agronomic and physical characteristics of selected soybean varieties.

MANITOBA GROUPING	COMPANY HEAT UNITS[1]	VARIETY	HILUM COLOUR[2]	SEED[3] SIZE
Short Season	2375	90A01	IY	S
Mid Season	**2450**	**OAC PRUDENCE**	**Y**	**L**
	2575	OAC Erin	Y	M
	2500	90A07	Y	M
	2500	OAC 01-12	IY	L
	2575	Dolly	Y	L
	2450	Jim	Y	M
Experimental Lines	-	CL987704	IY	L
Experimental Lines	-	OT05-20	Y	L
Experimental Lines	-	OT05-21	Y	L
Long Season	2550	Lotus	IY	L
	2600	Kaminchis	Y	M
	2600	OAC 01-13	IY	L

[1]Each company assigns a Heat Unit rating to each of their varieties that describes the maturity of their variety across Canada. Experience has shown that Company assigned Heat Unit ratings do not always reflect the actual maturity in Manitoba. Experimental lines are not assigned a HU rating until they become registered.

[2]Hilum Colour: Y=Yellow, IY=Imperfect Yellow

[3]Seed Size where S = Small sized seed 3440 to 4100 seeds/lb); M = Medium sized seed (2800 to 3440 seeds/lb); L = Large sized seed (less than 2800seeds/lb).

Source: Seed Manitoba-2007 (pp. 56-57)

Table 3.2. Summary of the sites, years, and varieties used in the study.

YEAR	**2005**	**2006**
SITES	**Carman, Morris, Rosebank**	**St. Adolphe, Morris, Rosebank**
SOYBEAN VARIETY	1. OAC Prudence	1. OAC Prudence
	2. 90A01	2. 90A01
	3. OAC Erin	3. OAC Erin
	4. 90A07	4. 90A07
	5. OAC 01-12	5. OAC 01-12
	6. Dolly	6. Dolly
	7. Jim	7. Jim
	8. OAC 01-13	8. OAC 01-13
	9. CL987704[1]	*9. OT05-20*[2]
	10. Lotus[1]	*10. OT05-21*[2]
	11. *Kaminchis*[1]	

[1] CL987704, Lotus, and Kaminchis were withdrawn from the test trials after 2005.
[2] OT05-20 and OT05-21 were new varieties developed specifically for tofu making.

The variety OAC Prudence, developed by Dr. Istvan Rajcan of the University of Guelph, has become the most widely grown food-grade soybean variety in Manitoba. It has been a very attractive soybean variety to Japanese buyers and non-GMO end-users (Roberts, 2006). OAC Prudence is a short season soybean with a yellow hilum, large-seed and high-protein content. It is ideal for food-grade status and identity-preserved export markets.

A commercial sample of Harovinton was included in the study because of its exceptional consistency in making quality tofu (Zarkadas *et al.,* 2007). Harovinton was developed by Dr. Richard Buzzell at Agriculture and Agri-Food Canada's Harrow (Ontario) Research Centre and has been a registered food-grade soybean since 1991 (Buzzell *et al.,* 1991). In 2006, Harovinton, which is referred to as the 'Asian Pearl' by Japanese buyers, was named seed of the year by Ontario's agricultural industry. The samples of Ontario-grown Harovinton soybeans obtained in 2005 and 2006 were provided by Wheatley Elevators Limited (Wheatley, ON).

3.2 Whole Seed Protein and Oil Analysis

Protein and oil content of whole seed soybean were measured on an Infratec near infrared (NIR) whole grain analyzer (Infratec™ 1241 Grain Analyzer, Foss Analytical, Brampton, ON). The NIR calibrations used for determining protein and oil content of the soybeans were developed by the Industry Services Laboratory at the Canadian Grain Commission, Winnipeg.

3.3 Moisture Analysis

Moisture content of whole seed soybeans was determined using a Seedburo automatic flow-through moisture tester (Model 1200A-CAN, Seedburo Equipment Company, Chicago, IL).

3.4 Seed Size Determination

Seed size was determined by randomly selecting and weighing 100 whole soybean seeds. Duplicate 100 seed weights were taken and averaged to determine the final 100 seed weight.

3.5 Determination of Sucrose Content

Sucrose content was measured using a FOSS 6500 NIR spectrometer (FOSS Electric Multispec Division, York, UK) equipped with a calibration developed at the AAFC, Harrow, ON. The NIR analysis was performed on ground soybean samples. Samples were ground for 30 seconds using a water cooled, Knifetec™ 1095 Sample Mill equipped with the manufacturers 'sharp' blade (FOSS, Eden Prairie, MN, USA).

3.6 Soymilk Preparation

The laboratory-scale procedure for soymilk preparation was adapted from Mullin *et al.* (2001) and is summarized in Figure 3.1. Due to limited sample size only one replication was performed. The method used a constant water to protein ratio of 18:1 in order to differentiate between soybean varieties for protein quality rather than protein quantity (Poysa and Woodrow, 2002).

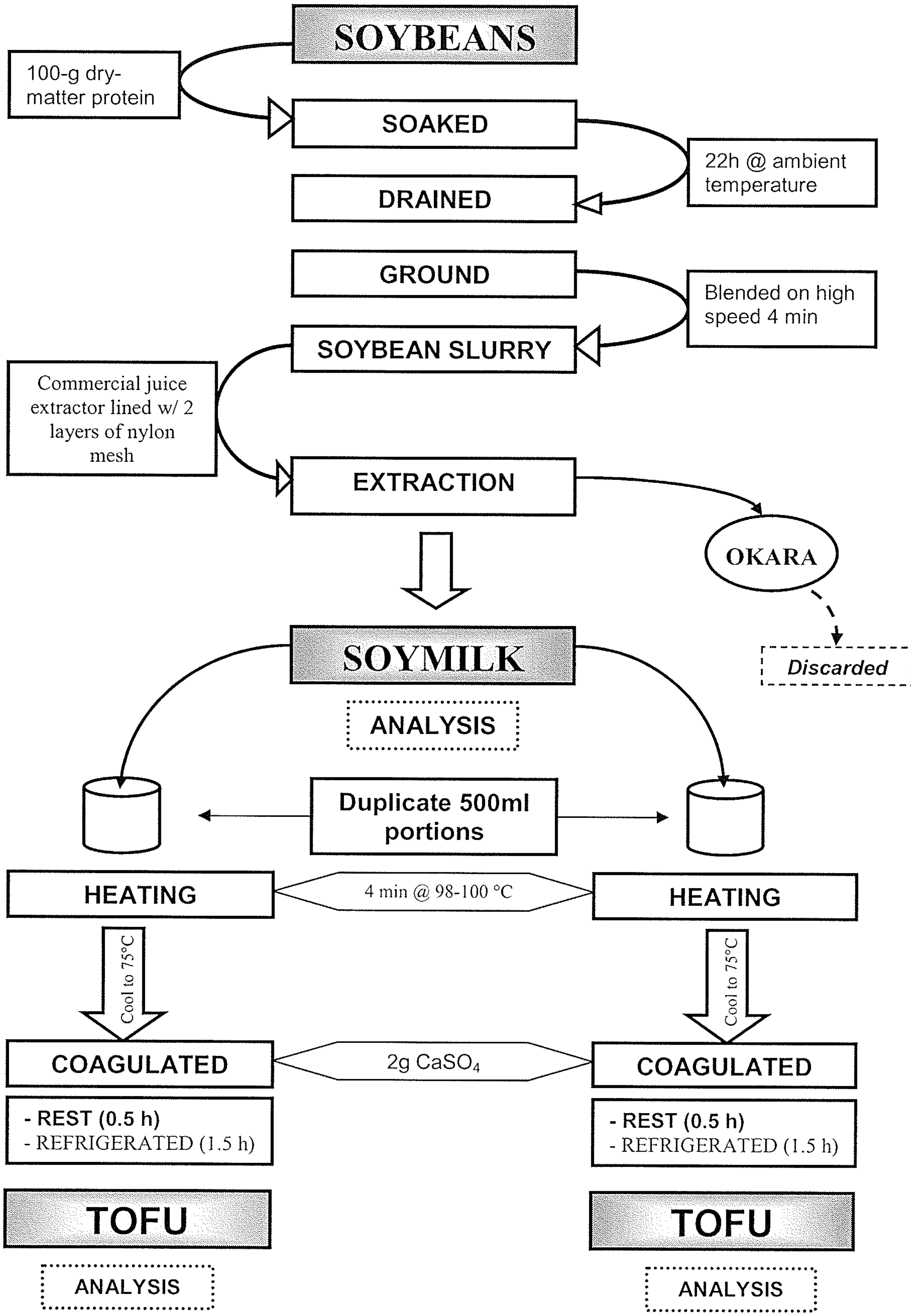
A sample of soybeans containing 100 g dry matter protein was required for soymilk preparation and was calculated as follows:

$$\text{Weight of Soybeans `as is'} = 100\ (100/\%\ \text{protein})(100/\%\ \text{dry matter})$$

The soybean sample containing 100 g dry matter protein was weighed and soaked in a 2L beaker in an excess of distilled water (1100 mL) for 22 h at room temperature. The soaked soybeans were then drained and rinsed with cold tap water. The soybeans were patted dry with a paper towel and then re-weighed to determine the water uptake. The Water Uptake Factor (WUF) was determined by dividing the drained weight of the soaked soybeans by the initial weight of the raw soybeans. From the initial sample weight and the soaked sample weight the additional amount of water needed to obtain a water to dry protein ratio of 18:1 was calculated as follows:

$$\left[Water\ Required\ (mL) = 1800 - (soaked\ weight - sample\ dry\ weight) \right]$$

Figure 3.1. Flow diagram for labraory-scale soymilk and tofu preparation.



The soaked soybeans plus 800 mL of the additional water requirement were put into a 4-L Heavy Duty Waring blender (Model CB15, Waring Laboratory, Torrington, CT), and processed at high speed for 3.5 min. The remaining water was added to the slurry and the blender was processed at high speed for a further 30 sec.

The slurry was further processed using a commercial juice extractor (Chesher Vitamat Commercial Juicer, Mississauga, ON), which had been lined with two layers of fine mesh (SEFAR NITEX 03-100/44, Sefar Filtration Inc., Kansas City , MO). The extracted material was collected in a 2 L plastic pitcher and passed through the mesh-lined juicer a second time. After both extractions, the juicer lid was removed and the fibrous material (okara) was scraped from inside the juicer and collected. The okara was then passed through the mesh-lined extractor one more time to remove any remaining liquid. The material from the three extractions was combined and considered to be the final soymilk product.

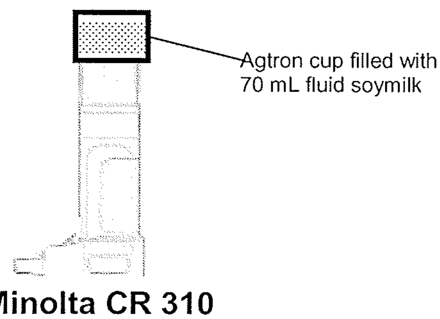
3.7 Determination of Soymilk Yield and Quality

The extracted soymilk was weighed to determine yield and was reported as gram of soymilk produced per gram of dry whole soybean.

Colour was determined by pouring a 70 mL aliquot of soymilk into an Agtron sample cup (Agtron Inc., Reno, NV) and placing the cup on top of a Minolta colorimeter (Model CR 310, Konica Minolta, Ramsey, NJ) (Figure 3.2) and $L^*a^*b^*$ values were recorded. A whiteness index (WI) was calculated by subtracting the b^* value from the L^* value (WI =

L* - b*). The a* value was not part of the equation because it typically has a value very close to zero (± 1) (Appendix 1) and does not significantly impact colour (Oliver *et al*, 1992). Four readings were taken of each sample. Samples were stirred between measurements.

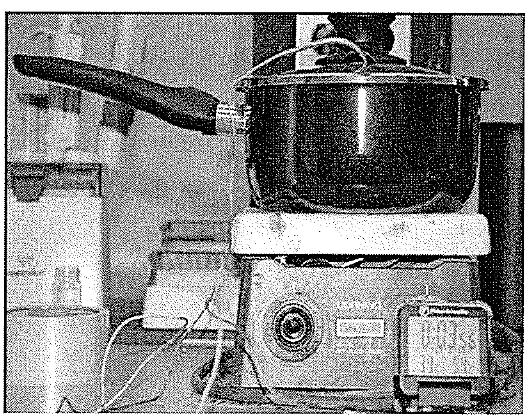
Figure 3.2. Diagram of soymilk analysis using a Minolta colorimeter.



3.8 Tofu Preparation

The laboratory-scale procedure for tofu preparation was adapted from Mullin *et al.* (2001). Figure 3.3 summarizes the method used to prepare the tofu from the soymilk. Duplicate 500-mL portions of soymilk were placed in 2.25-L Teflon coated saucepans. A magnetic stirring bar (7.62 x 1.91cm) was added to each saucepan and the covered saucepan was placed on a hotplate stirrer. The hot plate was set at a medium-high temperature setting and the stirring bar was activated in order to prevent the soymilk from burning. A 21.6 cm metal probe connected to digital thermometer (Model 15-077-29, Control Company, Friendswood, TX) was placed through the lid into the soymilk (Figure 3.3). The soymilk was slowly heated to 98°C at which point the heat was reduced in order to hold the soymilk between a temperature of 98 - 100°C for 4.0 min. The soymilk was then poured into a 1-L plastic measuring cup and intermittently stirred with a spatula until it cooled to 75°C.

Figure 3.3. Teflon pot with digital thermometer and probe.

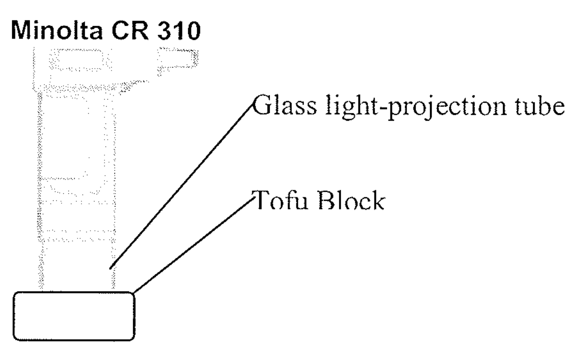


The coagulant was prepared by dissolving 2 g of food-grade calcium sulfate dihydrate ($CaSO_4 \cdot 2H_20$) (EMD Chemicals Inc., Gibbstown, NJ) in 20 mL of distilled water. The cooled soymilk and coagulant were simultaneously poured in a 1 L glass beaker. The mixture was immediately stirred to ensure proper dispersal of the coagulant. The mixture was left to rest for 30 min at room temperature and then refrigerated at approximately 4°C for 1.5 hr.

3.9 Determination of Tofu Yield and Quality

After cooling in the refrigerator, the tofu was removed from the beaker by carefully running a knife between the outside of the tofu and the inside edge of the beaker. Once removed, tofu yield was determined by weighing the tofu block.

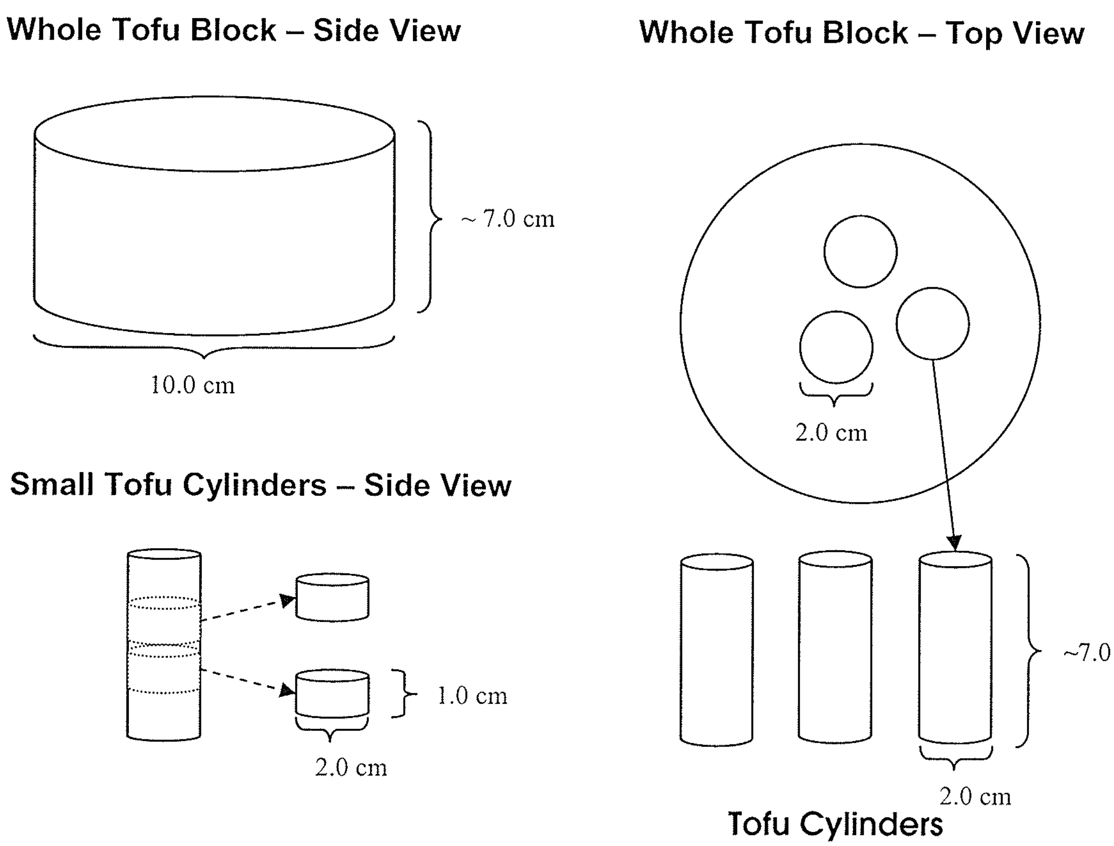
Figure 3.4. Diagram of tofu colour analysis using a Minolta colorimeter.



Tofu colour was measured by fitting the Minolta colorimeter with a glass light-projection tube and then gently pressing the colorimeter into the tofu block (Figure 3.4). Enough pressure was put on the colorimeter to ensure the entire surface of the light-projection fitting tube was touching the tofu block without compromising the structural integrity of the tofu. Colour was determined on each block four times by moving the colourimeter to different areas on the top surface of the block.

The tofu was prepared for texture analysis by first cutting three cylinders from the middle portion of the tofu block using a 2.0 cm diameter stainless steel cutting tube. Two smaller cylinders were cut from the middle 1.0 cm of each cylinder using a double bladed knife with thin blades that were spaced 1.0 cm apart. This produced six small cylinders measuring 1.0 cm × 1.0 cm (Figure 3.5).

Figure 3.5. Steps taken in preparing tofu for texture analysis.



Texture was measured using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 25 kg load cell and TA-4 probe (3.81 cm diameter cylinder w/ radius edge, acrylic, 3.5 cm tall). Hardness was defined as the peak force (Newtons) needed to obtain 80% deformation of the sample. Each 2.0 × 2.0 × 1.0 cm tofu cylinder was analyzed by placing it directly under the center of the probe. An example of a typical curve produced by the Stable Microsystems-Texture Expert software (Stable Micro Systems, Surrey, UK) can be found in Appendix 2.

3.10 Statistical Analyses

Main effects of site-year and variety were analyzed using PROC MIXED procedure (SAS 9.1.3, SAS Institute 2007). The data were treated as a randomized complete block (RCB) design with site-year serving as the block and variety serving as the treatment. Site-year by variety interaction was used as the error term for the whole seed and soymilk data. Since there were two observations for the tofu data, the residual error could be used as the measure sampling error. In addition, contrast statements (Year effect 2006 vs.2005; Site effect Morris vs. Rosebank; and Site by Year Interaction) were used to analyze the factorial effects (variety, site and variety by site effects) of the main effect of site-year. The sums of degrees of freedom for the factorial effects did not equal the degrees of freedom for the main effect of site-year because only two locations (Morris and Rosebank) were used in both years.

A Dunnett's two tailed t-test was used to compare each experimental mean (Manitoba soybean varieties) with the control mean (Harovinton) using the PROC MIXED procedure (SAS 9.1.3, SAS Institute 2007). Each year was analyzed separately and all varieties grown in each year were included. The data were treated as a randomized complete block (RCB) design with site serving as block and variety as the treatment. The model included effects of site and variety. Site by variety interaction was used as the error term for the soymilk data. Since there were two observations for the tofu data, the residual error represented a measure of sampling error. Since the objective of this study was to compare Manitoba-grown soybean varieties to a well established food-grade variety and there was no requirement to compare Manitoba varieties with each other.

Simple correlations between seed characteristics and soymilk and tofu characteristics were performed using PROC CORR (SAS 9.1.3, SAS Institute 2006) in order to determine the relationship between seed characteristics and soymilk and tofu yield and quality characteristics.

CHAPTER 4

4 Results and Discussion

The analysis of variance tables included in the results and discussion section contain the main effects of site-year and variety as well as an analysis of the factorial effects (Year, Site, and Site by Year Interaction) of the main effect of site-year. The analysis of the factorial effects is not considered complete because it does not include data for the 2005 Carman location nor the 2006 St. Adolphe location. They could not be included since both site-years were grown in only one year and therefore could not be analyzed. Although partial, the factorial effects can still be used to identify potential sources of variance. However, the interpretation of the significance of the factorial effects needed to be done with caution since not all data points were used to calculate the main effect of site-year.

4.1 Seed Characteristics

4.1.1 Effect of Variety and Site-year on Seed Size

The main effects of variety ($P < 0.0001$) and site-year ($P < 0.05$) were significant for the seed size of Manitoba-grown soybeans (Table 4.1; Figure 4.1). The factorial effect of site was significant ($P < 0.05$) where as the year and site by year interaction were not, suggesting that site had a strong influence on the main effect of site-year.

Table 4.1. Analysis of variance results for the seed size of Manitoba-grown soybeans.

Source	ANOVA			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	4.6	3.25	0.0168
Year (Y) (2006 vs 2005)	*1*		*3.41*	*0.0736*
Site (S) (Morris vs Rosebank)	*1*		*5.93*	*0.0202*
S × Y	*1*		*0.48*	*0.4938*
Variety	7	19.9	14.21	<.0001
Error	34	1.4		

NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

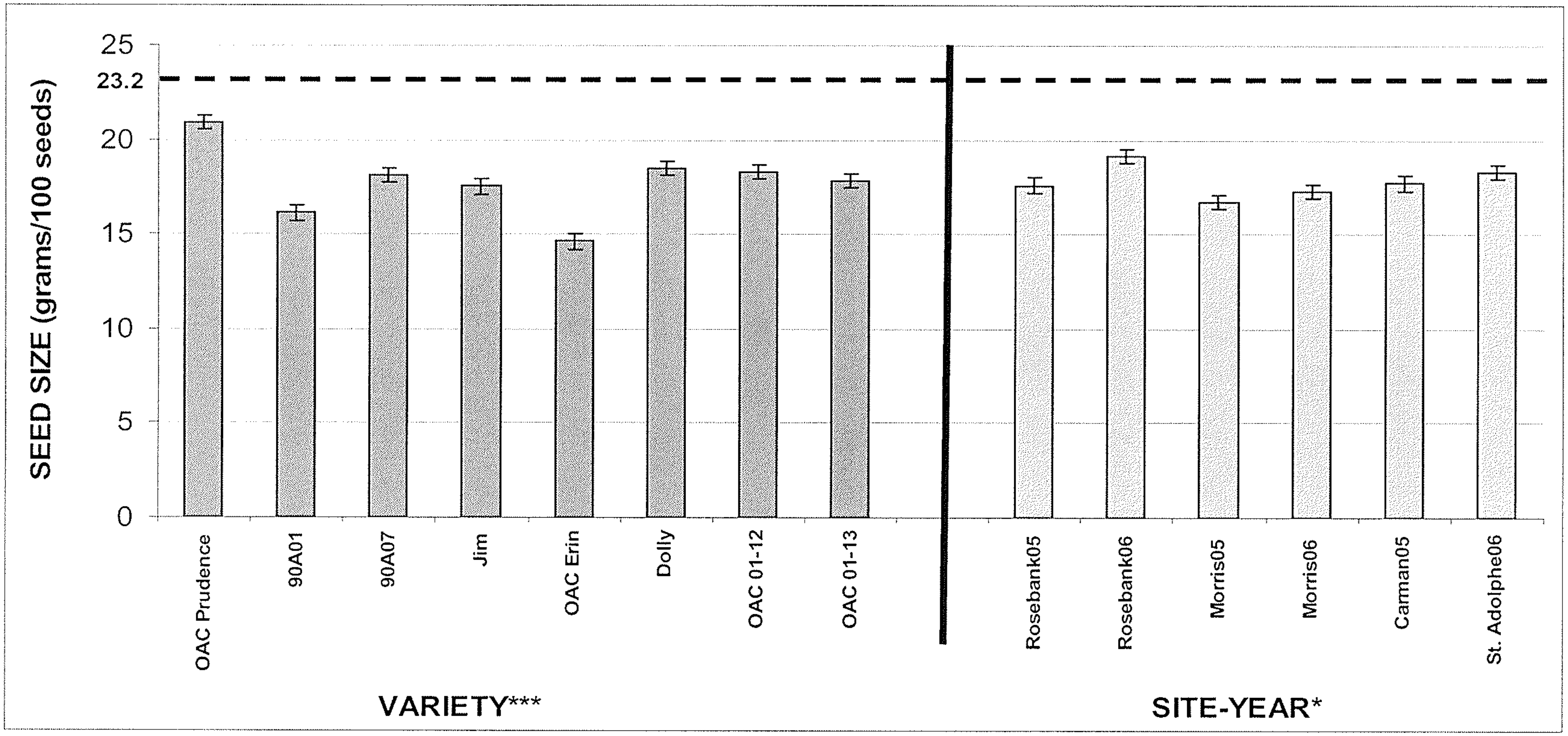
These findings support the results by Poysa and Woodrow (2002) and Bhardwaj *et al.* (1999). In both studies, both variety and location had significant effects on seed size. Poysa and Woodrow also found a significant year effect which they attributed to differences in temperature and rainfall between the years. They concluded that hot and dry conditions resulted in reduced seed size.

The mean seed size of individual varieties as well as the mean seed size at each location is shown in Figure 4.1. This figure provides a visual assessment of the variability between varieties and site-years and a graphic comparison of Manitoba soybean mean seed size to the seed size of the commercial Harovinton soybean samples. The Manitoba-grown varieties shown in Figure 4.1 include only those that were grown in both crop years. Overall, the Manitoba-grown soybeans were smaller in size than the commercial

Harovinton soybean samples used as a comparison. This finding is not unexpected given that Harovinton has gained much of its popularity in Asia because of its large seed size.

Figure 4.2a and 4.2b show the mean seed size of Manitoba-grown soybeans for the 2005 and 2006 crop years respectively. All varieties grown in 2005 (Figure 4.2a) were significantly smaller than the commercial Harovinton sample (Appendix 3, Table 1a). Of the varieties grown in 2006 there were three varieties (OAC Prudence, OT05-21, and OT05-20) that were not significantly different than Harovinton (Appendix 3, Table 1b). The varieties OT05-21 and OT05-20 were entered into the Manitoba soybean trials for the first time in 2006 and were bred to be large-seeded specifically for tofu production.

Figure 4.1. Mean[1] seed size of Manitoba-grown soybeans for variety and site-year.



[1]For Variety means n=6 site-years; for site-year means n=8 varieties

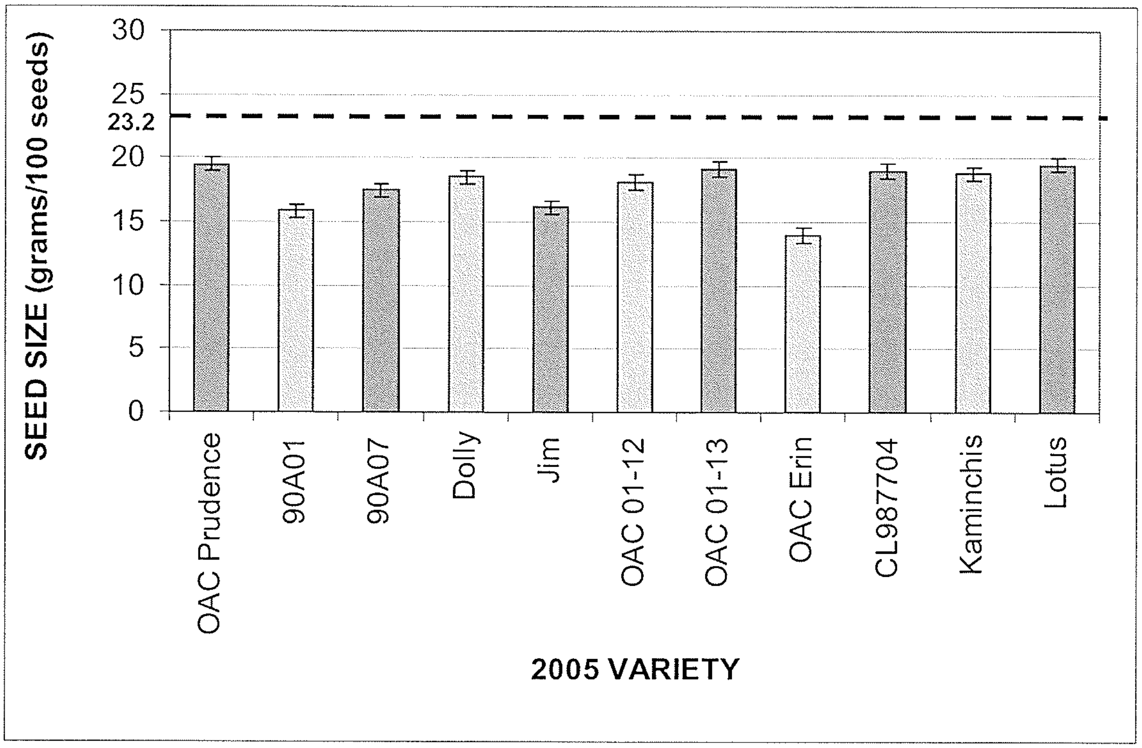
*** $P < 0.001$

*$P < 0.05$

Variety and Site-Year significance levels from Table 4.1

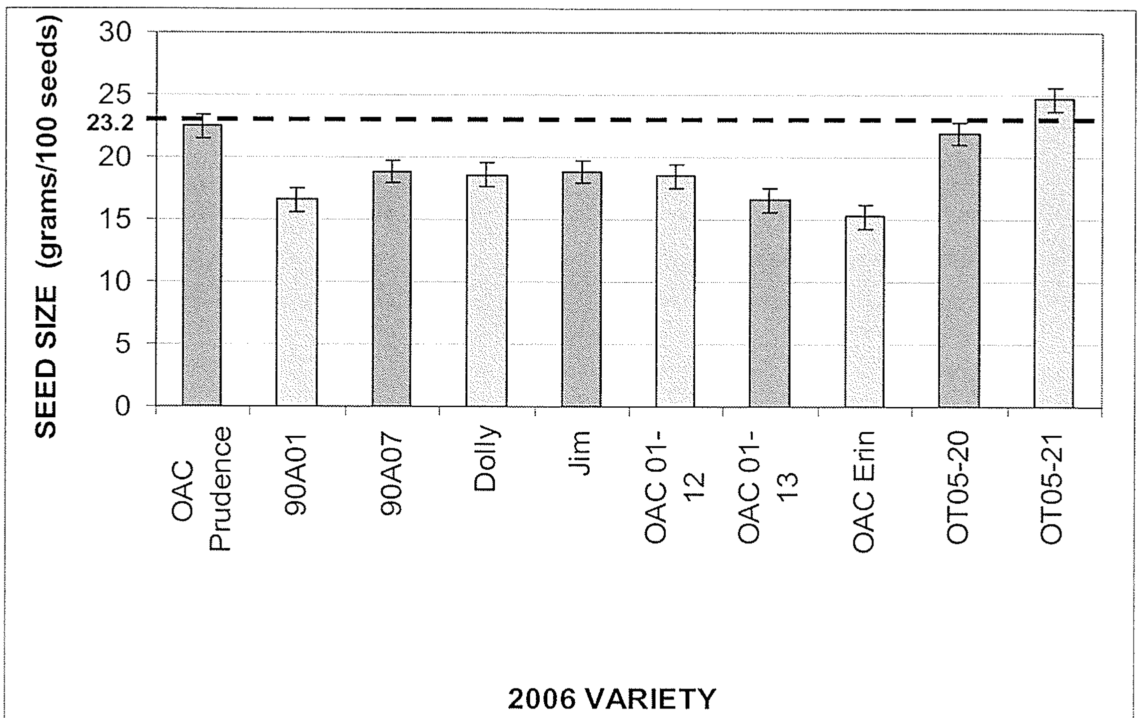
(Dashed line represents mean seed size for the commercial Harovinton sample.)

Figure 4.2a. Mean[1] seed size of soybeans grown in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean seed size for the commercial Harovinton sample.)

Figure 4.2b. Mean[1] seed size of soybeans grown in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean seed size for the commercial Harovinton sample.)

4.1.2 Effect of Variety and Site-year on Seed Protein Content

Protein and oil content of individual soybean varieties by site-year are presented in Appendix 4. Site-year ($P < 0.0001$) and variety ($P < 0.0001$) significantly affected the protein content of Manitoba-grown soybeans ($P < 0.0001$)(Table 4.2). These findings are in agreement with the findings from several other studies (Min *et al,* 2005; Poysa and Woodrow, 2002; Aziadekey *et al.,* 2002).

Table 4.2. Analysis of variance results for the protein content of Manitoba-grown soybeans.

Source	ANOVA df	Mean Square	F Value	Pr > F
Site-year	5	4.6	6.66	< 0.0001
Year (Y) (2006 vs 2005)	*1*		*0.09*	*0.7625*
Site (S) (Morris vs Rosebank)	*1*		*25.04*	*< 0.0001*
S × Y	*1*		*11.30*	*0.0013*
Variety	7	8.4	12.28	< 0.0001
Error	34	0.7		

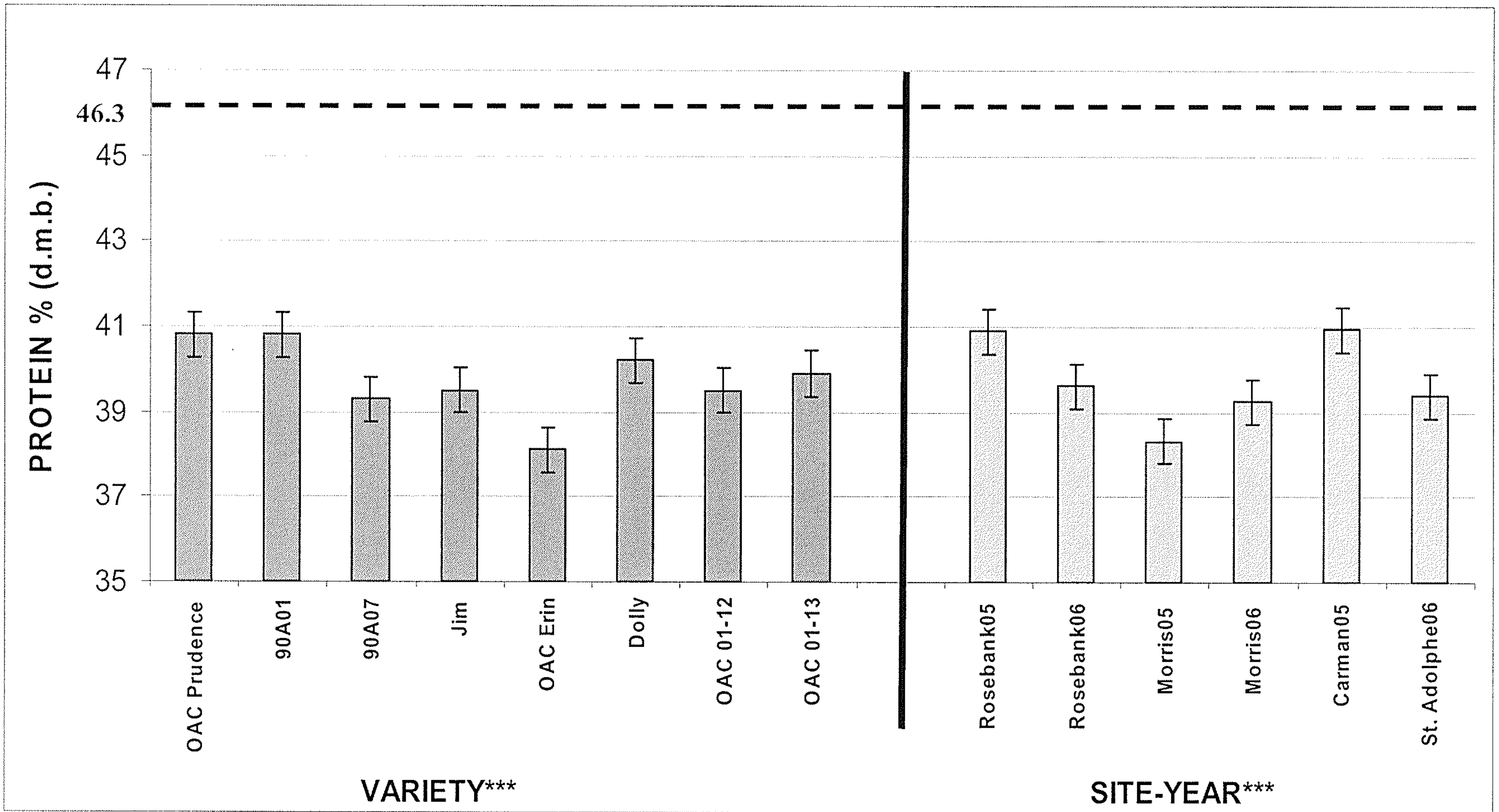
NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

As with seed size, the overall mean protein content of all individual varieties and site-years was lower than the commercial Harovinton samples (Figure 4.3). However, it is too early to conclude that high protein soybeans cannot be grown in Manitoba. The high-protein Harovinton soybean variety was the product of years of development in an extensive breeding program whereas most Manitoba-grown varieties are relatively new in comparison.

Results from the Dunnett's test performed on each year show that in 2005 there were three varieties (CL987704, Kaminchis, and Lotus) were not significantly different than Harovinton in protein content (Appendix 3, Table 2a). However, these three varieties were removed from the trials after 2005 because they were not well suited for Manitoba's growing conditions. Interestingly, the varieties OT05-20 and OT05-21, which were bred specifically for tofu, did not have particularity high protein content (41.3% and 42.8%, respectively) (Appendix 3, Table 2b). Soybeans bred for tofu production will typically have a protein content in excess of 44%.

The lower protein content of Manitoba soybeans was not unexpected, since high protein soybean varieties typically have two major downfalls to them: (1) they usually are lower yielding and (2) they typically require a longer growing season. Until more work is done to breed short season, high protein soybean varieties it is quite likely that Manitoba-grown soybeans will be lower in protein than varieties grown in areas with longer growing seasons.

Figure 4.3. Mean[1] protein content of Manitoba-grown soybeans for variety and site-year.



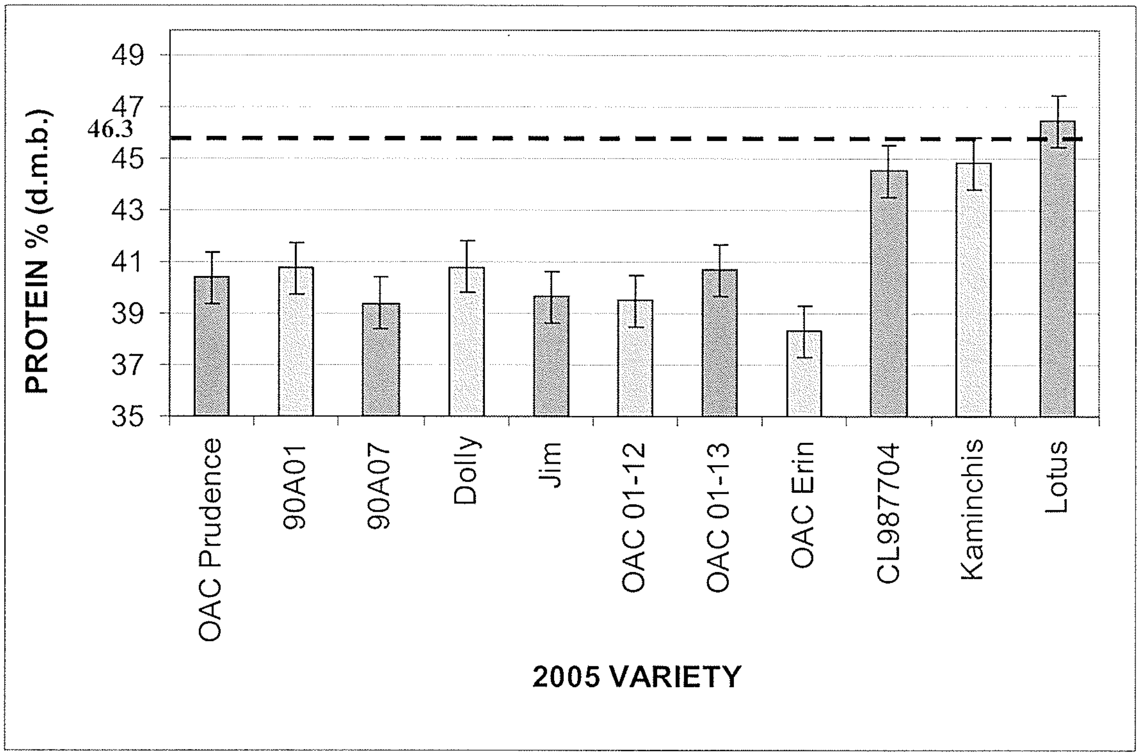
[1]For Variety means n=6 site-years; for site-year means n=8 varieties

*** $P < 0.001$

Variety and Site-Year significance levels from Table 4.2

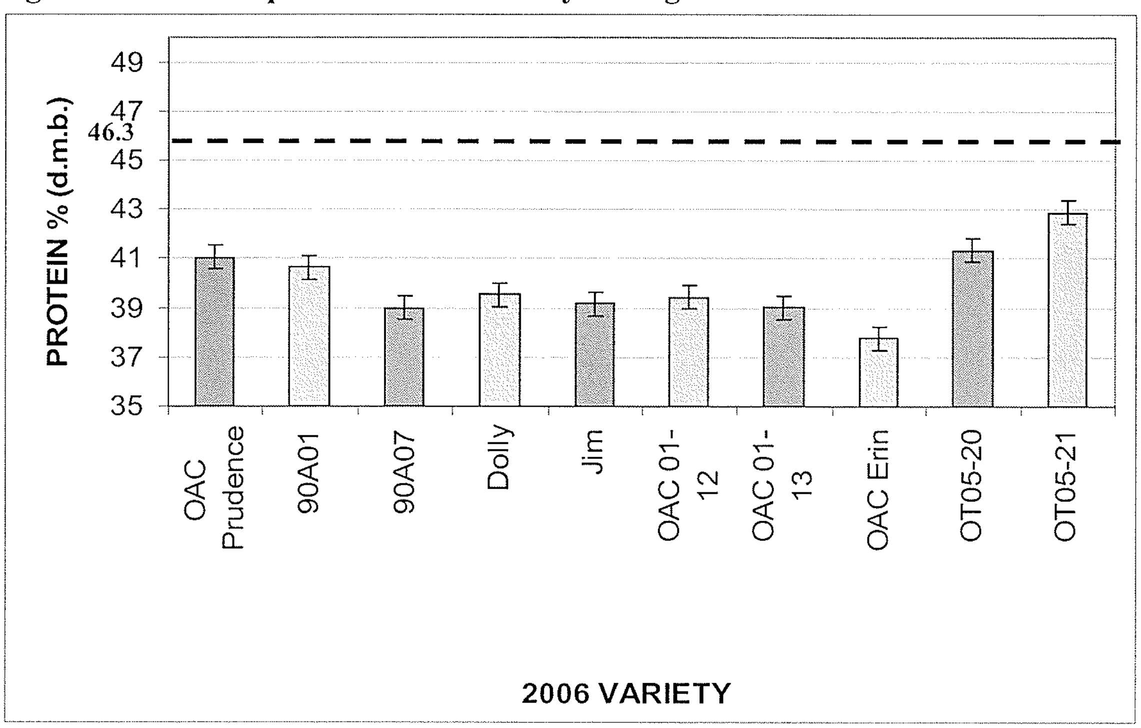
(Dashed line represents mean protein content for the commercial Harovinton sample.)

Figure 4.4a. Mean[1] protein content for soybeans grown in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean protein content for the commercial Harovinton sample.)

Figure 4.4b. Mean[1] protein content for soybeans grown in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean protein content for the commercial Harovinton sample.)

4.1.3 Stability of Manitoba-grown Soybean Seed Size and Protein Content

An important factor for processors to consider when selecting soybeans for soymilk and tofu is the stability of the seed size and protein content over years and locations (Poysa and Woodrow, 2002). Processors adjust their processing methods primarily based on the seed size and protein content of the soybeans they are using. Therefore the more consistent the seed size and protein content the less changes in processing procedures they will have to make (Poysa and Woodrow, 2002).

The stability of seed size and protein content can be evaluated by plotting the seed size and protein content of different soybean varieties over growing location and crop year (Figure 4.5a, 4.5b and 4.6a, 4.6b). Ideally, a soybean variety's relative rank for seed size and protein content will remain the same regardless of growing location or crop year. The seed size and protein content of soybeans grown in Manitoba were relatively stable across all growing locations. Although growing locations did affect the absolute seed size and protein content of Manitoba-grown soybeans, the ranking from largest to smallest stayed the same. The same trends were observed for seed size and protein content plotted across the six site-years (Figure 4.7a and 4.7b).

Figure 4.5a. Stability of protein content across Manitoba growing locations in 2005.

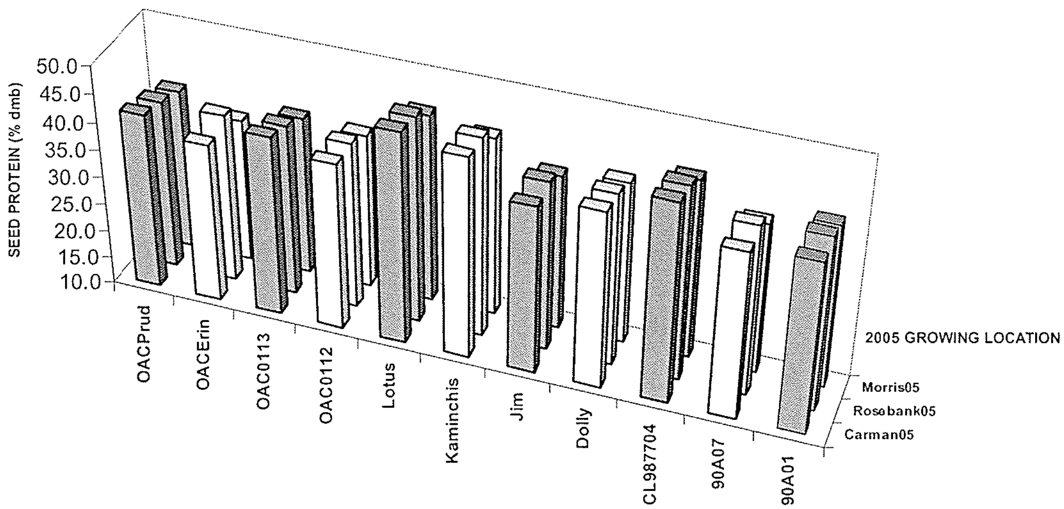
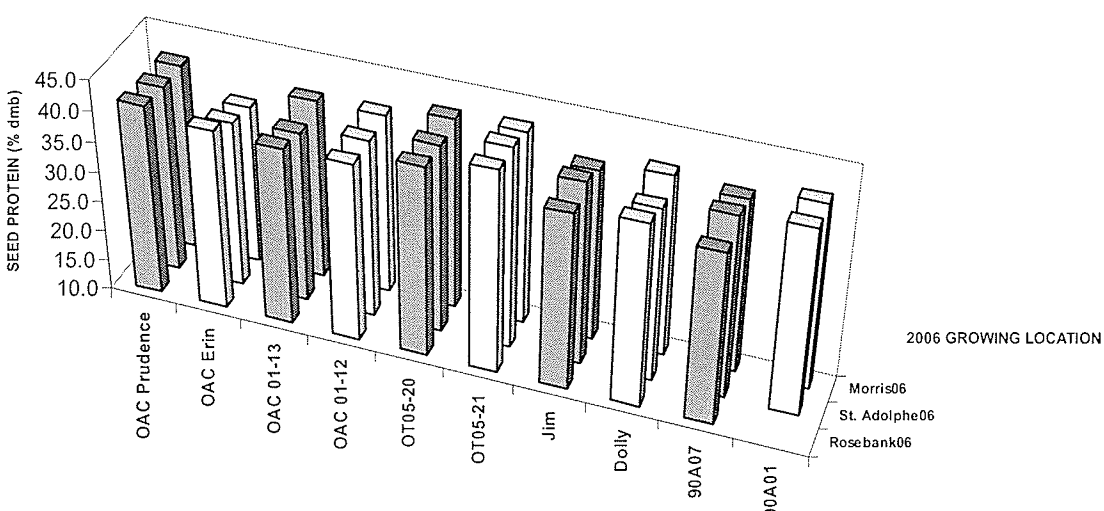


Figure 4.5b. Stability of protein content across Manitoba growing locations in 2006.



90A01 was not grown at the Rosebank location in 2006

Figure 4.6a. Stability of seed size across Manitoba growing locations in 2005.

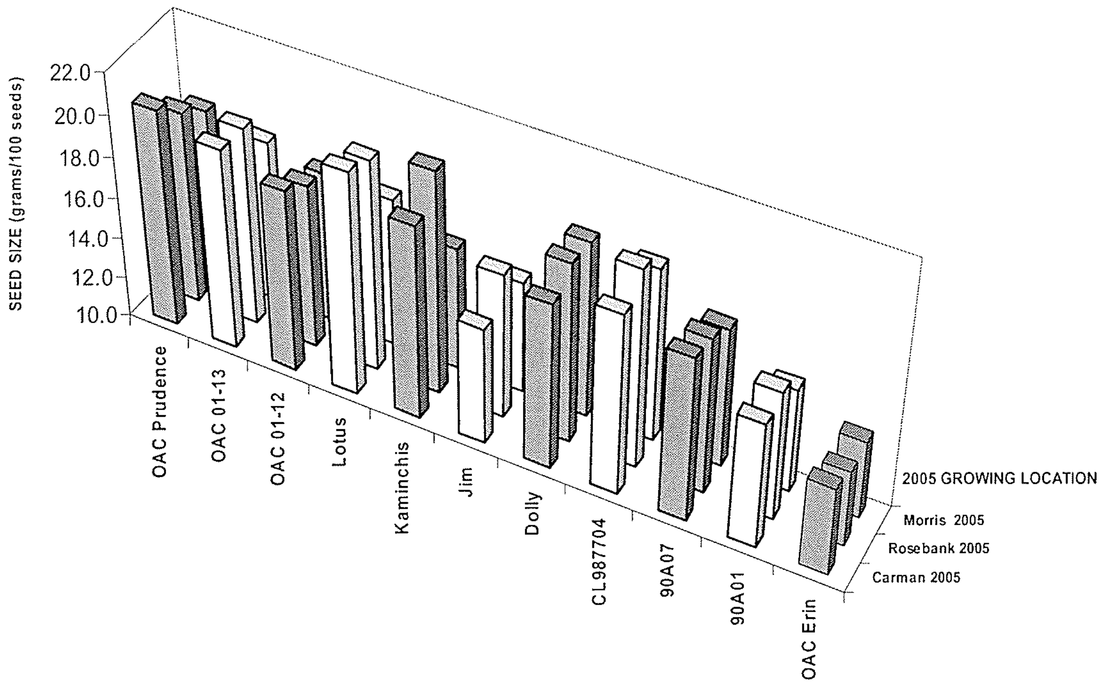
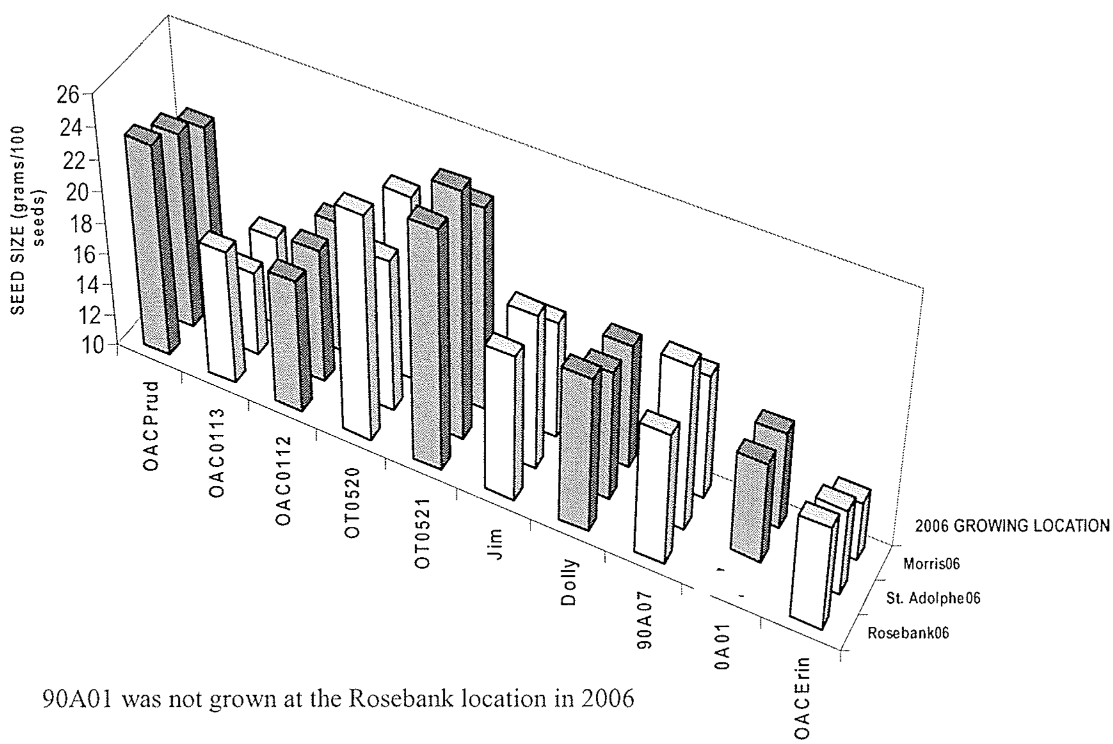


Figure 4.6b. Stability of seed size across Manitoba growing locations in 2006.



90A01 was not grown at the Rosebank location in 2006

Figure 4.7a. Stability of protein content of eight Manitoba-grown soybean varieties by site-year.

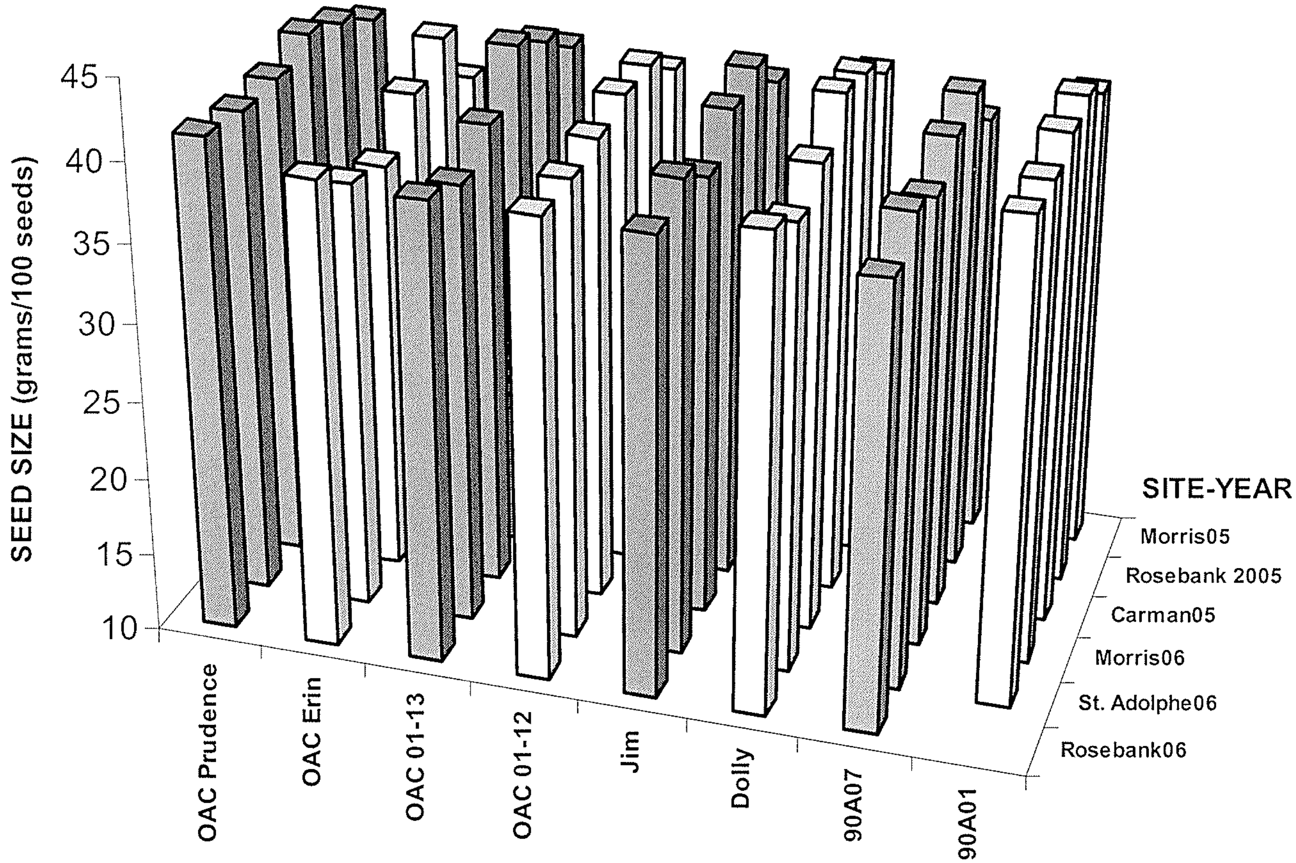
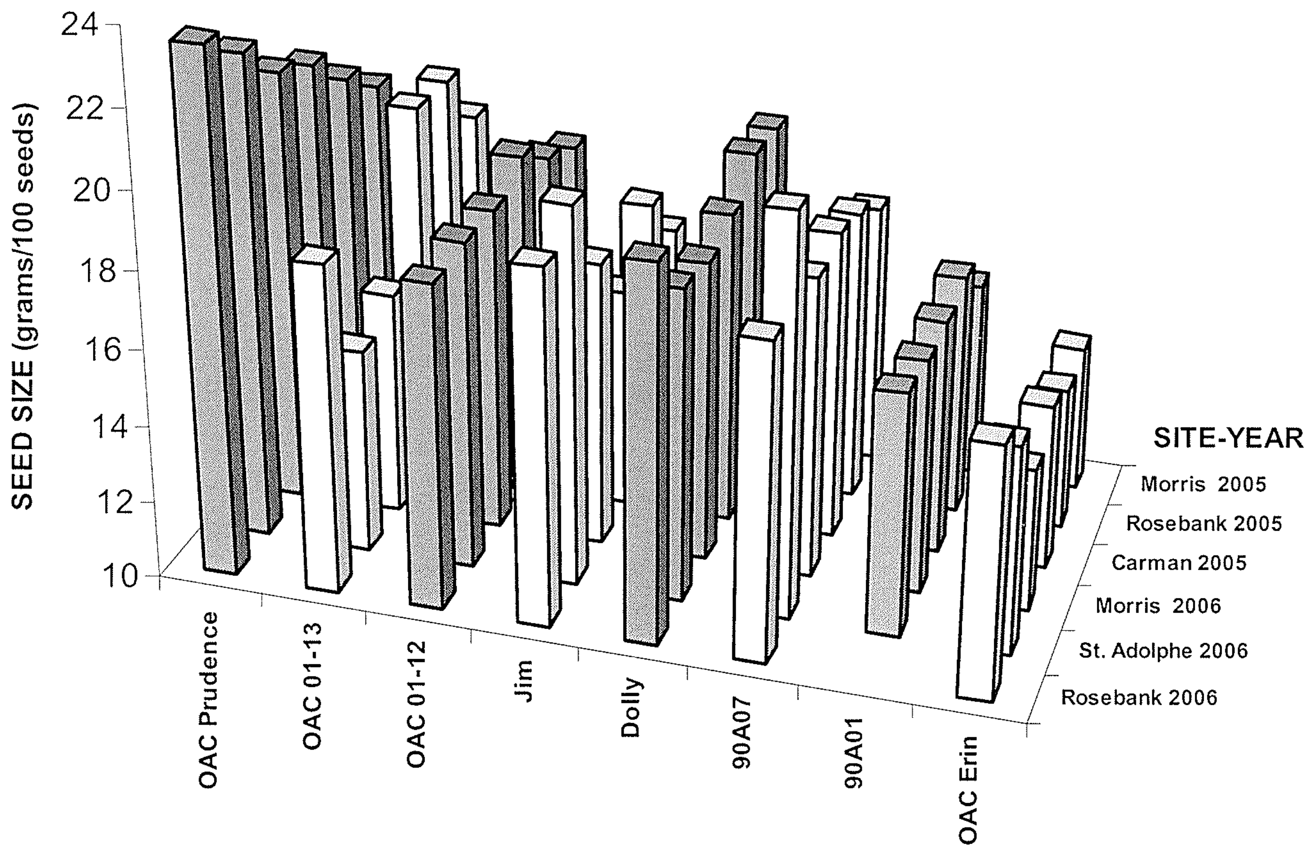


Figure 4.7b. Stability of seed size of eight Manitoba-grown soybean varieties by site-year.

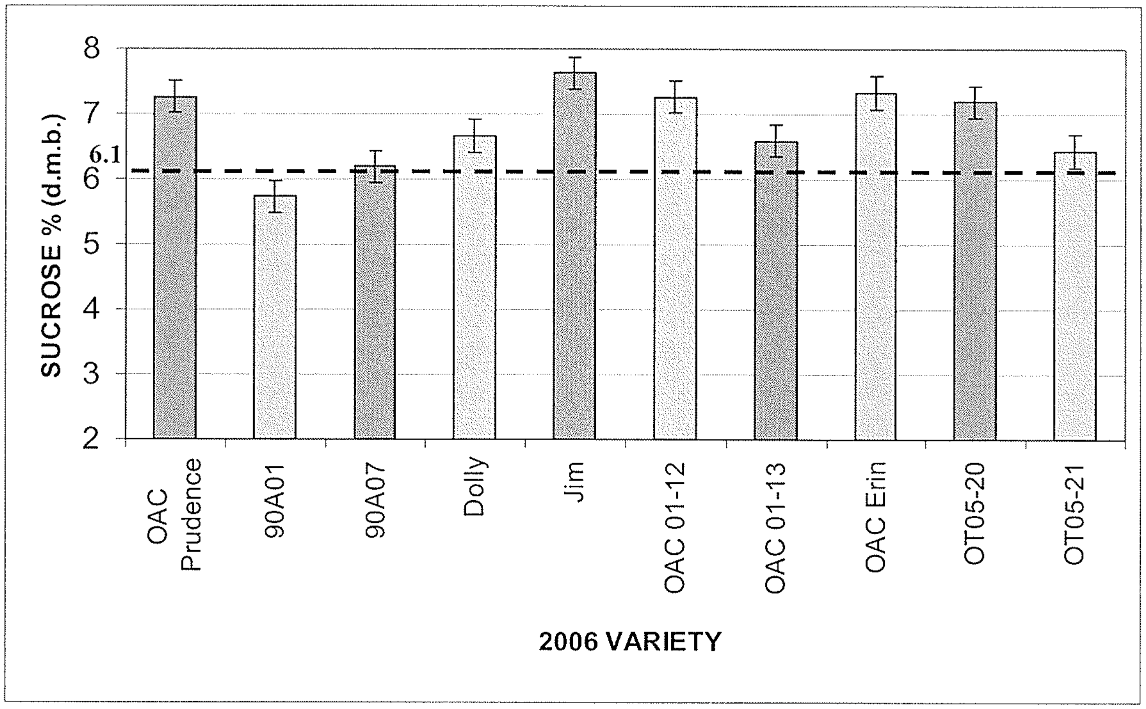


4.1.4 Effect of Variety on the Sucrose Content of Manitoba-Grown Food-Grade Soybeans

Sucrose levels of Manitoba soybeans were measured for the 2006 crop year only (Figure 4.8). The varieties Jim, OAC 01-12, OAC Erin, OAC Prudence and OT05-20 all had significantly higher sucrose levels ($P < 0.05$) than the commercial Harovinton sample, while the remaining samples had sucrose levels that were not significantly different from the commercial Harovinton sample (Table 4.3).

Poysa and Woodrow (2002) found protein content to be inversely related to sucrose content. This finding offers a possible explanation for high sucrose levels in Manitoba-grown soybeans as compared to the commercial Harovinton soybeans given that the Harovinton sample had much higher protein content than the Manitoba-grown soybeans.

Figure 4.8. Mean[1] sucrose content of soybeans grown in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean seed sucrose for Harovinton standard.)

Table 4.3. Statistical comparison of the sucrose content of Manitoba-grown soybeans in 2006 to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	[1]*Difference of LS Means*	[2]*SE*	[3]*Pr > | t |*
Harovinton	6.3	-	-	-
OAC Prudence	7.3	1.0	0.2186	0.0024
90A01	5.7	-0.6	0.2464	NS
90A07	6.2	-0.1	0.2186	NS
Jim	7.6	1.3	0.2186	<0.0001
Dolly	6.7	0.4	0.2186	NS
OAC 01-12	7.3	1.0	0.2186	0.0012
OAC 01-13	6.6	0.3	0.2186	NS
OAC Erin	7.3	1.0	0.2186	0.0012
OT05-21	6.4	0.1	0.2186	NS
OT05-20	7.2	0.9	0.2186	0.0046

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

4.2 Soymilk Yield and Colour

4.2.1 Effect of Site-year and Variety on Soymilk Yield

The main effects of site-year and variety both were significant for soymilk yield ($P < 0.001$ and $P < 0.05$, respectively) (Table 4.4). The factorial effects of year and site were both significant ($P < 0.05$) while the site by year interaction was not significant. These findings were also observed by Poysa and Woodrow (2002) and Aziadekey *et al.* (2002). In contrast, Bhardwaj *et al.* (1999) found that variety and location had no effect on soymilk yield. These discrepancies are likely due to differences in the methods used to extract soymilk from the soybeans.

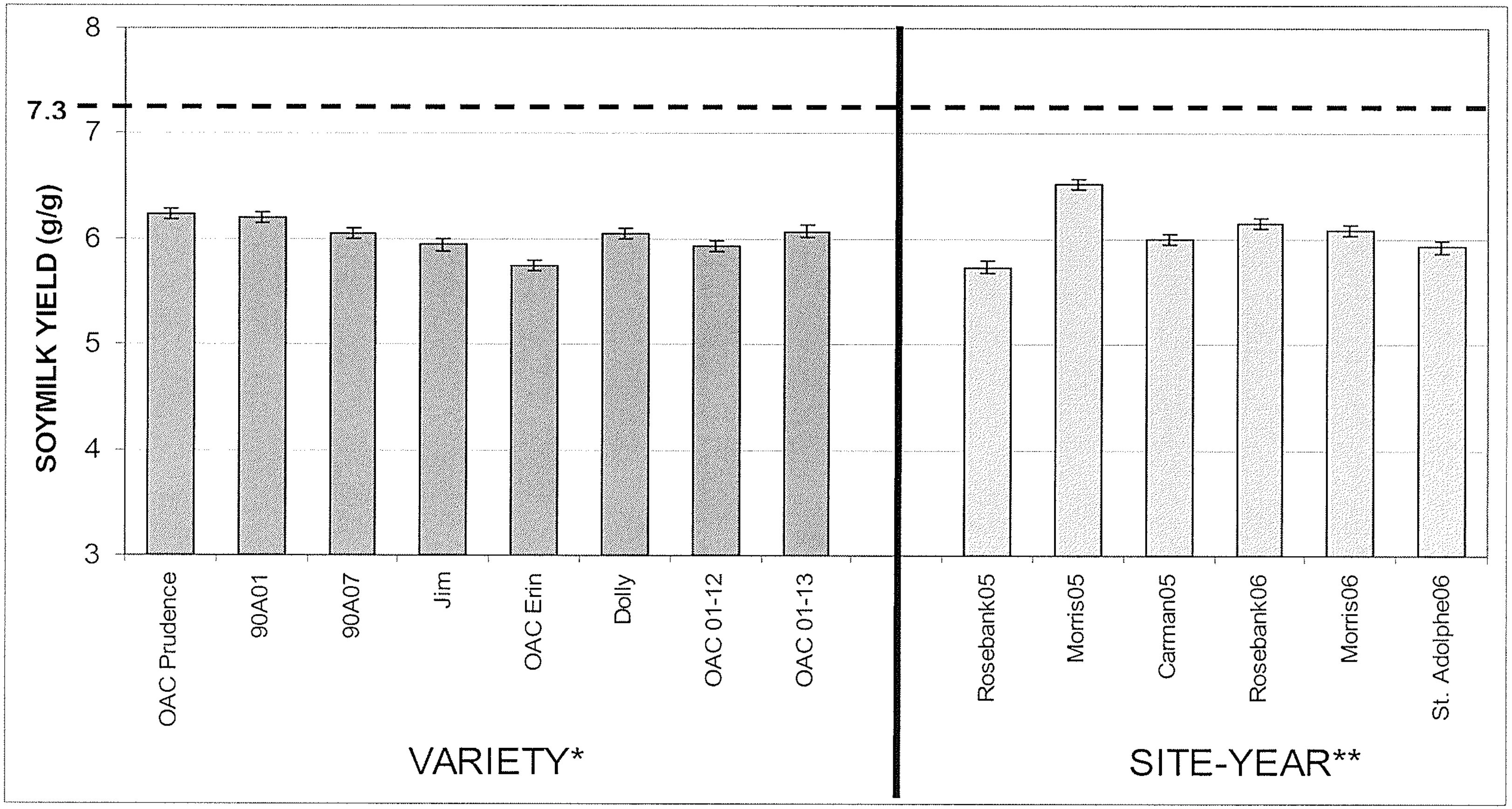
Table 4.4. Analysis of variance results for the yield of soymilk made from Manitoba-grown soybeans.

Source	*ANOVA*			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	0.32	5.64	0.0007
Year (Y) (2006 vs 2005)	*1*		*5.18*	*0.0294*
Site (S) (Morris vs Rosebank)	*1*		*7.14*	*0.0116*
S × Y	*1*		*0.41*	*0.5248*
Variety	7	0.14	2.47	0.0374
Error	33	0.06		

NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

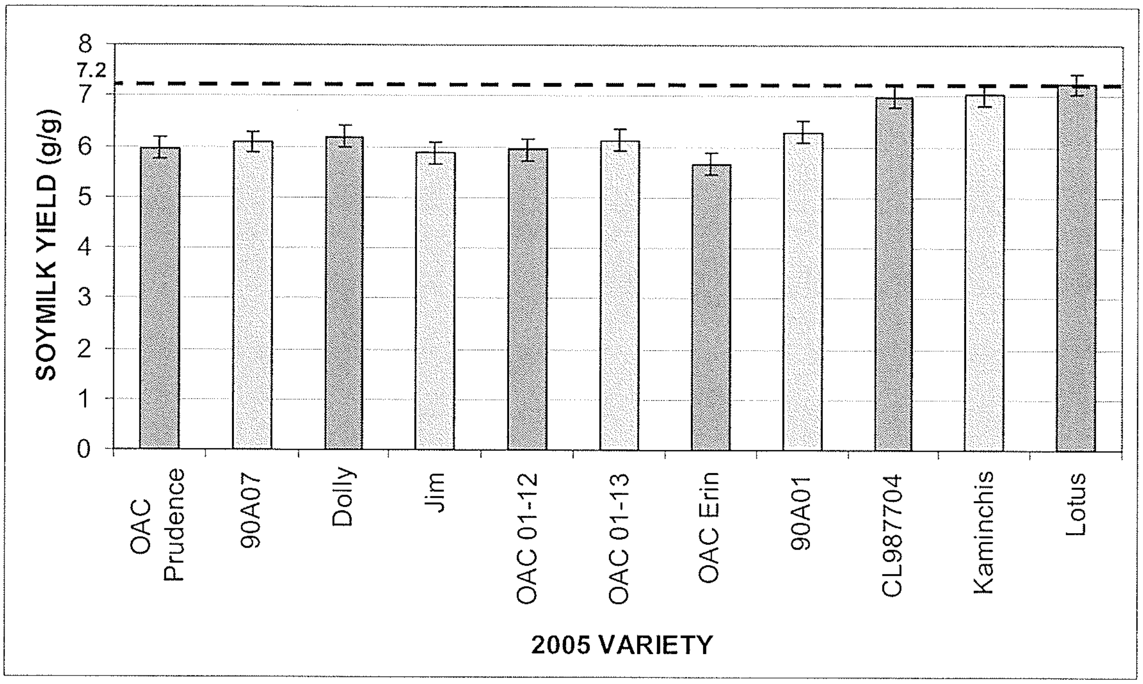
Figure 4.9 compares the mean soymilk yields of the Manitoba-grown varieties and Manitoba site-years to the mean soymilk yields of the commercial Harovinton samples. The Manitoba-grown varieties and site-years produced lower soymilk yields than the commercial Harovinton samples. However, in 2005, three Manitoba-grown varieties (CL987704, Kaminchis, and Lotus) were found not to be significantly different than the commercial Harovinton sample for soymilk yield (Figure 4.10a). These results can likely be attributed to the fact that CL987704, Kaminchis, and Lotus were also not significantly different than Harovinton in protein content. A positive relationship between seed protein content and soymilk yield had previously been reported by Poysa and Woodrow (2002), which would suggest that soybeans with similar protein contents should also have similar soymilk yields. Further evidence of the positive relationship between protein content and soymilk yield was found in 2006, when all Manitoba-grown soybean varieties evaluated had both significantly lower protein content (Figure 4.2b) and significantly lower soymilk yield (Figure 4.10b) than the commercial Harovinton sample.

Figure 4.9. Mean[1] soymilk yield of Manitoba-grown soybeans for variety and site-year.



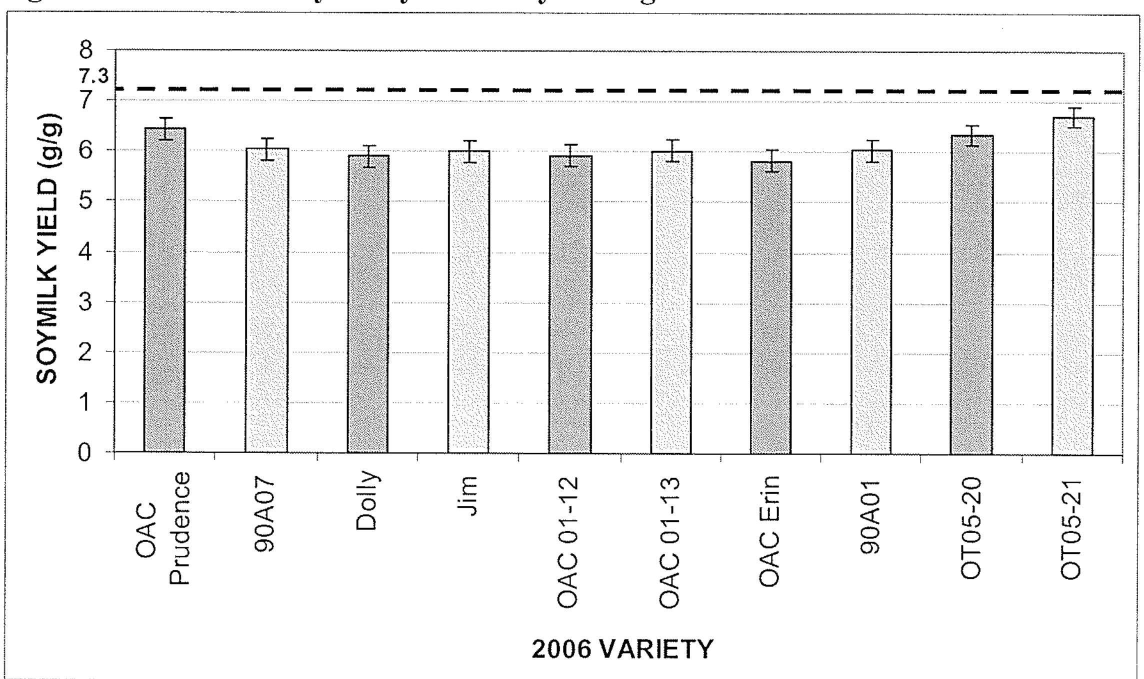
[1]For Variety means n=6 site-years; for site-year means n=8 varieties
(Dashed line represents mean soymilk yield for the commercial Harovinton samples.)
Variety and Site-Year significance levels from Table 4.4
* $P < 0.05$
** $P < 0.001$

Figure 4.10a. Mean[1] soymilk yield of soybeans grown in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean soymilk yield for the commercial Harovinton samples.)

Figure 4.10b. Mean[1] soymilk yield of soybeans grown in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean soymilk yield for the commercial Harovinton samples.)

4.2.2 Effect of Site-year and Variety on Soymilk Colour

Processors desire the colour of soymilk to have a high degree of whiteness. In this study the degree of whiteness was calculated by subtracting the b* from the L* values (Oliver *et al,* 1992). The a* value was not incorporated into the calculation because its value typically is between -1 and +1 and therefore is not considered to be significant in defining the whiteness value of soymilk (Appendix 1).

The main effects of site-year ($P < 0.0001$) and variety ($P < 0.0001$) both had a significant effect on soymilk whiteness value (Table 4.5). The factorial effects of year ($P < 0.0001$) and site ($P < 0.0205$), as well as the site by year interaction ($P < 0.0001$) were all significant. Poysa and Woodrow (2002) also found site ($P < 0.0001$), year ($P < 0.0001$), and location ($P < 0.05$) effects to be significant; however, Bhardwaj *et al.* (1999) found only variety ($P < 0.01$) to have a significant effect.

Table 4.5. Analysis of variance results for the whiteness value of soymilk made from Manitoba-grown soybeans.

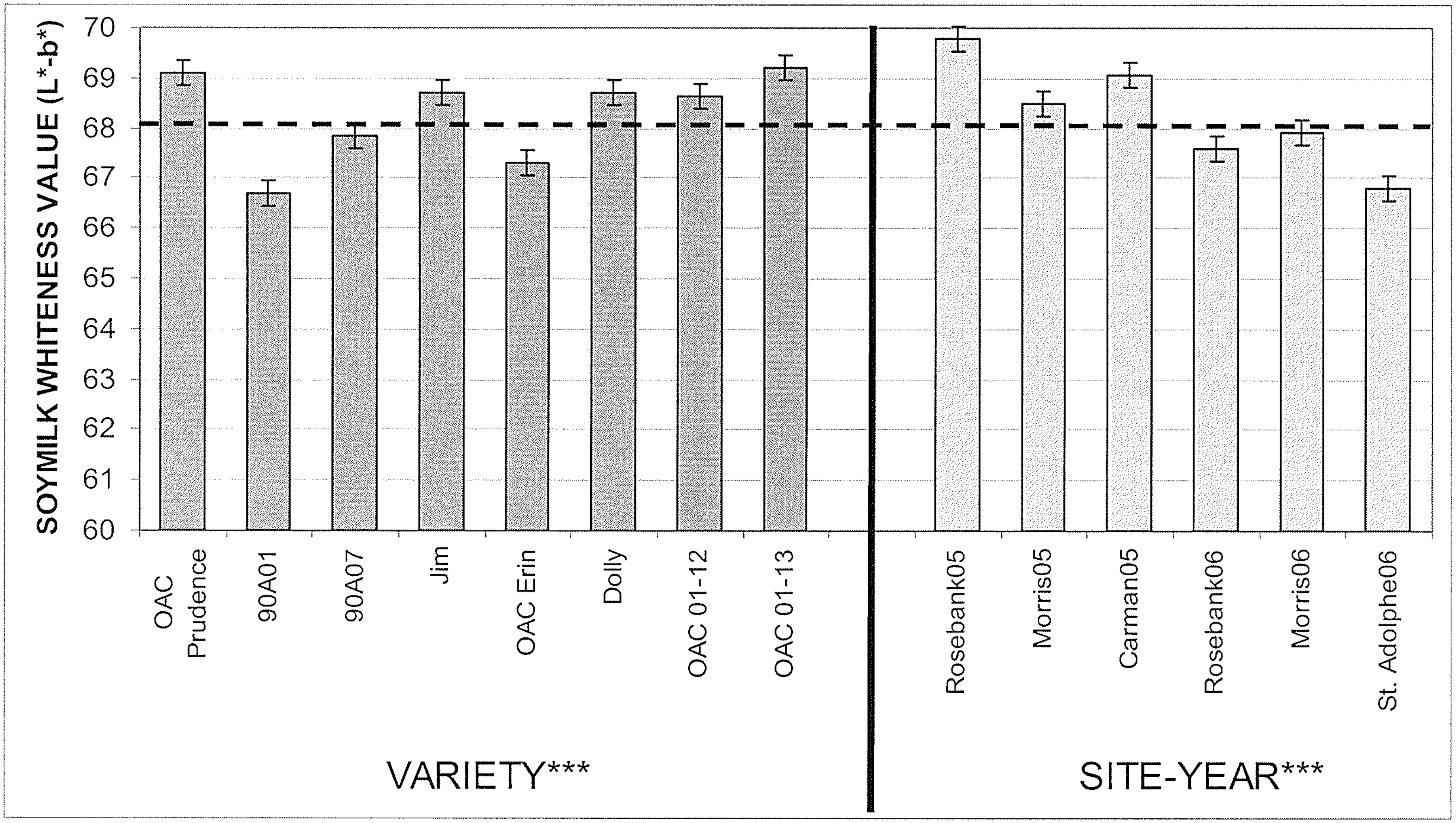
Source	ANOVA			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	9.60	41.71	<0.0001
Year (Y) (2006 vs 2005)	*1*		*77.63*	<0.0001
Site (S) (Morris vs Rosebank)	*1*		*5.93*	*0.0205*
S × Y	*1*		*30.36*	<0.0001
Variety	7	4.35	18.93	<0.0001
Error	33			

NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

The mean whiteness value of soymilk made from Manitoba-grown soybean varieties comparable to the whiteness value of soymilk made from the commercial Harovinton soybean sample (Figure 4.11). The same was true for the mean soymilk whiteness value of Manitoba-grown soybeans grouped by site-year (Figure 4.11). In 2005, the varieties Dolly, Jim, OAC 01-12, OAC 01-13, and OAC Prudence produced soymilk with whiteness values that were significantly greater (P <0.05) than the commercial Harovinton sample (Figure 4.12a)(Appendix 3, Table 4a). All of the remaining varieties evaluated in 2005 were not significantly different in soymilk whiteness values compared to the commercial Harovinton sample. In 2006, soymilk whiteness values were slightly lower than in 2005; however, 8 of the 10 varieties evaluated produced soymilk with whiteness values that were not significantly different from Harovinton soymilk (Figure 4.12b)(Appendix 3, Table 4b) . The varieties 90A01 and OAC Erin were the only varieties in 2006 with significantly lower whiteness values than the commercial

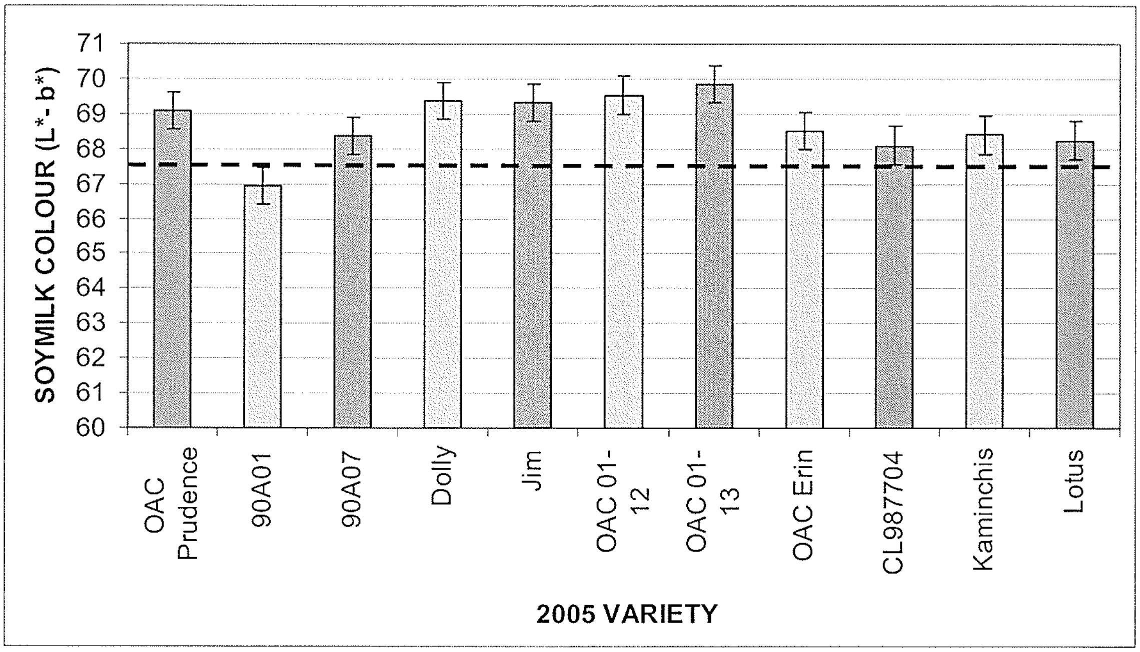
Harovinton sample. Although colorimetric readings found differences in soymilk whiteness values there is still a question as to whether or not these differences would be detected by a trained panel.

Figure 4.11. Mean[1] soymilk whiteness of Manitoba-grown soybeans for variety and site-year.



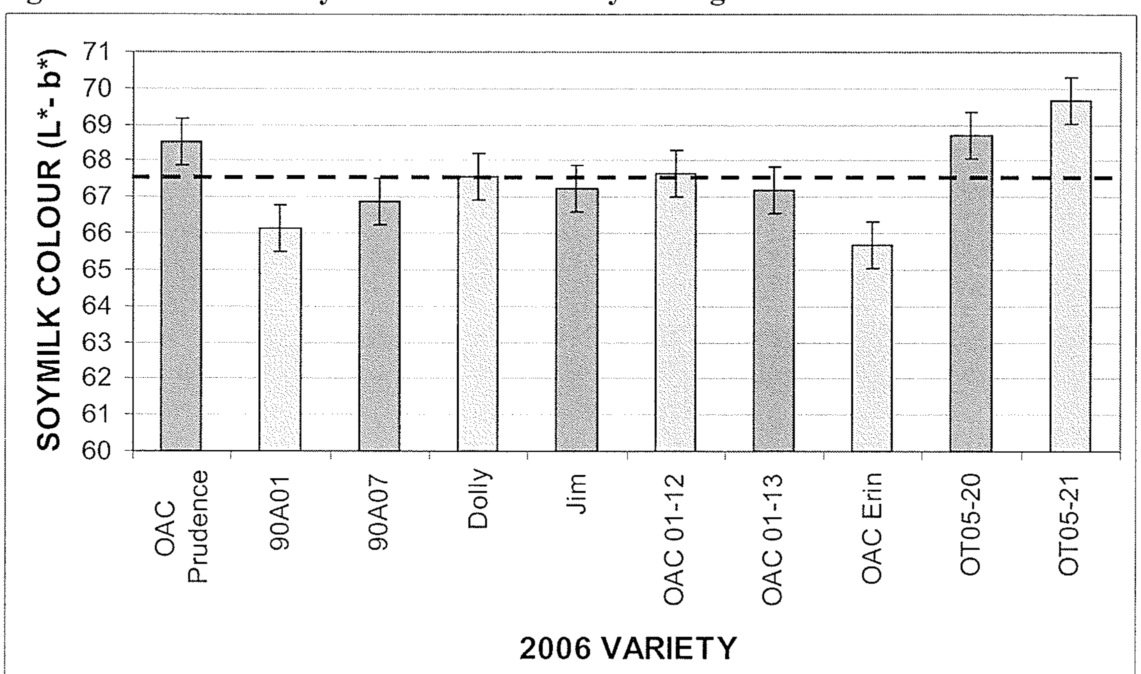
[1]For Variety means n=6 site-years; for site-year means n=8 varieties
(Dashed line represents mean soymilk whiteness for the commercial Harovinton samples.)
Variety and Site-Year significance levels from Table 4.4
*** $P < 0.0001$

Figure 4.12a . Mean[1] soymilk whiteness of soybeans grown in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean soymilk whiteness for the commercial Harovinton samples.)

Figure 4.12b . Mean[1] soymilk whiteness of soybeans grown in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean soymilk whiteness for the commercial Harovinton samples)

4.3 Effect of Site-year and Variety on Tofu Yield

Tofu yield, as with soymilk yield, is of economic importance to manufacturers. In this study, tofu yield was reported as grams of tofu produced per gram of soybean seed. As stated earlier in Section 3.11, there were two observations for the tofu data, thus the residual error was used as the error term. Unlike the ANOVA tables for seed and soymilk characteristics, the ANOVA tables for tofu not only include the main effects of site-year and variety, but also the effect of the site-year by variety interaction.

The main effects of site-year, variety and the site-year by variety interaction were significant for tofu yield ($P < 0.0002$, $P < 0.0435$, and $P < 0.0001$ respectively) (Table 4.5). These findings are similar to the findings of Poysa and Woodrow (2002); however, they are contrary to the results of Bhardwaj *et al.* (1999) who found that location had no significant effect on tofu yield. These differences are possibly due to differences in tofu preparation methods.

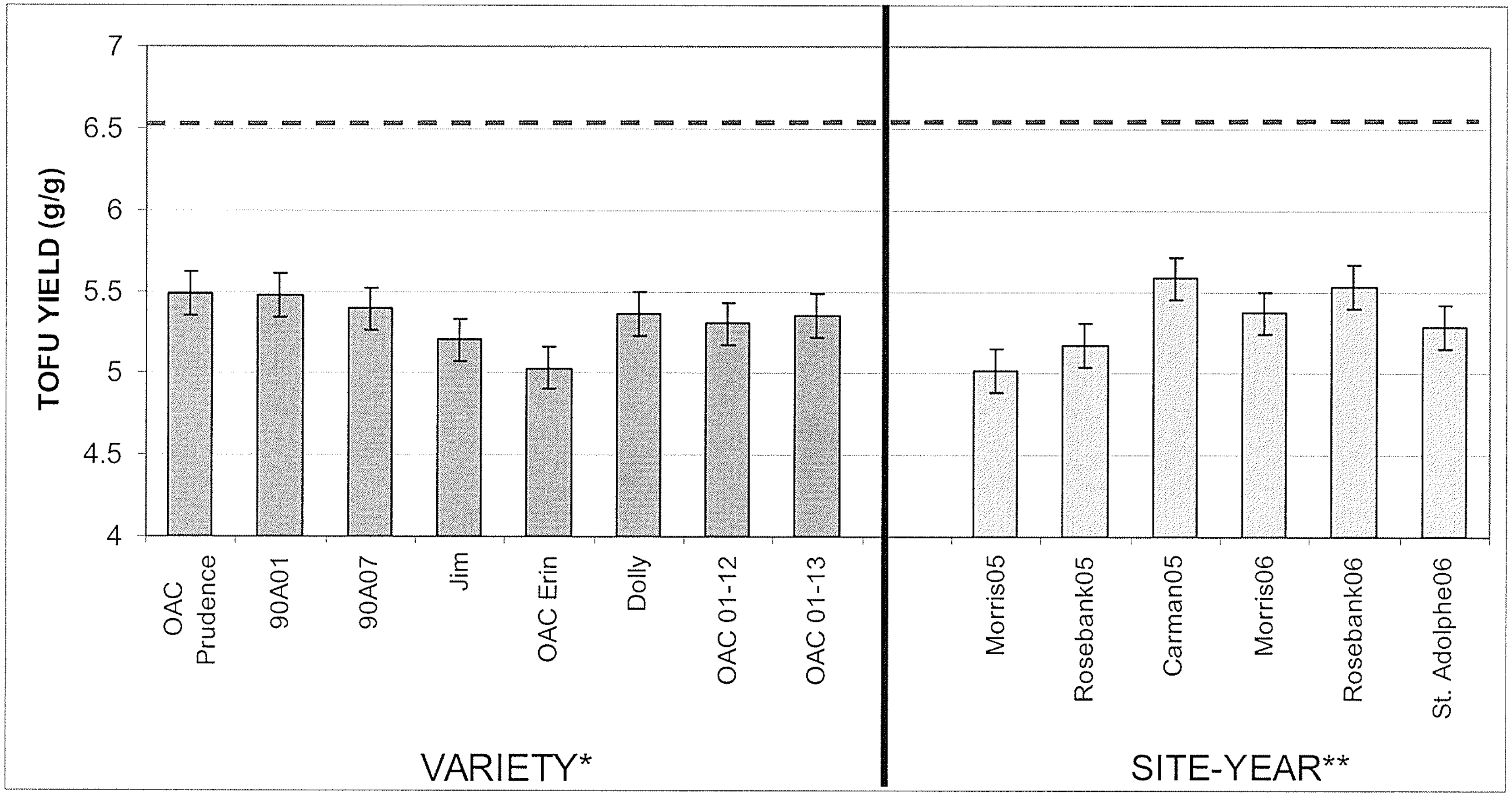
Table 4.6. Analysis of variance results for the yield of tofu made from Manitoba-grown soybeans.

Source	*ANOVA*			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	0.70	6.57	0.0002
Year (Y) (2006 vs 2005)	*1*		*12.67*	0.0009
Site (S) (Morris vs Rosebank)	*1*		*4.98*	*0.0305*
S × Y	*1*		*0.05*	0.8232
Variety	7	0.25	2.38	0.0435
Site-Year × Variety	33	0.11	47.87	<0.0001
Error	46	0.002		

NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

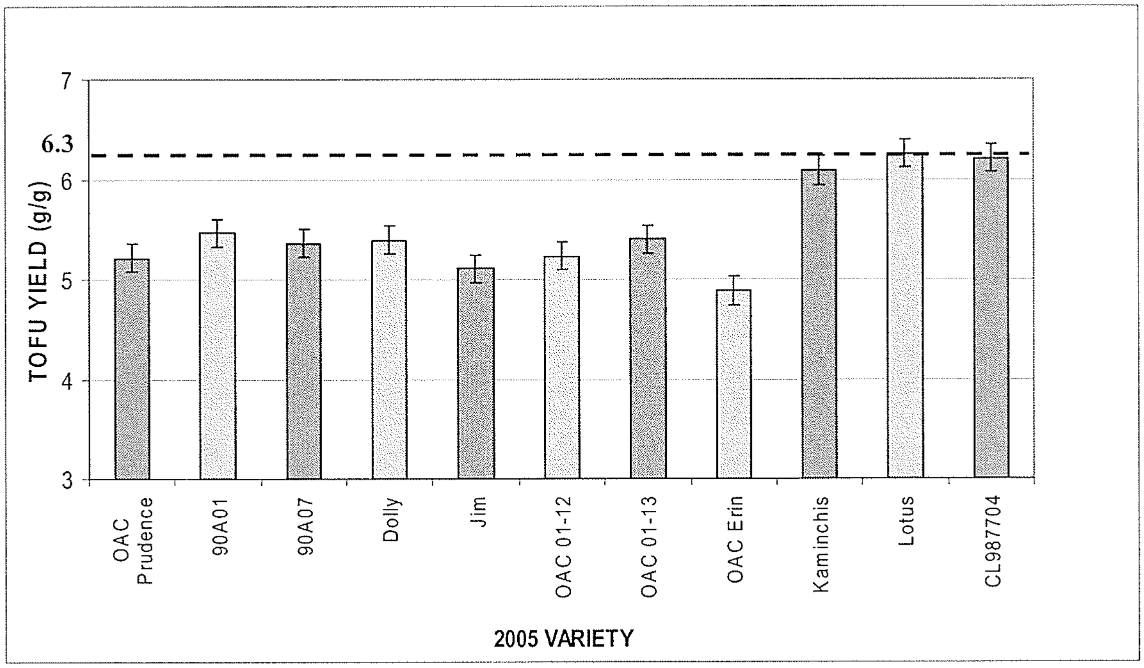
The results for tofu yield were similar to the results for soymilk yield. Tofu yield has been shown to be largely dependent on soybean seed protein content (Aziadekey *et al.*, 2002; Poysa and Woodrow, 2002; Bhardwaj *et al*, 1999). Figure 4.13 shows the mean values for tofu yield of the eight Manitoba-grown soybean varieties and six Manitoba site-years. As this figure demonstrates, the mean tofu yield of the Manitoba-grown varieties and site-years is lower than that of the mean tofu yield of the commercial Harovinton soybean samples. In 2005 and 2006 the exact same observations were made for tofu yield as were earlier reported for soymilk yield (Figure 4.14a and 4.14b). The only varieties in these two years that had tofu yields that were not significantly different from the commercial Harovinton samples were the high-protein varieties CL987704, Kaminchis and Lotus (Appendix 3, Tables 5a and 5b). The soymilk and tofu yield results from this study highlight the strong positive relationship between seed protein content and yield of soymilk and tofu.

Figure 4.13. Mean[1] tofu yield of Manitoba-grown soybeans for variety and site-year.



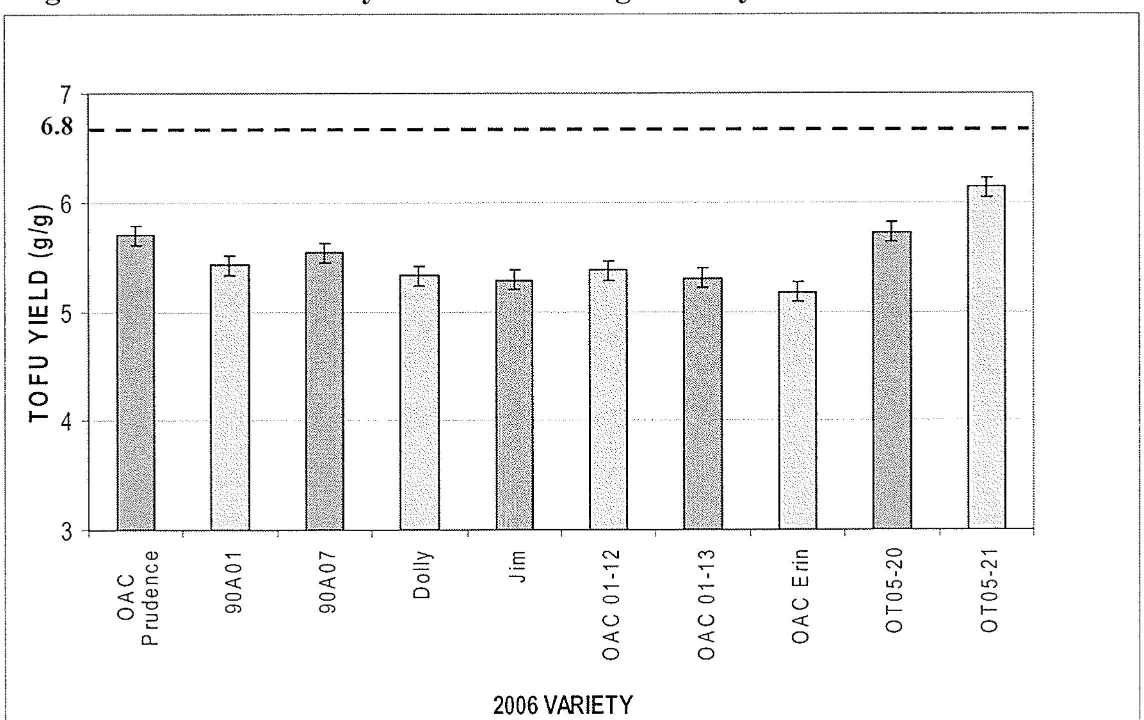
[1]For Variety means n=6 site-years; for site-year means n=8 varieties
(Dashed line represents mean tofu yield for the commercial Harovinton samples.)
Variety and Site-Year significance levels from Table 4.5
* $P < 0.05$
** $P < 0.01$

Figure 4.14a. Mean[1] tofu yield of Manitoba-grown soybeans in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean tofu yield for the commercial Harovinton samples.)

Figure 4.14b. Mean[1] tofu yield of Manitoba-grown soybeans in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean tofu yield for the commercial Harovinton samples.)

4.4 Effect of Site-year and Variety on Tofu Whiteness

Processors desire tofu that has a high degree of whiteness (Wang and Chang, 1995). The whiteness value for tofu was derived in the same manner as it was for soymilk whiteness ($L^* - b^*$) (Oliver *et al,* 1992). The main effects of site-year, variety, and the site-year by variety interaction were all significant for tofu whiteness ($p < 0.0004$). The factorial effects of site-year year ($P < 0.0031$) and site ($P < 0.0007$) were both found be significant contributors, while the site by year interaction was not significant. Poysa and Woodrow (2002) found variety, year and the year by location interaction significantly affected tofu colour, while Bhardwaj *et al.* (1999) and Aziadekey *et al.* (2002) found only variety to significantly effect tofu colour.

Table 4.7. Analysis of variance results for the whiteness value of tofu made from Manitoba-grown soybeans.

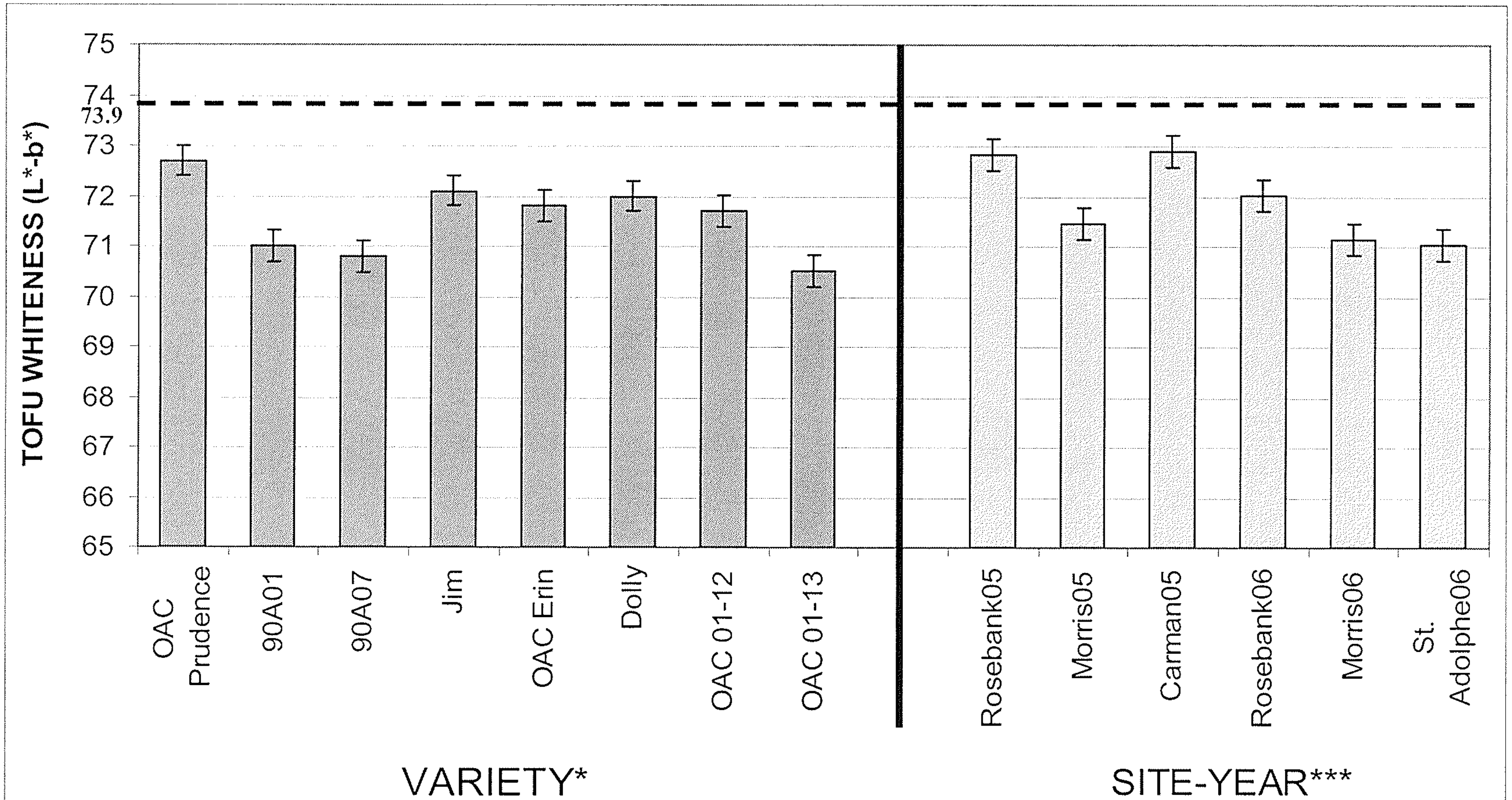
Source	ANOVA			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	14.45	12.63	0.0004
Year (Y) (2006 vs 2005)	*1*		*9.77*	*0.0031*
Site (S) (Morris vs Rosebank)	*1*		*13.39*	*0.0007*
S × Y	*1*		*0.55*	*0.4629*
Variety	7	6.14	5.37	<0.0001
Site-Year × Variety	33	1.14	12.74	<0.0001
Error	46	0.08		

NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

Figure 4.15 shows the mean values for tofu whiteness of the eight Manitoba-grown soybean varieties and six Manitoba site-years. As this figure demonstrates, Manitoba-grown soybeans had lower whiteness values than that of the commercial Harovinton sample. In this study, seed protein content was found to be positively correlated with tofu whiteness (r =0.68), which may explain why the high-protein commercial Harovinton sample produced tofu with a higher degree of whiteness than the lower-protein Manitoba soybean varieties. However, this explanation is contradicted by the findings of both Poysa and Woodrow (2002) and Bhardwaj *et al.* (1999) who found seed protein content to be negatively correlated with tofu whiteness.

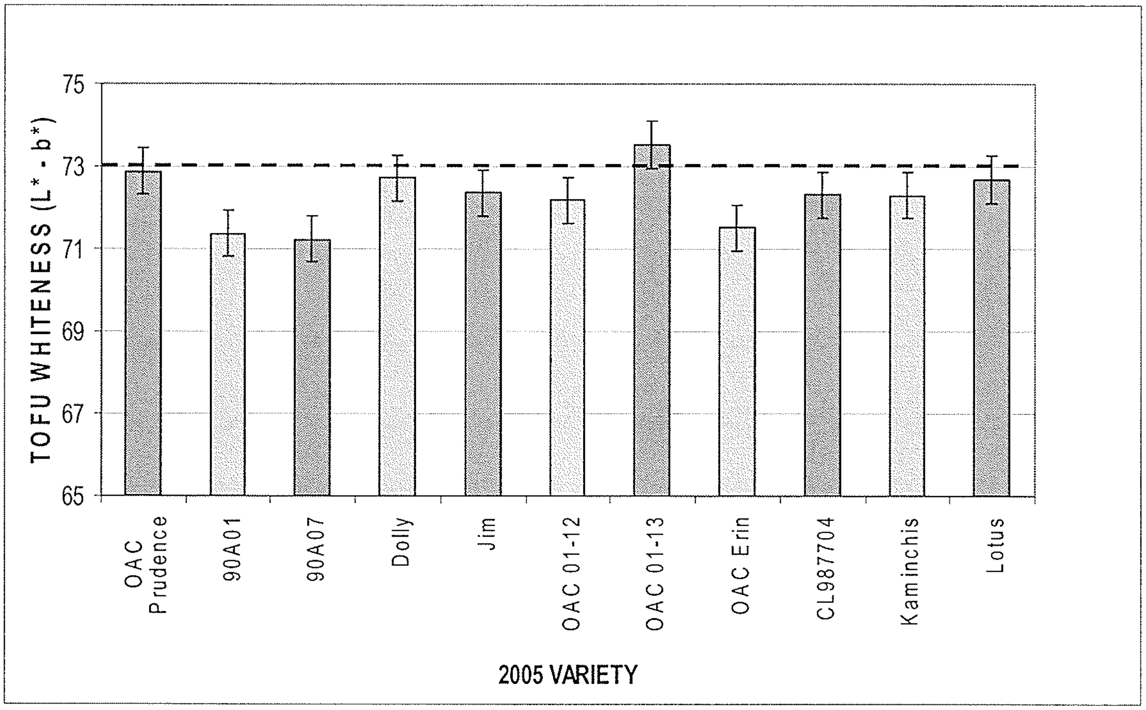
There was a stark contrast in tofu whiteness values between the 2005 and 2006 growing seasons. In 2005, the varieties OAC Prudence, Jim, Dolly, OAC 01-12, OAC 01-13, CL987704, Kaminchis, and Lotus were not significantly different in tofu whiteness than the commercial Harovinton sample (Figure 4.16a; Appendix 3, Table 6a). However, in 2006 all varieties evaluated had significantly lower tofu whiteness ($P < 0.05$) than the commercial Harovinton sample (Figure 4.16b; Appendix 3, Table 6b). The difference between years corresponds with the significant year effect ($P=0.0031$)(Table 4.7) on tofu whiteness. Although previous research has found year to have a significant effect on tofu colour (Poysa and Woodrow, 2002), an explanation as to why has not been explored. It is possible that differences in rainfall and temperature from year to year contributed to differences in tofu whiteness.

Figure 4.15. Mean[1] tofu whiteness of Manitoba-grown soybeans for variety and site-year.



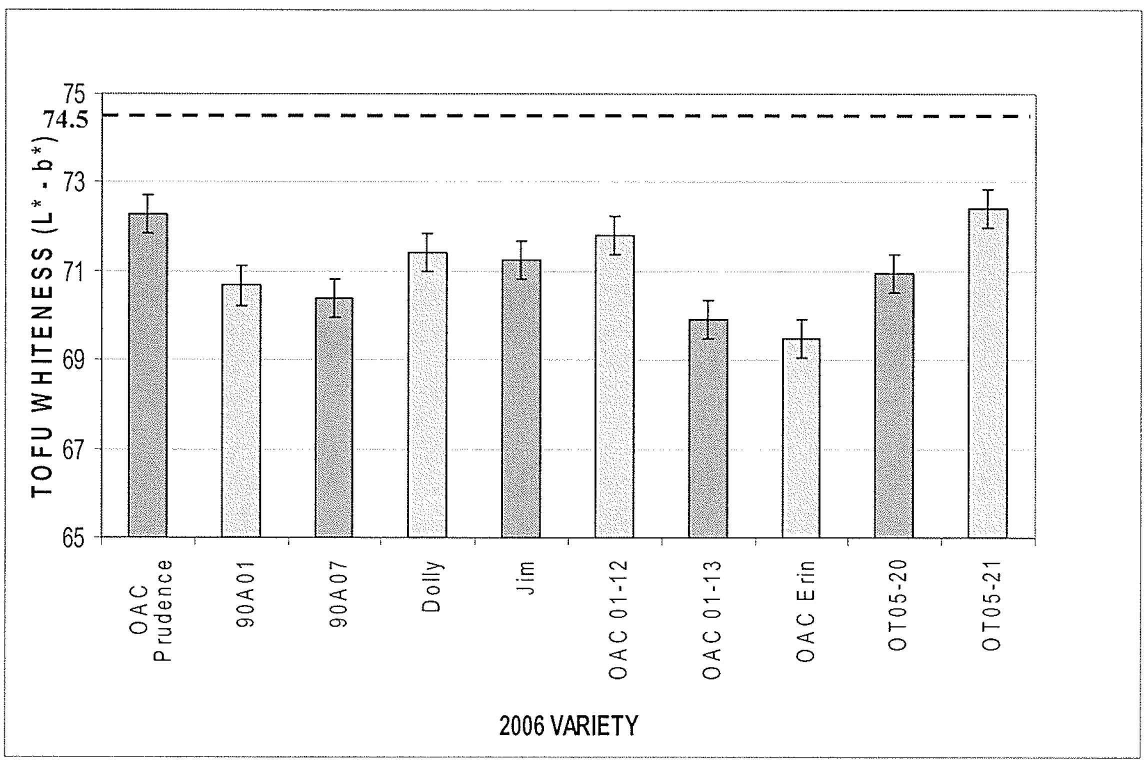
[1]For Variety means n=6 site-years; for site-year means n=8 varieties
(Dashed line represents mean tofu whiteness for Harovinton standard.)
Variety and Site-Year significance levels from Table 4.7
** $P < 0.01$
*** $P < 0.0001$

Figure 4.16a. Mean[1] tofu whiteness of Manitoba-grown soybeans in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean tofu whiteness for Harovinton standard.)

Figure 4.16b. Mean[1] tofu whiteness of Manitoba-grown soybeans in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean tofu whiteness for Harovinton standard.)

4.5 Effect of Site-year and Variety on Tofu Texture

As described earlier, there are many different types of tofu that are classified by hardness. It is generally understood that the ability of soybean seed to form a firm gel is very important characteristic that processors look for (Aziadekey *et al.*, 2002). A soybean variety which can produce firmer tofu at a given water to protein ratio compared to another variety is considered to be more valuable because it can be used for making a greater volume of tofu of a defined hardness (Poysa *et al.*, 2006). In this study, the texture of tofu was measured as compression force (N) with greater force measurements being viewed as desirable.

The main effects of site-year ($P < 0.0001$), variety ($P < 0.0028$), and site-year by variety ($P < 0.0001$) were significant for tofu texture (Table 4.8). The factorial effects of site-year were all significant with year ($P < 0.0001$) and the site by year interaction ($P < 0.0007$) having a stronger effect than site ($P < 0.0348$) (Table 4.8). Aziadekey *et al.* (2002) also found variety, location, and variety by location interaction to be significant. However, these findings differ from Poysa and Woodrow (2002) who found that only variety had significant effects on tofu texture and Bhardwaj *et al.,* who found only location to have a significant effect on tofu texture. The variation in results found among researchers is possibly due to a number of factors. Variation occurs depending on the texture analyzer used, the sample preparation and the method by which the tofu was prepared. Even if similar methods were used, there can be a great deal of error among laboratories (Mullin et *al.,* 2001).

An advantage of the method used in this study is that it measures texture on silken tofu, which is not pressed. Pressed tofu has a layer if skin that is firmer than the interior portion of the tofu block (Yuan and Chang, 2007). Pressing affects textural analysis by increasing hardness based on the method of pressing rather than the quality of the soybean.

Table 4.8. Analysis of variance results for the hardness of tofu made from Manitoba-grown soybeans.

Source	ANOVA			
	df	*Mean Square*	*F Value*	*Pr > F*
Site-year	5	27.90	11.58	<0.0001
Year (Y) (2006 vs 2005)	*1*		*21.47*	*<0.0001*
Site (S) (Morris vs Rosebank)	*1*		*4.73*	*0.0348*
S × Y	*1*		*13.33*	*0.0007*
Variety	7	13.57	4.02	0.0028
Site-Year × Variety	33	15.90	12.04	<0.0001
Error	46	1.84		

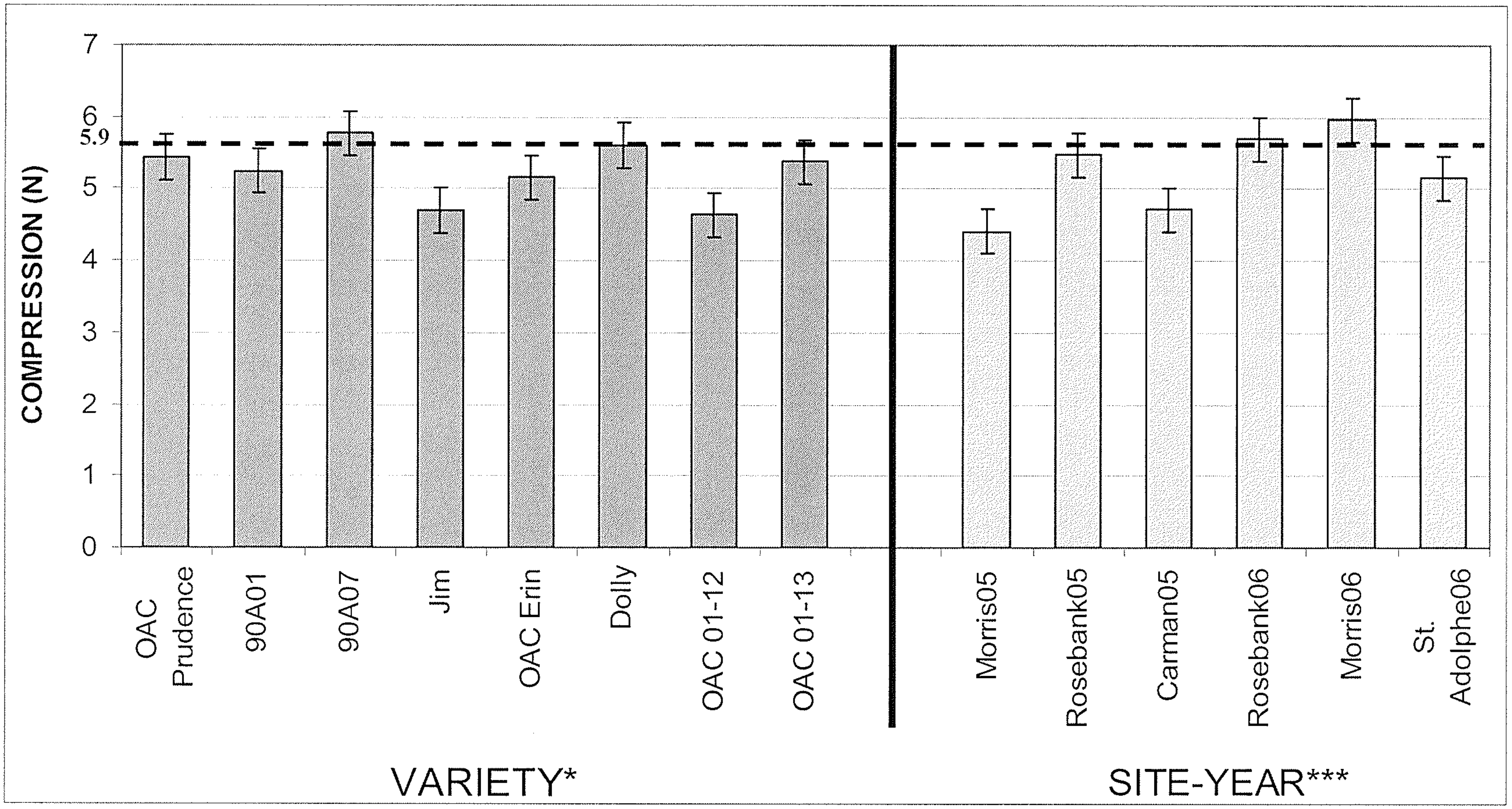
NOTE: In a true site by location study the degrees of freedom (df) for site-year should be equal to the sum of the df for site, year and site by year interaction. The F-values for year, site, and the site by year interaction were calculated based on data from only two locations (Morris and Rosebank) and therefore do not entirely account for the effect of site-year.

Figure 4.17 illustrates the differences in hardness between the eight Manitoba-grown soybeans varieties and six Manitoba site-years. As mentioned earlier, Manitoba varieties had much lower average seed protein than the commercial Harovinton samples (Figure 4.3) yet the hardness of tofu made from Manitoba soybean varieties compared well to Harovinton. These findings are in contrast to the traditional belief that higher seed protein content will produce tofu with a greater hardness.

Poysa and Woodrow (2002) and Azidekey *et al.* (2002) suggested that the tofu hardness was influence by factors such as the 11S and 7S protein subunit concentrations rather than seed protein quantity. When Poysa *et al.* (2006) studied the effects of the soy protein subunit composition on tofu hardness they found it to have a significant effect. In particular, Poysa *et al.* (2006) found that increased levels of the 11S group greatly increased tofu hardness. Given these findings it is possible that Manitoba-grown soybeans contain higher levels of the 11S soy protein group, therefore giving them the ability to form firmer tofu. An examination of the protein subunit composition of Manitoba-grown soybeans would need to be conducted in order to test this hypothesis.

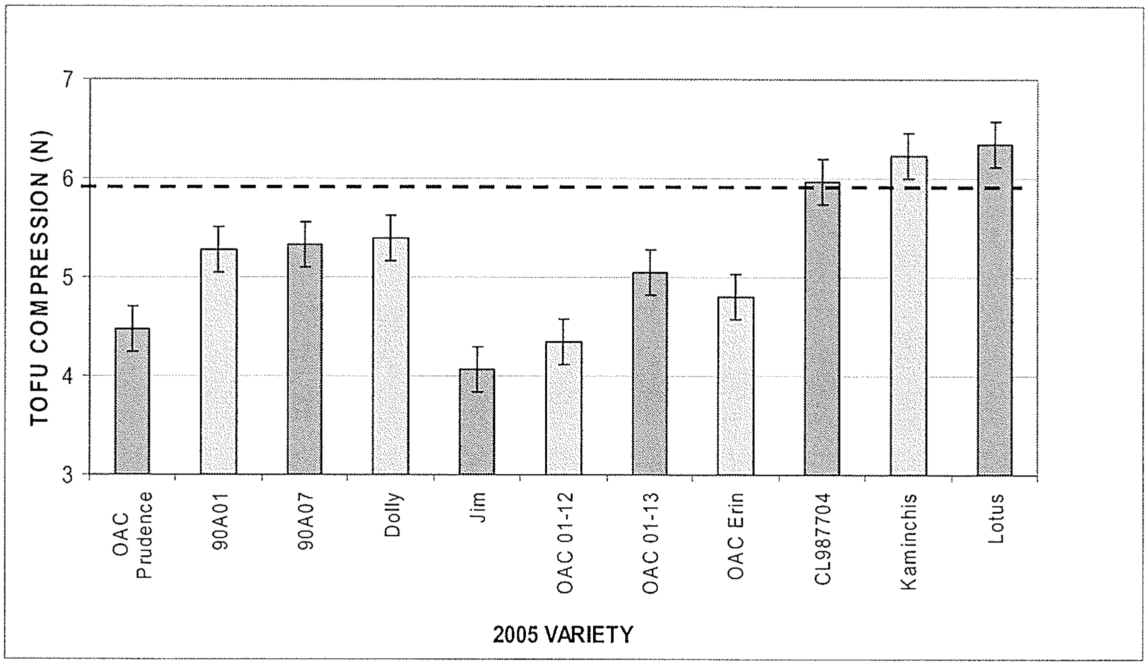
In both 2005 and 2006 Manitoba-grown soybeans compared well to the commercial Harovinton sample for tofu hardness. In 2005, the varieties 90A01, 90A07, Dolly, OAC 01-13, OAC Erin, Cl987704, Kaminchis, and Lotus were not significantly different than the commercial Harovinton samples (Figure 4.18a; Appendix 3, Table 7a). In 2006, all of the varieties evaluated were not significantly different than the commercial Harovinton samples (Figure 4.18b; Appendix 3, Table 7b).

Figure 4.17. Mean[1] tofu hardness (as measured by compression) of Manitoba-grown soybeans for variety and site-year.



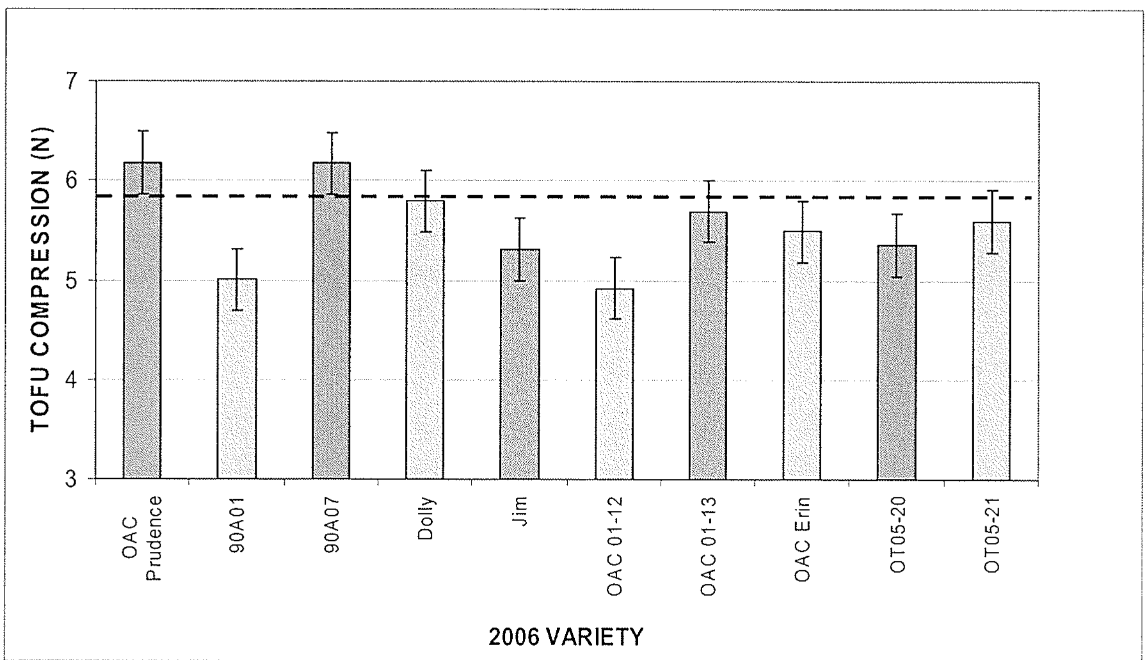
[1]For Variety means n=6 site-years; for site-year means n=8 varieties
(Dashed line represents mean tofu hardness for Harovinton standard.)
Variety and Site-Year significance levels from Table 4.7
*P < 0.05
*** P < 0.0001

Figure 4.18a. Mean[1] tofu hardness (as measured in compression) of Manitoba-grown soybeans in 2005.



[1]Mean of 3 site-years in 2005
(Dashed line represents mean tofu hardness for Harovinton standard.)

Figure 4.18b. Mean[1] tofu hardness (as measured in compression) of Manitoba-grown soybeans in 2006.



[1]Mean of 3 site-years in 2006
(Dashed line represents mean tofu hardness for Harovinton standard.)

4.6 Correlations: Seed Characteristics and Soymilk and Tofu Characteristics

There is a general belief by soybean breeders and processors that large-seeded, high-protein soybeans will produce higher yielding and higher quality soymilk and tofu (Bhardwaj *et al.*, 1999). To determine if this theory holds true for Manitoba soybeans, simple correlations were performed to examine the relationship between seed characteristics and protein content and soymilk and tofu characteristics (Table 4.9).

A significant correlation (r = 0.50) was found between the seed size and seed protein. This is in agreement with the generally accepted belief that high-protein soybean seeds are typically larger in size. Seed size and protein were significantly correlated to soymilk yield (r = 0.40, r = 0.71; respectively) and tofu yield (r = 0.56, r = 0.62), which further explains the findings that higher protein soybean varieties were producing higher yields. Positive correlations were also found between seed size and protein content and soymilk colour (r = 0.54, r = 0.42) and tofu colour (r = 0.49, r = 0.68).

The most interesting result found was the lack of a significant correlation between protein content and tofu hardness. This finding is in agreement with Aziadekey *et al.* (2002), but not with Poysa and Woodrow (2002) and Bhardwaj *et al.* (1999) who both found positive correlations between seed protein and tofu hardness (r = 0.59 and r = 0.48, respectively).

These results confirm that large-seeded, high-protein soybeans will result in both higher soymilk and tofu yields higher soymilk and tofu whiteness. However, these results do not indicate that these soybeans will produce firmer tofu. As previously mentioned, the lack of a correlation between seed protein and tofu compression hardness in this study suggests that although Manitoba-grown soybeans are lower in protein quantity, they may contain higher quality protein.

Table 4.9. Simple correlations from observations on eight Manitoba-grown soybean varieties in six site-years.

SEED CHARACTERISTICS	SOYMILK		TOFU		
	Yield	Colour	Yield	Colour	Hardness
Seed Size	0.40**	0.54**	0.56***	0.49**	NS
Protein	0.71***	0.42**	0.62***	0.68***	NS

* *P < 0.01
*** P < 0.0001
NS = Not Significant

5 Conclusions, Limitations and Recommendations for Future Research

5.1 Conclusions

One of the objectives of this study was to adapt a method to determine the quality of soymilk and tofu made from Manitoba-grown food-grade soybeans. This objective was met by adapting the method by Mullin *et al.* (2001) to better suit the equipment and space available for this research. Also, changes such as closer temperature control during the mixing of the coagulant with the soymilk were made to help improve repeatability. The method was then successfully applied to evaluate the effects of site-year and soybean variety on soymilk and tofu quality (Objective 2) and to compare the yield and quality of soymilk and tofu made from Manitoba-grown soybeans with soymilk and tofu made from a commercial sample of Harovinton, a high quality Canadian food-grade soybean variety (Objective 3).

Both site-year and variety main effects were found to significantly affect soybean seed characteristics (protein), soymilk colour, and tofu hardness ($P < 0.0001$). Analysis of the factorial effects of site-year for samples grown in the same sites for both years showed that site was significant for all seed, soymilk and tofu characteristics, while year was significant only for soymilk and tofu characteristics. The site by year interaction was significant for seed protein, soymilk colour, and tofu hardness.

Manitoba soybean varieties had smaller seed size and were typically lower in protein than the commercial Harovinton sample. However, the sucrose levels of the Manitoba soybean varieties grown in 2006 were higher than the commercial Harovinton sample, which is viewed as a desirable characteristic by processors. Overall, the Manitoba-grown soybean varieties were found to have comparable soymilk colour and tofu hardness to the commercial Harovinton sample.

The varieties CL987704, Kaminchis, and Lotus, which were only grown in 2005, were not found to be significantly different from the commercial Harovinton sample for seed protein, soymilk yield and whiteness, and tofu yield, whiteness and hardness. No other varieties tested in 2005 or 2006 compared as well to the commercial Harovinton sample. Although removed from the varietal trials after 2005, the varieties CL987704, Kaminchis, and Lotus did demonstrate that high quality soybeans could be grown in Manitoba and may serve as cross-breeding varieties for future soybean varietal development for Manitoba.

The varieties OT05-20 and OT05-21, grown in the 2006 varietal trials, were of particular interest because they were developed specifically for tofu production. These two varieties along with OAC Prudence performed the best of all Manitoba-grown varieties grown in 2006. The seed size, soymilk whiteness and tofu hardness of these three varieties were not found to be significantly different from the commercial Harovinton sample.

The results from this study indicate that the major limitation of Manitoba-grown soybeans is their inability to produce large soymilk and tofu yields compared to the commercial Harovinton sample. As previously mentioned, soymilk and tofu yield are of economic importance to processors and with the exception of CL987704, Kaminchis, and

Lotus, Manitoba-grown soybeans produced significantly lower soymilk and tofu yields than the commercial Harovinton sample. A correlation between seed protein and soymilk and tofu yield was found in this study, which possibly explains why Manitoba-grown varieties produced lower soymilk and tofu yields than the high-protein commercial Harovinton sample.

The most promising result for the Manitoba-grown soybean varieties was how well they compared to Harovinton in tofu hardness. Wang and Chang (1995) demonstrated that high quality tofu could be made from soybeans with smaller size seeds despite soybean processors preference for larger soybeans. The same result was shown in this study, where smaller size seeds produced tofu with a hardness that was not significantly different from tofu made from larger commercial Harovinton soybeans. This study also found that seed protein content was not significantly correlated to tofu hardness suggesting that Manitoba-grown soybeans may have high protein quality. A further investigation into the composition of the protein found in Manitoba-grown soybeans is needed.

It is evident that in order to improve the soymilk and tofu yield and quality characteristics of soybeans grown in Manitoba it will be necessary to select varieties that contribute optimum seed and end use quality. The results from this study indicate that there is promise for Manitoba to become a significant producer of soybeans. Comparison of the Manitoba-grown soybean varieties with the commercial Harovinton sample demonstrated that high quality food-grade soybeans can be produced in Manitoba, but further varietal development is needed. In particular, it is important to develop varieties with characteristics similar to CL987704, Kaminchis and Lotus, but better suited to Manitoba growing conditions (i.e. adapted to a shorter growing season).

5.2 Limitations and Recommendations for Future Research

A better evaluation of factors affecting soymilk and tofu quality could have been performed had the study been a true variety by environment by crop year study. Ideally the experimental design would have involved the same varieties across the same locations over the two crop years. Despite our inability to have the same locations for both crop years due to flooding at the Carman location in 2006, this design limitation was overcome by grouping growing sites and years into site-years. Doing this allowed for statistical analyses to be performed that provided useful information regarding the effects of growing environment.

The effect of soybean storage conditions on soymilk and tofu quality is not well understood; however, soybean processors believe that the soymilk and tofu quality of soybeans decreases with extended storage periods (more than one year) (Jim Grey, personal communication, July 26, 2006). The time from which the soybeans used in this study were harvested and evaluated was longer in 2005 than in 2006. Although, soybeans were evaluated within a year of being harvested in 2005, they were stored for approximately four months longer than in 2006. This could have resulted in a decrease in the quality of soymilk and tofu produced from these soybeans. Although it is difficult to know whether the extended storage of the 2005 soybeans had an effect on their quality, it should still be considered as a possible limitation. Further research on the effects of storage conditions on soybean quality would help to understand optimal storage conditions as well give insight into compositional changes that occur over time.

Another limitation of this study was that instrumental colour and texture measurements were not correlated to scores from a trained sensory panel. Doing this would help to determine if the differences found were of the magnitude to be perceived by a trained panel. Performing a study to correlate texturometer and colorimeter measurements to trained panelist scores would allow realistic guidelines to be established in determining the quality characteristics of new soybean varieties.

Results from this study suggested that Manitoba-grown soybeans may be high in protein quality. Soybean protein quality is related to the protein subunit composition and is believed to have an effect on soymilk and tofu yield and quality. In order to verify the protein quality of Manitoba-grown soybean varieties it would be necessary to determine the 11S and 7S protein subunit composition and relate it to soymilk and tofu quality and yield.

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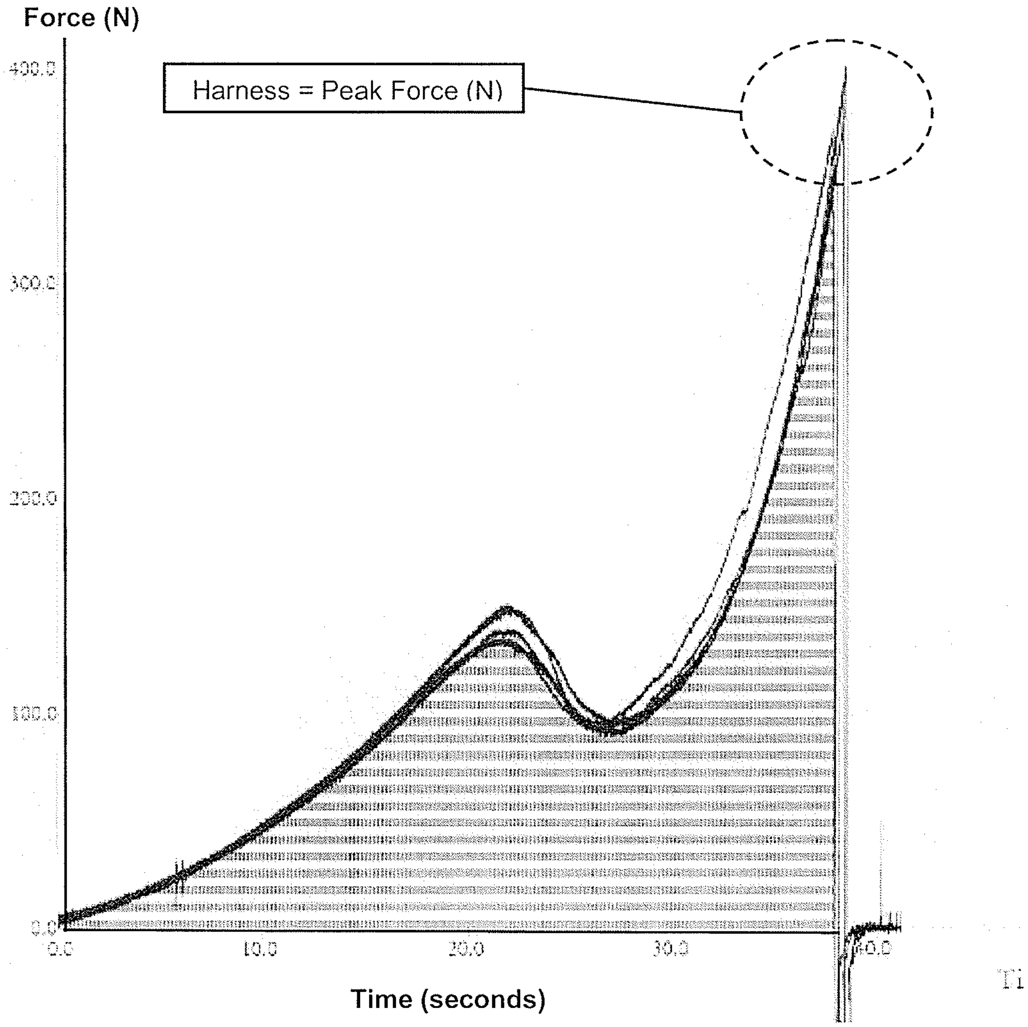
APPENDIX 1

Example of L*a*b* values from soymilk made from Harovinton soybeans.

	Repeated Measure	Rep 1 L*	Rep 1 a*	Rep 1 b*	Rep 2 L*	Rep 2 a*	Rep 2 b*	Rep 3 L*	Rep 3 a*	Rep 3 b*
Harovinton *(Standard)*	*1*	84.60	-0.33	16.96	84.62	-0.41	16.75	84.46	-0.3	16.88
	2	84.57	-0.32	17.03	84.59	-0.42	16.78	84.44	-0.29	16.93
	3	84.54	-0.34	17.09	84.57	-0.36	16.80	84.41	-0.26	16.97
	4	84.51	-0.34	17.14	84.55	-0.39	16.84	84.38	-0.28	17.00
MEAN		**84.56**	**-0.33**	**17.09**	**84.58**	**-0.40**	**16.81**	**84.42**	**-0.28**	**16.97**

APPENDIX 2
A typical peak force curve using a TA-XT2 texture analyzer.



TA-XT2i Settings

Mode: Measure force in compression

Pre-test speed: 2.0 mm/second

Test Speed: 0.2 mm/second

Post-test Speed: 5.0 mm/second

Distance: 80%

Force: Newtons

APPENDIX 3
Results from Statistical Analysis Using Dunnett's Two-tailed t-test

Table 1a. Statistical comparison (α = 0.05) of the seed size of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*[1]Difference of LS Means*	*[2]SE*	*[3]Pr > | t |*
Harovinton	23.2	-	-	-
OAC Prudence	19.5	-3.7	0.7	0.0003
90A01	15.9	-7.3	0.7	<0.0001
90A07	17.5	-5.7	0.7	<0.0001
Jim	16.1	-7.1	0.7	<0.0001
Dolly	18.5	-4.7	0.7	<0.0001
OAC 01-12	18.1	-5.1	0.7	<0.0001
OAC 01-13	19.1	-4.1	0.7	<0.0001
OAC Erin	13.9	-9.3	0.7	<0.0001
CL987704	19.0	-4.2	0.7	<0.0001
Kaminchis	18.8	-4.4	0.7	<0.0001
Lotus	19.5	-3.7	0.7	0.0003

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 1b. Statistical comparison of the seed size of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*[1]Difference of LS Means*	*[2]SE*	*[3]Pr > | t |*
Harovinton	23.3	-	-	-
OAC Prudence	22.4	-0.9	0.9	NS
90A01	16.6	-6.7	1.0	<0.0001
90A07	18.8	-4.4	0.9	0.0009
Jim	18.6	-4.3	0.9	0.0009
Dolly	18.8	-4.7	0.9	0.0005
OAC 01-12	18.5	-4.8	0.9	0.0004
OAC 01-13	16.6	-6.7	0.9	<0.0001
OAC Erin	15.2	-8.1	0.9	<0.0001
OT05-21	24.6	1.4	0.9	NS
OT05-20	21.9	-1.3	0.9	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 2a. Statistical comparison (α = 0.05) of the protein content of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	[1]*Difference of LS Means*	[2]*SE*	[3]*Pr* > | *t* |
Harovinton	46.2	-	-	-
OAC Prudence	40.4	-5.8	0.6	<0.0001
90A01	40.8	-5.4	0.6	<0.0001
90A07	39.4	-6.8	0.6	<0.0001
Jim	39.7	-6.5	0.6	<0.0001
Dolly	40.8	-5.4	0.6	<0.0001
OAC 01-12	39.5	-6.7	0.6	<0.0001
OAC 01-13	40.7	-5.5	0.6	<0.0001
OAC Erin	38.3	-7.9	0.6	<0.0001
CL987704	44.6	-1.6	0.6	NS
Kaminchis	44.9	-1.3	0.6	NS
Lotus	46.5	0.3	0.6	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 2b. Statistical comparison of the protein content of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr* > | *t* |
Harovinton	46.2	-	-	-
OAC Prudence	41.1	-5.1	0.7	<0.0001
90A01	40.7	-5.5	0.8	<0.0001
90A07	39.0	-7.2	0.7	<0.0001
Jim	39.2	-7.0	0.7	<0.0001
Dolly	39.5	-6.7	0.7	<0.0001
OAC 01-12	39.4	-6.8	0.7	<0.0001
OAC 01-13	39.0	-7.2	0.7	<0.0001
OAC Erin	37.8	-8.4	0.7	<0.0001
OT05-21	42.9	-3.3	0.7	0.0009
OT05-20	41.3	-4.9	0.7	<0.0001

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 3a. Statistical comparison (α = 0.05) of the soymilk yield of Manitoba-grown soybeans in 2005 (n=11)to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*[1]Difference of LS Means*	*[2]SE*	*[3]Pr > | t |*
Harovinton	7.2	-	-	-
OAC Prudence	6.0	-1.2	0.2	<0.0001
90A01	6.3	-0.9	0.2	0.0007
90A07	6.1	-1.1	0.2	<0.0001
Jim	5.9	-1.3	0.2	<0.0001
Dolly	6.2	-1.0	0.2	0.0002
OAC 01-12	5.9	-1.3	0.2	<0.0001
OAC 01-13	6.1	-1.1	0.2	<0.0001
OAC Erin	5.7	-1.5	0.2	<0.0001
CL987704	7.0	-0.2	0.2	NS
Kaminchis	7.0	-0.2	0.2	NS
Lotus	7.2	0	0.2	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 3b. Statistical comparison of the soymilk yield of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr > | t |*
Harovinton	7.3	-	-	-
OAC Prudence	6.4	-0.9	0.2	0.0013
90A01	6.0	-1.3	0.2	<0.0001
90A07	6.0	-1.3	0.2	<0.0001
Jim	6.0	-1.3	0.2	<0.0001
Dolly	5.9	-1.4	0.2	<0.0001
OAC 01-12	5.9	-1.4	0.2	<0.0001
OAC 01-13	6.0	-1.3	0.2	<0.0001
OAC Erin	5.8	-1.5	0.2	<0.0001
OT05-21	6.7	-0.6	0.2	0.028
OT05-20	6.3	-1.0	0.2	0.0005

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 4a. Statistical comparison (α = 0.05) of the soymilk whiteness of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	[1]*Difference of LS Means*	[2]*SE*	[3]*Pr* > | *t* |
Harovinton	67.5	-	-	-
OAC Prudence	69.4	1.9	0.3	0.0004
90A01	66.9	-0.6	0.3	NS
90A07	68.4	0.9	0.3	NS
Jim	69.3	1.8	0.3	0.0002
Dolly	69.4	1.9	0.3	<0.0001
OAC 01-12	69.4	1.9	0.3	<0.0001
OAC 01-13	69.8	2.3	0.3	<0.0001
OAC Erin	68.4	0.9	0.3	NS
CL987704	68.1	0.6	0.3	NS
Kaminchis	68.4	0.9	0.3	NS
Lotus	68.3	0.8	0.3	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 4b. Statistical comparison of the soymilk whiteness of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr* > | *t* |
Harovinton	68.9	-	-	-
OAC Prudence	68.5	-0.4	0.7	NS
90A01	66.2	-2.7	0.8	0.0169
90A07	66.9	-2.0	0.7	NS
Jim	67.2	-1.7	0.7	NS
Dolly	67.6	-1.3	0.7	NS
OAC 01-12	67.7	-1.2	0.7	NS
OAC 01-13	67.2	-1.7	0.7	NS
OAC Erin	65.7	-3.2	0.7	0.0014
OT05-21	69.7	0.7	0.7	NS
OT05-20	68.7	-0.2	0.7	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 5a. Statistical comparison (α = 0.05) of the tofu yield of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	[1]*Difference of LS Means*	[2]*SE*	[3]*Pr > | t |*
Harovinton	6.3	-	-	-
OAC Prudence	5.2	-1.1	0.2	0.0008
90A01	5.5	-0.8	0.2	0.0034
90A07	5.4	-0.9	0.2	0.0010
Jim	5.2	-1.1	0.2	<0.0001
Dolly	5.4	-0.9	0.2	0.0014
OAC 01-12	5.2	-1.1	0.2	0.0002
OAC 01-13	5.4	-0.9	0.2	0.0016
OAC Erin	4.9	-1.4	0.2	<0.0001
CL987704	6.2	-0.1	0.2	NS
Kaminchis	6.1	-0.2	0.2	NS
Lotus	6.3	0	0.2	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 5b. Statistical comparison of the tofu yield of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr > | t |*
Harovinton	6.8	-	-	-
OAC Prudence	5.7	-1.1	0.2	0.0003
90A01	5.4	-1.4	0.2	<0.0001
90A07	5.5	-1.3	0.2	<0.0001
Jim	5.3	-1.5	0.2	<0.0001
Dolly	5.3	-1.5	0.2	<0.0001
OAC 01-12	5.4	-1.4	0.2	<0.0001
OAC 01-13	5.3	-1.5	0.2	<0.0001
OAC Erin	5.2	-1.6	0.2	<0.0001
OT05-21	6.1	-0.7	0.2	0.0310
OT05-20	5.7	-1.1	0.2	0.0004

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 6a. Statistical comparison (α = 0.05) of the tofu whiteness of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*[1]Difference of LS Means*	*[2]SE*	*[3]Pr > | t |*
Harovinton	73.3	-	-	-
OAC Prudence	73.1	-0.2	0.5	NS
90A01	71.4	-1.9	0.4	0.0026
90A07	71.2	-2.1	0.4	0.0013
Jim	72.4	-0.9	0.4	NS
Dolly	72.7	-0.6	0.4	NS
OAC 01-12	72.2	-1.1	0.4	NS
OAC 01-13	73.5	0.2	0.4	NS
OAC Erin	71.5	-1.8	0.4	0.0058
CL987704	72.3	-1.0	0.4	NS
Kaminchis	72.3	-1.0	0.4	NS
Lotus	72.7	-0.6	0.4	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 6b. Statistical comparison of the tofu whiteness of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr > | t |*
Harovinton	74.5	-	-	-
OAC Prudence	72.3	-2.2	0.5	0.0018
90A01	70.7	-3.8	0.6	<0.0001
90A07	70.4	-4.1	0.5	<0.0001
Jim	71.2	-3.3	0.5	<0.0001
Dolly	71.4	-3.1	0.5	<0.0001
OAC 01-12	71.8	-2.7	0.5	0.0002
OAC 01-13	69.9	-4.6	0.5	<0.0001
OAC Erin	69.5	-5.0	0.5	<0.0001
OT05-21	72.4	-2.1	0.5	0.0030
OT05-20	70.9	-3.4	0.5	<0.0001

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 3 (Cont'd)

Table 7a. Statistical comparison (α = 0.05) of the tofu hardness (compression force) of Manitoba-grown soybeans in 2005 (n=11) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	[1]*Difference of LS Means*	[2]*SE*	[3]*Pr > | t |*
Harovinton	5.9	-	-	-
OAC Prudence	4.5	-1.4	0.4	0.0286
90A01	5.3	-0.6	0.4	NS
90A07	5.3	-0.6	0.4	NS
Jim	4.1	-1.8	0.4	0.0009
Dolly	5.4	-0.5	0.4	NS
OAC 01-12	4.3	-1.6	0.4	0.0050
OAC 01-13	5.1	-0.8	0.4	NS
OAC Erin	4.8	-1.1	0.4	0.0731
CL987704	6.0	0.1	0.4	NS
Kaminchis	6.2	0.3	0.4	NS
Lotus	6.3	0.5	0.4	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

Table 7b. Statistical comparison (α = 0.05) of the tofu hardness (compression force) of Manitoba-grown soybeans in 2006 (n=10) to a commercial Harovinton sample.

VARIETY	*LS Means Estimate*	*Difference of LS Means*	*SE*	*Pr > | t |*
Harovinton	5.9	-	-	-
OAC Prudence	6.2	0.3	0.4	NS
90A01	5.0	-0.9	0.3	NS
90A07	6.2	0.3	0.3	NS
Jim	5.3	-0.6	0.3	NS
Dolly	5.8	-0.1	0.3	NS
OAC 01-12	4.9	-1.0	0.3	NS
OAC 01-13	5.7	-0.2	0.3	NS
OAC Erin	5.5	-0.4	0.3	NS
OT05-21	5.6	-0.3	0.3	NS
OT05-20	5.4	-0.5	0.3	NS

[1]Difference between LS Mean of Manitoba-grown soybean variety and the LS Mean of the commercial Harovinton sample.
[2]Standard error of the difference of LS Means
[3]The Dunnett's procedure was used to adjust for multiple comparisons.

APPENDIX 4
Protein and Oil Content (% dmb) of Manitoba-Grown Food-Grade Soybeans

	SITE-YEAR											
	Carman05		Morris05		Rosebank05		Morris06		Rosebank06		St. Adolphe06	
VARIETY	Prot	Oil	Prot	Oil	Prot	Oil	Prot	Oil	Prot	Oil	Prot	Oil
OAC Prudence	41.9	19.4	40.7	19.9	39.0	21.0	40.9	21.2	41.4	20.8	40.9	21.1
OAC Erin	38.8	20.3	40.5	19.5	36.0	21.8	36.3	23.0	39.7	21.7	37.3	23.1
OAC 01-13	42.6	19.2	41.0	20.3	38.8	21.0	39.7	21.6	39.3	21.9	38.1	22.7
OAC 01-12	40.4	19.9	40.3	20.0	38.1	21.6	39.7	22.0	39.3	22.3	39.3	22.5
Lotus*	48.3	17.5	47.4	17.6	44.1	18.9	-	-	-	-	-	-
Kaminchis*	46.4	17.6	46.1	18.2	42.4	18.7	-	-	-	-	-	-
Jim	40.3	19.1	40.9	19.1	38.1	20.4	38.2	21.9	39.1	21.5	40.2	21.2
Dolly	42.0	19.4	41.3	20.1	39.4	21.1	39.9	21.9	40.2	21.8	38.5	22.9
CL987704*	46.2	17.4	45.3	18.0	42.5	19.3	-	-	-	-	-	-
90A07	40.3	20.6	40.9	21.0	37.3	22.2	38.7	23.0	38.3	23.5	40.0	22.4
90A01[1]	41.3	20.1	41.6	20.3	39.7	21.0	40.6	22.7	-	-	40.7	22.8
OT05-21**	-	-	-	-	-	-	41.9	20.4	43.4	19.9	43.2	20.0
OT05-20**	-	-	-	-	-	-	41.6	19.8	41.4	20.3	41.0	20.3

[1]Not grown at Rosebank06.
*Grown in 2005 only.
**Grown in 2006 only.