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Soy has been used as a human food for more than 2000 years, especially in the Pacific Basin. According to many sources, soy was introduced into the United States in the early 1900s as a forage crop. Since then it has become a major protein staple in North America for animal feed as well as a source of vegetable oil for human consumption and is now grown for seed and processing instead of grazing. However, not long after the introduction of soy into North America, two scientists (Osborne and Mendel, 1917) made the significant observation that soybeans had to be treated with heat in order for the protein to support the growth of laboratory rats. Subsequent studies found that compounds present in soybean interfered with the absorption of the soy protein and slowed the rate of growth of rats and quail. Further investigation led to the isolation and characterization of what are now referred to as the Kunitz trypsin inhibitor and the Bowman-Birk Inhibitor, which inhibits both trypsin and chymotrypsin. Trypsin and chymotrypsin are compounds called enzymes that are secreted by the pancreas into the stomach and are crucial for digesting or breaking down foods into nutrients that can be absorbed into the bloodstream. The Bowman-Birk Inhibitor (BBI) binds to the trypsin and chymotrypsin in the stomach and inhibits their digestive activity. The process is much more complicated than I have described but the concept is correct. It is understandable that animal nutritionists would be concerned about the presence of BBI in soy meal, as it would slow the rate of growth of an animal that was using the soy meal as a protein and energy source. Thankfully, for the feed industry, simply toasting the soy meal deactivates the BBI and then there is no growth inhibition.

In many parts of the world soy has been used as a base for healthy food products such as tofu and there are many studies attesting to the health benefits of soy and soy-based food products, most notably to a group of compounds present in soy called isoflavones. Many studies in Asia and other areas demonstrated the health benefits in soy-based foods that contained BBI. Epidemiological evidence indicates that diets containing high amounts of soybean products are associated with low cancer incidence and mortality rates, particularly for breast, colon, and prostrate cancers.

Fast forward to this century, several studies now point to the BBI as the compound that is protecting against these cancers and so presence of BBI in soy and soy-based foods for human

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diets is now potentially a positive attribute instead of being seen as detrimental.

The initial objective of this research was to screen short season soybean breeding lines and varieties (both GM and conventional) for presence and quantity of a Bowman-Birk Inhibitor (BBI) in order to allow breeders to select germplasm with the highest levels of BBI for new variety development (and exclude material that has little to know BBI present in the seed). The range of BBI present in soybean varieties or breeding lines grown in Manitoba from Dr. Elroy Cober's soy germplasm development program was determined. Dr. Cober is a scientist employed by Agriculture and Agri-food Canada. The research funding was to determine BBI in whole seeds but we also wanted to see if the BBI was present in bean sprouts as well as in food products related to tofu.

The manufacture of tofu, also called bean curd, is done by coagulating soy milk and then pressing the resulting curds into soft white blocks. There are many different varieties and ways to make tofu so the first step was to use a standardized tofu manufacturing process that duplicates, on a laboratory bench, a typical process used by manufacturing companies in Asia. This method was developed by Dr. Judith Fregeau-Reid at Agriculture and Agri-food Canada. The second method for tofu production utilized a "made-for-home-consumer" appliance (soyabella soy milk maker and tofu kit). Tofu has a low calorie count, high concentration of protein and iron, and depending upon the coagulants used in the manufacturing process, a high calcium and magnesium content. Tofu manufacture is outlined in Figure 1.

The first step is to make soy milk. This begins with soaking the soy bean in water at various lengths of time, cooking/heating in some processes, grinding and then filtering. This results in two fractions – the Okara (seed hulls/fibre, fat, nutrients) and

Figure 1. Soy Milk and Tofu Manufacturing Process

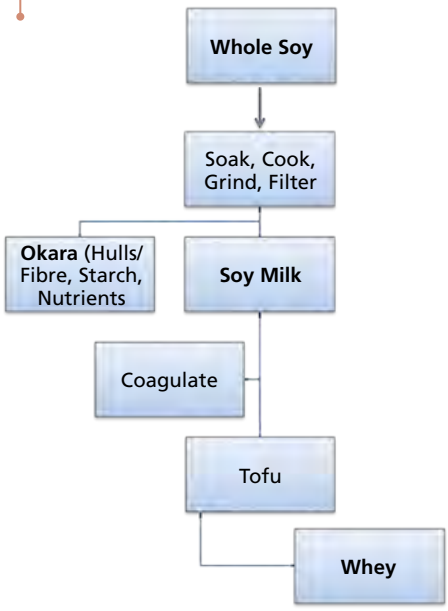

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Figure 2. Bowman-Birk inhibitor levels present in mature seed of soybean varieties grown in Manitoba.

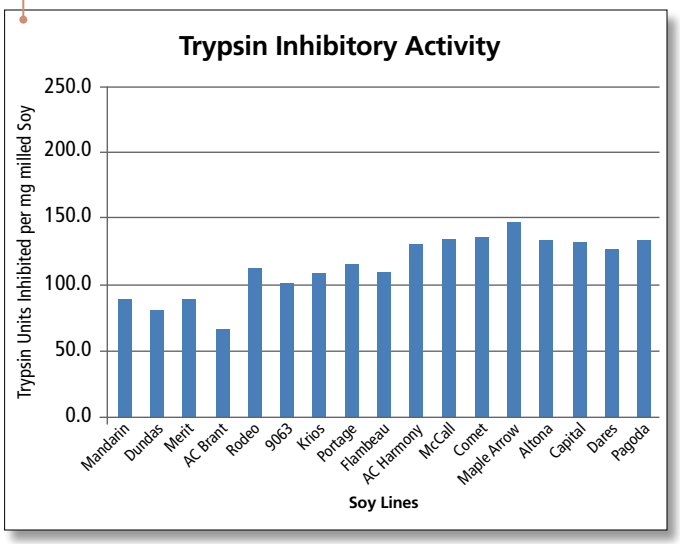
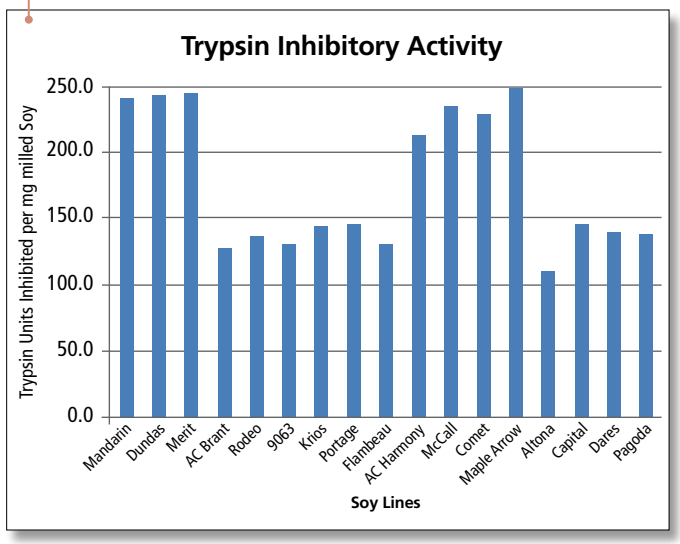


Figure 3. Bowman-Birk inhibitor levels in 4-day-old soybean sprouts.



soy milk. The soy milk is coagulated using different processes and reagents and pressed into tofu. The slurry that remains is the whey fraction.

Figure 2 shows the Bowman-Birk (Trypsin) Inhibitor Levels present in the mature seeds of some of the soybean varieties provided by Dr. Elroy Cober from research plots in Manitoba. The BBI levels (expressed as Trypsin Units Inhibited/gm of soy) ranged from 60–148 with the majority of the varieties having greater than 100 TUI/mg of seed.

Figure 3 shows the BBI levels present in 4-day-old soybean sprouts. BBI levels increased dramatically in Mandarin, Dundas and Merit and also increased in AC Harmony, McCall, Comet and Maple Arrow. BBI levels decreased in 4-day-old sprouted seed for the remaining varieties. The data for the 8-day-old sprouted material is not presented as it was not significantly different from the 4-day-old sprouts.

As mentioned earlier, the Okara fraction is the material that remains after the soybeans are soaked, ground and filtered to produce soy milk required for tofu. The BBI levels in the Okara fraction were lower than those of mature seeds for the majority of the varieties evaluated. This is expected as the majority of protein would be present in the soy milk or soy whey fractions.

This research shows that there are differences in BBI present in mature seed of different varieties of soy as well as in sprouted soybean. While the differences are significant, we are unsure as to BBI dose required in order to affect tumour size or reduce incidence of cancer. Understanding dosage amount and frequency will be important in order to select for optimum concentrations of BBI future varieties.

As our knowledge of the role of BBI for prevention or cure of cancer and other maladies grows, we may find that BBI present in infant formulas or children's cereals is not acceptable due to their trypsin/chymostrypsin binding activities but that as we pass through other life stages (puberty, menopause as examples) the role of BBI in health becomes more important and that it is desirable to have high levels of BBI in food products. We now know that there is soybean germplasm available that can provide BBI and that it is not necessarily destroyed in all food processing activity. If BBI is undesirable in infant formulas, it can be denatured using heat. If it is desired in food products, likely gentle heating similar to the tofu process will not lead to degradation, but more study is required to verify this. In order to conduct this research, the Manitoba Pulse Growers Association has funded us for a project titled *Evaluation of Soybeans for Biomedical and Functional Food Utilization* and we want to thank the Manitoba Pulse Growers Association for providing this funding.

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