DATA ANALYSIS OF THE CORRELATION BETWEEN PROCESSING VARIABLES AND CONCENTRATIONS OF ISOFLAVONES IN SOYMILK

by

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Abstract

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Data analysis of the correlation between processing variables and concentrations of isoflavones in soymilk

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Soybeans and soyfoods are rich in isoflavones. The importance of isoflavones is widely appreciated and is the subject of intense research and discussions. The most common isoflavones are genistin, genistein, daidzin, and daidzein. Genistein and daidzein are the non-glucoside forms of genistin and daidzin, respectively. The chemical structures of these non-glucoside forms are very similar to the human estrogen. In soybeans the concentrations of the glucoside forms of isoflavones are relatively higher than the corresponding non-glucoside compounds. When soyfoods are made, processing procedures can affect the concentrations of the isoflavones.

The main objective of this study is to determine the effects of processing variables on concentrations of genistin, daidzin, genistein and daidzein in soymilk. The variables that were considered are type and pH of soaking solution, soaking temperature, grinding temperature, and addition of glucosidase enzyme.

The three soaking solutions are water, hydrochloric acid (HCl), and acetic acid ($HC_2H_3O_2$) solutions. The three soaking temperatures used are 5 °C, 21 °C, and 55 °C. The pH of HCl and $HC_2H_3O_2$ solutions are 2, 4, and 6. The two grinding temperatures used are 21 °C and 80 °C.

High-Performance-Liquid-Chromatography (HPLC) was used to determine isoflavone contents of soymilk samples. The data were analyzed using a statistical package called Statistical Product and Service Solutions (SPSS). Statistical analysis was conducted to evaluate correlations between processing variables and the concentrations of isoflavones in soymilk. In addition, pair-sample t-test was conducted to evaluate whether or not there are differences in the means of isoflavone concentrations in soymilk treated with and without glucosidase enzyme.

The major findings of this study are: the pH of $HC_2H_3O_2$ solution significantly affected the yield of genistein ($p < 0.05$). A negative correlation was found between soaking temperature and the concentrations of genistin and daidzein in soymilk made from beans that were soaked in water ($p < 0.05$). There were strong correlations between grinding temperature and the concentrations of genistein, daidzin, daidzein ($p < 0.01$), and genistin ($p < 0.05$) in soymilk made from beans that were soaked in HCl solution. The results of pair-sample t-test showed that the addition of glucosidase enzyme significantly increased the yield of the four isoflavones in soymilk.

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CHAPTER ONE

INTRODUCTION

Public awareness and interest in healthy living and good nutrition have increase significantly in the past 20 years. From 1975 to 1985, there was about a threefold increase in awareness of the relation between salt and high blood pressure, and public knowledge of the link between saturated fat and heart disease has doubled (McGinnis & Ernst, 2001). In 1999, the Food and Drug Administration (FDA) approved a health claim for the cholesterol-lowering properties of soy protein (FDA, 1999). The health claim is that, diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease (FDA, 1999). A year later, the American Heart Association (AHA) revised dietary guidelines (Krauss et al., 2000). The revised dietary guidelines provide population-wide recommendations for cardiovascular disease prevention and treatment that are supported by decades of research (2000). The recommendations are based on an overall balanced and nutritious dietary pattern.

Even though increasing public awareness of the importance of good nutrition through governmental founded programs and technologically advanced medical care, significant health problems still exist in the United States. According to the World Health Organization's compilation of cancer data from 45 countries around the world collected from 1994 to 1997, the incidence of cancer in females in the United States was ranked 7th in the world, whereas China was ranked 37th and Japan was ranked 42nd. The United States was ranked 14th in female breast cancer incidence, whereas Japan was ranked 43rd and China was ranked 44th. In 2001, data show that 700,142 Americans died of heart diseases and 553,768 Americans died of cancers (Cancer statistics, 2004). Currently, the

top five causes of death in United Stated are heart disease, cancer, cerebrovascular disease, chronic lung diseases, and accidents (McGinnis & Ernst, 2001). Almost 70% of all deaths in United States are from heart disease, cancer, and stroke (2001). McGinnis and Foege reported that 20 - 33% of cardiovascular deaths, 20 - 60% of fatal cancers, and 50 - 80% of diabetes mellitus cases are associated with dietary factors or sedentary lifestyles (McGinnis & Foege, 1993). Over the past years, increasing evidence suggests that dietary consumption of fruits and vegetables may help reduce the incidence of several chronic diseases (Fournier et al, 2001). Soybeans, in particular, have been recognized as having a potential role in the prevention and treatment of osteoporosis, cardiovascular disease, and hormone-dependent cancers (Miller, 1990).

Diets high in animal fat and red meat are associated with an increased risk of cancer and other chronic diseases (Miller et al. 1994). Differences in the incidence of cancer in Asian and American cultures may be due to fundamental differences in diet (Garlock, 2000). Americans consume a typical Western diet consisting of high-fat foods while those in Asian cultures typically consume a low-fat diet (Garlock, 2000). Consumption of soy products such as tofu, miso, and tempeh is common in Asian cultures (Garlock, 2000). According to Fournier et al. (2001), countries with high daily intakes of soy products have the lowest incidence rates of hormone-dependent cancers. Figure 1 shows daily soy product consumption by country (Fournier et al., 2001).

With the increased emphasis on healthy diets, soy consumption in United States has changed. This change was presented by increased sales of soy products in the United States. According to FDA data, U.S. retail sales of soyfoods were $852 million in 1992

and were projected to rise to $3.714 billion in 2002 (Henkel, 2000). Figure 2 shows retail sales of soyfoods in the United States (Henkel, 2000).

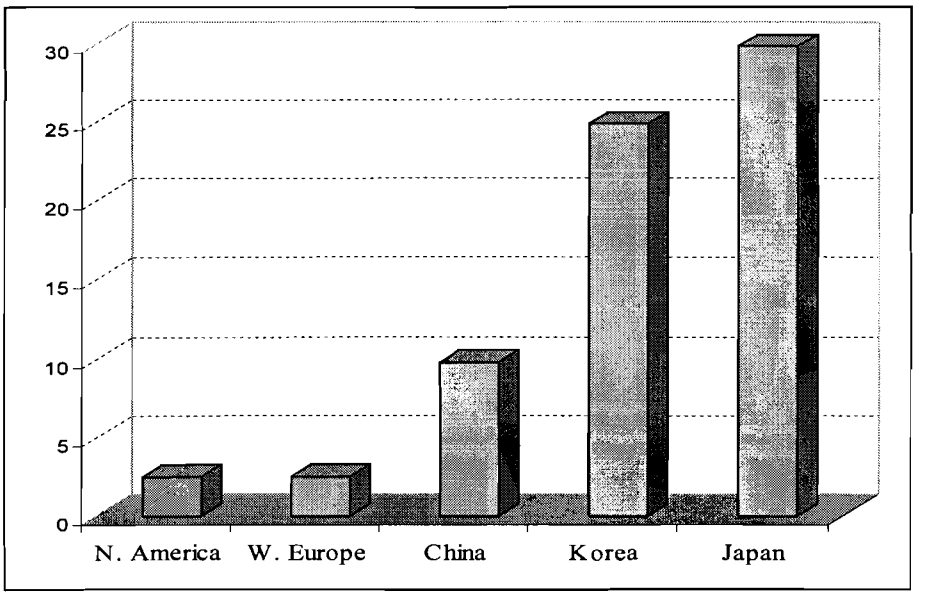


Figure 1. Daily soy product consumption in g/day by country

Source: Fournier et al., 2001

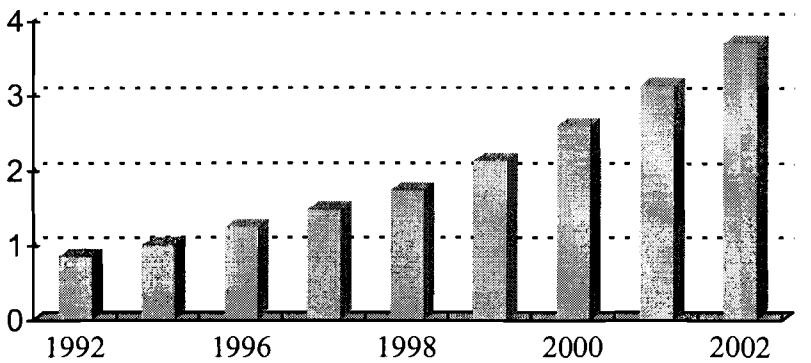


Figure 2. U.S. retail sales of soyfoods in billion dollars from 1992 to 2002

Source: Henkel, 2000

Soybeans contain a group of compounds called isoflavones. Isoflavones are categorized as phytoestrogens (Knight & Eden, 1994). Phytoestrogens occur naturally in many plants and have structural and functional similarities to the human estrogen, 17β - estradiol (1994). Increasing evidence has indicated that isoflavones in soybeans have potential benefits on human health, particularly to several chronic diseases such as osteoporosis, cardiovascular disease, breast cancer, and prostate cancer (Potter et al., 1998; Alekel et al., 2000; Nagata et al., 1998; Tonstad et al., 2002; Dai et al., 2001; Kolonel et al., 2000; & Lee et al., 2003).

The most common isoflavones are genistin, genistein, daidzin, and daidzein. Genistein and daidzein are the non-glucoside forms of genistin and daidzin, respectively. The concentrations of the glucoside forms of isoflavones are relatively higher than the corresponding non-glucoside compounds in soybeans. The effects of soybean processing techniques on the contents of isoflavones have been investigated (Wang & Murphy, 1996; Grun et al., 2001; & Eisen et al., 2003). According to Wang and Murphy (1996), soaking and heat processing in tempeh production significantly reduces the isoflavones by 12% and 49%, respectively. In tofu production the process of coagulation causes 44% loss of isoflavones. The alkaline extraction process causes 53% loss of isoflavones in soy protein.

The objectives of this study are, to investigate the effects of type and pH of soaking solution, soaking temperature, grinding temperature, and addition of glucosidase enzyme on concentrations of isoflavones in producing soymilk. The three soaking solutions are water, hydrochloric acid (HCl) solution, and acetic acid ($HC_2H_3O_2$) solution. The pH of HCl and $HC_2H_3O_2$ solutions are adjusted at three different levels of 2, 4, and 6.

The three soaking temperatures are 5 °C, 21 °C, and 55 °C. The two grinding temperatures are 21 °C and 80 °C.

Specific Aims

This research study is to perform statistical analysis on the data generated in Dr. Ana M.Q. VandeLinde's laboratory. There are four research questions that this study attempts to answer:

A. Is there any significant correlation between pH of HCl and $HC_2H_3O_2$ solutions and concentrations of isoflavones in soymilk?

B. Is there any significant correlation between soaking temperature and concentrations of isoflavones in soymilk made from soybeans that are soaked in water, HCl, and $HC_2H_3O_2$ solutions?

C. Is there any significant correlation between grinding temperature and concentrations of isoflavones in soymilk made from beans that are soaked in water, HCl, and $HC_2H_3O_2$ solutions?

D. How does the addition of glucosidase enzyme affect the concentrations of isoflavones during the production of soymilk?

CHAPTER TWO

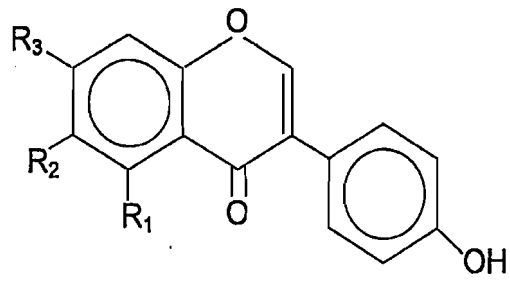
LITERATURE REVIEW

This chapter begins with a discussion of the different forms of isoflavones, their consumption, absorption, and metabolism. Studies of the effects of soy isoflavones on human health are then discussed with emphasis on certain selected chronic diseases including osteoporosis, cardiovascular disease, breast cancer, and prostate cancer.

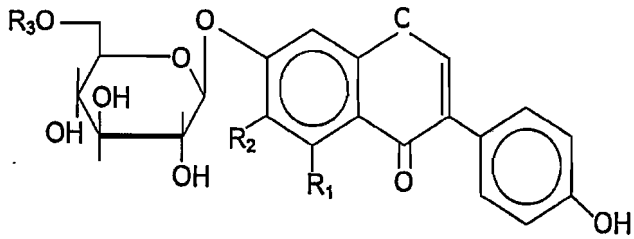
Background on Soy Isoflavones

Isoflavones are groups of compounds found in a variety of grains, legumes, and vegetables. In particular, legumes such as soybeans provide the most abundant source of isoflavones (Munro et al., 2003). Soy isoflavones are considered as phytoestrogens. Phytoestrogens are plant-derived estrogens that may have estrogenic or anti-estrogenic effects. These phytoestrogens offer potential alternative therapies for a range of hormone-dependent diseases including breast cancer, prostate cancer, osteoporosis, and cardiovascular diseases (Setchell & Cassidy, 1999).

Isoflavones exist in two forms. One form contains glucose and the other form called aglycones does not contain glucose. The glycone form of the isoflavones is biologically active. The three most common isoflavone aglycones in soybeans are genistein, daidzein, and glycitein. These are the aglycone of genistin, daidzin, and glycitin, respectively. In soybean, the glycoside forms are present in higher concentration than the aglycone forms (Naim et al., 1974). When the glycosides are esterified with either malonyl or acetic acid, 12 different isoflavones are formed. Figure 3 shows the chemical structures of these isoflavones (Messina, M.J. & Messina, V., 2003).



Isoflavone Aglycones	R_1	R_2	R_3
Daidzein	H	H	OH
Genistein	OH	H	OH
Glycitein	H	OCH_3	OH



Isoflavone glycosides	R_1	R_2	R_3
Daidzin	H	H	OH
Malonyl daidzin	H	H	$COCH_2COOH$
Acetyl daidzin	H	H	$COCH_3$
Genistin	OH	H	OH
Malonyl genistin	OH	H	$COCH_2COOH$
Acetyl genistin	OH	H	$COCH_3$
Glycitin	H	OCH_3	H
Malonyl glycitin	H	OCH_3	$COCH_2COOH$
Acetyl glycitin	H	OCH_3	$COCH_3$

Figure 3. Chemical structures of common isoflavones.

Source: Messina, M.J. & Messina, V., 2003

Of all the plants investigated, soybeans contain the highest concentration of isoflavones. The concentration of isoflavones in soybeans varies with the variety of soybeans, geographic location, soil type, crop year, and environmental growing conditions (Wang & Murphy, 1994). In addition, processing of soybeans either at home or within the food industry affects the concentration of isoflavones in soy products (Reinli & Block, 1996). Zhou and Erdman (1997) indicated that processing of soybeans for the manufacturing soy-containing food products increases hydrolysis of isoflavone glucosides, resulting in higher concentrations of the aglycones.

Products Containing Soybeans

In general, soyfoods can be divided into two categories: fermented soyfoods and non-fermented soyfoods. Fermented soyfoods include miso, tempeh, shoyu or soy sauce, and natto; soymilk and tofu are non-fermented soyfoods. According to Hutchins and colleagues (1995), the fermented soyfoods contain proportionally higher concentrations of aglycone forms of isoflavones because fermentation partially hydrolyzes isoflavone glucosides.

There are five commonly consumed soyfoods in America (Munro et al., 2003). The most commonly consumed soyfood is soymilk. Soymilk can be processed from whole soybeans or full-fat soy flour (Smith, 1978). Traditionally soymilk is made from good quality whole soybeans. The beans are thoroughly washed and soaked in water for several hours depending on the temperature of the water. The beans are then ground with the addition of enough water to give the desired slurry. The slurry is heated at near boiling for 15 to 20 minutes. The warm slurry is then filtered to remove the insoluble residue. The milky liquid is soymilk. Soymilk can adapt to any recipe. The plain, less sweet soymilk works best for sauces, gravies, and soups. One cup of plain-unfortified soymilk contains 80 calories and seven grams of protein. One cup of fortified soymilk contains 130 calories and 10 grams of protein (Soyfood facts, n.d.). The nutrition facts of soymilk are listed in Table 1 (Soyfood facts, n.d.).

Tofu is the second common consumed soyfood in America (Munro et al., 2003). Tofu is made by precipitating the protein called curd from soymilk. Coagulants such as calcium sulfate are used to form the curd. The curd then is pressed to release amber liquid called whey.

Table 1

Nutrition facts of soymilk, tofu, miso, and tempeh

	Unfortified soymilk (1cup/serving)	Fortified soymilk (1 cup/serving)	Tofu (½ cup/serving)	Miso (1 Tbs/serving)	Tempeh (½cup/serving)
Calories	80	130	97	35	165
Total fat (g)	4	4	6	1	6
Saturated fat (g)	0.5	0.5	0.8	0.5	1
Total carbohydrates (g)	4	13	4	5	14
Protein (g)	7	10	10	2	16
Cholesterol (mg)	0	0	10	0	10
Sodium (mg)	39	105	10	626	5
Dietary fiber (g)	3	0	0	1	7
Calcium (mg)	10	200	204	11	77
Potassium (mg)	345	440	222	28	305
Phosphorus (mg)	129	150	185	26	171
Folate (mcg)	4	4	42	6	43

Source: www. soyfoods.org/facts/facts.html (Retrieved November 12, 2004)

The firmness of tofu changes depending on the type of coagulants, the amount of coagulants, and the amount of whey removed (Tofu, n.d.). Half a cup of tofu contains 97 calories and 10 grams of protein (Table 1).

Miso is made from soybeans, salt, water, and *koji*. *Koji* is a microorganism that are used to trigger fermentation. Rice or barley can also be used to make miso. The common use of miso is to enhance the flavor of soups, dips, and dressings. The color and the flavor of miso vary wisely depending on the proportion of salt added to soybeans, the addition of rice or barley, and the length of fermentation (Miso, n.d.). For example, the higher the ratio of rice to soybeans, the lighter the color of miso and the sweeter the miso. The longer the fermentation period, the better the flavor of miso (Smith, 1978). One table spoon of miso contains 35 calories and two grams of protein (Table 1).

Tempeh is a very popular Indonesian soyfood that undergoes a sequence of soaking, cooking, inoculation, and fermentation of soybeans (Garlok, 2000). *Rhizopus oligosporus* is the mold responsible for tempeh fermentation (2000). During incubation, *Rhizopus oligosporus* produces a white mycelium throughout the soy grits, resulting in a formed, unified, white tempeh cake (2000). Tempeh is also a good source of dietary fiber and soy protein. Half a cup of tempeh contains 165 calories, 16 grams of protein, and seven grams of dietary fiber (Table 1).

In 1999, researchers at the US Department of Agriculture (USDA) and Iowa State University have created an online database that lists the isoflavone content of foods (USDA, 2002). Table 2 summarizes the isoflavone content of selected soyfoods (Messina, M.J. & Messina, V., 2003).

Table 2

Isoflavone, protein, and energy content of selected soyfoods

Food (USDA* No.)	Isoflavones (μg/g)	Protein/100g	Kcal/100g	Isoflavone/Protein (mg/g)
Tofu, firm (16126)	247	8.0	77	3.1
Tofu, regular (16427)	236	8.1	76	2.9
Silken, firm (16162)	279	6.9	62	4.0
Natto (16113)	589	17.7	212	3.3
Soymilk (16120)	97	2.8	33	3.5
Miso (16112)	426	11.8	206	3.6
Tempeh (16114)	435	18.5	193	2.3
Soynuts, dry roasted (16111)	1284	39.6	450	3.2
Soyflour, defatted (16117)	1312	47.0	329	2.8
Soyflour, full fat (16115)	1779	34.5	436	5.2
Soybeans, raw (16108)	1284	36.5	416	3.5
Soybeans, cooked (16109)	547	16.6	173	3.3

Source: Messina, M.J. & Messina, V., 2003

Absorption and Metabolism of Isoflavones

The chemical form of isoflavones and their metabolites may influence the extent of their intestinal absorption. Research indicates that the aglycone forms of isoflavones are more readily absorbed and more bioavailable than its glycoside forms (Setchell, 2000; Izumi et al., 2000). It has also been reported that intestinal microflora plays a key role in the metabolism and bioavailability of isoflavones (Borriello et al., 1985; Setchell et al., 1984).

After ingestion, soy isoflavones are hydrolyzed in the large intestine by intestinal glycosidases, resulting in the removal of the sugar moiety to release their aglycones, daidzein, genistein, and glycitein (Izumi et al., 2000). Following absorption of the aglycones, these compounds and their metabolites are readily conjugated in the liver with glucuronic acid and/or sulfate, and then circulated enterohepatically with potential metabolism and reabsorption in the intestine. Conjugated aglycones of isoflavones are excreted predominantly in the urine (Munro et al., 2003). Any isoflavones that are not absorbed in the intestine are excreted in an unconjugated form in feces (Adlercreutz et al., 1995). Daidzein may be further metabolized by resident microflora in the gastrointestinal tract to equol and *O*-desmethylangolensin. Similarly, genistein may be metabolized to 6'-hydroxy- *O*-desmethylangolensin (Munro et al., 2003). Figure 4 and Figure 5 show the metabolism of daidzein and genistein, respectively (2003).

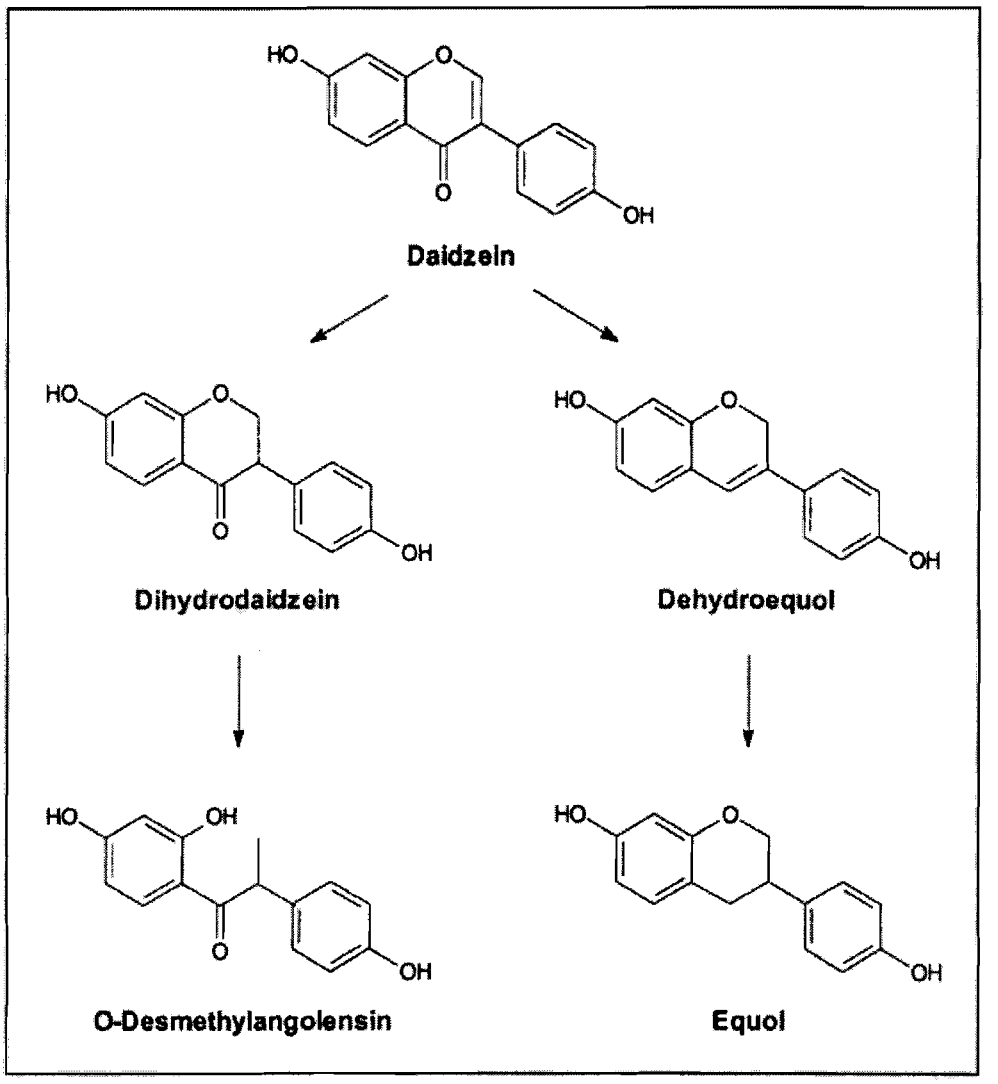


Figure 4. The metabolism of daidzein.

Source: Munro et al., 2003

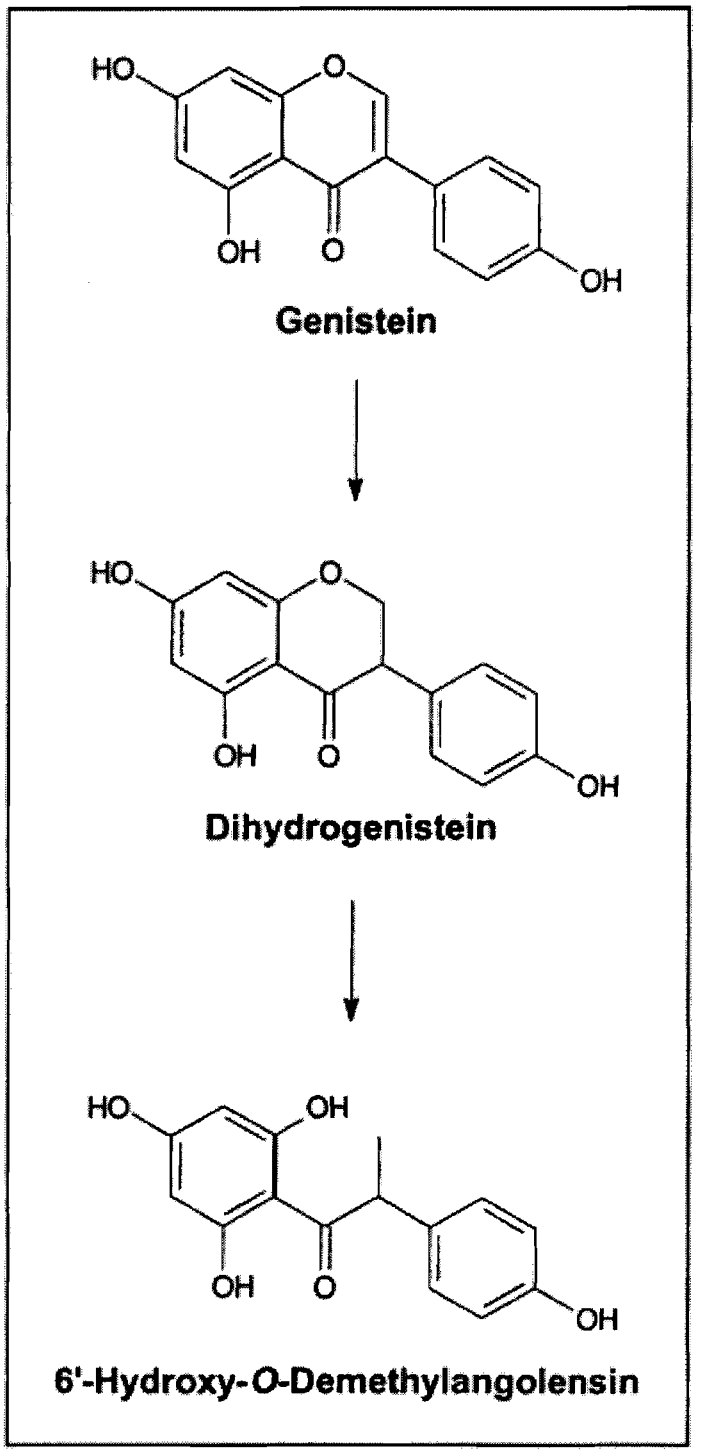


Figure 5. The metabolism of genistein.

Source: Munro et al., 2003

The absorption, distribution, and excretion (ADE) of soy isoflavones may be influenced by an individual's age, gender, and cultural group. Interindividual variability has been showed in some studies (Xu et al., 1995; Morton et al. 1997; Xu et al., 2000). Lu and Anderson (1998) investigated the ADE of isoflavones between males and females and found inconsistent results. Hendrich et al. (1999) suggested that metabolic diffferences may be attributed to differences in the subpopulation's microflora, intestinal transit time, pH, or redoxpotential in the intestines of research subjects. Other factors such as diet, surgery, medication, bowel disease, and host immunity may also influence isoflavone metabolism (Knight & Eden, 1995).

Effects of Isoflavones on Human Health

Bone Health and Osteoporosis

The development of osteoporosis is a major concern for postmenopausal women. The decreased availability of estrogen among postmenopausal women are believed to be a cause of accelerated bone loss and the increased susceptibility to develop osteoporosis (Goldwyn et al., 2000). Hormone replacement therapy is the treatment of choice for postmenopausal women suffering from a reduction in bone mass (Goldwyn et al. 2000). Although the mechanisms are not entirely clear, it has been proposed that estrogen (a) reduces the sensitivity of bone tissue to the resorptive effects of parathyroid hormone; (b) blocks the release of interleukin-1, a potent bone resorption agent; and/or (c) directly modulates osteoblast activity (Kurzer & Xu, 1997).

Ipriflavone is a synthetic isoflavone lacking estrogenic activity but is strikingly similar in chemical structure to daidzein and genistein (Setchell & Cassidy, 1999). Ipriflavone is approved as an alternative to hormone replacement therapy for preventing

bone loss in estrogen-deficient conditions (Setchell & Cassidy, 1999). Unfortunately, hormone replacement therapy may have long-term negative effects that include an increased risk of breast cancer and of developing thromboembolisms (Goldwyn et al., 2000). This led researchers to investigate the effects of isoflavones on preventing bone-related diseases such as osteoporosis.

In 1998, Potter et al. investigated the effects of soy isoflavones on bone mineral density (BMD) and bone mineral content (BMC) in the lumbar spine among 66 postmenpausal women ages 41 to 83 years. All the subjects were randomly assigned to three dietary groups: the control group had a diet with 40 grams of protein obtained from casein and nonfat dry milk daily; the ISP56 group had a diet with 40 grams of isolated soy protein containing 56 milligrams of isoflavones daily; and the ISP90 group had a diet with 40 grams of isolated soy protein containing 90 milligrams of isoflavones daily. Potter and colleagues (1998) reported that after six months of treatment, the bone mineral density and content in the lumbar spine in the subjects of ISP90 group increased significantly compared with the subjects belonging to the control group. They also found that the ratio of total cholesterol to high density lipoprotein (HDL) cholesterol was improved with respect to cardiovascular risk in the subjects of both ISP56 and ISP90 groups. The results from this study indicated that soy protein at both isoflavone concentrations was effective in modulating the risks of both cardiovascular disease and osteoporosis in postmenopausal women (1998).

In another similarly designed study, Alekel et al. (2000) examined the effect of soy protein with isoflavones on BMD and BMC in lumbar spine among 69 perimenopausal women. All the subjects were randomly assigned to three dietary groups.

The control group had a diet containing 40 grams of whey protein daily, the SP+ group had a diet with soy protein containing 80.4 milligrams of isoflavones daily, and the SP- group had a diet with soy protein containing 4.4 milligrams of isoflavones daily. The results of this study found that the SP+ diet had a significantly positive effect on both BMD and BMC in lumbar spine, compared with both SP- and control groups. There was no effect on spinal loss in the subjects of both SP- and control groups. This study suggested that soy products containing isoflavones could serve as an alternative or adjunct treatment for women at risk of osteoporosis, especially for women who are poor candidate for hormone replacement therapy.

Two long-term studies have been conducted on the effects of isoflavones in postmenopausal women (Vitolins et al., 2002; Lydeking-Olsen et al., 2002). Both studies were conducted for a period of two years. In the study of Vitolins et al. (2002), the base diet supplied 25 g of soy protein daily as recommended by the Food and Drug Administration (FDA, 1999). The 25 g of soy protein base diet was supplemented with three different levels of isoflavones: 5 mg/day, 42 mg/day, and 58 mg/day. The results showed that there was no difference on the total body BMD in total body among the three groups. By contrast, Lydeking-Olsen et al. (2002) found that the BMD and the BMC in lumbar spine increased 1.1% and 2.2% respectively in subjects who consumed 500 mL soymilk supplemented with 85 mg of isoflavones per day. For the subjects of another group who consumed the same amount of soymilk with less than 1 mg isoflavone per day, the BMD and BMC in lumbar spine decreased by 4.2% and 4.3%, respectively. The results from both studies revealed somewhat conflicting data with regard to the effect of isoflavones (Setchell & Lydeking-Olsen, 2003). Setchell and Lydeking-Olsen (2003)

indicated that more long-term studies are needed before definitive conclusions can be reached regarding the effectiveness of phytoestrogens on bone health.

Cardiovascular Diseases

Cardiovascular disease is a broad term used to describe many different conditions of the heart. Preventing or reducing the increase in serum cholesterol is associated with a decreased risk for cardiovascular disease (Setchell & Cassisy, 1999). In 1999, the U.S. Food and Drug Administration approved a health claim that diets low in saturated fat and cholesterol (that include 25 grams of soy protein a day) may reduce the risk of heart disease (FDA, 1999).

The exact mechanism underlying the hypocholesterolemic effect of soy protein remains intangible and is almost certain to be multifactorial (Setchell & Cassisy, 1999). Consumption of soy products has the effect of increasing fecal bile acid excretion and altering the rate of synthesis of bile acids, one of the primary mechanisms responsible for the regulation of cholesterol homeostasis (1999). Moreover, lipoprotein metabolism is influenced by estrogenic hormones (1999). Soy isoflavones may influence lipoprotein metabolism by upregulating low density lipoprotein (LDL) receptor activity (1999).

Nagata et al. (1998) reported a relationship between soy product intake and serum total cholesterol concentration in 1,242 men and 3,596 women in Japan. They reported a strong inverse relationship between serum cholesterol and daily intake of soy products in men and women after controlling for age, smoking status and intake of total energy, total protein and total fat.

In another study, Jenkins et al. (2002) reported the effects of three diets on several parameters associated with cardiovascular health. Thirty seven healthy hyperlipidemic

men and 36 postmenopausal women participated in this study. The parameters that they observed were total cholesterol, estimated coronary artery disease (CAD) risk, ratio of total cholesterol to HDL cholesterol, and ratio of LDL cholesterol to HDL cholesterol. Each participant was assigned to three different groups. The control group was given daily a low fat dairy diet. The second group was given daily a high isoflavone soyfood diet containing 50 g soy protein. This diet contains 73 mg isoflavones. The third group was given daily a low isoflavone soyfood diet containing 52 g soy protein which has 10 mg isoflavones. No significant differences were found among participants in the high-and low-isoflavone soy diets. Compared with the control group, however, both soy groups resulted in significantly lower total cholesterol, estimated CAD risk, ratio of total cholesterol to HDL cholesterol, and ratio of LDL to HDL cholesterol. The results suggested that substitution of soyfoods, regardless of isoflavone concentration, may improve many lipid and non-lipid risk factors for CAD and thus justify the use of soyfoods as part of a dietary strategy to reduce CAD risk.

Tonstad et al. (2002) studied the effect of soy protein on serum lipid, lipoprotein, and homocysteine concentrations in 130 men and women who had initial LDL cholesterol concentrations greater than or equal to 4 mmol/L. Participants consumed either 30 grams or 50 grams of isolated soy protein daily as a beverage for 16 weeks. Equivalent grams of casein were also given to the participants in the control groups. Their results showed that in both soy protein groups, LDL cholesterol concentrations were significantly reduced without increasing lipoprotein concentrations when compared with both control groups.

Breast Cancer

Interest in research on the relationship between soy intake and risk of breast cancer was stimulated by the observation that relatively low breast cancer mortality rates are observed in women living in Asian countries where soyfoods are commonly consumed (Messina, 1999). In Japan for example, the breast cancer mortality rate is only about one-quarter of that in the United States (1999). Almost a decade earlier Barnes et al. (1990) reported potential antiestrogenic effects of soy isoflavones in mice. Their study showed that the soy isoflavones reduced the number of 7, 12-dimethylbenz (a) anthracene-induced mammary tumors. These early observations provided the basis for the hypothesis that soy intake decreases breast cancer risk. Since then numerous epidemiologic studies have examined the relation between soy intake and breast cancer risk (Greenstein et al., 1996; Dai et al., 2001; Shu et al., 2001; Wu et al., 2002; Keinan-Boker et al., 2004).

The results of the Iowa Women's Study involving 34,000 women conducted by Greenstein et al. (1996) found that, after eight years of follow up, tofu intake was associated with a modest decrease in breast cancer risk among postmenopausal women. The results, however, were not statistically significant.

Dai and colleagues (2001) found an association between soyfood intake and breast cancer risk in a population-based case control study of Chinese women in Shanghai, China. This study used a food frequency questionnaire to obtain information. The results suggested that regular soyfood intake, particularly very high intake of

soyfoods, may be associated with a reduced risk of breast cancer, especially among those individuals testing positive for the presence of both the estrogen receptor and the progesterone receptor (2001).

Results from two case control studies, one of Chinese women (Shu et al., 2001) and the other of Asian-American women (Wu et al., 2002) showed that women with higher intakes of soy isoflavones from soyfoods during their teen age years were significantly less likely to develop breast cancer later in life than those consuming diets lower in soy isoflavones.

The inverse relationship between soy isoflavones and risk of breast cancer in women is not supported by all studies, however. The results of a recent large population study of Dutch women aged 49 to 70 years old conducted by Keinan-Boker et al. (2004) found that a high intake of isoflavones or mammalian lignans was not significantly related to breast cancer risk. According to the report of Messina & Loprinzi (2001), the research results attempting to show an association between soyfood intake and breast cancer risk are inconsistent, more epidemiologic studies may need to be considered.

Prostate Cancer

Prostate cancer is a hormone dependent cancer. This cancer is the second most common cancer in the United States (Goldwyn et al., 2000) and the sixth most common cause of cancer death (Messina, 2003). Prostate cancer mortality rates vary noticeably among countries. For example, prostate cancer mortality rates are three times higher in developed countries than in developing countries. Mortality rates are known to be especially low in Asia (2003). Biological mechanisms have been proposed that soy isoflavones have preventative roles in the development of prostate cancer that involves

inhibition of angiogenesis, inhibition or stimulation of regulatory proteins in the cell cycle, and inhibition of a signal transduction pathway involving epidermal growth factor (Lee, et al., 2003).

Results from three recent case-controlled studies using food frequency questionnaires to investigate the relationship between consumption of soy products and the incidence of developing prostate cancer have been equivocal because some results lack statistical significance (Strom, et al., 1999; Kolonel, et al. 2000; Lee, et al. 2003). In a study by Strom et al. (1999), 83 cases and 107 controls were examined. Cases involved patients treated at the University of Texas M.D. Anderson Cancer Center for prostate cancer diagnosed between January 1996 and February 1998. The controls were frequency matched to cases on age and race. The principal findings showed there were inverse relationships between genistein, daidzein and prostate cancer risks. The results of this study suggested that genistein and daidzein may reduce prostate cancer risks.

Lee and colleagues (2003) observed the effect of soyfood consumption and two isoflavones (genistein and daidzein) on the risks of prostate cancer in 133 cases and 265 age- and residential community-matched controls in China. Their results indicated reduced risks of prostate cancer associated with consumption of soyfoods and isoflavones. Interestingly, significant relationship was found in the subjects who consumed soyfoods and the isoflavone daidzein.

In another multicenter case-control study by Kolonel et al. (2000), the relationship between vegetables, fruits, legumes and decreased risks of prostate cancer was examined among 1,619 cases and 1,618 controls involving African-American, White, Japanese, and Chinese men. Controls were frequency-matched to cases on the basis of ethnicity, age,

and region of residence in a ratio of approximately 1:1. Some interesting findings were found in this study. First, there was little epidemiological evidence for a protective effect of fruits and tomatoes against prostate cancer. Second, the intake of yellow-orange vegetables such as carrots was inversely related to the risks of prostate cancer. Last, for all legumes including soyfoods, there was an inverse relationship between legumes consumption and the risks of prostate cancer with statistical significance in the African-American and Chinese men. Overall, these results suggest that legumes and certain types of vegetables may protect against development of prostate cancer.

CHAPTER THREE

METHODOLOGY

This chapter includes information about the materials and reagents, the procedure for sample preparation, and the instrument used to generate data that are utilized in this study. The experimental data were provided by Dr. Ana M.Q. VandeLinde at the University of Wisconsin-Stout. The emphasis of this study is on the statistical analyses addressed of the data to address the research questions identified in Chapter one.

Materials and Reagents

All of the solvents, reagents, and isoflavones standards were purchased from either Sigma Chemical Co. (St. Louis, Mo) or Indofine Chemicals Inc. (Somerville, NJ). Milli-Q water, water filtered with a micropore filtering system, was used for the preparation of solvents and samples used in High-Performance-Liquid-Chromatograph (HPLC) analytical procedures. Soybeans were purchased from local supermarket.

Soymilk Preparation

A flow diagram for producing soymilk samples is shown in Figure 6. In the soaking process, soybeans were soaked until they weighed about 2.2 times the original weights at three different soaking temperatures and under three different acidic conditions. The three soaking temperatures used were 5 °C, 21 °C, and 55 °C. The three soaking solutions were water, hydrochloric acid (HCl) solution, and acetic acid ($HC_2H_3O_2$) solution. The pH of HCl and $HC_2H_3O_2$ solutions were adjusted at three different levels of 2, 4, and 6. To make the slurry, water was added to the soaked soybeans until the weight was 3.5 times of the original weight of dried soybeans. Two

different grinding temperatures used were 21 °C and 80 °C. In addition, glucosidase enzyme was added to the slurry of six soymilk samples to determine the effect of glucosidase enzyme on the concentrations of isoflavones in soymilk. A total of 35 soymilk samples were analyzed in triplicates. Table 3 summarizes the different variables used in preparing the soymilk samples.

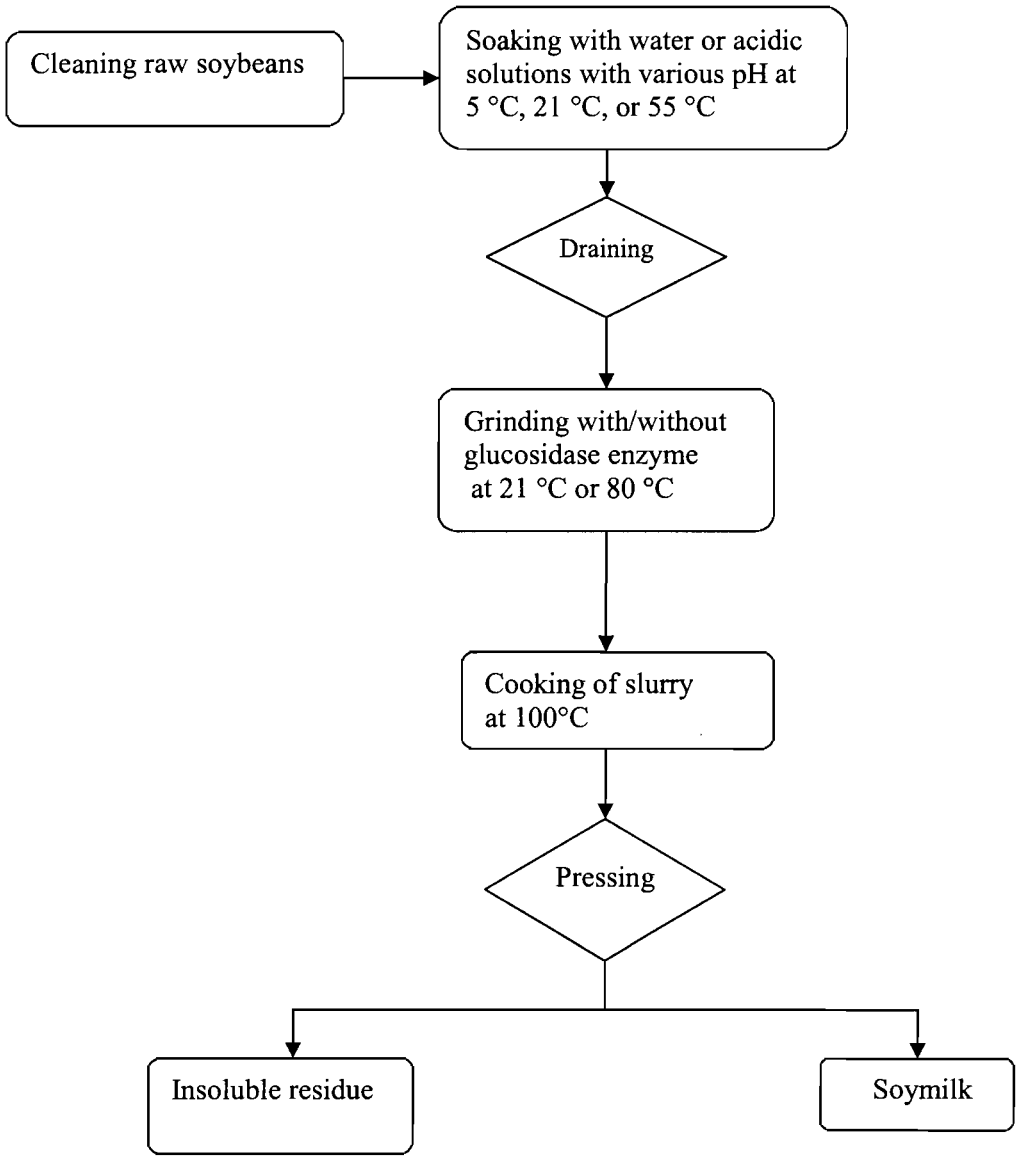


Figure 6. Flow diagram for production of soymilk

Table 3
Selected variables for producing soymilk samples

Soaking Solution	Soaking Temp (°C)	Grinding Temp (°C) with or without glucosidase
water	5, 21, 55	21, 80
HCl pH at 2, 4, 6	5, 21, 55	21, 80
$HC_2H_3O_2$ pH at 2, 4, 6	5, 21, 55	21, 80

Instrumentation

High-Performance-Liquid-Chromatography (HPLC) was used to determine the isoflavone contents of soymilk samples by determining a linear relationship of concentration and peak areas of isoflavone standard solutions. The HPLC system was equipped with a Millipore/Waters model 710A WISP auto-injector and a Millipore/Waters model M6000A pump. The column Novapak C18 was purchased from Millipore Corporation (Milford, MA). The instrument conditions for detection of isoflavones at ambient temperature were: (a) mobile phase: methanol and 0.01M ammonium acetate buffer with pH 4.6, (b) flow rate at 0.40mL/minute, (c) injection volume of 15 mL, and (e) detection at 254 nm. HPLC procedure was conducted with a linear gradient from 25% to 60% methanol in 20 minutes, followed by an isocratic hold period of 25 minutes. Peak identification was carried using standard isoflavones: genistin, genistein, daidzin, and daidzein. Peak areas were converted into concentrations of these four isoflavones in milligrams per gram of dry soybean (mg/g). Table 4 shows the means of concentrations of these four isoflavones in the soymilk samples.

Table 4

Mean (± s, mg/g) concentrations of genistin, genistein, daidzin, and daidzein in soymilk samples

Soaking solution	Solution pH / Soaking Temp. (°C) / Grinding Temp. (°C)	Genistin	Genistein	Daidzin	Daidzein
water	7 / 5 / 21	0.3765 ± 0.0078	0.1063 ± 0.0006	0.1939 ± 0.0062	0.0875 ± 0.0011
water	7 / 21 / 21	0.4261 ± 0.0099	0.0411 ± 0.0037	0.2815 ± 0.0003	0.1499 ± 0.0025
water	7 / 55 / 21	0.3129 ± 0.0291	0.1926 ± 0.0041	0.1300 ± 0.0222	0.0092 ±0.0031
water	7 / 21 / 80	0.3898 ± 0.0198	0.2899 ± 0.0074	0.2889 ± 0.0137	0.1358 ± 0.0195
water glucosidase added	7 / 21 / 21	0.4437 ± 0.0211	0.3400 ± 0.0147	0.3336 ± 0.0090	0.2473 ± 0.0206
HCl	2 / 5 / 21	0.3524 ± 0.0110	0.0908 ± 0.0058	0.1817 ± 0.0095	0.0999 ± 0.0076
HCl	4 / 5 / 21	0.3165 ± 0.0192	0.1860 ± 0.0079	0.1404 ± 0.0113	0.0384 ± 0.0337

Table 4 (continued)

Soaking solution	Solution pH / Soaking Temp. (°C) / Grinding Temp. (°C)	Genistin	Genistein	Daidzin	Daidzein
HCl	6 / 5 / 21	0.3702 ± 0.0112	0.0857 ± 0.0026	0.1863 ± 0.0053	0.1017 ± 0.0042
HCl	2 / 21 / 21	0.2144 ± 0.0144	0.2490 ± 0.0106	0.0913 ± 0.0124	0.0913 ± 0.0106
HCl	4 / 21 / 21	0.3341 ± 0.0196	0.1966 ± 0.0119	0.1631 ± 0.0130	0.0160 ± 0.0120
HCl	6 / 21 / 21	0.3511 ± 0.0087	0.1821 ± 0.0020	0.1696 ± 0.0034	0.0016 ± 0.0006
HCl * (* one trial)	2 / 55 / 21	0.3502	0.2633	0.1660	0.1293
HCl	4 / 55 / 21	0.3718 ± 0.0425	0.2316 ± 0.0529	0.1786 ± 0.0267	0.1015 ± 0.0666
HCl	6 / 55 / 21	0.3895 ± 0.0214	0.1085 ± 0.0173	0.2370 ± 0.0220	0.0108 ± 0.0153

Table 4 (continued)

Soaking solutuon	Solution pH / Soaking Temp. (°C) / Grinding Temp. (°C)	Genistin	Genistein	Daidzin	Daidzein
HCl	2 / 21 / 80	0.4074 ± 0.0313	0.3284 ± 0.0338	0.3038 ± 0.0203	0.1767 ± 0.0453
HCl	4 / 21 / 80	0.3908 ± 0.0251	0.0443 ± 0.0102	0.3068 ± 0.0145	0.1989 ± 0.0317
HCl	6 / 21 / 80	0.3925 ± 0.0680	0.3996 ± 0.1342	0.3099 ± 0.0599	0.2001 ± 0.0613
HCl glucosidase added	2 / 21 / 21	0.4183 ± 0.0296	0.2536 ± 0.0078	0.3223 ± 0.0205	0.1293 ± 0.0221
HCl glucosidase added	4 / 21 / 21	0.4338 ± 0.0178	0.3128 ± 0.0052	0.3043 ± 0.0104	0.1977 ± 0.0223
HCl glucosidase added	6 / 21 / 21	0.4573 ± 0.0233	0.3481 ± 0.0054	0.3201 ± 0.0112	0.2325 ± 0.0224
$HC_2H_3O_2$	2 / 5 / 21	0.2998 ± 0.0281	0.1125 ± 0.0126	0.1342 ± 0.0093	0.0826 ± 0.0127

Table 4 (continued)

Soaking solution	Solution pH / Soaking Temp. (°C) / Grinding Temp. (°C)	Genistin	Genistein	Daidzin	Daidzein
$HC_2H_3O_2$	4 / 5 / 21	0.3330 ± 0.0067	0.1636 ± 0.0022	0.1872 ± 0.0102	0.0110 ± 0.0021
$HC_2H_3O_2$	6 / 5 / 21	0.3563 ± 0.0266	0.2194 ± 0.0328	0.1858 ± 0.0236	0.0505 ± 0.0365
$HC_2H_3O_2$	2 / 21 / 21	0.3324 ± 0.0061	0.0199 ± 0.0019	0.2192 ± 0.0051	0.1827 ± 0.0031
$HC_2H_3O_2$	4 / 21 / 21	0.3370 ± 0.0052	0.1896 ± 0.0044	0.1755 ± 0.0068	0.0330 ± 0.0064
$HC_2H_3O_2$	6 / 21 / 21	0.3370 ± 0.0323	0.2483 ± 0.0655	0.1747 ± 0.0248	0.0793 ± 0.0491
$HC_2H_3O_2$	2 / 55 / 21	0.4405 ± 0.0249	0.0156 ± 0.0026	0.3108 ± 0.0060	0.1810 ± 0.0024
$HC_2H_3O_2$	4 / 55 / 21	0.4055 ± 0.0218	0.1392 ± 0.0076	0.2799 ± 0.0148	0.0128 ± 0.0084

Table 4 (continued)

Soaking solution	Solution pH / Soaking Temp. (°C) / Grinding Temp. (°C)	Genistin	Genistein	Daidzin	Daidzein
$HC_2H_3O_2$	6 / 55 / 21	0.3238 ± 0.1008	0.2498 ± 0.0063	0.2058 ± 0.0059	0.1065 ± 0.0046
$HC_2H_3O_2$	4 / 21 / 80	0.3735 ± 0.0239	0.3411 ± 0.0153	0.2979 ± 0.0092	0.1783 ± 0.0140
$HC_2H_3O_2$	6 / 21 / 80	0.3561 ± 0.0212	0.1175 ± 0.0027	0.3247 ± 0.0047	0.0869 ± 0.0093
$HC_2H_3O_2$ glucosidase added	4 / 21 / 21	0.4148 ± 0.0103	0.3247 ± 0.0089	0.3080 ± 0.0080	0.1979 ± 0.0298
$HC_2H_3O_2$ glucosidase added	6 / 21 / 21	0.4205 ± 0.0182	0.3223 ± 0.0098	0.3201 ± 0.0082	0.1788 ± 0.0075

Data Analysis

The data were analyzed using a statistical package called Statistical Product and Service Solutions (SPSS). Statistical analysis was conducted to evaluate the following: (1) correlation between the pH of soaking solutions and the concentrations of isoflavones, (2) correlation between soaking temperature and the concentrations of isoflavones, and (3) correlation between grinding temperature and the concentrations of isoflavones. In addition, pair-sample t-test was conducted to evaluate whether or not there are differences in the means of isoflavone concentrations in soymilk treated with and without glucosidase enzyme.

CHAPTER FOUR

RESULTS AND CONCLUSIONS

Table 5a to 5c show the means of isoflavone concentrations in soymilk made from beans that were soaked in water. Table 6a to 6c show the means of isoflavone concentrations in soymilk made from beans that were soaked in HCl solution. Table 7a to 7c show the means of isoflavone concentrations in soymilk made from beans that were soaked in $HC_2H_3O_2$ solution.

Table 8 to 10 show the Pearson correlation results between the concentrations of isoflavones and the pH of soaking solutions, soaking temperature, and grinding temperature in soymilk.

Table 11 shows the results of pair sample t-tests of the concentrations of isoflavones in soymilk samples made from the slurry that was treated with glucosidase enzyme and soymilk samples that were not treated with glucosidase enzyme (the other processing variables were held constantly).

Table 5a

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in water

N	Genistin	Genistein	Daidzin	Daidzein
12	0.3763 ± 0.0455	0.1569 ± 0.0977	0.2223 ± 0.0689	0.0950 ± 0.0580

Table 5b

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in water

Soaking temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
5	3	0.3765 ± 0.0078	0.1057 ± 0.0006	0.1937 ± 0.0060	0.0870 ± 0.0010
21	6	0.4079 ± 0.0243	0.1650 ± 0.1363	0.2832 ± 0.0103	0.1422 ± 0.0147
55	3	0.3130 ± 0.0291	0.1920 ± 0.0044	0.1293 ± 0.0223	0.0087 ± 0.0032

Table 5c

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in water

Grinding temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
21	9	0.3718 ± 0.0516	0.1128 ± 0.0658	0.2003 ± 0.0656	0.0817 ± 0.0611
80	3	0.3897 ± 0.0198	0.2893 ± 0.0075	0.2883 ± 0.0136	0.1350 ± 0.0195

Table 6a

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in HCl

pH	N	Genistin	Genistein	Daidzin	Daidzein
2	13	0.3534 ± 0.0882	0.1777 ± 0.1274	0.2172 ± 0.0931	0.1362 ± 0.0458
4	12	0.3536 ± 0.0394	0.2391 ± 0.0699	0.1969 ± 0.0691	0.0883 ± 0.0820
6	11	0.3740 ± 0.0364	0.2012 ± 0.1460	0.2242 ± 0.0659	0.0869 ± 0.0873

Table 6b

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in HCl

Soaking temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
5	9	0.3457 ± 0.0261	0.1202 ± 0.0491	0.1689 ± 0.0231	0.0794 ± 0.0359
21	18	0.3486 ± 0.0729	0.2829 ± 0.0952	0.2237 ± 0.0919	0.1136 ± 0.0902
55	9	0.3962 ± 0.0436	0.1353 ± 0.1086	0.2339 ± 0.0646	0.1140 ± 0.0694

Table 6c

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in HCl

Grinding temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
21	27	0.3473 ± 0.0611	0.1547 ± 0.0789	0.1813 ± 0.0590	0.0764 ± 0.0592
80	9	0.3973 ± 0.0402	0.3571 ± 0.0765	0.3064 ± 0.0324	0.1914 ± 0.0429

Table 7a

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in $HC_2H_3O_2$

pH	N	Genistin	Genistein	Daidzin	Daidzein
2	6	0.3161 ± 0.0255	0.0658 ± 0.0512	0.1763 ± 0.0472	0.1322 ± 0.0556
4	12	0.3622 ± 0.0341	0.2078 ± 0.0826	0.2345 ± 0.0577	0.0583 ± 0.0731
6	12	0.3433 ± 0.0495	0.2083 ± 0.0647	0.2218 ± 0.0643	0.0803 ± 0.0338

Table 7b

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in $HC_2H_3O_2$

Soaking temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
5	9	0.3297 ± 0.0315	0.1646 ± 0.0495	0.1687 ± 0.0296	0.0474 ± 0.0367
21	15	0.3472 ± 0.0237	0.1829 ± 0.1164	0.2379 ± 0.0654	0.1116 ± 0.0642
55	6	0.3647 ± 0.0791	0.1940 ± 0.0609	0.2413 ± 0.0428	0.0592 ± 0.0516

Table 7c

Mean (± s, mg/g) concentrations of isoflavons in soymilk made from beans soaked in $HC_2H_3O_2$

Grinding temperature (°C)	N	Genistin	Genistein	Daidzin	Daidzein
21	24	0.3406 ± 0.0449	0.1673 ± 0.0773	0.1945 ± 0.0422	0.0693 ± 0.0577
80	6	0.3648 ± 0.0223	0.2288 ± 0.1229	0.3108 ± 0.0158	0.1322 ± 0.0513

Table 8

Pearson correlation data of isoflavone concentrations in soymilk made from beans soaked in water

Processing variables		Genistin	Genistein	Daidzin	Daidzein
pH	Pearson correlation	-	-	-	-
	Sig. (2-tailed)	-	-	-	-
Soaking temperature (°C)	Pearson correlation	-0.678 *	0.295	-0.562	-0.693 *
	Sig. (2-tailed)	0.015	0.352	0.057	0.012
Grinding temperature (°C)	Pearson correlation	0.178	0.818 **	0.578 *	0.416
	Sig. (2-tailed)	0.580	0.001	0.049	0.179

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

N = 12

Table 9

Pearson correlation data of isoflavone concentrations in soymilk made from beans soaked in HCl

Processing variables		Genistin	Genistein	Daidzin	Daidzein
pH	Pearson correlation	0.138	0.092	0.031	-0.280
	Sig. (2-tailed)	0.423	0.593	0.858	0.099
Soaking temperature (°C)	Pearson correlation	0.338 *	-0.120	0.259	0.133
	Sig. (2-tailed)	0.044	0.485	0.127	0.441
Grinding temperature (°C)	Pearson correlation	0.365 *	0.755 **	0.719**	0.677 **
	Sig. (2-tailed)	0.029	0.000	0.000	0.000

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

N = 36

Table 10

Pearson correlation data of isoflavone concentrations in soymilk made from beans soaked in $HC_2H_3O_2$

Processing variables		Genistin	Genistein	Daidzin	Daidzein
pH	Pearson correlation	0.162	0.521 **	0.222	-0.237
	Sig. (2-tailed)	0.393	0.003	0.239	0.208
Soaking temperature (°C)	Pearson correlation	0.286	0.111	0.380 *	0.010
	Sig. (2-tailed)	0.126	0.559	0.038	0.957
Grinding temperature (°C)	Pearson correlation	0.234	0.280	0.779 **	0.418 *
	Sig. (2-tailed)	0.214	0.133	0.000	0.022

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

N = 30

Table 11

Pair-sample t-test data

Pair of isoflavones	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Genistin without / with	-.0984	.0598	.0141	-.1281	-.0686	-6.980	17	.000
Genistein without / with	-.1606	.1161	.0274	-.2183	-.1029	-5.870	17	.000
Daidzin without / with	-.1353	.0584	.0138	-.1643	-.1062	-9.827	17	.000
Daidzein without / with	-.1104	.0813	.0192	-.1508	-.0700	-5.764	17	.000

Results of Research Questions

The first question that is addressed in this study was; Is there any significant correlation between the pH of HCl and $HC_2H_3O_2$ solutions and the concentrations of isoflavones? Since water has a constant pH at about 7, no correlation can be made as shown in Table 8. Table 9 shows no significant correlation between the pH of HCl solution and the concentrations of isoflavones.

Table 10 shows the average concentrations of isoflavones in soymilk that were made by soaking the beans in $HC_2H_3O_2$ solution at the various pH levels. The concentration of genistein increases with the increasing pH of $HC_2H_3O_2$ solution. The mean values of genistein concentration are 0.0658 ± 0.0512 mg/g at the pH of 2, 0.2078 ± 0.0826 mg/g at the pH of 4, and 0.2083 ± 0.0647 mg/g at the pH of 6. In Table 16, the only significant correlation found was between the pH of $HC_2H_3O_2$ solution and the concentration of genistein. The Pearson correlation is significant at the 0.01 level ($p < 0.01$).

The second question addressed was; Is there any significant correlation between the soaking temperature and the concentrations of isoflavones in soymilk made from soybeans were soaked in water, HCl, and $HC_2H_3O_2$ solution? In Table 8, results from soymilk made from beans that were soaked in water show a negative correlation between soaking temperature and the concentrations of genistin and daidzein. Pearson correlation is significant at the level of 0.05 ($p < 0.05$). In Table 5b, the data show that increasing the soaking temperature from 5 °C to 21 °C results in increasing the concentrations of genistin and daidzein. At 5 °C the mean value of the concentration of genistin is 0.3765 ± 0.0078 mg/g and it increased to 0.4079 ± 0.0243 mg/g at 21°C. The mean daidzein

concentration at 5 °C is 0.0870 ± 0.0010 mg/g and it increased to 0.1422 ± 0.0147 mg/g at 21°C. Interestingly, the concentrations of genistin and daidzein decrease from 21 °C to 55 °C. The mean value of the concentration of genistin is 0.3130 ± 0.0291 mg/g at 55 °C and the mean value of the concentration of daidzein is 0.0087 ± 0.0032 mg/g at 55 °C.

Data in Table 6b show that increasing the soaking temperature from 5 °C to 55 °C results in increasing the concentration of genistin when soybeans were soaked in HCl solution. The mean value of genistin concentration is 0.3457 ± 0.0261 mg/g at 5 °C, 0.3486 ± 0.0729 mg/g at 21 °C, and 0.3962 ± 0.0436 mg/g at 55 °C. In Table 9, the only significant correlation was found between the soaking temperature and the concentration of genistin at the 0.05 level

($p < 0.05$).

In Table 7b, data show that increasing soaking temperature results in increasing concentration of daidzin when the soybeans were soaked in $HC_2H_3O_2$ solution. The mean values are 0.1687 ± 0.0296 mg/g at 5 °C, 0.2379 ± 0.0654 mg/g at 21 °C, and 0.2413 ± 0.0428 mg/g at 55 °C. Table 10 shows that the only significant correlation was found between the soaking temperature and the concentration of daidzin at the 0.05 level ($p < 0.05$). The positive correlation indicates that the concentration of daidzin increases with increasing soaking temperature.

The third question addressed in this study was; Is there any significant correlation between grinding temperature and the concentrations of isoflavones in soymilk made from soybeans soaked in water, HCl, and $HC_2H_3O_2$ solutions? In Table 8, the results from soymilk made from beans soaked in water show significant correlation between the grinding temperature and the concentrations of genistein and daidzin. The data in

Table 5c show that the mean values of the concentration of genistein are 0.1128 ± 0.0658 mg/g at 21 °C and 0.2893 ± 0.0075 mg/g at 80 °C. The Pearson correlation is significant at the level of 0.01 ($p < 0.01$) shown in Table 8. The mean values of the concentration of daidzin are 0.2003 ± 0.0656 mg/g at 21 °C and 0.2883 ± 0.0136 mg/g at 80 °C. The Pearson correlation is significant at the level of 0.05 ($p < 0.05$) shown in Table 8.

In Table 6c, the results obtained from soymilk made from beans soaked in HCl solution show that grinding temperature strongly affects concentrations of the four isoflavones. The concentrations of these four isoflavones increase with the increasing grinding temperature. Table 9 shows significant correlations between the grinding temperature and concentrations of these four isoflavones. The Pearson correlations are significant at the level of 0.05 for the concentration of genistein ($p < 0.05$) and at the level of 0.01 for the concentrations of genistein, daidzin, and daidzein ($p < 0.01$).

In Table 10, the results obtained from soymilk made from beans that were soaked in $HC_2H_3O_2$ solution show that there is significant correlation between grinding temperature and the concentrations of daidzin and daidzein. The data in Table 7c show that increasing the grinding temperature results in increasing the concentrations of daidzin and daidzein. The mean values of the concentrations of daidzin are 0.1945 ± 0.0422 mg/g at 21 °C and 0.3108 ± 0.0158 mg/g at 80 °C. The mean values of the concentration of daidzein are 0.0693 ± 0.0577 mg/g at 21 °C and 0.1322 ± 0.0513 mg/g at 80 °C. The positive correlation indicates that the concentrations of daidzin and daidzein increase with increasing grinding temperature.

The fourth question addressed was; Does the addition of glucosidase enzyme affects the concentrations of isoflavones in soymilk? In other words, is there any

significant differences in the means of isoflavone concentrations in soymilk made from the slurry that was treated with and without glucosidase enzyme? Table 11 shows that significant differences in the means of isoflavone concentrations were found in treated and untreated soymilk. The statistical results indicate that the addition of glucosidase enzyme may increase the concentrations of genistin, genistein, daidzin, and daidzein in producing soymilk.

Conclusions

The variables that were considered in this study are type and pH of soaking solution, soaking temperature, temperature during grinding of soybeans, and addition of glucosidase enzyme. The following conclusions can be drawn from this study:

In soymilk made from beans that were soaked in water, there is an inverse association between the soaking temperature and the concentration of genistin and daidzein. In addition, a strong correlation was observed between the grinding temperature and the concentrations of genistein and daidzin.

In soymilk made from the beans soaked in HCL solution, the pH of the solution did not affect the profile of isoflavones. However, a significant correlation was detected between soaking temperature and the concentration of genistin. Furthermore, the grinding temperature is a strong predictor of the profile of isoflavones.

In soymilk made from beans soaked in $HC_2H_3O_2$ solution, the pH of $HC_2H_3O_2$ solution is correlated with the concentration of genistein only. The soaking temperature influences the concentration of daidzin in producing soymilk. Moreover, the concentrations of daidzin and daidzein were also influenced by grinding temperature.

REFERENCES

Adlercreutz, H., Fotsis, T., Kurzer, M.S., Wahala K., Makela, T., & Hase, T. (1995). Isotope dilution gas chromatographic-mass spectrometric method for the determination of unconjugated lignans and isoflavones in human feces, with preliminary results in omnivorous and vegetarian women. *Analytical Biochemistry, 225,* 101-108.

Alekel, D.L., Germain, A.S., Peterson, C.T., Hanson, K.B., Stewart, J.W., & Toda, T. (2000). Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *American Journal of Clinical Nutrition, 72,* 844–852.

Barnes, S., Grubbs, C., Setchell, K.D.R., & Carlson, J. (1990). Soybeans inhibit mammary tumors in models of breast cancer. In: Pariza, M.W., Aeschbacher, H.U., Felton, J.S., & Sato, S. (Eds). *Mutagens and carcinogens in the diet* (pp. 239-253). New York: Wiley Liss.

Borriello, S.P., Setchell, K.D.R., Axelson, M., & Lawson, A.M. (1985). Production and metabolism of lignans by the human faecal flora. *Journal of applied bacteriology, 58,* 37-43.

Cancer statistics (2004). Retrieved December 10, 2004, from: http://www.cancer.org/downloads/PRO/Cancer%20Statistics%202004.ppt

Dai, Q., Shu, X.O., Jin, F., Potter, J.D., Kushi, L.H., Teas, J., Gao, Y.T., & Zheng, W. (2001). Population-based case control study of soyfood intake and breast cancer risk in Shanghai. *British Journal of Cancer, 85* (3), 372-378.

Eisen, B., Ungar, Y., & Shimoni, E. (2003). Stability of isoflavones in soy milk stored at elevated and ambient temperatures. *Journal of Agricultural and Food Chemistry, 52*, 2212-2215.

FDA approves new health claim for soy protein and coronary heart disease. (October 20, 1999). Retrieved September 29, 2004, from: http://www.cfsan.fda.gov/~lrd/tpsoypr2.html

Fournier, D.B., Erdman, J.W.Jr., & Gordon, G.B. (2001). Soy and cancer prevention. In: Bendich, A. & Deckelbaum,R.J. (Eds). *Primary and secondary preventive nutrition* (pp. 3-17). Totowa, NJ: Humana Press Inc.

Garlock, L.A. (2000). *The effect of various acidic solutions on the concentration of genistein in tempeh.* Unpulished master's thesis, University of Wisconsin-Stout, Menomonie, WI.

Goldwyn, S., Lazinsy, A., & Wei, H. (2000). Promotion of health by soy isoflavones: Efficacy, benefit, and safety concerns. *Drug Metabolism and Drug Interactions, 17* (1-4), 261-285.

Greenstein, J., Kushi, L., & Zheng, W. (1996). Risk of breast cancer associated with intake of specific foods and food groups. *American Journal of Epidemiology, 145*, S36 (abstr).

Grun, I.U., Adhikari, K., Li, C., Li., Y., Lin, B., Z., J., & Fernando, L.N. (2001). Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. *Journal of Agricultural and Food Chemistry, 49*, 2839-2843.

Henkel, J. (May-June, 2000). Soy: Health claims for soy protein, questions about other components. Retrieved December 10, 2004, from:

http://www.fda.gov/fdac/features/2000/300_soy.html

Hutchins, A.M., Slavin, J.L., & Lampe J.W. (1995). Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented soy products. *Journal of the American Dietetic Association, 95*, 545-551.

Izumi, T., Piskula, M.K., Osawa, S. Obata A., Tobe K., Saito M., Kataoka S., Kubota Y., & Kikuchi M. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition, 130,* 1695-1699.

Jenkins, D.J.A., Kendall, C.W.C., Jackson, C.J.C., Connelly,P.W., Parker, T., Faulkner, D., Vidgen,E., Cunnane, S.C., Leiter, L.A., & Josse, R.G. (2002). Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *American Journal of Clinical Nutrition, 76,* 365-372.

Keinan-Boker, L., Schouw, Y.T.D., Grobbee, D.E., & Petra HM Peeters, P.H.M. (2004). Dietary phytoestrogens and breast cancer risk. *American Journal of Clinical Nutrition, 79*, 282–288.

Knight, D.C., & Eden, J.A. (1994). A review of the clinical effects of phytoestrogens. *Obstet Gynecol, 87*, 897-904.

Knight, D.C., & Eden, J.A. (1995). Phytoestrogens: A short review. *Maturitas, 22*, 167-184.

Kolonel, L.N., Hankin, J.H., Whittemore, A.S., Wu,A.H., Gallagher, R.P., Wilkens,L.R., John, E.M., Howe, G.R., Dreon, D.M., West, D.W., & Paffenbarger, Jr. R.S. (2000).Vegetables, fruits, legumes and prostate cancer: a multiethnic case-

control study. *Cancer Epidemiology Biomarkers & Prevention, 9*, 795–804.

Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Jr, Kris-Etherton, P., Goldberg, I. J., Kotchen, T. A., Lichtenstein, A. H., Mitch, W. E., Mullis, R., Robinson, K., Wylie-Rosett, J., St Jeor, S., Suttie, J., Tribble, D. L., & Bazzarre, T. L. (2000) AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation, 102,* 2284-2299.

Kurzer, M.S., & Xu, X. (1997). Dietary phytoestrogens. *Annual Review of Nutrition, 17,* 353-381.

Lee, M.M., Gomez, S.L, Chang, J.S., Wey, M., Wang R.T., & Hsing, A.W. (2003). Soy and Isoflavone Consumption in Relation to Prostate Cancer Risk in China. *Cancer Epidemiology Biomarkers & Prevention, 12,* 665-668.

Lu, L.-J.W., & Anderson, K.E. (1998). Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans. *American Journal of Clinical Nutrition, 68*, 1500S-1504S.

Lydeking-Olsen, E., Jensen, J.-B.E., Setchell, K.D.R., Damhus, M., & Jensen, T.H. (2002). Isoflavone-rich soymilk prevents bone-loss in the lumbar spine of postmenopausal women: a 2 year study. *Journal of Nutrition, 132*, 582S (abstract).

McGinnis, J.M. & Ernst, N.D. (2001). Preventive nutrition: A historic perspective and future economic outlook. In: Bendich, A. & Deckelbaum,R.J. (Eds). *Primary and secondary preventive nutrition* (pp. 3-17). Totowa, NJ: Humana Press Inc.

McGinnis, J.M. & Foege, W.H. (1993). Actual causes of death in the United States. *Journal of the American Medical Association, 270*, 2207-2212.

Messina, M.J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition, 70,* 439S-450S.

Messina, M.J. (2003). Emerging evidence on the role of soy in reducing prostate cancer risk. *Nutrition Review, 61* (4), 117-131.

Messina, M.J. & Loprinzi, C.L. (2001). Soy for breast cancer survivors: A critical review of the literature. *Journal of Nutrition, 131*, 3095S-3108S.

Messina, M.J. & Messina, V. (2003). Provisional recommended soy protein and isoflavone intakes for healthy adults: rationale. *Nutrition Today, 38*(3), 100-109.

Miller, A.B. (1990). Diet and cancer: A review. *Acta Oncologica, 29,* 87-95.

Miller, A.B., Berrino, F., Hill, M., Pietinen, P., Riboli, E., & Wahrendorf, J. (1994). Diet in the aetiology of cancer: A review. *European Journal of Cancer, 30A,* 207-220.

Miso (n.d.). Retrieved November 12, 2004, from: http://www.soyfoods.org/facts/MISO.pdf

Morton, M.S., Matos-Ferreira, A., Abranches-Monteiro, L. Correia, R., Blacklock, N., Chan, P.S., Cheng, C., Lloyd, S., Chieh-ping, W., & Griffiths, K. (1997). Measurement and metabolism of isoflavnoids and lignans in the human male. *Cancer Letters, 114*, 145-151.

Munro, I.C., Harwood, M., Hlywka, J.J., Stephen, A. M., Doull, J., Flamm, W. G., & Adlercreutz, H. (2003). Soy isoflavones: A safety review. *Nutrition Reviews, 61*(1), 1-33.

Nagata, C., Takatsuka, N., Kurisu, Y., & Shimizu, H. (1998). Deceased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. *Journal of Nutrition, 128* (2), 209-213.

Naim, M., Gestetner, B., Bondi, A., & Birk, Y. (1974). Soybean isoflavones: Characterization, determination, and antifungal activity. *Journal of Agricultural and Food Chemistry, 22,* 806-810.

Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., & Erdman, J.W. Jr. (1998). Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *American Journal of Clinical Nutrition, 68(suppl),* 1375S–1379S.

Reinli, K., & Block, P. (1996). Phytoestrogen content of foods: a compendium of literature values. *Nutrition and Cancer, 26,* 123-148.

Setchell, K.D.R. (2000). Absorption and metabolism of soy isoflavones- from food to dietary supplements and adults to infants. *Journal of Nutrition, 130,* 654S-655S.

Setchell, K.D.R., Borriello, S.P., Hulme, P., Kirk, D.N., & Axelson, M. (1984). Non-steroidal oestrogens of dietary origin: Possible roles in hormone dependent disease. *American Journal of Clinical Nutrition, 40,* 569-578.

Setchell, K.D.R., & Cassisy, A. (1999). Dietary isoflavones: Biological effects and relevance to human health. *Journal of Nutrition, 129,* 758S-767S.

Setchell, K.D.R., & Lydeking-Olsen, E. (2003). Dietary phytoestrogens and their effect on bone: Evidence from in vitro and in vivo, human observational, and dietary intervention. *American Journal of Clinical Nutrition, 78(suppl),* 593S-609S.

Shu, X.O., Jin, F., Dai, Q., Wen, W., Potter, J.D., Kushi, L.H., Ruan, Z., Gao, Y.T., & Zheng, W. (2001). Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. *Cancer Epidemiology Biomarkers & Prevention, 10* (5), 483-488.

Smith, A.K. (1978). Soybeans: *Chemistry and technology*. Westport, CN: the Avi Publishing Company, Inc.

Soyfood facts (n.d.). Retrieved November 12, 2004, from: http:// www. soyfoods.org/facts/facts.html

Strom, S.S., Yamamura, Y.D., Cherie M., Spitz, M.R., Babaian, R.J., Pillow, P.C., & Hursting, S.D. (1999). Phytoestrogen intake and prostate cancer: a case-control study using a new database. *Nutrition and Cancer, 33* (1), 20-25.

Tempeh (n.d.). Retrieved November 12, 2004, from: http:// www.soyfoods.org/facts/TEMPEH.pdf

Tofu (n.d.) Retrieved November 12, 2004, from: http:// www.soyfoods.org/facts/TOFU.pdf

Tonstad, S., Smerud, K., & Hoxie, L. (2002). A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. *American Journal of Clinical Nutrition, 76*, 78–84.

USDA-Iowa State University Database on the Isoflavone Content of Foods. (2002, March). Retrieved October 5, 2004, from: http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html

Vitolins, M., Anthony, M., Lenschik, L., Bland, D.R., & Burke, G.L. (2002). Does soy protein and its isoflavones prevent bone loss in peri- and postmenopausal women? Results of a two year randomized clinical trial. *Journal of Nutrition, 132*, 582S (abstract).

Wang, H., & Murphy, P. (1994). Isoflavone composition of American and Japanese

soybeans in Iowa: Effects variety, crop year, and location. *Journal of Agricultural and Food Chemistry, 42*, 1674-1677.

Wang, H., & Murphy, P. (1996). Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry, 44*, 2377-2383.

Wu, A.H., Wan, P., Hankin, J., Tseng, C.C., Yu, M.C., & Pike, M.C. (2002). Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. Carcinogenesis, 23 (9), 1491-1496.

Xu, X., Duncan, A.M., Wanagen, K.E., & Kurzer, M.S. (2000). Soy consumption alters endogenous estrogen metabolism in postmenopausal women. *Cancer Epidemiology, Biomarkers & Prevention, 9*, 781-786.

Xu, X., Harris, K.S., Wang, H.J., Murphy, P.A., & Hendrich, S. (1995). Bioavailability of soybean isoflavones depends upon gut microflora in women. *Human Nutrition-Clinical Nutrition, 125*, 2307-2315.

Zhou, J.R., & Erdman, J. (1997). Chemical effects of processing and food preparation on carotenoids and soy and garlic phytochemicals. In P. A. Lachance (Ed), *Nutraceuticals: designer foods III garlic, soy, and licorice* (pp. 23-37). Trumbull, CT: Food and Nutrition Press.