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NUTRITIONAL EVALUATION OF GERMINATED WHEAT AND ITS USE IN A NUTRITIONAL BAR

BY

FENG YANG ©

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled NUTRITIONAL EVALUATION OF GERMINATED WHEAT AND ITS USE IN A NUTRITIONAL BAR submitted by FENG YANG here in partial fulfillment of the requirements for the degree of MASTER in FOOD SCIENCE AND TECHNOLOGY.

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February 2, 2000

ABSTRACT

Consumption of sprouts in Canada and worldwide has increased. However, wheat sprout is vastly underutilized to fulfill its nutritional and economic functions. The primary objectives of this study were to evaluate a selected group of antioxidants in wheat grain before and after germination for various lengths of time, and to develop an acceptable functional food product containing germinated wheat at its maximum antioxidant levels.

To investigate the effect of germination on chemical and nutritional composition of wheat grain, wheat seed was first steeped in tap water for 24 h, followed by germination in the dark for another 7 days at 98% relative humidity and 16.5°C to achieve the optimal sprouting effect.

Vitamins C and E, and β-carotene content in wheat grain were almost undetectable. During germination, however, the concentrations of these antioxidant vitamins increased with increasing germination time and reached their peak levels at day 7 (550 μg/g for vitamin C; 10.92 μg/g for α-tocopherol; and 3.1 μg/g for β-carotene). In addition to these compounds, ferulic acid, another antioxidant, also increased following the germination and reached its highest concentration after 7 days (932.4 μg/g). This study suggested that antioxidants may play a crucial role in the break of dormancy and germination.

Using wheat germinated for 7 days as an ingredient, a "Nutritional Bar" was designed. Essentially, the bar consisted of a blend of roasted soybean nuts, crispy rice, freeze-dried germinated wheat powder, honey, raisins, and instant skim milk powder. The bar was formed using a cold press technique, and sensory evaluation showed that the bar

was liked by most consumers (31 out of 32). A selected nutrient composition of the bar was evaluated by an Esha nutritional analysis software. Each bar provided appreciable amounts of dietary fiber (23% of the daily suggested intake), vitamin B_{12} (> 100% of RNI), vitamin A (60% of RNI), folic acid (60% of RNI), potassium (28% of RNI), iron (20% of RNI), and calcium (10% of RNI). The bar was low in saturated fat (0.5 g per 45 g serving), and contained no cholesterol.

Since Alberta is one of the world's leading wheat growers, it makes economic sense that food scientists should find ways to add more value to this commodity. Sprouting of wheat grains and processing them into functional food ingredients is one excellent way of doing it.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Sprout History

In recent years, there has been an increasing awareness of the effect of sprouts on human health and disease. Medicinally and nutritionally, sprouts have a long history in the human food chain (Price, 1988). According to *The Book of Daniel* in *The Old Testament*, Nebuchadnezzar King of Babylon (630-562 B.C.) ate only cereal sprouts for seven years, and claimed that his mental clarity and sanity were restored during this period, allowing him to again rule his kingdom. In the 1700's, sailors were riddled by scurvy (lack of vitamin C) and suffered heavy casualties during their two to three years voyages. From 1772-1775, Captain James Cook had his sailors eat limes, lemons and varieties of sprouts which are rich in vitamin C. These plus other fresh fruits and vegetables and a continuous program of growing and eating sprouts were credited with the breakthrough in solving the scurvy problems, thus solving the marines' greatest casualty dilemma (Seibold, 1990).

1.2 Economic Impact

Although sprouts have been used for centuries, particularly in the Orient, it is only in recent years that sprouts and sprouting have become popular in the western world (Price, 1988). This is evident by the increased production and consumption of products such as mung bean and soybean sprouts and alfalfa sprout. It was reported that in 1970, germinated alfafa sprout consumption in California was 22.7 tonnes, but increased to 659 tonnes in 1979 and, with an estimated value of US$ 8.5 million, exceeded the farm value of Californian lettuce ($ 7.3 million) (Hesterman and Teuber, 1979). Lipton *et al.* (1981) have indicated that 50,000 tonnes of sprouts per annum are produced in the United States while unofficial figures estimated that sprout production in Hong Kong exceeds 150 tonnes/day (Price, 1988). Other legume and cereal grains are also excellent candidates for sprouting. Among these are kidney bean and wheat grain.

1.3 Nutritional Advantage of Sprout

It has been reported that certain vitamins (Sattar *et al.*, 1985), proteins (Alexander, 1983), amino acids and sugars (Chavan *et al.*, 1981) were increased during germination of food grains. Wheat is a major agricultural crop of Canada and the United States. North America exports more wheat than any other countries (Finney, 1978). However, studies on the influence of germination on the nutritional quality of wheat grains are scanty. Commercial wheat sprout production therefore has been greatly hampered.

Modern food processes used for wheat are selected on the basis of speed, storability and ease of preparation rather the nutrient content. Nutrients can be lost from wheat flour when germ and bran (with the aleurone layer) portions are removed. If "maximum nutrients for minimum costs" is desired, wheat should be eaten whole, or processed with time-proven traditional methods (as with wheat germination or fermentation). According to Health and Welfare Canada (1990), dietary levels should be increased for whole grains, fruits, and vegetables, and decreased for animal fat and protein, sugar and salt. The germinated seeds were commonly called sprouts (Alexander *et al.*, 1984). Wheat sprouts fall within the scope of this nutritional principle of whole food nutrition. Germination has important effects on chemical composition, nutritive value, and acceptability characteristics of wheat for human consumption (Cole *et al.*, 1983). In comparison to wheat grains, wheat sprouts have a greater nutritive value due to a better quality of protein, a more favorable distribution of amino acids, a higher content of polyunsaturated fatty acids, an increased bioavailability of essential minerals and trace elements, as well as a higher content of vitamins (Finney, 1978).

1.3.1 Wheat Kernel

Wheat kernel (Figure 1) is the seed from which the wheat plant grows. Each seed contains three nutrient rich parts, which are separated during the dry milling process to produce flour (Slavin *et al.*, 1997). These are:

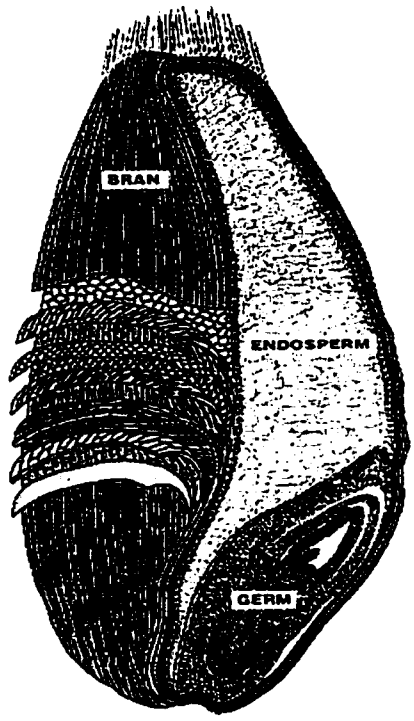


Figure 1 Inside a kernel of wheat

(Wheatfoods Electronic News, 1998)

i. Endosperm comprising 83% of the wheat kernel, is rich in protein, carbohydrate, iron, and B vitamins, such as riboflavin, niacin and thiamin. It is also a significant source of soluble dietary fiber.

ii. Bran constitutes 14.5% of the kernel. It is included in whole wheat but not in white flour. Bran is rich in riboflavin, niacin and thiamin, as well as some trace minerals and insoluble dietary fiber.

iii. Germ is the embryo or sprouting section of the seed, constituting 2.5% of the wheat kernel. It contains minimal quantities of high quality protein and a greater share of B-complex vitamins, vitamin E, lipid and trace minerals.

1.3.2 Wheat Germination

Wheat grains are generally classified as dormant at the time of cropping because they germinate poorly in ambient environment. The prime molecular mechanism triggering seed germination is not well understood (Fontaine *et al.*, 1995). Recently, it has been suggested that gibberellic acid (GA_3) may play an important role in seed dormancy breakage and germination process by enhancing embryo metabolism and growth, and inducing the hydrolytic enzymes in the germ (Yao, 1996). These enzymes, including amylase, protease, and lipase have the responsibility of using all of basic nutrient reserve to synthesize new molecules and tissue that will ultimately produce a new seedling. The activity of amylase is increased a hundred times during germination (Table 1). This enzyme hydrolyzes starch granule wall and randomly breaks the amylose and amylopectin chains in the starch granule, resulting in shorter-chain molecules such as dextrins as well as simpler molecules like glucose and maltose. This action is termed dextrinization (Dullus, 1984). Proteases contribute to the transformation of wheat gluten to free amino acids. The increase of cysteine or glutathione during germination prompts the proteolytic activities of the germ (Fontaine *et al.*, 1995). As a result, protease activity of the whole wheat grain is increased about ten fold during sprouting (Pomeranz, 1971).

Some germinated wheat enzymes, however, may facilitate the biosyntheses of antioxidants (Maillard and Berset, 1995).

Table 1 Effect of germination on the α- and β-amylase activities of wheat and barley[a]

Amylase	Barley		Hard Red Winter Wheat	
	Total	Free	Total	Free
(β-amylase units)				
Ungerminated	28	10 (1 hr)	23	7 (1 hr)
Germinated	28	19 (1 hr)	22	18 (15 min)
(α- amylase units)				
Ungerminated	0.04		0.04	
Germinated	54	53 (1 hr)	183	165 (15 min)

[a]Data in parentheses are extraction times.

One amylase unit is defined as the amount of enzyme that can hydrolyze one gram of starch in one hour. (Briggs *et al.*, 1981)

1.3.3 Chemical Changes during Germination

When wheat grain is steeped (soaked), the oxygen dissolved in the water is rapidly utilized and the grain begins to ferment (respire anaerobically), with the major products being ethanol and carbon dioxide. These are derived from stored sugars via the glycolytic pathway. A considerable proportion of the ethanol is leached into the steep liquor. Steep aeration (turning) reduces ethanol production and subsequently leads to more vigorous germination. Even so, grain ethanol levels continue to rise until chitting occurs, when the surface layers of the grain are broken and oxygen reaches the living tissues more easily. Oxygen uptake is limited by the surface moisture film of the grain, the husk and the pericarp. Also the microorganisms present in the surface layers actively compete for oxygen with the grain tissue. When the grain is chitted, ethanol formation ceases and residual ethanol is oxidized. Oxygen uptake and carbon dioxide production occur at approximately equal rates. The main respiratory substrate is carbohydrate. Elevated oxygen levels increase grain respiration and accelerate enzyme production (Briggs *et al.*, 1981). Antioxidants biosynthesized during this process, are believed to help protect polyunsaturated fatty acids in the cell membrane of the new seedling from oxidation (Seibold, 1990). Concentrations of various vitamins have been reported to be markedly increased when cereals are properly germinated (Table 2).

Table 2 Percentage increase in vitamin concentration of cereal sprouts

Material	Vitamin	Percentage of increase (compared to wheat grain)
Sprouted oat	Vitamin B_2	1300%
Baby green oat	Vitamin B_2	2000%
Sprouted wheat		
	Inositol	100%
	Biotin	50%
	Pantothenic acid	200%
	Vitamin B_6 (Pyridoxine)	500%
	Vitamin B_3 (Niacin)	500%
	Folic acid	600%
	Vitamin C	600%
Sprouted soybean	Vitamin C	553%

Source: Isga-sprouts Electronic News, 1999

1.4 Sprout Nutrients

According to many contemporary laboratory analyses, a wide variety of nutrients are contained in germinated wheat (Lemar and Swanson, 1976). Some of these nutrients are more concentrated than others. These nutrients are combined by nature to provide a uniquely potent food. It should be stated that the nutrient concentrations largely depend on the growing conditions and the growth stage at which the sprouts are harvested, rather than on the type of cereal sprouts (barley, rye, or wheat) analyzed (Seibold, 1990).

1.4.1 Dietary Fiber

Wheat sprout is high in dietary fiber, which has been extensively studied for the effects it may have on many bowel and non-bowel chronic diseases. There have been substantial amount of evidence suggesting that dietary fibers, such as those in the cereal sprouts and whole grains, may reduce colon cancer (Parke *et al.*, 1996). The mechanism for this protection is the subject of much debate, but it is thought that the increased stool bulk and enhanced colon emptying associated with the dietary fiber may reduce the exposure of the bowel to carcinogens and other toxic substances.

Soluble fiber is currently receiving much attention for its potential role in lowing serum cholesterol and blood glucose concentrations (Slavin *et al.*, 1997). Diets rich in soluble fibers such as wheat sprout helps reduce total cholesterol and low density lipoprotein (LDL) cholesterol in people with both high and normal blood cholesterol level (Ificinfo, 1998).

The Food and Nutrition Board of the National Academy of Science, the National Cancer Institute, the American Cancer Society, and the U.S. Department of Health and Human Services recommend 20-30 g of dietary fiber per day, while North Americans currently consume an average of only 11 g of dietary fiber daily (Seibold, 1990). As one of a few fiber-rich food supplements (Table 3), wheat sprout may be developed as a textural ingredient in engineered food (hamburger, chicken surimi, *etc.*) to improve textural quality and provide an array of vitamins, minerals and proteins.

Table 3 Total dietary fiber content of high fiber food

Fiber Food	Serving Size	Grams/ Serving	Grams/ 100 Grams
Dehydrated Wheat Sprout	5 grams (0.175 oz.)	1.9	37.5
Wheat Bran	5 grams	2.2	44.4
Oat Bran	5 grams	0.9	17.9
Whole Wheat (cooked)	1/3 cup	1.0	2.0
Prunes	1/2 cup	2.5	2.9
Brown Rice	1/2 cup	2.0	2.0

(Seibold, 1990)

1.4.2 Protein

Protein is a part of every living cell. It accounts for over half of the dry weight of the human body. The protein contained in wheat sprout is a nutritional resource that has barely been tapped. Dehydrated wheat sprout has 20 – 25% protein, making it higher than that in milk (3%), eggs (12%), and sirloin steak (16%). All proteins are not functionally equal, but, the protein in wheat sprout is superior to most of other plant sources, and is even superior to that of some animal foods (Pirie, 1969).

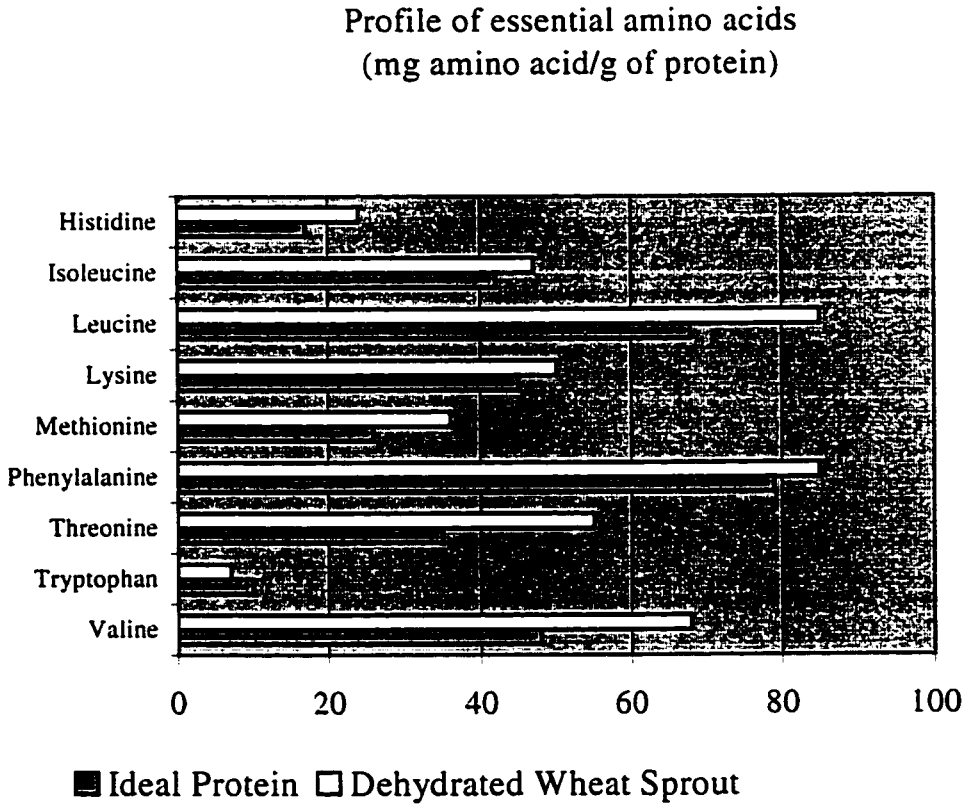
Proteins are built from some 22 different amino acids. Healthy adult humans can synthesize only 14 of these amino acids in adequate quantities. The remaining 8 amino acids must be obtained from foods, and are named essential amino acids (Yao, 1996).

In order for food proteins to be optimally used by the body, all of the essential amino acids must be present in suitable proportion. Proteins which meet this requirement are called complete proteins. Those which are deficient in one or more amino acids are called incomplete proteins. Most animal food proteins are considered complete proteins; plant proteins are usually considered incomplete. Grain foods tend to be low in the essential amino acid lysine, while beans often lack methionine. Eggs are believed to contain the most usable combination of amino acids, and hence often referred to as a "reference protein" (Reed, 1980).

After germination, wheat sprout contains all of the essential amino acids in the amounts which make the protein very usable (Tkachuk, 1979), unlike most plant proteins. Wheat sprout contains high levels of both methionine and lysine (Figure 2). The total free amino acid content after 122 h of germination has been found to be 10 times that of

untreated wheat grain at 16.5 °C. Glutamine and proline showed the largest increase; proline increased by 100-fold. (Tkachuk, 1979).

Figure 2 Comparisons of wheat sprout protein to ideal protein



(Seibold, 1990)

1.4.3 Bioavailability of Minerals and Trace Elements

In the literature, data indicate that minerals and trace elements in cereals and cereal products are mainly bound to fiber components (cellulose, hemicellulose and lignin, phenols, tannic acid and most importantly to phytic acid) (Harmuth-Hoene, 1987). Phytic acid may be one of the main factors responsible for reducing mineral bioavailability. During germination, phytase activity increases (Reddy *et al.*, 1982), which reduces phytic acid content, and changes the digestibility of the fiber and protein. Meanwhile, the increases of amino acids and ascorbic acid, which are potential ligands for chelation during germination, also contribute to a better bioavailability of the trace minerals (Lintschinger *et al.*, 1997).

1.4.4 Vitamin C

The major function of vitamin C is in the formation of collagen, which gives structural stability to connective tissues, which surround and support the bones and ligament, skin and other epithelial tissue. Vitamin C deficiency results in the inability to form collagen, which leads to the structural breakdown of tissues, including gums, bones, and blood cells. Injured and infected tissues cannot repair themselves if the body is low in vitamin C, making this nutrient essential for resistance to disease (immunity), healing of wounds, and formation of scar tissue (Reed, 1980).

Vitamin C is perhaps the best water-soluble antioxidant found in nature. It acts with other antioxidants, vitamin E, β-carotene and selenium to reduce free radicals, chemical substances, which damage cells and cellular membranes.

The most obvious link between immunity and vitamin C is that the cells involved in the immune response contain very high concentration of vitamin C of the order of 40 – 60 times the concentration found in the plasma. These high concentrations within the leucocytes are rapidly depleted by acute diseases, infection, trauma and chronic diseases (Basu *et al.*, 1996).

Vitamin C is stored in large amounts in the adrenal glands. Stress stimulates the release of adrenal hormones and neurotransmitters, and thus leads to the depletion of vitamin C. For this reason (along with many of the B complex vitamins, which are quite concentrated in cereal sprouts), vitamin C is considered an "anti-stress" nutrient (Baskin and Salem, 1997).

Higher than normal requirements for vitamin C have been recommended for smokers, elderly people, pregnant and nursing mothers, and for women taking birth control pills (Reed, 1980).

1.4.5 Vitamin E

It is well established that vitamin E comprises an important part of body's antioxidant defense system against free radicals such as reactive oxygen radicals (Aruoma, 1998). Although vitamin E is an excellent quencher of singlet oxygen, and reacts with $HO^{\cdot}$ at about the same rapid rate as many other biomolecules, its antioxidant activity *in vivo* is almost certainly due to its scavenging of peroxyl and alkoxyl radicals. It

has been repeatedly shown to react very fast with peroxyl radicals; its rate constants with these species are usually agreed to be the highest of all naturally occurring substances. This is the basis for its outstanding ability to act as a chain-breaking antioxidant (Gletsu and Basu, 1993).

$$LH + R^{\cdot} \rightarrow L^{\cdot}$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$\alpha\text{-tocopherol} + LOO^{\cdot} \rightarrow \alpha\text{-tocopherol}^{\cdot} + LOOH$$

$$\alpha\text{-tocopherol}^{\cdot} + LOO^{\cdot} \rightarrow LOO\text{-}\alpha\text{-tocopherol}$$

(LH – polyunsaturated fatty acid; $R^{\cdot}$ – free radical; $L^{\cdot}$ – pentadienyl radical; $LOO^{\cdot}$ – peroxyl radical; α-tocopherol$^{\cdot}$ – alpha -tocopherol radical; LOO-α-tocopherol – a resonance-stabilized conformation)

It is also demonstrated that rheumatoid arthritis patients exhibit low antioxidant status with depressed serum concentration of α-tocopherol and the oxidative damage has been implicated in the pathogenesis of rheumatoid arthritis. A study in the Annals of the Rheumatoid Disease reported that vitamin E can help in alleviating some of the stiffness experienced by arthritis sufferers (Gutteridge and Halliwell, 1994).

1.4.6 β-Carotene

β-carotene is an antioxidant. Like vitamins E and C, it can bind and reduce free radicals, which are proven to cause cell aging and, in some cases, cancer (Witz, 1991). β-carotene is also required for several components of a healthy immune system. There is strong evidence that β-carotene enhances the capability of the immune functions

including T and B lymphocyte proliferation and induction of cells capable of killing tumor cells (Basu et al. 1999).

Moreover, β-carotene has been demonstrated to reduce the risk of certain cancers, particular those of epithelial tissues, such as skin cancer and cervical cancer (Gutteridge and Halliwell, 1994). The American Cancer Society strongly recommends diets rich in β-carotene, which as shown in Table 4 is quite concentrated in wheat sprout.

Table 4 β-carotene content of selected vegetables

(in IU vitamin A activity)

Vegetable	Serving	IU per Serving	IU per 100 grams
Dehydrated Wheat Sprout	5 g (0.175 oz)	1,156	23,136
Carrots (raw)	1/2 cup	6,050	11,000
Kale (raw, finely chopped)	1/2 cup	4,565	8,300
Spinach (raw, finely chopped)	1/2 cup	2,230	7,964
Summer Squash	1/2 cup	410	390
Broccoli (raw, finely chopped)	1/2 cup	680	877
Cabbage (raw, finely chopped)	1/2 cup	60	133

(Seibold, 1990)

1.4.7 Phenolic Compounds

The health benefits of fresh fruits and vegetables, in the context of antioxidant properties, have largely been attributed to the antioxidant vitamins and carotenoids. More recently the polyphenolic components of plants have been shown to be highly efficacious antioxidants (Bourne and Rice, 1997). These compounds scavenge superoxide and hydroxyl radicals and inhibits various oxygenase enzymes such as lipoxygenase and cycloxygenase (Larson, 1997). Among the most widely distributed phenylpropanoids in plant tissues are the hydroxycinnamic acids, coumaric acid, caffeic acid, ferulic acids and vanillic acids.

Phenolic acids are part of wheat kernel cell walls linking cellulose to other polysaccharide components (Maillard and Berset, 1995). Phenylalanine ammonia lyase (PAL) is the key enzyme in phenolic biosynthesis. The major roles of phenolic acids in grains are to confer strength and rigidity to the cell walls of the husk, and enhance germination. They are also thought to limit access of microflora to otherwise fermentable polysaccharides. It has been observed that phenolic acids are effective in the protection against oxidative damage toward living cell membranes that contain polyunsaturated fatty acids during germination (Maillard and Berset, 1995). Accompanying hydrolysis and degradation of cell walls, large amounts of phenolic acids are released and synthesized, which enhance the resistance of low-density lipoprotein (LDL) to oxidation.

It has been suggested that hydroxycinnamates, such as ferulic acid and vanillic acid act mainly as peroxyl radical scavengers (Passwater, 1995). Ferulic acid might be a better radical scavenger of superoxide radical than vitamin C. It can, not only act as a

powerful antioxidant in aqueous solution in the way that vitamin C acts, but in a non-aqueous medium as vitamin E does (Castelluccio *et al.*, 1995). Besides, ferulic acid and caffeic acid are also potent inhibitors of allergic reactions (Nardini *et al.*, 1995). It was established that ferulic acid (FA) and isoferulic acid (IFA) are active components of the rhizome of *Cimicifuga* species used frequently as anti-inflammatory drugs in Japanese Oriental medicines (Hirabayashi *et al.*, 1995). By antibody sandwich enzyme-linked immunosorbent assay, it was found that FA and IFA can suppress the production of interleukin-8 (IL-8) which is the main cause of the local accumulation of neutrophils and modulates various inflammatory reactions.

1.5 Nutraceuticals and Functional Foods

"Functional Foods" and "Nutraceuticals" are broad terms that have attracted significant attention from scientific researchers, health professionals and journalists. Functional foods are generally considered foods containing significant levels of biologically active components that impart health benefits or desirable physiological effects beyond basic nutrition. Functional attributes of many traditional foods (germinated or fermented) are being discovered, while new food products are being developed to enhance or incorporate beneficial components, such as wheat sprout-added bread, breakfast cereal, and nutritional bar, etc.

Nutraceuticals are components of food materials which are known to have health benefits, e.g. antioxidants, vitamins, phenolic compounds or minerals. They may be used as ingredients for functional foods, or marketed as dietary supplement products.

With regard to the health food market, the total market for functional foods in the United States was between US$ 7.5-9 billion in 1996 (Hasler, 1996), the Japanese functional food market was over US$ 3 billion in 1993 (Wrick, 1993), the Chinese functional food market was approximately US$ 2.5 billion in 1996 (Dai and Luo, 1996), and the Canadian functional food market reached Can$ 1.0 billion in 1999 (Singtao, 1999). There is strong scientific evidence that a food supply enriched in naturally occurring healthy compounds could put a significant dent in the $200 billion annual cost of diet-related diseases in North America (Ificinfo, 1998). The demand for "healthy" food products is increasing.

"Phytochemical" is a more recent evolution of the term of nutraceutical. It emphasizes the plant sources of most of these protective, disease-preventing compounds, e.g. vitamins C, E, carotenoids, flavonoids, phenolic compounds which could be incorporated into "functional foods". The National Cancer Institute's experimental foods program has identified whole wheat, soy, flax, brown rice, and oats as phytochemical-containing foods worthy of further study because of their potential to block specific metabolic reactions leading to the formation of malignant tumors (Wrick, 1993).

A large body of credible scientific research is needed to confirm the benefits of any particular food or component in functional food. In order to realize the potential public health benefits of wheat sprout, it is necessary to have a clear understanding of its composition with particular reference of its antioxidant content. It is also important to determine the condition and the length of germination when the maximum levels of these antioxidants are reached.

1.6 Objectives of the Research

The primary objectives of this study were to evaluate antioxidant contents in wheat grain before and after germination for various lengths of time, and to develop a functional food product containing the germinated wheat. It is anticipated that germinated wheat, grown in Alberta, will become an important ingredient in products, such as chewing gum, nutritional bar and other functional foods. These products may help modify antioxidant status and thereby be protective against many chronic degenerative diseases, such as coronary heart disease and cancer.

CHAPTER 2

GERMINATION OF WHEAT GRAINS AND ANALYSES OF ANTIOXIDANT CONTENTS IN GERMINATED WHEAT

2.1 Introduction

In comparison to cereal grains and their-products, cereal sprouts are believed to have a greater nutritive value (Price, 1988). Several nutritive factors such as vitamin concentrations and bioavailability of trace elements and minerals increase during germination (Lintschinger *et al.*, 1997), while stacchyose and raffinose which are generally assumed to be responsible for flatulence, decrease during this process (Colmenares De Ruiz and Bressani, 1990). At the same time, sprouting of cereals, such as wheat modifies the taste and texture, and thus the sprouting wheat may become potentially a new food source to enhance human nutrition (Finney, 1978).

It has been reported that the nutrient concentrations of cereal sprouts depend mainly on the growing conditions (temperature, humidity, culturing media, steeping and germination time) and the growth stage at which the sprouts are harvested, rather than on the type of cereal (Price, 1988). However, reports on optimal germination time vary (Tkachuk, 1979). Some preferred 12 hours germination after 12 hours steeping; some germinated wheat for 5-7 days after 24-48 hours steeping; while some grew the sprouts in green house for another 5-7 days before the green grass was harvested. In these studies, no marker was used to determine the optimal duration for germination. The present study was undertaken to determine the optimal germination time of wheat grain in terms of its contents of some antioxidants.

2.2 Germination Process and Sample Preparation

Wheat, grown in 1997 at Forgotten Creek Farm, Alberta, was used. Control of microbial growth is critical in wheat germination process and hygiene conditions were maintained by such practices as treating germination vessels, holding containers and equipment with boiling water, and use of NaClO for grain and equipment treatment. A 1.25% NaClO surface disinfecting solution was prepared by diluting commercial household bleach (Javex). A thermometer was part of the assembly to monitor the temperature. Germination was carried out with tap water summarized in Table 5. Prior to germination, the wheat grains (approx. 5 kg) were submerged in 1.25% NaClO (seed: water ratio of 1:5, W/V) at room temperature for half an hour to disinfect any contaminating microorganisms on the grain surface. Tap water at approximately 10 °C

was then run over the grains for 1 hour to thoroughly rinse off residual NaClO. The disinfected grains were weighed into two batches, one steeped in tap water at 16.5 °C for 24 hours and the other for 48 hours, to investigate the relationship between steeping time and germination process. For sprouting, approximate 1 kg of wheat grains were placed into each aluminum foil pan with draining holes at the bottom to keep the grains at a constant moisture without overwatering them. Germination was accomplished in the dark incubators at 98% relative humidity and 16.5 °C for various lengths of time (1-7 days).

Germination at 16.5 °C instead of at ambient temperature, was found to produce more amino acids and antioxidants (Hesterman and Teuber, 1980). Light is not required for seed germination or sprouting and production of white or etiolated sprouts is usually carried out in total darkness, because activation of chloroplasts by light results in "greening" of the etiolated hypocotyledons (Price, 1988). The maintenance of high humidity (98%) helped to reduce the drying of the sprouts between watering periods. The grains were wrapped with sterile cheese-cloth to maintain the moisture. The total germination length included the time for steeping (soaking) and the time for sprouting. The grains were sprinkled with tap water for 15 min at 12 hours intervals and turned (aerated) by hand once every 24 hours in order to (i) prevent rootlets matting, (ii) ensure that on average all grains were treated as nearly evenly as possible, (iii) break up hot spots and (iv) "lighten the piece" (i.e. reduce its bulk density and ensure the grain bed saturated with water vapor).

The germinated wheat was harvested for antioxidant analyses by removing 250 g per lot at various times (1- 9 days). After the harvest, all sprouts were washed carefully with distilled water (3 x 800 ml) to remove the testas. All samples were freeze dried in a

REPP freeze drier (The Vir Tis Co. Inc., Gardiner, N.Y.) to a moisture content of 4%. Prior to drying, samples were frozen for 2 hours at –45°C. Drying occurred at –5°C and a pressure of 0.31 milli bar. Afterwards, the dry samples were ground in a UD-Cyclone mill and stored at –30°C in plastic zipper bags before the analysis. The latter included vitamin C, vitamin E, carotenoids such as β-carotene and lycopene, phenolic compounds, and superoxide dismutase.

Table 5 Germination conditions

Treatment	**Conditions**	**Time**
Disinfection	Submerging wheat grains in 1.25% NaClO at room temperature	0.5 h
Washing	Running tap water over the grains at approximately 10 °C	1 h
Steeping	Submerging the wheat grains in tap water at 16.5 °C	24 or 48 h
Germination	In the dark, at 98% relative humidity with temperature of 16.5 °C, the wheat grains were irrigated and turned by hand once every 24 hours.	1- 8 day

2.3 Antioxidant Vitamin Analysis

2.3.1 Vitamin C

Vitamin C was analyzed according to the AOAC (Association of Official Analytical Chemists) Official Method 967.21 with some modifications. Dehydrated wheat sprout instead of fresh one was used. The chemicals used in this assay included 2,6- DCPIP (dichlorophenolindophenol) solution, ascorbic acid standard solution (0.02 mg/ml), acetic acid glacial, deionized water and TCA (Trichloroacetic acid 5%).

One gram of ground freeze dried wheat flour (n=3) was homogenized with 5 ml of 5% TCA and subsequently diluted to 30 ml in a measuring cylinder with deionized water, and centrifuged. 5 ml of diluted supernatant was transferred into an Erlenmeyer flask containing 1 ml of glacial acetic acid. This mixture was then titrated with the diluted 2,6-DCPIP solution to a faint rose-pinked color. To prepare 2,6-DCPIP standard solution, 250 mg of 2,6-DCPIP (sodium salt) was first dissolved in 250ml water containing 210 mg $NaHCO_3$, and then diluted to 1L with water. The mixture was filtered into a dark (amber) glass bottle. The filtrate was stored in the cold until used. Just before use, it was accurately diluted to 1/16.

The titrations of 5 ml of deionized water with 1 ml of glacial acetic acid added for the blank (Bl), 5 ml of ascorbic acid standard solution with 1 ml of glacial acetic acid added for the standard (St) and 5 ml of germinated wheat sample (T) were carried out. The vitamin C content of the test sample was then calculated using the equation shown below:

Vitamin C of test sample (mg/100ml) = (T- Bl) / (St- Bl) x 2

2.3.2 Vitamin E

Analysis of vitamin E vitamers in germinated wheat comprised separation and individual quantification of the corresponding tocopherols.

Wheat samples of different germination times (n=3) were collected, freeze-dried, ground, packaged in zipper bags and stored at -30° C till use. All the reagents used were HPLC grade. The tocopherol content was measured as described by Zaspel and Csallany (1983). External standards (α-,γ-, δ- tocophrol) were purchased from SIGMA (Steinheim, Germany).

The tocopherol content of wheat sprout was determined according to Indyk (1988) with some modifications. For example, sodium hydroxide was chosen to substitute potassium hydroxide for practical purpose. Extraction was preceded by alkaline saponification. 1 g of each wheat samples were weighed into a 50 ml screw capped tubes. Ethanol (10 ml) containing 0.1% pyrogallol was added to each tube and vortexed. Sodium hydroxide solution (2 ml) (40% wt/vol) was added and the tubes were incubated for 30 min at 70°C. Following cooling, 10 ml of hexane: ethyl acetate (9:1, vol/vol) and 10 ml of 1% sodium chloride were added and the tubes inverted 10 times. The upper layer was taken and evaporated under vacuum at 60°C. The dried sample was then redissolved with 5 ml of methanol, vortexed, centrifuged at 1,000 x g for 10 min, and the clean upper layer was collected. To protect vitamin E from light, both standard and extract bottles were wrapped in aluminum foil and kept at +4°C for a maximum of 24 hours before analysis.

HPLC analysis was performed by isocratic elution. A Varian 5000 liquid chromatography system with a Shimadzu SIL-9A model autosampler (75µl/injection) was used along with a Supelcosil, 3 µm RP LC-18 column (150 mm x 4.6 mm) and a guard column (5 cm, 20-40 µm LC-18 packing). The flow rate was set at 1.5ml/min. The mobile phase consisted of methanol: acetonitrile (50:50 vol/vol). Detection was performed with a Shimadzu RF-535 fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. In the detection of tocopherols and tocotrienols, fluorescence detection is preferable to UV detection because of its higher sensitivity and specificity. There are very few lipid components in foods with the chromatographic properties and fluorescence characteristics of tocopherols and tocotrienols. A Shimadzu EZChrom chromatography data system (Kyoto, Japan) was used to integrate peak areas.

2.3.3 β-Carotene

Wheat samples of different germination times (n=3) were collected, freeze-dried, finely ground, packaged in zipper bags and stored at -30°C till use. All the reagents used were HPLC grade. The β-carotene content was measured as described by Heinonen *et al.* (1989). External standard (β-carotene) was purchased from SIGMA (St. Louis, MO, USA).

The β-carotene content of wheat sprout was determined after saponification (Indyk, 1988). The wheat samples were treated in the same manner as described for vitamin E determination (section 2.3.2). After the dried sample of extract was redissolved

and centrifuged, the clean upper layer was collected for β-carotene assay. The extracts changed color with increasing germination times from white and light yellow to yellow and brown. To protect β-carotene from light, both standard and extract bottles were wrapped in aluminum foil and kept at +4°C for a maximum of 24 hours before analysis.

HPLC analysis was carried out by isocratic elution. A Varian 5000 liquid chromatography system with a Shimadzu SIL-9A model autosampler (75µl/injection) was used along with a Supelcosil, 3 µm RP LC-18 column (150 mm x 4.6 mm) with a guard column (5 cm, 20-40 µm LC-18 packing). Solvent A was acetonitrile, tetrahydrofuran and H_2O (50: 20: 30); solvent B was acetonitrile, tetrahydrofuran and H_2O (50: 44: 6). The H_2O in each solvent contained 1% ammonium acetate and 0.35% acetic acid, which provided sufficient buffer capacity (assuming that acidity is the factor critical to recovery). Flow rate was set at 1ml/min. Detection was performed with a Varian UV-200 detector at an excitation wavelength of 453 nm. A Shimadzu EZChrom chromatography data system (Kyoto, Japan) was used to integrate peak areas.

2.3.4 Phenolic Acids

High performance liquid chromatography (HPLC) has found wide applications in the separation and identification of phenolic compounds in plant extracts (Mueller-Harvey *et al.*, 1982; Pussayanawin and Wetzel, 1987; and McMurrough *et al.*, 1984). The retention data of phenolic acids separated by HPLC have also been complied by Banwart *et al.* (1985).

Wheat samples of different germination times were collected, freeze dried, homogenized, packaged in zipper bags, and stored at -30°C till use. All the reagents used were HPLC grade. The phenolic acid contents were measured as described by Maillard and Berset (1995). External standards (ferulic acid, coumaric acid and vanillic acid) were purchased from SIGMA (Steinheim, Germany).

Extraction was preceded by alkaline saponification. The alkaline hydrolysis was preferred to an acid hydrolysis. Since alkaline hydrolysis greatly reduced the yield of cinnamic derivatives, better resolution resulted (Glennie, 1984).

The wheat sprout contents of phenolic acids were determined following the method described by Maillard and Berset (1995) with some modification. For example, to save NaOH solution, 0.25 g of each wheat samples (n=3) instead of 1 g were weighed into a 50 ml screw capped tubes and hydrolyzed with 12.5 ml (instead of 50 ml) of 2 N NaOH under N_2 at ambient temperature for 4 h. The mixture was then acidified with 2 N HCl at pH 1 and extracted three times with ethyl acetate (v/v). The ethyl acetate phase was collected and then evaporated to dryness at 40°C under vacuum and the residue was dissolved in 0.5 ml of methanol for HPLC analysis.

A Varian 5000 liquid chromatography system with a Shimadzu SIL-9A model autosampler (75µl/injection) was used along with a Supelcosil, 3 µm RP LC-18 column (150 mm x 4.6 mm) with a guard column (5 cm, 20-40 µm LC-18 packing). The method involved two linear gradients and an isocratic step. Solvent A was water adjusted to pH 2.6 by orthophosphoric acid and solvent B was acetonitrile. The first linear gradient began with 100% A – 0% B and went to 90% A -10% B in 10 min. From 10 min to 37 min, the composition was held unchanged. The second linear gradient ran from this

composition to 85% A – 15% B in 15 min. From 52 min to 90 min, the column was washed and re-equilibrated to initial conditions (100% A – 0% B). The solvent flow rate was set at 0.8 ml/min. Detection was performed with a Hewlett-Packard 1040 M photodiode array detector. The two main hydroxycinnamic acids were quantified at their maximal absorbances using an external standard method at wavelength of 322 nm. EZChrom chromatography data system (Kyoto, Japan) was used to integrate peak areas.

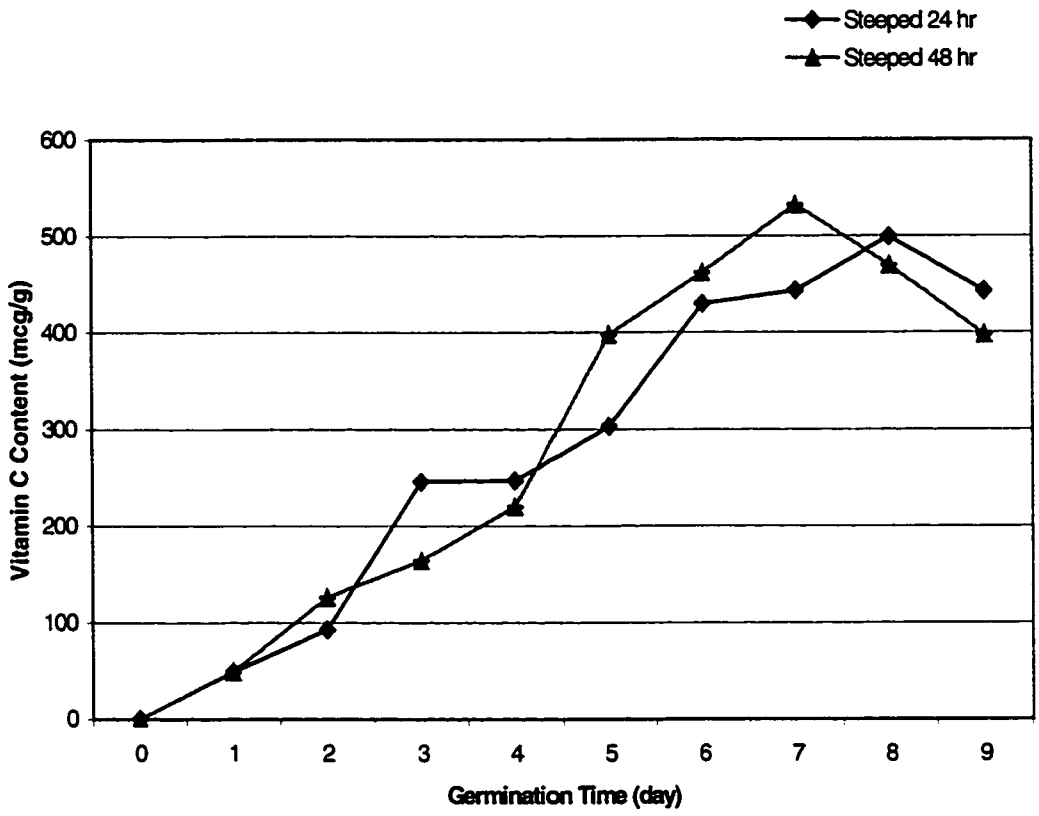
Peak retention times and areas were monitored and computed automatically. A calibration mixture was used to calculate the response factors by dividing the known weight of a phenolic acid standard by its corresponding peak area. Phenolic acids in the extract were quantified from peak areas through the calculated response factors.

2.4 Results

2.4.1 Vitamin C

Figure 4 shows that after steeping (soaking) in tap water for 24 h or 48 h, vitamin C content in wheat grain was greatly increased during the germination, while no vitamin C was found in dry wheat grain (control). However during sprouting, the vitamin content was gradually increased as the germination time increased by a almost linear characteristic curve and reached the peak at day 7 (550 μg/g). The steeping time had a positive effect on the production of vitamin C during wheat germination (Figure 3). More vitamin C was thus produced after 48 h than after 24 h steeping ($n=3$, $P<0.05$).

Figure 3 Effect of steeping & germination time on the vitamin C content of wheat grain

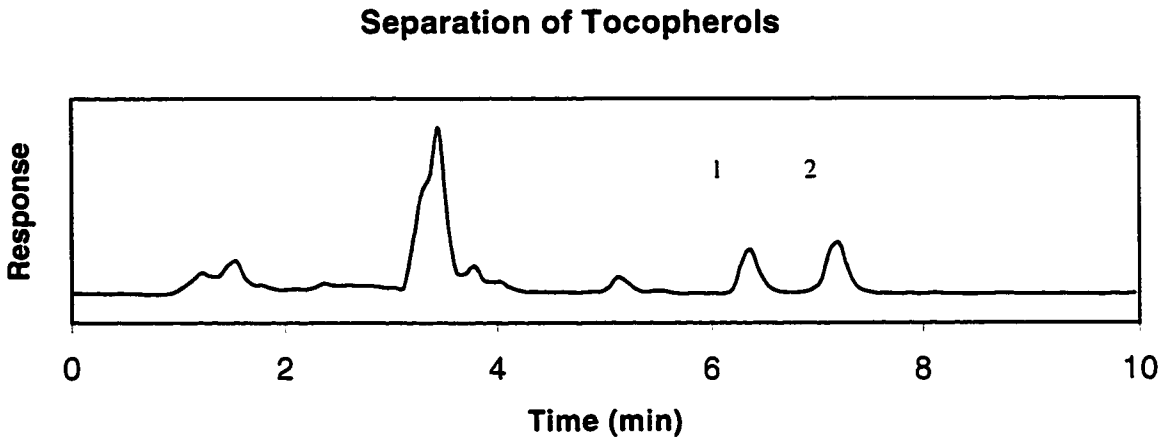


2.4.2 Vitamin E

The influence of germination on vitamin E content of wheat was examined. The chromatographic separation of the vitamin E vitamers in wheat grain is shown in Figure 4. This sample contained α- and γ-tocopherol. No δ-tocopherol was identified.

Both α- and γ-tocopherol were measured before and after germination for 1-9 days. After steeping for 24 h or 48 h, both α- (Figure 5) and γ- (Figure 6) tocopherol contents in wheat sprout were markedly increased during the germination process. Vitamin E content in ungerminated wheat grain (control) was very low, with 4.37 μg/g of α-tocopherol and 0.914 μg/g of γ-tocopherol. During sprouting, vitamin E content gradually increased as the germination time increased and reached the peak at day 8 (10.92 μg/g of α-tocopherol and 1.5 μg/g of γ-tocopherol). More vitamin E was produced after 24 h steeping than after 48 h steeping ($n=3$, $P<0.05$).

Figure 4 Nonaqueous reverse-phase chromatogram of germinated wheat



Peak identification: 1. γ-tocopherol; 2. α-tocopherol.

Figure 5 Relationship between the length of steeping & germination and α -tocopherol content of wheat

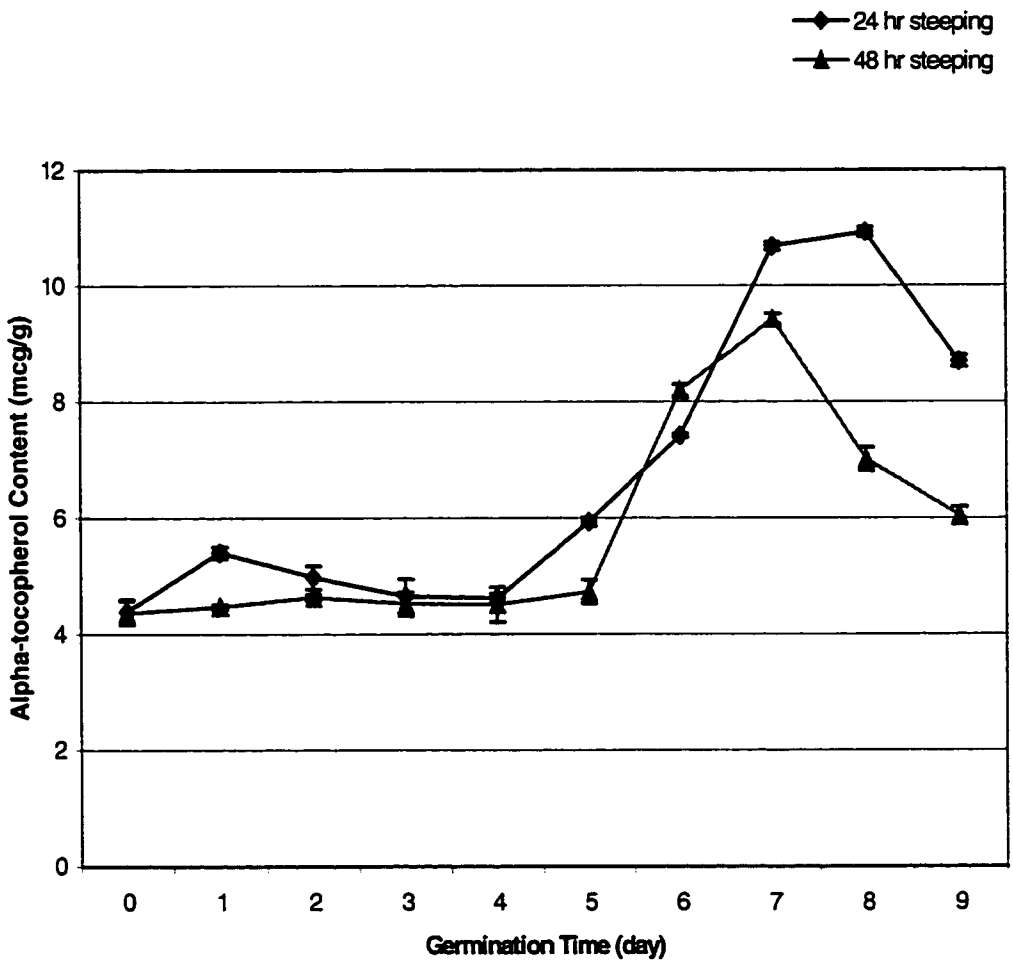
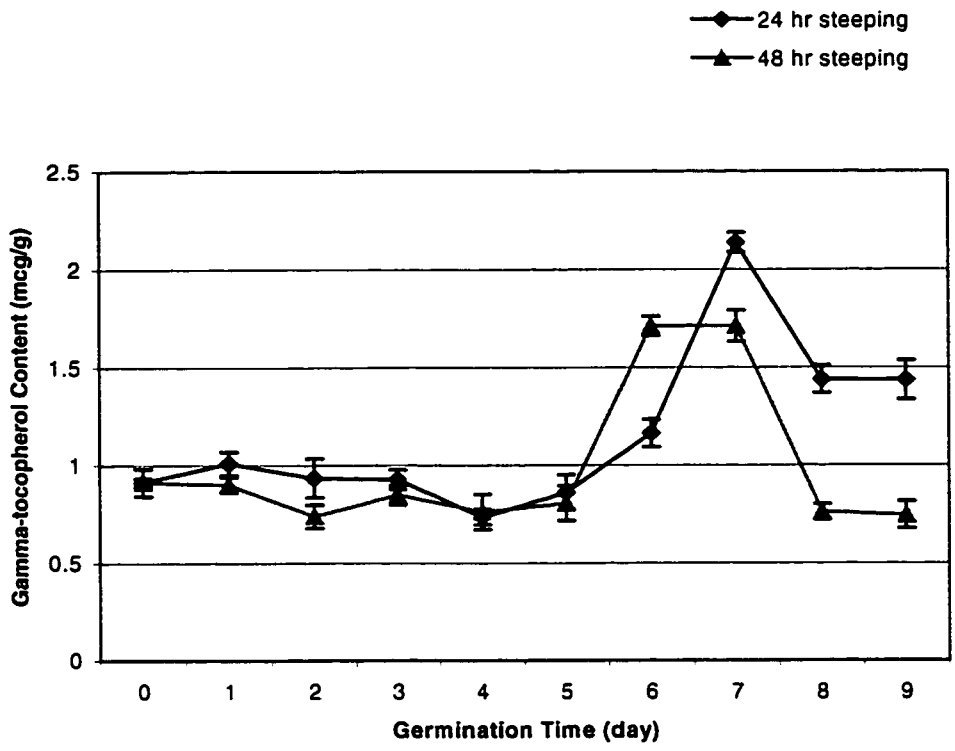


Figure 6 Relationship between the length of steeping & germination and γ- tocopherol content of wheat



2.4.3 β-Carotene

Figure 7, 8 show that after steeping for 24 h or 48 h, β-carotene content in wheat sprouts was greatly increased during the germination process. Original β-carotene content in wheat grain (control) was undetectable. During germination, β-carotene content gradually increased as germination time increased and reached the peak at day 8 As for vitamin C, steeping time has some effect on the production of β-carotene. After 48 h steeping, wheat sprout appeared to produce more β-carotene than when steeping for 24 h ($n=3$, $P<0.05$).

2.4.4 Phenolic Acids

The influence of germination on phenolic acid content of wheat was examined. The chromatographic separation of phenolic acids in wheat grain is shown in Figure 9. This sample contained two phenolic acid, ferulic acid and vanillic acid. No other polyphenols were detected.

Both ferulic acid and vanillic acid were measured before and after germination for 1-9 days. After steeping (soaking) for 24 h or 48 h, both ferulic acid (Figure 10) and vanillic acid (Figure 11) contents in wheat sprouts were markedly increased during germination. In the first four days, the total phenolic acid contents slightly decreased as most of the water-soluble free phenolic acids were leached out during the washing and steeping process. However, after five days of germination, both phenolic acid contents gradually increased and reached the peak at day 8 (932.4 μg/g for ferulic acid and 13

μg/g for vanillic acid). Figure 10 and 11 also show that steeping time has an effect on the germination process. After 24 h steeping, wheat sprout appeared to produce a little more phenolic acids than when steeping for 48 h.

Figure 7 Nonaqueous reverse phase chromatogram of germinated wheat

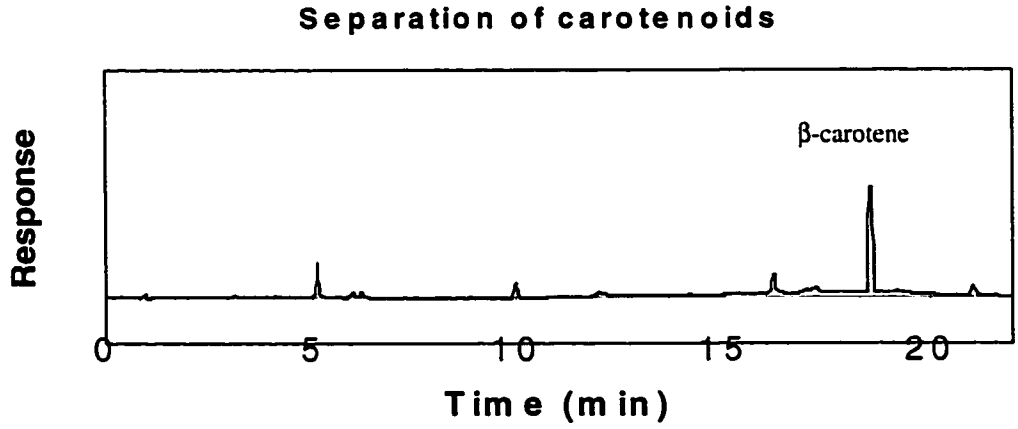


Figure 8 Effect of steeping & germination time on the β-carotene content of wheat grain

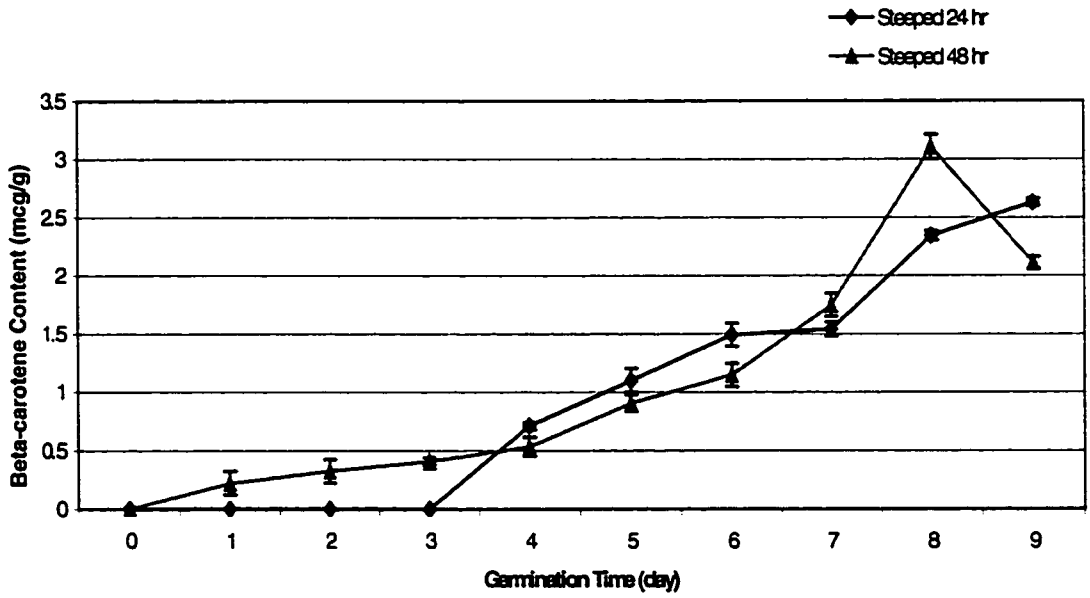
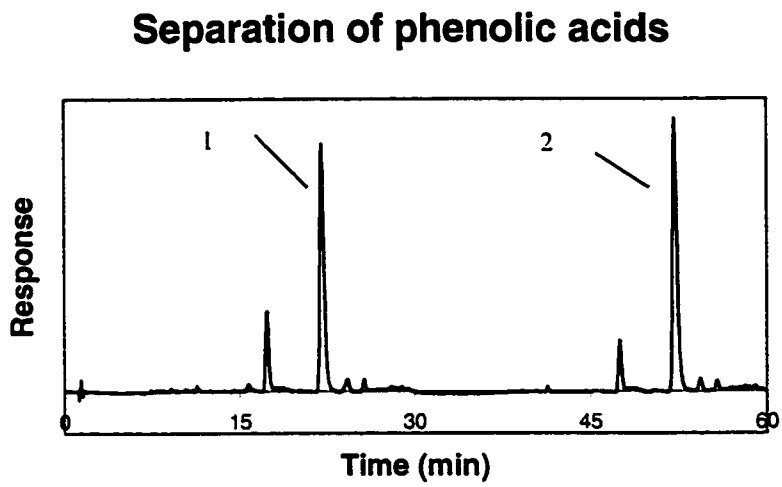


Figure 9 Reverse phase chromatogram of germinated wheat



Peak identification: 1. vanillic acid; 2. ferulic acid

Figure 10 Relationship between the length of steeping & germination and ferulic acid content of wheat

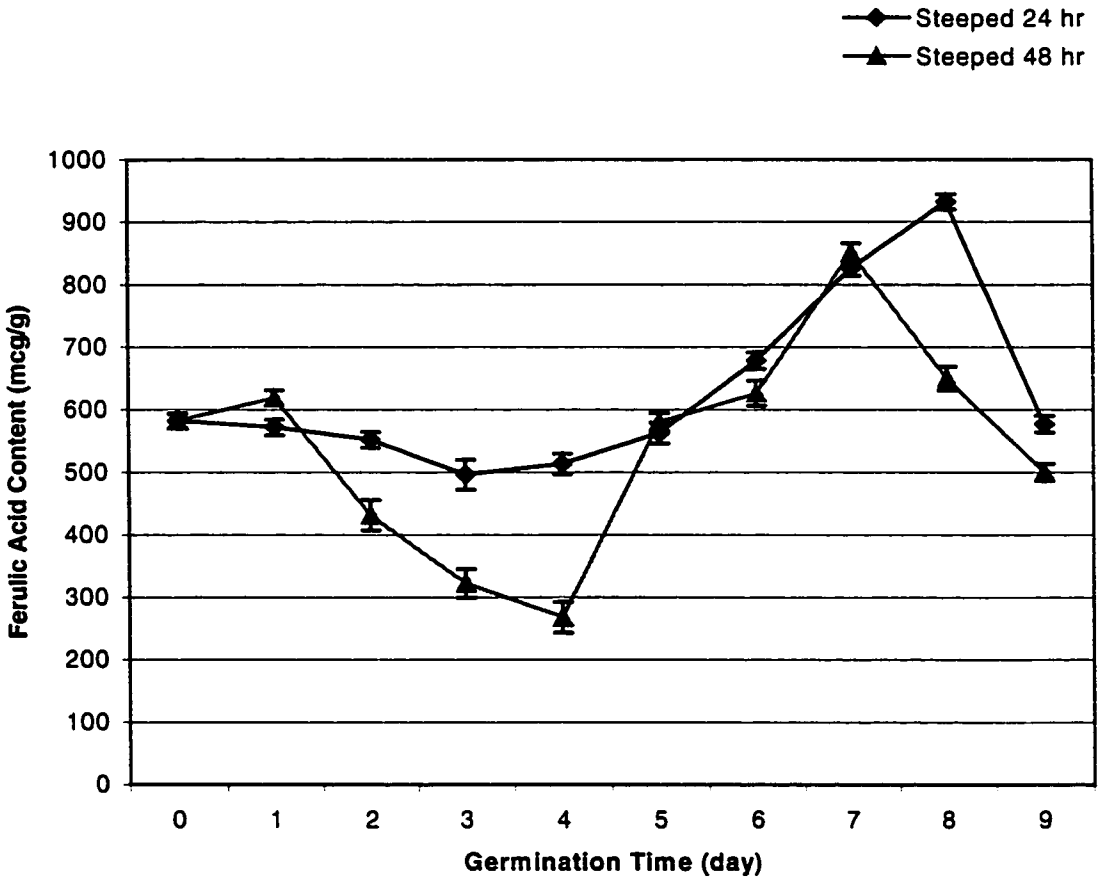


Figure 11 Relationship between the length of steeping & germination and vanillic acid content of wheat

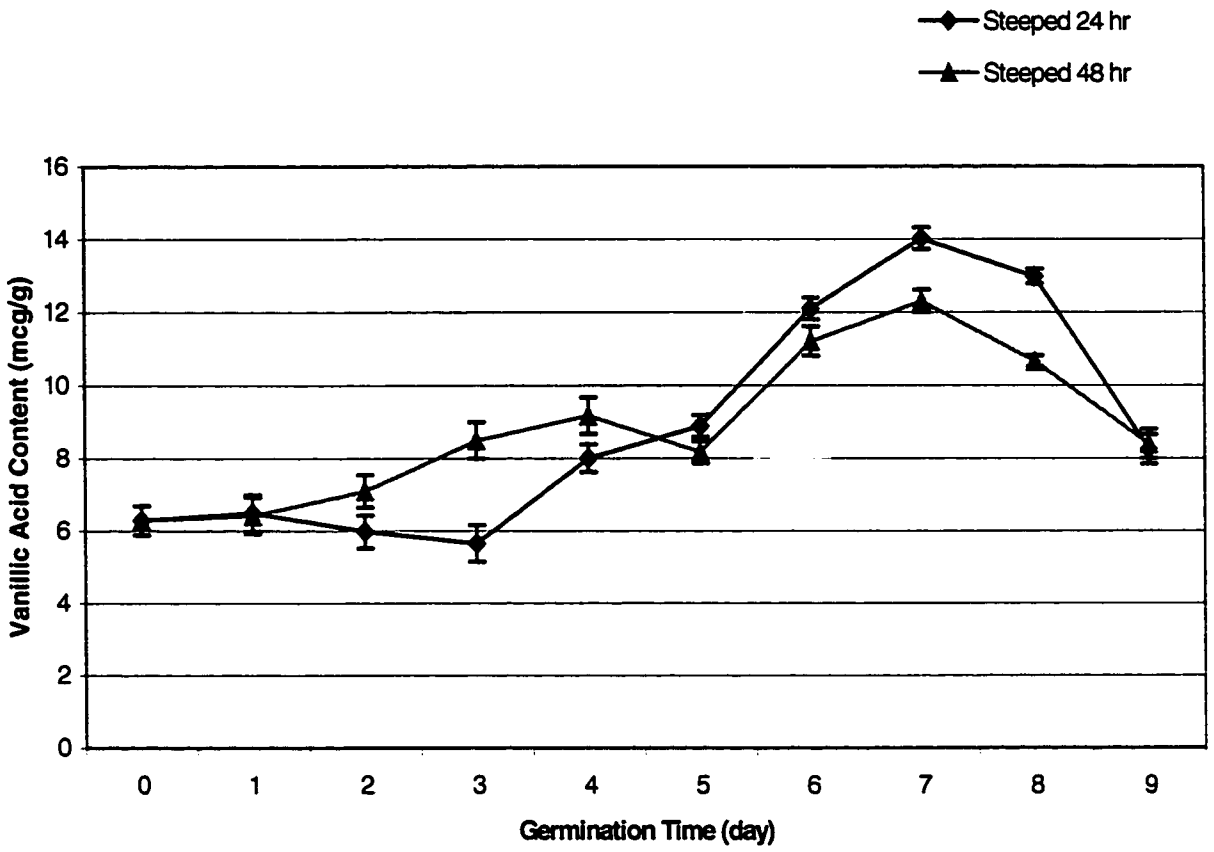


Table 6 Effect of steeping time on 7 day germination properties

*: Each value is significantly different (n=3, $P<0.05$).

	Steeped 24 h	Steeped 48 h
Vitamin C content	499.3±0.6 μg/g	469.8±0.2 μg/g *
α-tocopherol content	10.92±0.08 μg/g	7.04±0.2 μg/g *
γ-tocopherol content	1.5±0.07 μg/g	0.8±0.04 μg/g *
β-carotene content	2.6±0.04 μg/g	3.1±0.1 μg/g *
ferulic acid content	932.4±12.4 μg/g	650±19.3 μg/g *
vanillic acid content	12.9±0.2 μg/g	10.6±0.15 μg/g *
General Appearance	Dry, sweet smell, Yellow Not sticky	Wet, sour smell, Yellow-brown Sticky, bruised-looking

2.5 Discussion

This study examined the modifying effect of germination on the content of a selected group of antioxidants in wheat grain. The antioxidant included were vitamin C, vitamin E, β-carotene, and phenolic acids. Germination was carried out in the dark at 98% relative humidity with 16.5 °C for 1-9 days. The antioxidant content was increased with the length of germination; the peak level reached by each of the antioxidant was after 7^{th} day of germination following 24h steeping. These results are in agreement with others (Harmuth-Hoene, 1987; Heinonen *et al.*, 1989; and Cole *et al.*, 1983), who observed similar responses, especially for vitamin C and β-carotene. The increase of vitamin C content was characterized by an almost linear curve (Figure 4), which reached its peak concentration of 550 μg/g at day 7. Since this vitamin was found to be absent in dry wheat grain, its production may be taken as a representative parameter for metabolic germination process to improve the nutritional value of wheat grain (Lintschiger *et al.*, 1997). It was observed that the methanol extracts of β-carotene changed color from white and light yellow to yellow and brown as germination duration increased. This color change was in parallel with an increased concentration of β-carotene content during germination process.

Phenolic acids (Figure 10 and 11) were by far the largest contributor to the antioxidant contents in wheat sprout. In the first four days, free ferulic acid was leached out during watering process, however after 5 days, ferulic acid content accumulated due to phenolic biosyntheses and hydrolysis of polyphenolic compounds bound to cell walls (Hatcher and Kruger, 1997). It was reported that phenylalanine ammonia lyase (PAL) is

the key enzyme in these phenolic biosyntheses (Maillard and Berset, 1995). A significant decrease in tannin content during germination period may also contribute to the great increase of these phenolic acids (Cole *et al.*, 1983). It is believed that ferulic acid (FA) and isoferulic acid (IFA) are active components of the rhizome of *Cimicifuga* species used frequently as anti-inflammatory drugs in Japanese Oriental medicines (Hirabayashi *et al.*, 1995). By antibody sandwich enzyme-linked immunosorbent assay, it was found that FA and IFA can suppress the production of interleukin-8 (IL-8) which is the main cause of the local accumulation of neutrophils, and modulates various inflammatory reactions. This finding may open a new approach to some chronic inflammatory disease treatment by consuming germinated wheat.

The biosynthesis of antioxidants during germination is strongly dependent on time-dependent uptake of the trace elements and water (Lintschinger *et al.*, 1997). Germination starts with the uptake of water (imbibition) by a wheat kernel that has lost its post-harvest dormancy. Plant development is resumed once the embryo is fully imbibed. It was observed that after 12 hours of germination chitting occurred (See Figure 12). Within the second and third day of germination, the radicle and coleoptile (sprout) emerged from the seed. During this period, trace element uptake and antioxidant synthetic rates were relatively low as a result of saturation phenomena due to a balanced concentration gradients and/or a totally covered surface (Lintschinger *et al.*, 1997). After 4-5 days, the first three seminal roots were produced and then the coleoptile elongated dramatically, pushing the growing point upwards (Fowler, 1993). The fast growth of coleoptile and roots allowed a much faster uptake of water and trace elements essential to the biosynthesis of antioxidants. Possible ligands for the trace elements and a star-burst of

enzymes were produced, which could further enhance the biosynthesis of antioxidant in wheat grain. Consequently, there occurred an exponential increase in antioxidant levels of α- and γ- tocopherol, ferulic acid and vanillic acid. When the germination procedure was prolonged to 8 days, the length of wheat sprout (coleoptile) was 3 cm and the length of wheat root was 2 cm as shown in Figure 12. Grain at this point reached its 100% germination. Thereafter, since water and other nutrients for the further elongation of wheat sprout could not be sufficiently supplied, the sprout dried out quickly between 12 hours watering periods and antioxidant biosynthesis dramatically slowed down due to the loss of cell surface water.

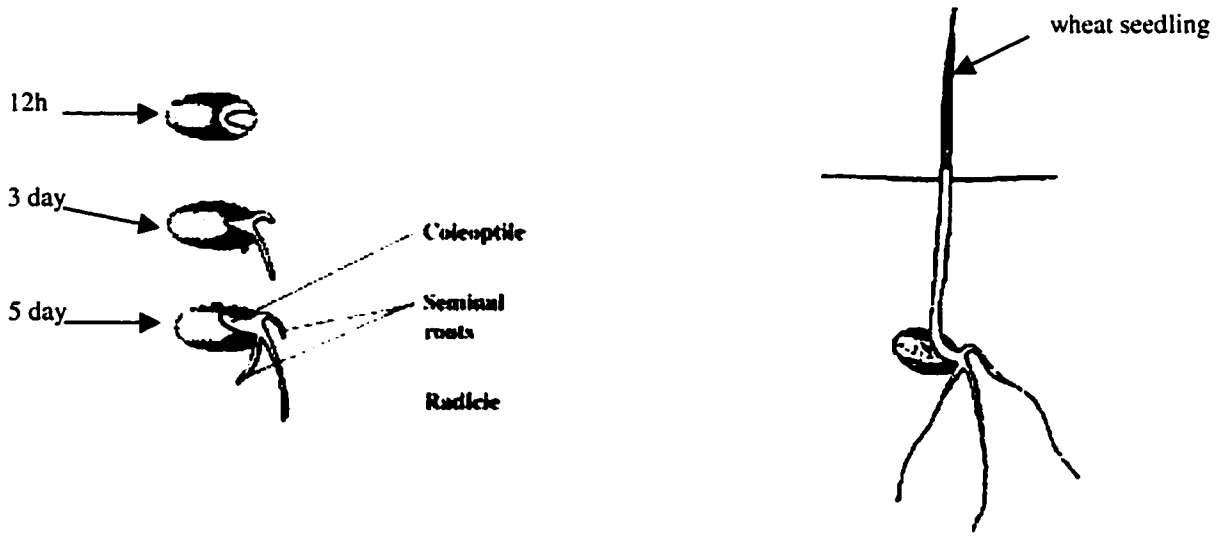
As shown in Table 6, the difference in antioxidant contents and general appearance of wheat sprout after 8 days germination were attributed to different steeping time (24 or 48 hours). It is possible that this steeping time related variation in antioxidant contents, vigor, and wholesomeness are either due to water sensitivity or lack of adequate oxygen. Excess steeping (i.e. more than 24-48 hours) even with vigorous aeration and water draining, results in excessive water absorption, slower sprouting rate and decay, which are particularly detrimental (Finney, 1978 and Price, 1988). Although 48 h steeping reduced the growth of wheat roots due to its higher surface water content (Finney, 1978), when germination times prolonged to 9-10 days, the sprout became rather sticky because of excessive metabolism. As a result, antioxidant levels may have been decreased quickly (e.g. β-carotene).

Optimal steeping period appears to be an important factor for water uptake. According to the factors, such as degree of sprout growth, hardness of sprout, sensory acceptability and antioxidant contents, this study suggests that the optimal condition for

germination to increase antioxidant quality of wheat grain is 7 days of seed germination following 24 hours steeping in dark and with twice watering per day.

Figure 12 Wheat germination

(Fowler, 1993)



α- and γ-tocopherol were the dominant vitamin E vitamers identified in this study. No δ-tocopherol (Figure 4) was found. These results are in agreement with others (Barnes,1983). Vitamin E concentration quantified in this study was, however, 10 folds higher than that (0.3 μg/g) published by Seibold (1990). Since only reversed phased HPLC was available for this study to separate vitamin E vitamers, no tocotrienols were identified. Thompson and Hatina (1979), Barnes and Taylor (1980) reported that HPLC on silica was the most suitable method to separate all the vitamin E vitamers (including 4 tocotrienols) from food products and reversed phase HPLC, on the other hand, failed to separate all the vitamers (Vatassery *et al.*, 1978 and Westerberg *et al.*, 1981).

Compared with the concentration (77 μg/g) determined by Seibold (1990), the β-carotene content quantified in this study is relatively low. There are several factors contributing to this big variation. First, in this study, antioxidant was extracted from freeze dried ground samples, whereas, theoretically wet non-treated samples are used for extraction to protect the antioxidant contents. Second, β-carotene are unstable in the presence of light and some may be oxidized during sample preparation or elution process. Third, the biosynthesis of antioxidants during germination is dependent on various parameters, such as type of seeds, temperature, light conditions, air exchange and moisture (Price, 1988 and Sattar *et al.*, 1989), absolute concentrations may vary between different experiment. Seibold (1990) cultivated the sprout in light for another several days after germination and found that light was a very important factor in the biosynthesis of β-carotene in germinated wheat. Farhangi and Valadon (1983) and Farhangi (1980) also reported that exposure of etiolated bean sprouts to artificial light for 24 hours increased provitamin A content; vitamin C content was unaffected but

provitamin A increased from 18 to 321 μg β-carotene/100 g fresh weight. It is possible that certain germination condition (e.g. light, temperature, soaking time, *etc.*) could be modified for wheat grain to increase the production of a specific antioxidant nutrient.

This study suggests that antioxidant (ascorbic acid, tocopherols, carotenoids, and phenolic compounds) may play a crucial role in seed dormancy breakage and germination (Fontaine *et al.*, 1995). Antioxidant compounds synthesized during germination are essential to the protection of the new seedling by reducing the rate of initiation or preventing the propagation of free radicals created during germination, and thereby inhibiting the oxidation of LDL and damage toward new seedling cell membranes (Osawa *et al.*, 1985). Phenolic compounds enjoyed the greatest share of antioxidant contents in germinated wheat. Synergistic effect between phenolic antioxidants has been reported, which can dramatically increase the antioxidant activity of germinated wheat (Maillard and Berset, 1995).

Generous consumption of various whole grains, fruits and vegetables, including their germination or fermentation products has been recommended by nutritional experts (Slavin *et al.*, 1997). Evidence to date suggests that the antioxidant nutrients can work together synergistically and contribute to the total antioxidant protection of human body. Tocopherols play the most effective role in scavenging free radicals; but their activities depend upon the presence of other factors, such as their reactivity and concentrations as well as the availability of their regenerating systems, including ascorbic acid, β-carotene and selenium. Ascorbic acid, on the other hand, requires cellular thiols such as glutathione in order to be regenerated from its radical form (Basu *et al.*, 1999).

It is possible that consumption of wheat sprout provides various highly concentrated antioxidants, including vitamin C, β-carotene, vitamin E and phenolic acids to the tissues. The wheat sprout may also scavenge free radicals in the body, lowers plasma cholesterol (LDL-cholesterol) in hypercholesterolemic subjects, and alters immune function by reducing the inflammatory response in patients with autoimmune diseases (Seibold, 1990).

CHAPTER 3

DEVELOPMENT OF A NUTRITIONAL BAR

3.1 Introduction

Results of the current research as presented in Chapter 2 and some other independent research (Seibold, 1990) have shown that wheat grains when sprouted create a large amount of antioxidants (vitamins C, E, β-carotene, phenolic acids), minerals, and dietary fibers. A greater understanding of the etiology of autoimmune diseases and their relationship with free radicals has opened the way to antioxidant therapy. Consumption of wheat sprout would provide a redox buffer, which is critical to this therapy.

The majority of current wheat studies have emphasized the utilization of wheat flours and the multiplicity of products derived from them. The National Cancer Institute's experimental foods program has identified whole wheat, soy, flax, brown rice, and oat as phytochemical-containing foods which have the potential to block specific metabolic reactions leading to some chronic degenerative diseases (Wrick, 1993). Moreover, development of food products from germinated wheat may be another way to further increase the versatility and utility of whole wheat food. Alberta has an abundant supply of wheat, which could be sprouted and processed as functional foods. However, presenting the sprouted wheat grains in a sensorily acceptable form is a challenge.

Traditional food products such as breakfast cereals, snacks, chewing gums, drinks, baked goods, *etc.* may be good carriers for these functional ingredients from the germinated wheat. The objective of this section was to design a nutritional bar in which freeze dried germinated wheat powder constituted an important element.

3.2 Materials and Methods

3.2.1 Materials

The use of food ingredients is based primarily on their functionality. The functional properties, which reflect mainly the quantity and quality of the protein, carbohydrate, vitamin, mineral and dietary fiber content, are required for selecting suitable utilization in food preparation (Hollingsworth, 1997).

Wheat sprout was chosen as a good source of various vitamins, protein and dietary fiber. It contains no flatulent factors, such as stachyose and raffinose, which are converted to vitamin C during germination (Wong and Yen, 1997). At the same time, as trypsin inhibitor activity is decreased, protein digestibility is increased.

The following ingredients were purchased from local stores:

Crushed, roasted, salted soybeans

Marshmallow

Crispy Rice

Instant skim milk powder

Honey

Low fat raisin granola

Chocolate flavor powder

Coconut Flavoring Extract

Vanilla Flavoring Extract

To investigate wheat and soybean complimentarity, crushed, roasted, salted soybeans were chosen, since it was reported that soybeans can supplement wheat protein and greatly increase their nutritive value (Finney, 1978).

3.2.2 Processing Method

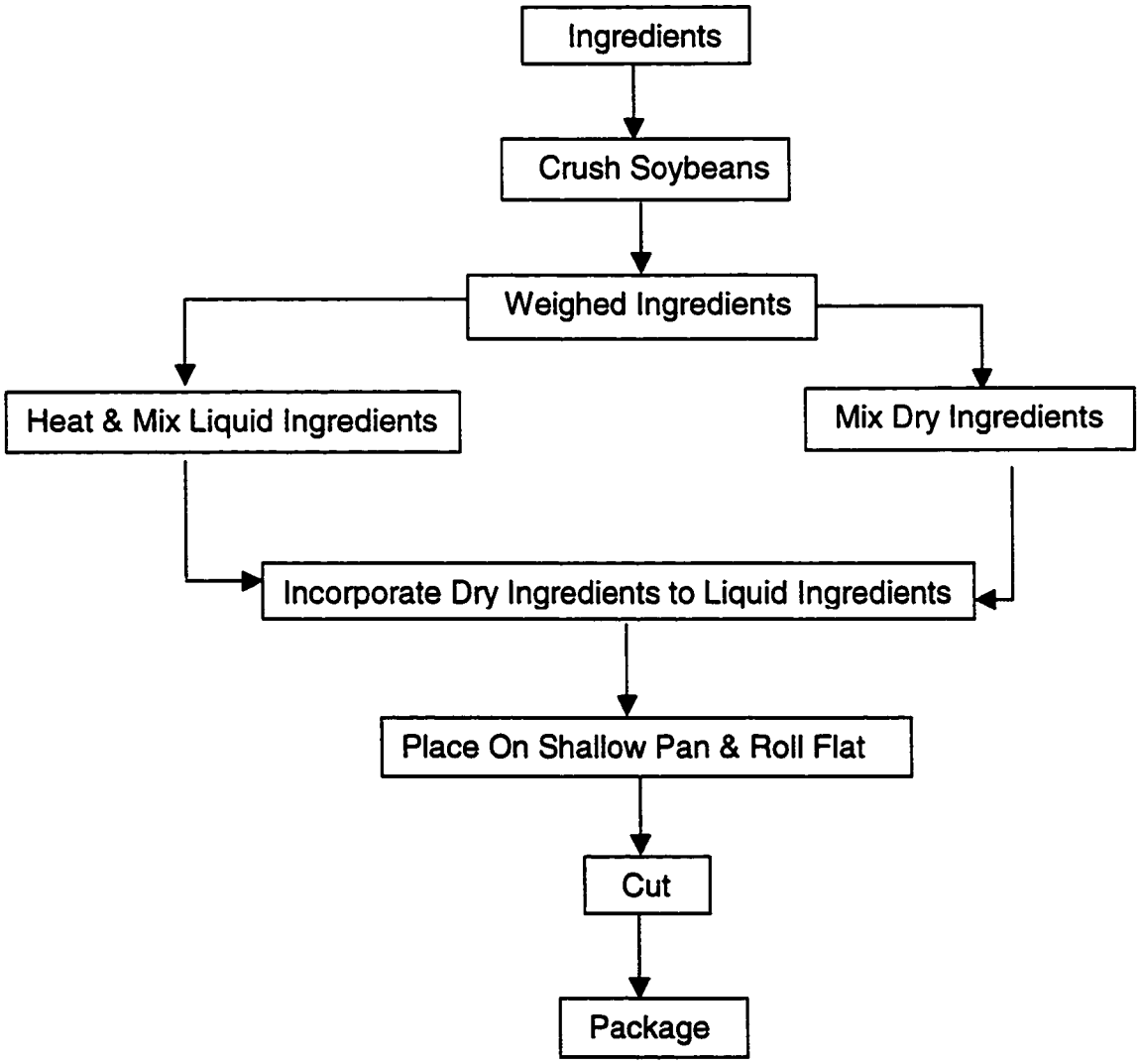
To minimize the thermal loss of heat-labile vitamins, such as riboflavin and vitamin C, wheat sprout was freeze dried and incorporated into the bar using a cold press technique.

The process for the snack bar is summarized in Figure 13. The following steps were followed:

1. All utensils were coated with vegetable oil.
2. Honey, chocolate powder, marshmallow, coconut flavoring were combined in a container placed in hot water bath at 70 °C, and mixed well.
3. The soybeans were crushed to small pieces using a grinder (Quaker City Mill, Model 4-E) by adjusting the tightness of the screw.
4. Soybeans, raisin granola, germinated wheat powder, crispy rice, skim milk powder were combined and mixed well.

5. Dry mixture from 4 was added to the melted ingredients in 2 and mixed until all ingredients were uniformly blended (approximately 3 minutes).
6. The mixture was pressed into a cold shallow pan and flattened with a rolling pin to 2 cm thickness.
7. After cooling for 60 min in ambient environment, the product was cut into a desired shape and size

Figure 13 Process flow chart for prototype nutritional bar



3.2.3 Nutritional Evaluation

An Esha Professional Food Processing and Nutritional Analysis Software (Version 7.21) was used in the nutritional analysis of the snack bar.

3.2.4 Sensory Analysis – A Pilot Evaluation

A pilot consumer acceptance test of the product was conducted with 32 untrained panelists recruited from students and staff in the Department of Agricultural, Food and Nutritional Science, University of Alberta. Using a hedonic scale with seven points ranging from dislike very much (=1) to like very much (=7), the overall acceptability of the product was evaluated. The participants were asked to taste the products following the sequence indicated on the questionnaire. The panelists were instructed to rinse their months with water before and after tasting. Nutritional and labeling information (Table 7 and Figure 14) was provided to encourage confidence and participation.

3.3 Results and Discussion

3.3.1 Product Design

The product was nutritionally designed, targeting the consumers who may be suffering from degenerative diseases, such as the loss of energy and acuity, indigestion, constipation, and obesity. There are several thousand food items stored in this

comprehensive database, from which a specific ingredient (e.g. crushed, roasted, salted soybeans) can be chosen for a recipe. This nutritional analysis software also provides a selected nutrient composition (e.g. vitamins A, E and C, etc.) of each ingredient designed for the bar. Once the list of ingredients is decided, the product can be analyzed for its nutritional breakdown. A selected nutrient composition of this bar was calculated and finally presented in a variety of ways, such as nutritional information spreadsheet (Table 7), percentage of macronutrient contents (Figure 14), nutritional labeling (Table 9) and protein quality information (Figure 15). It should be stated that the selected nutrient composition of wheat sprout was obtained from the Esha nutritional analysis database instead of first hand lab results. Because the nature of this research was to investigate antioxidant contents in wheat sprouts, other data, (e.g. dietary fiber, trace minerals, *etc.*) were not available when the nutritional bar was designed.

Figure 14 Percentage of macronutrients in one bar

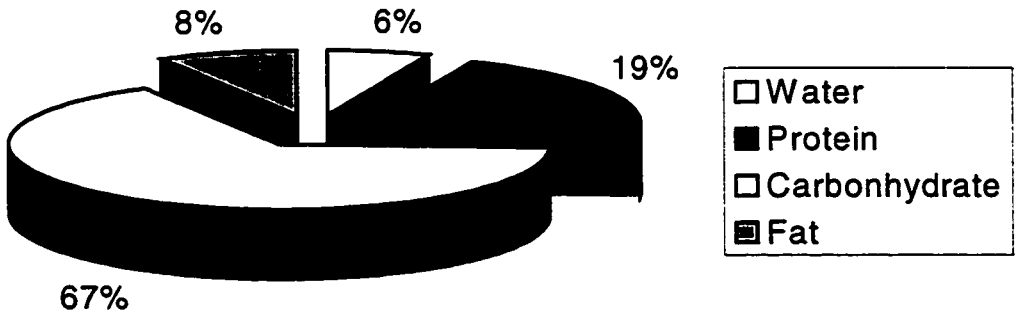


Table 7 Nutritional information spreadsheet

Nutritional bar July 16, 1999

Total Weight: 45.00 g (1.59 oz-wt.)
Serving Size: 45.00 g (1.59 oz-wt.)
Service: 1.00

Amount	Food Item	Vitamin A (IU)	B_{12} (µg)	C (mg)	E (mg)	Folic acid (µg)	Ca (mg)	Protein (g)
12.5 g	Soybean, Roasted	14.88	0	0.27	0.24	26.16	17.11	4.36
3 g	Chocolate-Flavor Chips	--	0	0.02	0.01	0.17	1.11	0.10
7.0 g	Marshmallows	--	0	0	0	0.07	0.20	0.12
5 g	Crispy Rice Cereal	--	0.01	2.65	0.01	24.70	0.90	0.32
3.5 g	Raisin Granola	0	0	0	0.05	0.46	2.52	0.29
4 g	Honey	0	0	0.02	0	0.09	0.26	0.01
5 g	Instant Skim Dry Powder Milk	--	0.20	0.28	0	2.49	61.50	1.76
5 g	Dehydrated Wheat Sprouts	1156.86	1.43	15.71	0.01	54.29	25.71	1.14
	Totals	1171.74	1.64	18.95	0.33	108.42	109.32	8.11

Amount	Food Item	Fe (mg)	Fiber (g)	P (mg)	K (mg)	Na (mg)	Se (µg)	Zn (mg)
12.5 g	Soybean, Roasted	0.48	2.19	45.01	182.28	20.21	2.37	0.39
3 g	Chocolate-Flavor Chips	0.09	0.17	3.84	17.73	6.30	0.08	0.05
7.0 g	Marshmallows	0.02	0.01	0.54	0.34	3.20	0.13	0
5 g	Crispy Rice Cereal	0.13	0.06	5.45	4.75	36.70	0.77	0.08
3.5 g	Raisin Granola	0.09	0.22	11.48	10.29	13.06	0.61	0.06
4 g	Honey	0.02	0.01	0.17	2.24	0.17	0.03	0.01
5 g	Instant Skim Dry Powder Milk	0.02	0	49.25	85.25	27.45	1.37	0.22
5 g	Dehydrated Wheat Sprouts	2.86	1.87	25.71	160.00	1.43	5.00	0.03
	Totals	3.70	4.53	141.46	462.88	108.51	10.35	0.83

3.3.2 Formulation

After preliminary experiments with developing a snack bar, modifications were made to the choice of ingredients for the bar. The water was replaced with honey, melted chocolate and marshmallow, which contributed to a decrease in water activity, increase in overall crispy mouthfeel of the bar, since most western consumers preferred crispiness or crunchiness to stickiness or softness (Sutherland *et al.*, 1986). Low fat raisin granola was used to mask the irregularity of the physical appearance of germinated wheat powder. Melted chocolate was also added to cover the plain whiteness of the wheat flour. To avoid allergic reaction to peanuts by some people, soybean nuts were chosen to increase protein content of the bar. Coconut flavoring was chosen to minimize the dominant grassy taste of germinated wheat, which was disliked by some volunteers. Crushed, roasted soybeans and germinated wheat powder were combined to increase the total amount of dietary fiber. Skim milk powder was added to improve total protein quality of the bar.

The following was the final formula chosen for a 45 g bar:

	g/bar	%
Crushed, roasted, salted soybeans	12.5g	27.9
Marshmallow	7.0g	5.6
Crispy Rice	5.0g	11
Instant skim milk powder	5.0g	11
Freeze dried germinated wheat powder	5.0g	11

Honey	4.0g	9
Low fat raisin granola	3.5g	7.8
Chocolate flavor powder	3.0g	6.7
Coconut Flavoring Extract	Trace quantity	

All ingredients present in this bar were natural products, with no artificial coloring or flavoring. By pilot sensory test and analyzing the ingredient lists of similar products purchased from local stores, it was concluded that this product was superior for its nutritional contents to the commercial products. It contained soybean, germinated wheat powder and skim milk powder, which provided complete protein and potential health benefit. Wheat and soybean complementation have been extensively studied, because it has long been recognized that soybean proteins effectively supplement wheat proteins in animal nutrition and greatly increase their nutritive value by the addition of lysine and other amino acids in which wheat proteins are relatively low (Finney, 1978). The water activity of the nutritional bar was 0.63, measured by the Aqualab machine (Decagon Device Inc., Pullman, Washington, USA). This value of water activity ensured the bar would have a quite long shelf life (Jelen, 1988). A large number of independent research on the medicinal effects of germinated cereal has been carried out in the United States and Western Europe. Several patented bakery products have appeared in the market, e.g. wheat sprout bread, breakfast cereals, and germinated wheat powder in small packets. However, no snack bar containing germinated wheat powder has been reported.

3.3.3 Sensory Evaluation of the Product

The following are the results of sensory evaluation of the nutritional bar:

Among the 32 panelists recruited there were 15 (47%) women and 17 (53%) men. Their age ranged from 25-44 years. Overall, 31 (97%) respondents said they would purchase this bar. The average score given to the bar was 6.2, which meant "like moderately". All female panelists stated that they would purchase the product and the majority of them (8 persons or 53%) indicated that they liked the product very much. Out of 32 panelists, 25 (78%) would like to buy the bars in boxes of 6 instead of an individual bar. The average price chosen was $0.77/bar. Three quarters of the panelists consumed this type of bar a few times a week.

Based on ingredient cost and estimated production cost, the product should be retailed at $0.91/bar, which was higher than the price the panelists indicated they would pay, i.e. $0.77/bar. However, it should be stated that the estimated retail price was extremely rough and much can be done to lower the ingredient and production costs. Nevertheless, the sensory panel has indicated very high market potential for the bar. Nutritional education especially the advertising on the clinical benefit of germinated wheat can further promote marketing.

It should be noted, however, that the panelists used in this study were limited to university students and professors, not general public or target consumers. A much wider market test must be done before definite conclusions can be made on the acceptability and market potential of the product.

3.3.4 Nutritional Analysis

Estimated nutritional composition of the nutritional bar using an Esha professional nutritional analysis software (version 7.21) is shown in Table 7.

3.3.4.1 Mineral and Trace Element Content

The ratio of Ca: P for this bar is 1: 1.29 and each bar provides 10% of RNI (recommended nutrient intake) for calcium (RNI=680 mg per day), and 20% for iron (RNI=13.2 mg per day), which are good amounts from a single source. Furthermore, the bar contains vitamin C and D, which help increase the absorption of calcium and iron.

The ratio of Na: K has been calculated to be 1: 4.27. This is a good value because most of processed foods on the market contain a high amount of sodium. The nutritional bar provides 28 % of RNI for potassium (RNI=1.65 g per day), which may help lower blood pressure in hypertensive patients.

3.3.4.2 Protein and Amino Acid Content

The protein content of the nutritional bar was quite high (19%) as shown in Figure 15, making it higher than the protein content in milk (3%), eggs (12%), and sirloin steak (16%). Germinated wheat flour (14%), and soybeans (54%) not only contribute to a large amount (68%) of protein for the bar, but excellent quality of complete protein of the bar as well. The amino acid profile of the bar is shown in Figure 15. The major essential

amino acids of concern are lysine, cysteine and methionine. Soybean contains high concentration of lysine but is low in cysteine and methionine, while wheat grain is usually high in cysteine and methionine but low in lysine. Cystein content in wheat grain increases during germination (Seibold, 1990). From the analytical results, it was shown that the protein in the nutritional bar was superior to most of other plant sources, and was even superior to that of some animal foods. The bar can provide sufficient lysine, methionine and cysteine to the consumers. The data revealed that complimentarity between soybean and wheat sprout protein could be further investigated to benefit human health and economy.

3.3.4.3 Dietary Fiber Content

According to the NCI (National Cancer Institute) and CDA (Canadian Diabetes Association), the daily suggested intake of fiber is 20-30%. The nutritional bar provides 23% of the RNI (RNI=20g per day) for dietary fiber. Dehydrated wheat powder was the main source of dietary fiber (41%). When compared with commercial products which do not contain fiber strands, the prototypes could attract more senior customers. Since the primary deficiency in western society is fiber and many senior people suffering from constipation look for products that are high in fiber, this bar should satisfy this requirement.

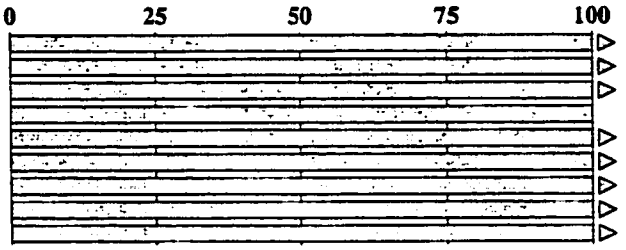
Figure 15 Amino acid profile

Nutritional Bar July 16, 1999

Total Weight: 45.00 g (1.59 oz-wt.)
Serving Size: 45.00 g (1.59 oz-wt.)
Serves: 1.00
Cost: --

Protein Quality

Amino Acid	Actual Ratio / Ideal Ratio	Score
Histidine	23.56 / 19	124%
Isoleucine	45.22 / 28	162%
Leucine	75.96 / 66	115%
Lysine	57.99 / 58	100%
Methionine + Cystine	28.27 / 25	113%
Phenylalanine + Tyrosine	80.54 / 63	128%
Threonine	40.45 / 34	119%
Tryptophan	11.74 / 11	107%
Valine	50.41 / 35	144%



Protein Quality Score, based on limiting value (Lysine) = 100%.*

* Results may not be completely accurate; some data is missing. View the Spreadsheet report to see if any missing values would significantly affect results.

FAO/WHO/UNU suggested Amino Acid requirement patterns.
Ratios are in milligrams of amino acid per gram of Protein (mg/g Protein).

3.3.4.4 Vitamin and Antioxidant Content

A single bar provides 1.64 μg of vitamin B_{12}, which exceeds the RNI (RNI=1 μg per day) for normal adults, pregnancy and lactation; it provides 60% for vitamin A (RNI=900 RE per day), 62% for folic acid (RNI=175 μg per day), 32% for riboflavin (RNI=1 mg per day). Pregnancy or lactation increases the risk and incidence of folic acid, vitamin B_{12} and riboflavin deficiency. Folate deficiency is the most common cause of megaloblastic anemia in infants and children (Health and Welfare Canada, 1990). This bar may well accommodate these pregnant or lactating female customers. Plant foods are generally considered to be devoid of vitamin B_{12}. For this reason, this bar may be developed for vegetarian who consume no animal products and are often advised to take vitamin B_{12} supplements. This may be done by replacing all animal-based ingredients with similar vegetarian-approved ingredients.

In addition, this nutritional bar contains soybeans, which are high in isoflavones. The principle isoflavones found in soybeans are diadzein, genistein, and glycetein. Several lines of evidence suggest a biological role in cancer prevention for both isoflavones and protease inhibitors from soybeans. Oriental women, who consume considerably larger amounts of soy products than American women, generally have low rates of breast cancer. This soy-based nutritional bar may also be developed for those postmenopausal women, as evidence showed consumption of about 200 mg of isoflavones daily induced an estrogenic response and increased the number of superficial cells of the vaginal epithelium (Wrick, 1993).

By comparing antioxidant contents in wheat sprout quantified in Chapter 2 with data provided by the Esha nutritional analysis software, it was found that there were no α-tocopherol and phenolic acids in Esha sprout. Therefore, each 45g of this nutritional bar provided additional 54.6 μg of α-tocopherol, 4.66 mg of ferulic acid and 65 μg of vanillic acid. It was found that FA and IFA can suppress the production of interleukin-8 (IL-8) which is the main cause of the local accumulation of neutrophils, and modulates various inflammatory reactions (Hirabayashi *et al.*, 1995). This finding may open a new approach to some chronic inflammatory disease treatment by consuming this nutritional bar.

Besides, this bar is an excellent source of all the specific nutrients needed for the synthesis of immune cells and their products (cytokines, antibodies, *etc.*) include protein, vitamin A, vitamin C, iron, folic acid and pyridoxine (Barrett, 1983).

However, Esha sprout had higher vitamin C (3.14 mg/g: 0.55 mg/g) and β-carotene (77 μg/g: 2.6 μg/g) contents. Since in the experiments carried out in Chapter 2, antioxidant was extracted from freeze-dried ground samples (theoretically wet non-treated samples are used for extraction to protect the antioxidant contents), antioxidants in dehydrated wheat sprout were more easily oxidized during mechanical processing and chemical treatment. Additionally, since vitamin C and β-carotene are unstable in the presence of heat and/or light. Moreover, since the biosynthesis of antioxidants during germination is dependent on various parameters, such as type of seeds, temperature, light conditions, air exchange and moisture (Price, 1988 and Sattar *et al.*, 1989), absolute antioxidant concentrations may vary between different experiment. Farhangi and Valadon (1983) and Farhangi (1980) found that exposure of etiolated bean sprouts to artificial

light for 24 hours increased provitamin A content from 18 to 321 μg β-carotene/100 g fresh weight, and concluded that biosynthesis of β-carotene may be closely related to the photosynthesis of sprout. It is recommended that some germination conditions could be modified for wheat grain to increase a specific antioxidant production.

3.3.4.5 Lipid Content and Lipid Class

The nutritional bar is low in saturated fat (0.5 g per 45 g serving), and does not contain cholesterol. Each 45 g bar provides 0.21 g of ω-3 fatty acids and 1.61 g of ω-6 fatty acids. The ratio of PUFA (polyunsaturated fatty acids): MUFA (monounsaturated fatty acids): SAFA (saturated fatty acids) is 3.64: 2.56: 1. Studies by Renaud (1987) showed that increasing the intake of food rich in polyunsaturated fatty acids decreased the clotting activity of platelets and their aggregation by thrombin, in both men and women. This nutritional bar may become a good supplementing source of unsaturated fatty acids.

3.3.5 Proposed Industrial Scale Production of the Nutritional Bar

3.3.5.1 Commercial Scale Processing

The bench-scale processing of the bar was a simple cold-press method in which dry ingredients were mixed and blended with melted ingredients. The final mixture was then

spread and rolled in a shallow pan before cooling and cutting into designed shapes and sizes.

For a commercial scale production, the following processing procedure may be considered:

All free-flowing major dry ingredients (crushed soybeans, crispy rice, germinated wheat powder, raisin granola, skim milk powder) are automatically weighed from separate feed hoppers through an adjustable gate into the same mixing bowl that is stationary on a scale and trolley (Stathmos Scale MFG. Ltd., Revagen, Sweden). The coconut flavoring is added to this bowl, and the content is mixed by an M-802 Hobart mixer with a flat beater for 5 min at speed level 2.

Using a steam-jacketed, wall-mounted tilting Kettle, the honey, marshmallow, chocolate powder are mixed and heated to 70°C, a temperature at which marshmallow and chocolate powder melt. The kettle temperature is automatically regulated, and a scraper mixer and wing whip beater are used for mixing. The warm liquid mixture is then added to the dry ingredients in the bowl. The bowl is returned to the Hobart mixer, and these ingredients are mixed for 10 min at speed level 2. The mixture is either manually or automatically placed on the APV conveyor belt. A pin spreader distributes the mixture on the belt, and a roller compresses it to a 2.0 cm thickness. The slab is cut by an Ac-U-Arc cross- cutting machine manufactured by APV Baker (Rosemont, Illinois, USA). The slitter of this machine makes longitudinal cuts of a 3.0 cm width, and the guillotine cutter cuts across the slab every 11.0 cm. This cutting pattern gives bar dimensions of 11.0 cm x 3.0 cm x 2.0 cm. The cut slabs are then packaged.

Since many of the ingredients are sticky, such as melted marshmallow, chocolate, and honey, equipment that comes into contact with the product must be coated with a teflon such as PTFE (Booth, 1990). Coated mixing bowl, kettle, beaters, conveyor belts, rollers, and cutters will help the process run smoothly and efficiently.

3.3.5.2 Quality Control Program

For an industrial scale production, the most important factor to ensure a high quality product is high quality ingredients and standardized operation procedures (SOP).

i. Ingredients

The best way to ensure all ingredients are of high quality is to use reputable suppliers. However, it is mostly up to the company to inspect the products to ensure high standard materials. The raw materials should be checked every time they are received for any signs of contamination. Any enterprise involved in producing an article for commercial gain needs to know the optimum conditions required to produce that product in a minimum of time, must be able to define the product and be able to constantly achieve consistent quality. Hazard Analysis Critical Control Point (HACCP) is a pro-active process control system by which food quality and safety are ensured. It provides a structured and critical approach to the control of identified hazards, including safety hazards such as toxins, contamination, foreign bodies, decomposition, microbiological and non-safety hazards such as product quality, product substitution, etc. (Food Safety and Inspection Services, 1993). It has the potential to identify areas of concern where failure has not yet been experienced, making it particularly useful for new product

development. A HACCP check list is provided in Table 8, which may be applied to commercial geminated wheat production.

Table 8 HACCP check list for wheat sprouting

Control Point	Hazard	Control Measures
Raw material: Dried wheat grains	Mold or bacterial growth due to damp storage conditions	Dry storage/humidity control
Quality of grains	Contamination by foreign matter	Inspection, sieving and washing
Soaking and germination	Growth of contaminating microorganisms on the surface	Surface sterilization by NaClO
Recycled soaking water	Contamination from water supply	Disinfection of water supply with the addition of H_2O_2
Growth of wheat sprouts	Excessive microbial proliferation	Use of disinfected tap water
	Contamination from dirty growth containers	Cleaning and disinfecting containers with NaClO
Harvesting	Contamination during harvesting	Cleaning and disinfecting scoops, hands, gloves and containers with NaClO
Storage	Microbial growth Use of out of date product	Freeze drying and vacuum packaged Date label and stock rotation

The aim in sprout production is to produce an etiolated germinated seedling (soybeans, mungbeans, alfafa, wheat, barley *etc.*) which has not yet produced true leaves, the primary interest being the sprout (hypocotyl or cotyledon) region. Long root systems are totally undesirable and optimum sprout length and thickness is required. There are no defined standards for sprout quality right now, although such standards are desirable. The harvested sprout should have reasonable keeping qualities after harvest, and prevention of water loss and maintenance of turgidity after harvest is a prime requirement. The basic factors affecting wheat grain germination also need to be taken into account in the commercial production system. Optimum sprout production is influenced by the following factors (Price, 1988):

(a) Wheat grain source.

(b) Viability of grain.

(c) Duration of grain steeping and temperature of steeping water.

(d) Watering regime, frequency, duration and water temperature.

(e) Removal of wasted gases during germination.

(f) Optimization of gas ratios of O_2, CO_2 and N_2.

(g) Room temperature and humidity.

(h) Light.

(i) Hormone, growth regulators.

(j) Harvest time.

(k) Post-harvest storage and longevity.

(l) Post-harvest treatment.

(m) Packaging

(n) Microbial and toxicological factors.

ii. Process

The standardization of the process are normally decided and approved by members of the Quality Control team. GMP (good manufacturing practice) requires participation of all floor staff as well as management staff. The process should be closely monitored. Each time a lot is produced, Q.C. (quality control) staff should record in writing whether or not procedures were followed. If deviations occur, they must be documented and reported.

iii. Product

The bars should be randomly chosen, and tested before packaging. Water activity is measured to ensure that it remains around 0.65. Shelf life of the product may be estimated using the Schaal test (Graf and Sauguy, 1991). This involves incubating the packaged sample at 63 °C, and measure the time it takes for sensory quality to decrease and mold to grow. One day of incubation at 63 °C is approximately equivalent to 6- 10 days at 21 °C. Due to the low a_w (0.63) and moisture content, generally, microbiological analysis is not mandatory. However, if consumer complaints occur, a SPC (standard plate count) test should be carried out.

Besides the physical testing, a trained sensory panel is responsible for determining whether or not the product has deviated from preset specifications. The sensory attributes

for the nutritional bar include: general appearance (any possible detection of staleness); natural odor and flavor; mouthfeel.

Statistical methods employed in quality assurance include sequential sampling and operation characteristic curves to determine the acceptance level of defects and foreign materials in the ingredients.

iv. Hazard Analysis Critical Control Points

Critical control points are those which may prevent contamination, or prevent acceptance of contaminated products.

The first critical control point is raw material inspection. There are always some outward signs of contamination upon receipt of the materials. These includes ensuring that all packages are properly sealed, no sign of pest infestation, no signs of staleness. On the same note, the storage and transportation equipment should also be checked for hygiene and contamination by pests. All conditions should be carefully observed and documented.

The second critical point involves floor staff. Anyone handling food materials or entering food processing area should follow proper hygiene requirements: hand washing, headgear, lab coat, mask or protective gloves wearing. Routine medical examination should be carried out for floor staff.

The third critical point is properly sanitizing equipment. Cleaning procedures should be standardized and strictly followed. Cleaning agents should be provided and renewed in time, to ensure all equipment can not only be easily cleaned but cleanable.

The fourth critical point is packaging materials. All materials that will come in contact with food materials should be of food grade, and free of pest or other contamination.

Another control point is the weighing and mixing of ingredients to ensure compliance with product formulation. Scales, metal detectors and thermometers of coolers should be routinely checked and calibrated.

3.3.5.3 Nutritional Labeling

In the USA, FDA regulates food products according to their intended use and the nature of claims made on the package. Two types of statements or claims may be allowed on food and dietary supplement labels (FDA, 1998):

1. Structure and function claims describing effects on normal function of the body and;
2. Disease risk reduction (health) claims implying relationship between components in the diet and a disease or health condition, as approved by FDA and supported by significant scientific agreement.

Proposed nutritional labeling of the bar is shown in Table 9.

The Percentage of Recommended Daily Intake (RDI) is based on 2000 calories daily intake, which is the minimal recommended intake for adults (Health and Welfare Canada, 1990).

Table 9 Nutritional labeling

Nutritional Bar

7/16/99

Nutrition Facts

Serving Size (45g)
Servings Per Container

Amount Per Serving	
Calories 170	Calories from Fat 30
	% Daily Value*
Total Fat 3.5g	5%
Saturated Fat 0.5g	3%
Cholesterol 0mg	0%
Sodium 110mg	5%
Total Carbohydrate 28g	9%
Dietary Fiber 5g	18%
Sugars 14g	
Protein 8g	
Vitamin A 60% •	Vitamin C 30%
Calcium 10% •	Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

For the nutritional bar marketed in Canada, on the package, according to federal labeling requirements for pre-packaged foods under the Canadian Food and Drug Act (FDA), the Food and Drug Regulations (FDR), the Consumer Packaging and Labeling Act (CPLA), and the Consumer Packaging and Labeling Regulations (CPLR), all of the mandatory information is provided in both official languages, French and English. The

label of the boxes and of the individual bar includes all of the following required information:

1. The common name for the product is Biogenic Nutritional Bar.
2. The weight of the individual bar is 45 grams, and a box has a net quantity of 270 grams.
3. The name and address of the responsible party who produces the bars.
4. The list of ingredients, in descending order of proportion by weight, in the bar.
5. A shelf life, which is around 3 months, due to its low a_w.
6. The label also displays nutritional information and claims, if any, according to "Nutritional Quality of the Product" (Agriculture and Agri-Food Canada, 1995).

To make the package more user-friendly, it is suggested that the labels have a 14-point type size, including instructions on how to open the package, and a 1-800 number for consumer service. With regard to color, important information is in bright yellow and large print with background in dark green, which indicates the bioactive germinated wheat content. A sans serif type is used, since it is a little easier to read. The use of both upper and lower case letters also applies.

3.4 Conclusion

Using wheat germinated for 7 days, a "Nutritional Bar" was designed. Essentially, the bar consisted of a blend of roasted soybean nuts, crispy rice, freeze-dried germinated wheat powder, honey, raisin, and instant skim milk powder. The bar was formed using the cold press technique, and sensory evaluation showed that the bar was liked by most consumers (31 out of 32).

A selected nutrient composition of the bar was determined by the Esha nutritional analysis software. Each bar provided appreciable amounts of dietary fiber (23% of the daily suggested intake), vitamin B_{12} (> 100% of RNI), vitamin A (60% of RNI), folic acid (62% of RNI), riboflavin (32% of RNI), potassium (28% of RNI), iron (20% of RNI), and calcium (10% of RNI). The bar was low in saturated fat (0.5g per 45g serving), and contained no cholesterol.

The nutritional composition of this prototype nutritional bar was initially developed with the desire to nutritionally accommodate consumers suffering from degenerative diseases. However, further modification could be made for some specific consumer groups, such as pregnant, lactating, postmenopausal female and senior consumers.

CHAPTER 4

GENERAL CONCLUSIONS AND RECOMMENDATIONS

4.1 General Conclusions

Consumption of sprouts in Canada and worldwide has increased. However, wheat sprout is vastly underutilized for nutritional or food application. In this study, the effect of germination on antioxidant contents of wheat grain was investigated. Wheat grain was first soaked in tap water for 24 h, followed by germination in the dark at 98% relative humidity and 16.5 °C for 7 days to achieve optimal sprouting effect.

High performance liquid chromatography techniques (HPLC) and 2,6-dichlorophenolindophenol titration were applied to determine the antioxidant contents in wheat grain before and after germination. A strong relation has been shown between the increase of a selected group of antioxidant levels and the length of germination time. Vitamins C and E, and β-carotene contents in dry wheat grain were almost undetectable. During germination, however, the concentrations of these antioxidant vitamins were increased with increasing germination time and reached to their peak levels at day 7 (500 μg/g for vitamin C; 10.92 μg/g for α-tocopherol; and 2.6 μg/g for β-carotene). In addition to these antioxidants, ferulic acid content of wheat grain was also increased following its

germination. Ferulic acid is an antioxidant; it reached to its highest concentration at day 7 (932.4 μg/g). These finding suggested that these antioxidants may play a crucial role in seed dormancy breakage and germination.

In the countries with well-developed economy, the consumers have higher purchasing power and are more concerned about their health and nutritional value of the food they consume. Almost all the participants (97%) in a survey conducted by Food Marketing Institute indicated that they would like to ensure their health by changing eating habits (Food Marketing Institute, 1997). Meanwhile, these countries are facing a rapid increase in aging population. As people get older, they become more concerned about their health, creating great demand for such products that will keep them well and slow down the natural process of aging (Gerber, 1989). The marked increase in antioxidant vitamins in wheat grain due to sprouting is of nutritional importance to these persons who prefer natural enhancement of nutrients to artificial enhancement.

Moreover, a greater understanding of the etiology of degenerative diseases and their relationship with free radicals has opened the way to the chronic disease treatment through dietary modulation. That is constant consumption of whole fruits, grains, vegetables and their germinated or fermented products such as germinated wheat, which is rich in various antioxidants.

For this research, a snack bar was chosen as the sensory carrier of germinated wheat. Nutritional bar is one of increasingly popular functional products in North America, because it can very conveniently satisfy hunger and the craving for frivolous food, while providing certain nutrients (e.g. vitamins, trace minerals, dietary fiber, *etc.*)

raising the daily intakes to the recommended level, which might not be met with meals alone.

Five g of wheat germinated for 7 days was incorporated into each 45 g of this nutritional bar. Essentially, this snack bar consisted of a blend of roasted soybean nuts, crispy rice, freeze-dried germinated wheat powder, honey, raisin, and instant skim milk powder. The bar was formed using the cold press technique and designed to be packaged with laminated polypropylene aluminum foil. A selected nutrient composition of the bar was calculated by the Esha professional nutritional analysis software. Each bar provided appreciable amounts of dietary fiber (23% of the daily suggested intake), vitamin B_{12} (> 100% of RNI), vitamin A (60% of RNI), folic acid (62% of RNI), riboflavin (32% of RNI), potassium (28% of RNI), iron (20% of RNI), and calcium (10% of RNI). The bar was low in saturated fat (0.5 g per 45g service), and contained no cholesterol.

A pilot sensory evaluation showed that the bar was liked by most consumers (97%) and preferred by females.

Degenerative diseases are common health problems afflicting these industrialized nations, as a result of overly simplified, narrow food and nutritional sources. This Nutritional Bar was developed as a possible relief for the common symptoms of aging or other degenerative diseases: tiredness, joint pain, high blood pressure, indigestion, constipation, etc. However, further modification could be made for some specific consumer groups, like pregnant, lactating, postmenopausal female and senior consumers.

Since Alberta is one of the world's leading wheat growers, it makes economic sense that food scientists should find ways to add more value to this commodity.

Sprouting of wheat grains and processing them into functional food ingredients is one excellent way of doing it.

4.2 Recommendations

For the nutritional bar, to achieve a better nutritional profile, the product formula can be altered: For example, to increase protein content, soybean protein or whey could be added; inclusion of various nuts, like almonds and walnuts could be considered; to increase fiber content, dry fruits or vegetables can also be added.

Since sensory panelists were all recruited from university, additional consumer tests in supermarkets or other communities where panelists represent a wider cross section of people or target consumer groups, will be necessary. A chocolate coating could be designed to cover the uneven surface of the bar and increase the acceptability of its appearance. The inclusion of dry fruits (e.g. raisins, apple) was liked by most consumers and its proportion could be increased.

For commercial-scale sprout production, hydroponic techniques may be considered. Economic considerations demand that larger batches of grain should be germinated, using less manpower. Thus germination is increasingly mechanized to process larger quantities of grain. One of the mechanized processes is "pneumatic malting" in which grain germinates in a bed 1 meter or more in depth; it is turned mechanically, and is attemperated and ventilated with a forced flow of "conditioned" air, i.e. air at a controlled temperature and optimal O_2/CO_2 ratio, and is as nearly saturated

with water vapor as possible (Godom and Willm, 1994). Artificial light may be applied to activate photosynthesis and increase vitamin A production (Seibold, 1990).

A practical way to kill pathogens will need to be developed, possibly by using warm water with the addition of hydrogen peroxide or ozone.

Where permitted by customers and local legislation, growth regulators, such as gibberellic acid (GA_3), ρ-chlorophenoxyacetic acid (CPA), indole-acetic acid (IAA) or ethylene can be used as additives, which is established to (i) break grain dormancy; (ii) promote germination; (iii) produce shorter-rooted or "rootless", larger diameter sprouts (Cheng and Chua, 1980).

Germination conditions to tailor the germinated product for bakery and other foods should be investigated. The germinated wheat may be fractionated, or its components extracted, to be used as functional ingredients with unique functional, nutritional and sensory properties. The processing methods for these purposes and the properties of the products should be thoroughly investigated so that their applications in the nutraceutical and functional food industries can be promoted and economic benefits fully realized.

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