Manitoba Pulse & Soybean Growers www.manitobapulse.ca

pulse beat

The Science Edition

January 2017

On-Farm Network › Agronomy › Breeding Nutrition & End-Use

Developing production tools and market demand for profitable and sustainable farms through local research.

Research is a core activity of Manitoba Pulse & Soybean Growers (MPSG). It is fundamental to supporting the development of our production resources and knowledge, as well as our efforts to increase local market demand for soybeans and pulses. MPSG invests approximately 60% of its annual budget into a well-rounded research program within four broad areas: agronomy, breeding, nutrition, end-use, and most recently, the On-Farm Network. The aim of the program is to identify and address production constraints and marketing opportunities to support profitable and sustainable pulse and soybean farms in Manitoba.

A successful research program not only addresses the issues, but also makes the information available. This new *Pulse Beat* issue – *The Science Edition* – was created to report on the progress made through MPSG's research investment to its members and industry stakeholders. It is a compilation of results from recently completed projects that were supported by farmer check-off dollars. Many of the following projects are also supported by other organizations. These collaborations not only strengthen MPSG's connections within the industry, but also maximize the value of MPSG check-off.

If you are looking for more extensive reports, visit our website, manitobapulse.ca. The staff team at MPSG is working to update the website with current results, so keep checking out the *Research* and *On-Farm Network* pages for new information over the winter.

We want to hear from you, the farmers. Tell us about your production issues and opinions on research needs in our province.

Laryssa Grenkow

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ON-FARM NETWORK

On-Farm Evaluations of Single vs. Double Inoculation Strategies in Eastern Manitoba

Only two of 25 on-farm trials showed higher soybean yield with double versus single inoculation on fields with at least two years history of soybean in eastern Manitoba.

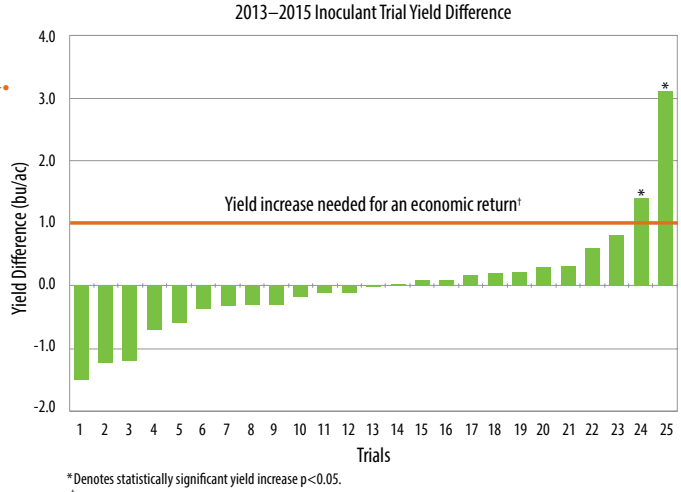
ADEQUATE NODULATION IS required for soybeans to acquire a large proportion of their nitrogen (N) requirements through biological N fixation. Because the soybean-specific N-fixing bacteria (*Bradyrhizobium japonicum*) is not native to Prairie soils, supplemental inoculation with products containing compatible rhizobia is required for soybeans grown on land with a limited history of soybean. The conventional practice for inoculating soybeans in Manitoba, typically referred to as "double inoculation," uses two inoculant formulations or placements, i.e. use of a seed applied liquid inoculant plus an in-furrow granular or liquid inoculant to ensure adequate levels of rhizobium are introduced in the case that a single inoculant failed due to desiccation or bridging in the tank. Other more established soybean growing regions of the Northern Great Plains mainly use a single inoculant or no inoculant at all. As soybean field history continues to grow in Manitoba and because the cost of inoculant ranges from $3–4/ac for liquid, to $10–12/ac for granular, there was a need to develop a local data set on the agronomic and economic implications of single and double inoculation strategies.

Twenty-five on-farm field trials were established from 2013 to 2015 comparing single inoculation (seed applied liquid only) vs. double inoculation (seed applied liquid with the addition of in-furrow granular or liquid inoculant) in Eastern Manitoba on fields with a history of at least two soybean crops. At each site, the farmer replicated each treatment strip six times. Averaged across all 25 on-farm trials, soybeans yielded 37.5 bu/ac and there was no yield benefit to double inoculation over single inoculation. At individual trial sites, there was only a statistically significant and economic yield response to double inoculation at two of 25 trials (see graph below). There was no specific response factor (seeding date, plant population, soil fertility, pH, salts, or inoculant product) that we found to explain the two responsive sites.

Overall, the frequency of yield response to seed applied liquid plus in-furrow inoculation (double inoculation) was low for fields with soybean history. These results have been incorporated into new production recommendations found in MPSG's *Soybean Fertility Fact Sheet* to help farmers assess the risk of moving towards a single inoculation strategy on a field-by-field basis based on field history and risk factors. The practice of double inoculation is still encouraged for fields with little or no soybean history. In addition to the 25 trials in this study, two trial locations did not meet the criteria of at least two previous soybean crops in the field history, and were excluded from this analysis. In these two trials, there was a significant yield advantage to the double inoculation strategy supporting the current double inoculation strategy for fields with less than two previous soybean crops.

This inoculant trial will be tested on-farm in western Manitoba starting in 2017. Current research is also underway to validate a soil test to determine the levels of rhizobia present in soils from Manitoba fields after soybeans have been grown for more than two years. In addition, a new on-farm trial was initiated in 2016 comparing the use of a single seed applied liquid inoculant vs. no inoculant on fields that have had at least three successful soybean crops.

Yield difference between 25 on-farm trials comparing seed planted with and without in-furrow inoculant from 2013 to 2015.



*Denotes statistically significant yield increase $p<0.05$.
†Economic return based on $10/ac cost of granular inoculant and $10/bu price for soybeans

PRINCIPAL INVESTIGATOR Manitoba Pulse & Soybean Growers

ACKNOWLEDGEMENT Tone Ag Consulting

MPSG INVESTMENT $97,650

DURATION 3.5 years

on-farm network

Phosphorus Fertilization Beneficial Management Practices for Soybean in Manitoba

Seed yield increase to phosphorus (P) fertilization was rarely observed, regardless of P fertilizer rate, placement or soil test level.

Dr. Don Flaten and graduate student Gustavo Bardella presenting results at 2015 MPSG SMART field day, Carman, MB.

SOIL SAMPLES TESTING below the critical P concentration have increased concurrently with the increase in soybean acreage in Manitoba. Soybeans remove relatively large amounts of P in harvested seed (~0.83 lbs P_2O_5/bu); however, P fertilizer application for soybean is not common, as farmers often do not observe a yield response. Although P fertilizer is placed in the seed row for many crops, some crops do not tolerate high rates and crop removal rates often exceed fertilizer application rates, depleting soil P reserves. As soybeans gain prominence in rotations, basic fertility recommendations for soybean and strategies for maintaining soil fertility need to be established. Two studies investigated the maximum safe rate of seed-placed P fertilizer for soybean and soybean yield response to P fertilizer placement and rate, and soil test P level.

Field trials at 10 locations in Manitoba (2013–2015) measured soybean yield response and seedling toxicity of P fertilizer rates (0, 20, 40 and 80 lb P_2O_5/ac) applied in side band, seed row or broadcast. Locations varied in soil texture and seeding equipment, which are important factors affecting fertilizer toxicity risk. Half of the trial sites had soil P test levels in the 0–10 ppm Olsen P range, a level at which many crops would have high probability of response to P fertilizer.

Plant stand reduction caused by seed-placed fertilizer toxicity was rare, but was most common in soils with medium to coarse textures or when low-disturbance or low seed-bed utilization seeding equipment was used. Seed-placed P reduced plant stand at six of 28 site-years, but usually only at the 80 lb P_2O_5/ac rate; rates of 20 and 40 lb P_2O_5/ac reduced emergence at one and two site-years, respectively. Soybean yield was reduced by P fertilizer in two site-years where, in both cases, seed-placed fertilizer was applied at 80 lb P_2O_5/ac and the plant stand was reduced below 100,000 plants/ac. Phosphorus fertilizer increased yield at only one of 28 site-years, where 40 and 80 lb P_2O_5/ac both increased yield by ~15%, compared to the control, regardless of placement.

Considering these results, the recommended maximum safe rate of seed-placed P_2O_5 (10 lbs/ac) probably underestimates soybeans' tolerance to seed-placed fertilizer in most situations. However, it is difficult to define a new value since there are many factors that can increase the risk of fertilizer toxicity: dry soil, sandy soil texture, narrow seeder opener type and wide row spacing.

To evaluate the response of soybean to background levels of soil P fertility, i.e. with no additional fertilizer added during the soybean production year, a second field study was conducted over seven site-years at three locations in Manitoba during 2013–2015. The sites for this study had been used for a previous long-term study, in which different rates of monoammonium phosphate (11-52-0) had been annually applied over nine years creating a range of soil P levels (7 to 93 ppm Olsen P). Studies conducted elsewhere have shown that soybean grown on high P fertility soils can produce greater yields. However, despite the differences in soil P in this study, there were no yield responses to soil test P at any site-year.

Although there is little chance of reward for P fertilization for soybeans, depletion of soil P caused by P removal without replacement may be detrimental to yields of other crops in the rotation. Soybeans present farmers with an opportunity for P fertilizer application flexibility, because there is no urgent need to apply P fertilizer at any particular rate or placement for this particular crop. However, to maintain P fertility, rotational fertilization could mean applying P in the soybean year of the rotation, where the best placement is side banding since it minimizes fertilizer toxicity risk, facilitates higher rates and places fertilizer below the soil surface, preventing erosion and run-off losses. Another strategy would be to apply higher than usual rates of P, in the form of commercial fertilizer or livestock manure, to other crops in the rotation.

PRINCIPAL INVESTIGATOR Don Flaten, Department of Soil Science, University of Manitoba

MPSG INVESTMENT $64,653

CO-FUNDER $129,306 – Western Grains Research Foundation, Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

DURATION 3 years

AGRONOMY

Soybean Aphid Control by Natural Enemies in Manitoba

Soybean fields adjacent to cereal fields were positively associated with lacewings, the aphid predators most associated with soybean aphid suppression.

Dr. Costamagna and a malaise trap used to assess predator movement in field experiments.

UNABLE TO OVERWINTER in Manitoba, soybean aphids migrate from the U.S. every summer and, on occasion, populations reach levels that cause economic yield loss. Although aphid control can be satisfactorily achieved by using registered, non-selective insecticide, a number of natural enemies (or predators), including species of ladybeetles, minute pirate bugs, damsel bugs, hoverflies and lacewings commonly found in Manitoba, also play an important role in controlling aphid populations. The action threshold (250 aphids per plant and populations increasing) accounts for aphid suppression by predators; however, a lack of understanding of the dynamics of predator movement reduces our ability to predict this component of the threshold. This study investigated the dispersal capacity and effectiveness of natural enemies in suppressing soybean aphids, and identified vegetation surrounding soybean crops that can be a source of predators in Manitoba.

A preliminary study assessed the effectiveness of predators on aphid suppression by infesting plants within soybean and alfalfa fields with soybean aphids that were: 1) open to predation; 2) protected from ground predators; or 3) completely protected from predation. Despite larger numbers of predators in alfalfa, aphids were significantly reduced in both crops. Foliar predators also contributed more than ground predators to aphid suppression.

A mark-release-recapture study investigated ladybeetle movement between soybean and alfalfa. Although most movement was away from soybeans, likely due to the low natural aphid levels, ladybeetles moved rapidly between both crops suggesting that alfalfa may act as a source of predators for soybean when infested with aphids.

Following up on these results, 27 field experiments were set up in the Interlake, Central and Eastman regions in 2013 and 2014 to determine the source and track the movement of aphid predators. Soybean plants within commercial soybean fields were infested with aphids and were: 1) left open or 2) protected from foliar predators to determine the level of aphid suppression by predators. Sweep nets and visual plant counts were used to quantify natural aphid populations and predators. Bi-directional malaise traps (see photo) were placed parallel to the edge of the soybean field to monitor movement of predators from outside sources. Overall, naturally occurring soybean aphids were very low; however, protection from predation resulted in a 3.6 fold increase in aphid populations. Predator populations were dominated by minute pirate bugs and hoverflies, and, to a lesser extent, green lacewings, damsel bugs, ladybeetles and brown lacewings.

Although aphid populations were significantly lower in the presence of predators compared with predator exclusion controls, results indicated that the strength of aphid suppression also varied by landscape. Land-cover types within a two kilometre radius of each soybean field were quantified and were dominated by cereals (wheat, oats, and barley), canola, corn and soybeans and to a lesser extent, natural vegetation. The proportion of cereals in the landscape showed a consistent negative association with soybean aphid abundance under predation at multiple spatial scales. Cereals were positively associated with green lacewings, which was the predator most associated with aphid suppression. By mid- to late-July, when soybean is still susceptible to aphid damage, most cereal crops are senescing, meaning that predators move from cereals to other habitats searching for prey; thus cereal crops can act as sources of natural enemies.

By contrast, the positive association of aphids with a percentage of canola and native vegetation in the landscape suggests that these habitats interfere with aphid suppression. The inverse mechanism as with cereals may be operating here, i.e. canola and native vegetation may compete for predators with soybeans, as they will have prey populations developing at similar times.

These results support previous studies in North America and confirm the role of generalist predators in controlling pest populations. Further research is required to identify the mechanisms of aphid suppression associated with cereal and alfalfa crops, i.e. understand how time during the growing season and the proportion of habitat and distances between habitat and soybeans affect effective predator populations.

PRINCIPAL INVESTIGATOR Alejandro Costamagna, Department of Entomology, University of Manitoba

MPSG INVESTMENT $36,950

CO-FUNDER $43,050 – Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

DURATION 3.5 years

Manitoba Soybean Cyst Nematode Survey (2014/15)

SCN was not present in sampled soybean fields in Manitoba, but farmers should continue to monitor fields as the threat is imminent.

Dr. Tenuta describing root symptoms of SCN.

HETERODERA GLYCINES (Soybean Cyst Nematode, SCN) is recognized as the major pest of soybean worldwide. SCN has rapidly moved northward from the mid-U.S. and is now present in some counties in North Dakota and Minnesota, which border Manitoba. Recently, the Canadian Food Inspection Agency declassified SCN as a regulated pest in Canada, which means that surveying for SCN is no longer conducted by the federal agency. Since early detection and precise identification is important for timely control measures to be implemented, surveys were initiated by the Soil Ecology Laboratory in the Department of Soil Science at the University of Manitoba.

In 2014–2015, 28 commercial soybean fields most likely to be infested (i.e. along the Red River Valley, with a long/frequent history of soybean) were selected for sampling. Each field was sectioned into areas that could be responsible for SCN introduction such as entrance ways, headlands near ditches and depressions or drainage ways. From these fields, a total of 205 soil samples were taken to the lab for analysis for the presence of SCN. Nematode cysts were recovered from 32 soil samples; however, the samples yielded one to a few cysts each, with the majority of cysts being empty and broken. Furthermore, most cysts were round and not lemon-shaped, circumfenestrate rather than bifenestrate cone tops, the later possible indicators of SCN. Only six of these cysts samples yielded DNA suitable for molecular PCR (polymerase chain reaction) analysis and all DNA samples tested were negative for SCN.

Through the most recent and past (2012–2013) surveys, a total of 76 commercial soybean fields in Manitoba have been sampled and have all tested negative for the presence of SCN. Because SCN is present in neighbouring states, surveys will continue to be conducted every two to three years, with the next survey commencing in summer 2017.

Figure 1. SCN-damaged areas of the field.

In addition, outreach activities will continue to ensure farmers are aware of how to scout for and identify SCN. Because SCN damages roots, nodulation and nutrient and water uptake will be impeded resulting in yellowed, stunted plants, which closely resemble symptoms of iron deficiency chlorosis (Figure 1). Ultimately, maturity and yield will be affected. To proactively manage SCN, the Soil Ecology Lab recommends to scout high risk fields regularly, (i.e. those with >3 years of soybean and focus your scouting on headlands, depressions and near fences, approaches and sloughs). Use of resistant soybean varieties and control of alternate host plants (e.g. chickweed, wild mustard, Shepherd's Purse and other less common species) will help contain and slow the expansion of SCN-infected areas and maintain populations at levels that do not cause economic damage.

Source: Albert Tenuta, OMAFRA

White, lemon-shaped cysts on roots

Source: Kris Lambert, Univ. Illinois

Adult soybean cyst nematode

PRINCIPAL INVESTIGATOR Mario Tenuta, Department of Soil Science, University of Manitoba

MPSG INVESTMENT $15,333

CO-FUNDER $30,667 – Western Grains Research Foundation, Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

DURATION 1.5 years

AGRONOMY

Soybean Crop Rotation in Manitoba

Crop rotation affects soybean N and P acquisition by symbiotic microbes and soybeans do not provide nitrogen credit to subsequent wheat crops.

LIKE OTHER LEGUMES, soybean's capacity to acquire phosphorus (P) and nitrogen (N) is related to symbiotic relationships with soil fungi and bacteria, respectively. Arbuscular mycorrhizal fungi (AMF) behave as an extension of the root itself, and can access P otherwise unavailable by penetrating soil pores too small for roots. *Bradyrhizobium japonicum* bacteria, housed in soybean root nodules, fix atmospheric N, reducing requirements of soil or fertilizer N. The effectiveness of these two microbes on soybean crop nutrition are affected by environmental and management factors.

To explore the effect of preceding crop on soybean yield, mycorrhizal colonization, and N fixation, two-year cropping sequence field trials were set up at Carman, Portage la Prairie and St. Adolphe in 2012–2014. Canola, corn, soybean and wheat were grown prior to soybean. Even within the short timeframe, there were differences in N fixation between sequences: soybeans grown on corn stubble generally had a higher proportion of N fixation (70.5–77.6%) and soybeans grown on canola had lower fixation (39.0–67.6%) than the other sequences. The low residual soil N levels after corn compared to canola explains these results: under levels of high soil N, soybean are less dependent on rhizobium as an N source and corn, having a high carbon to N ratio, immobilized more soil N, leaving little for the proceeding soybeans (see graph).

AMF colonization on soybean was also greater when grown on corn (53.5%) or soybean (54%) residue compared to wheat (45.1%) or canola (41.8%) residue. Because AMF do not colonize canola roots, soil AMF populations decline in a year where canola is grown. Colonization was also reduced by high residual soil P levels, but unlike soil N, was not correlated to preceding crop.

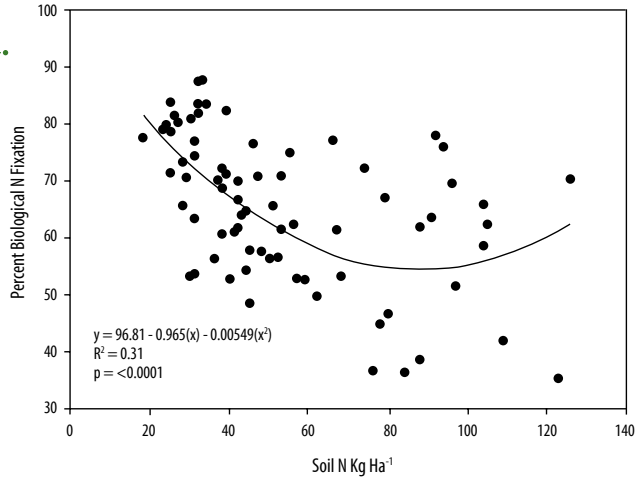
Preceding crop also affected soybean yield, though trends were not consistent. Although crop sequence had no effect at two of five site-years, corn-soybean and wheat-soybean sequences proved to be more consistent across site-years than canola-soybean and soybean-soybean sequences. Short-term rotation choices may have little effect on yield, but we can use the knowledge of soil microbes' role in P and N acquisition when growing soybeans on low fertility soils to maximize crop nutrition.

Legume residue decomposition can also contribute soil N so fertilizer recommendations are often adjusted to include an "N credit" for subsequent crops. Soybean, however, removes relatively high levels of N in the harvested grain and N credits vary widely with soil/climatic conditions and subsequent crop. Crop rotation studies on the Canadian Prairies have traditionally excluded soybean, but due to the rapid expansion in acreage in Manitoba, N credits for soybean needed to be quantified with a second field experiment.

Wheat, fertilized with 0, 28, 54, 80 or 107 lbs N/ac was planted on either soybean or canola stubble. The N credit of soybean is determined by comparing the amount of N fertilizer required for the wheat crop that follows the canola reference crop to produce the same yield as the wheat crop grown after soybean. In three of the four site-years, yields of wheat following soybeans was the same or lower compared to wheat following canola. Where wheat responded to N fertilizer, the yield of wheat following canola was always higher than following soybeans.

The lack of response to N fertilizer in these trials could have been due to the "masking effect" of the high organic matter levels (resulting in soil N mineralization) or the loamy textured soil (resulting in leaching of N fertilizer). Interestingly, soybean residues returned less N to the soil (22–41 lbs N/ac) than canola (29–80 kg N/ha). In addition, canola had a lower C:N ratio compared to soybean, and combined with a shorter growing season (i.e. longer period of residue decomposition) it was not surprising then that canola residue immobilized less soil N. Overall, soybean did not provide an N credit to the wheat crop, but rather, showed negative N balance.

Effect of residual soil N on biological N fixation in soybean averaged across five site-years.



PRINCIPAL INVESTIGATOR Yvonne Lawley, Department of Plant Science, University Of Manitoba

MPSG INVESTMENT $134,225

CO-FUNDER $134,225 – Growing Forward 2 Growing Innovation: Agri-Food Research and Development Initiative

DURATION 5 years

Evaluation of Nutritional, Physico-Chemical and Cooking Quality Traits in Manitoba-Grown Dry Beans for Breeding Use

Quality traits were affected by interactions between cultivar, location and year, but cultivar accounted the most variability in all traits except for phytic acid content.

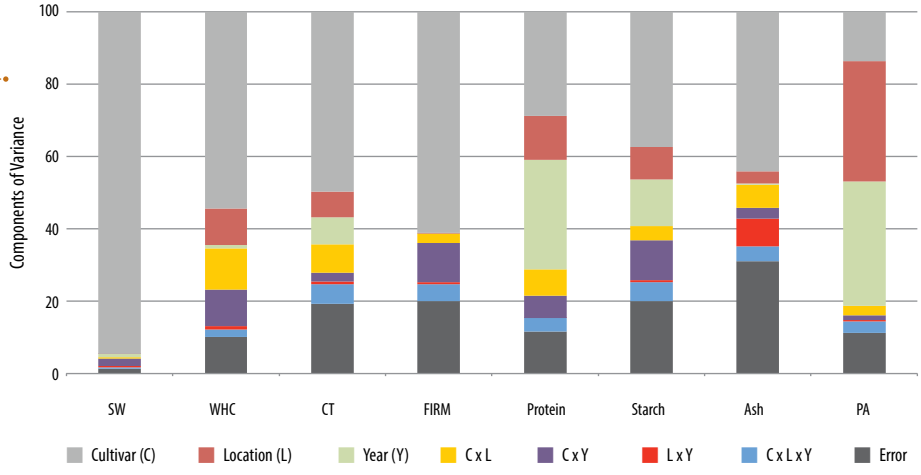
RELEASES OF CULTIVARS with high yield, disease resistance and marketable quality attributes are crucial to sustain dry bean production. While much attention is given to yield gains and disease resistance, quality and food value breeding have been largely limited to visual screening. Quality attributes of dry beans for human consumption include, but are not limited to: chemical composition (protein, moisture, ash, starch, phytic acid content), cooking quality (cooking time, firmness of cooked beans) and physical characteristics (seed weight, water hydration capacity). The quality of dry beans depends on many factors including cultivar, growing location and year. However, there is little information available on the effects of these factors and their interactions on dry beans grown in Manitoba. The quality traits in the breeding materials needed to be fully characterized to assist breeders with improving quality of beans in their breeding programs.

Twenty dry bean breeding lines, which are frequently used in crossing in the AAFC Morden Dry Bean Breeding Program, were evaluated for genetic variation in quality traits. These lines were grown at both Morden and Portage la Prairie in Manitoba in 2013 and 2014 to quantify the variability in quality across environment, as well.

Cultivar, growing location and year all had a significant effect on seed weight, water hydration capacity, cooking time, protein, starch and phytic acid content. Most of the traits were also significantly affected by various levels of interactions between cultivar, location and year. Cultivar accounted for most of the variability in all quality characteristics except for phytic acid content (see below).

Seed weight was negatively correlated with protein and ash content, but it positively correlated with starch content. Cooking time was negatively correlated with protein, ash and phytic acid content, but it positively correlated with firmness. Phytic acid level in beans was positively correlated with ash content.

Knowledge gained from this study will help bean breeders to select parental lines for crossing and cultivar development for desirable traits within various market classes. The Canadian Grain Commission recently initiated a complementary study, investigating the effect of cultivar and growing location on dietary fibre (soluble, insoluble and total fibre) and anti-nutritional factors (trypsin inhibitor activity, oligosaccharides), which rounds out the comprehensive assessment of important quality attributes.



Contributions to variance of quality traits of beans grown in Manitoba at two locations in 2013 and 2014. SW = seed weight; WHC = water hydration capacity; T = cooking time; FIRM = firmness of cooked seed; and PA = phytic acid.

PRINCIPAL INVESTIGATOR Ning Wang, Grain Research Laboratory, Canadian Grain Commission

MPSG INVESTMENT $36,000

DURATION 2 years

BREEDING

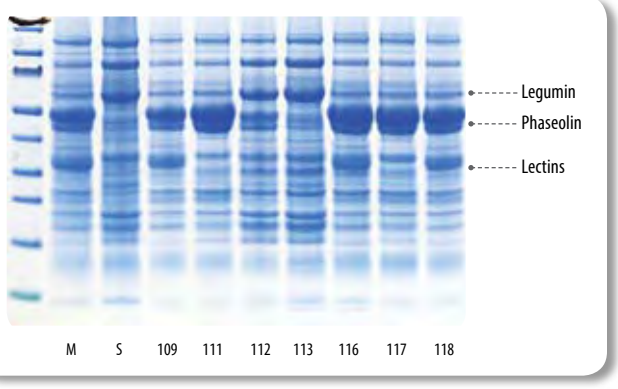
Genetic Improvement of Protein Quality in Manitoba-Adapted Dry Beans

Crossing a locally adapted cultivar with a germplasm line high in sulphur-containing amino acids increased protein quality.

DRY BEANS HAVE a high protein content; however, their protein quality is often considered poor compared to other plant sources, such as soybean. The key factor limiting the protein quality in dry beans is the sub-optimal content of sulphur-containing amino acids, particularly methionine. Using breeding methods to improve bean protein quality could lead new value-added food applications and identity preserved marketing opportunities. This study developed dry bean germplasm (breeding materials) with improved protein quality while maintaining important agronomic traits such as yield and anthracnose resistance.

Dry bean germplasm line SMARC1N-PN1 lacks the major seed storage proteins, which leads to increased cysteine and methionine. SMARC1N-PN1 was crossed with the locally-adapted navy bean cultivar Morden003 to develop a recombinant inbred line population. In 2013, a population of 182 lines were grown at Morden for seed increase and in 2014, the population was grown in field trials at Morden, MB and London, ON. After screening with protein electrophoresis (see below) and amino acid profiles, it was discovered that there were significant variations in polypeptide composition and amino acid content. Protein electrophoresis revealed that 61 of the 182 lines had Morden003 type phaseolin and lectin; 59 lines had Morden003 type phaseolin and SMARC1N-PN1 type lectin; 38 lines had SMARC1N-PN1 type phaseolin and Morden003 type lectin; and 16 lines had SMARC1N-PN1 type phaseolin and lectin. Amino acid analysis revealed significant increase of sulphur-containing amino acid (cysteine and methionine) concentration in 16 lines that exhibited protein patterns of SMARC1N-PN1.

Lines with desirable agronomic traits and anthracnose resistance were also identified. Morden003 is resistant to races 73 and 105 and SMARC1N-PN1 is resistant to race 73, but susceptible to race 105. Forty lines were resistant to both races, with the rest of the lines being susceptible to either one or both races. Lines with increased sulphur-containing amino acid content, acceptable yield, maturity and anthracnose resistant traits with be further tested in field trials as well as used for crossing for cultivar development.



Representative samples and electrophoresis results showing protein profiles of Morden003 (M), SMARC1N-PN1 (S), and selected lines with protein patterns same as Morden003 (Lines 109, 116, 118), and SMARC1N-PN1 (Line 113); lacking of phaseolin in SMARC1N-PN1 and similar lines leads to increased sulphur-containing amino acid content.

PRINCIPAL INVESTIGATOR Dr. Anfu Hou, Agriculture and Agri-Food Canada

MPSG INVESTMENT $36,000

DURATION 3 years

BREEDING

Development of Molecular Markers for Common Bacterial Blight and Anthracnose in Dry Beans

Molecular markers for CBB resistance (SU91) were improved based on candidate genes (SU91-CG10 and SU91-CG14) and anthracnose resistance genes and markers ($SAH18_{1100}$ and BM161) were identified in Manitoba-adapted dry bean cultivars.

COMMON BACTERIAL BLIGHT (CBB) and anthracnose are economically important diseases of dry beans across Canada. Antibiotic streptomycin seed treatment or foliar applied copper products can be used to control CBB and DCT (diazinon, captan, thiophanate-methyl) can be used to control anthracnose. A more cost effective and environmentally friendly solution is to breed genetic resistance into new cultivars. Resistance genes for these diseases are available, but conventional selection and pyramiding of resistance genes to multiple diseases have not been effective in developing cultivars with both CBB and anthracnose resistance because of the complex genetic control of resistance genes involved and slow screening process. Most of the major resistance genes and quantitative trait loci (QTL) for anthracnose and CBB resistance have been located on genetic maps and associated with closely linked molecular markers. These markers may be used for selecting individual lines from a cross carrying the genes of interest, speeding up the process of breeding for multi-disease resistance cultivars. This research studied the effectiveness of the available molecular markers for selection of resistant breeding lines and also developed new markers better associated with resistance.

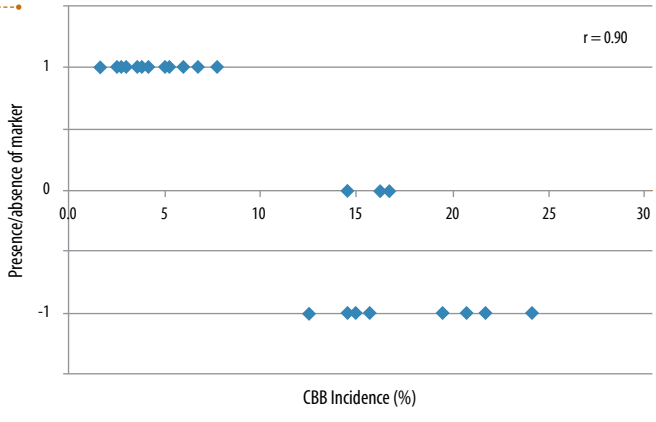
A combination of conventional breeding and marker-assisted selection was used to transfer CBB resistance genes into Manitoba-adapted cultivars with anthracnose and bean common mosaic virus (BCMV) resistance. Previously, crosses were performed between CBB resistant Ontario variety OAC-Rex (navy) and Manitoba varieties: anthracnose resistant Morden003 (navy), CBB and anthracnose susceptible Black Violet (black) and AC Pintoba (pinto).

QTLs conferring CBB resistance in OAC Rex are located on chromosomes Pv08 and Pv04 and linked to the SU91 and PV-ctt001 markers, respectively. Based on candidate gene approach, more reliable markers to SU91 have been developed at Harrow. These markers were used to screen the navy, black and pinto populations for CBB resistance at field trials in Morden, MB and Harrow, ON. A strong correlation between the incidence of CBB leaf infection and the presence of marker SU91 was found (see graph), however, the correlation between CBB and marker PV-ctt001 was low and insignificant.

Three of the 114 navy lines had improved resistance to both CBB and anthracnose, whereas ~50% of the lines exhibited strong resistance to anthracnose. Eleven black and seven pinto had resistance to CBB and four of the seven pinto beans were also presumed resistant to BCMV. There was good correlation between field evaluation and the presence of the marker alleles associated with resistance to anthracnose races 73 and 105 ($SAH18_{1100}$ and BM161).

A second study, using populations from the Morden003/OAC Rex crosses, mapped two race-specific (73, 105) resistance gene loci at the *Co-3* locus on Pv04, flanked by markers BM161 and $SAH18_{1100}$. The map generated in this research also showed strong linkage of the anthracnose resistance loci to markers SW12, PVctt001 and SF10, which were associated with the *Co-3* and *Co-10* loci. A weak, distant linkage of marker SB12 to the *Co-3* locus was also detected.

The new anthracnose molecular markers identified and the CBB, anthracnose, BCMV resistant lines developed possess desirable yield and seed characteristics, and can be used in crossing for future dry bean improvement.



CBB incidence at Morden in 2010–2011 and the presence/absence of the SU91 marker associated with CBB resistance of lines. Lines negative for the resistant allele were rated as -1; those segregating for the marker were given a value of 0; and those homozygous for SU91 as 1.

PRINCIPAL INVESTIGATORS Anfu Hou, Kangfu Yu, Ali Navabi, Frederic Marsaolais, Agriculture & Agri-Food Canada

MPSG INVESTMENT $30,000

DURATION 3 years

The Effect of Pulses on the Sensation of Fullness and Food Intake in Children

Adding puréed pulses to a meal does not affect food intake but increases consumption of dietary fibre and protein.

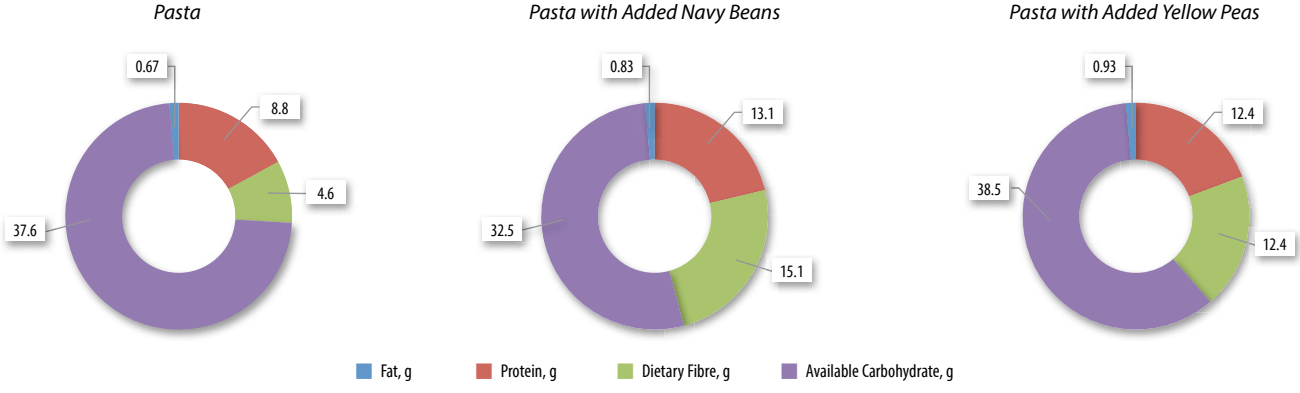
THE INTAKE OF fibre for many Canadian children is suboptimal and protein may also often be lacking, leading to a diet high in carbohydrates and fat. Adding pulses to a meal could increase desired nutrient intake, as well as lower available carbohydrate intake while changing the overall amount of food consumed. This study investigated the effect of adding puréed pulses to a meal on satiation (feeling of fullness during a meal), satiety (feeling of being full after a meal), total food intake and gastrointestinal comfort in children aged nine to 14.

Two hours after eating a standardized breakfast, children fed on one of three pasta dishes: pasta with just tomato sauce (control), pasta with tomato sauce and puréed navy beans or pasta with tomato sauce and puréed yellow peas. Children were asked to eat until they felt comfortably full. At regular time intervals after eating, as well as just before eating the pasta, they filled out questionnaires about their appetite, enjoyment of the food and overall physical comfort. Children were then asked to eat a meal of pizza until they were comfortably full and fill out one final questionnaire.

Adding puréed pulses to the pasta meal did not affect palatability or subsequent food intake at the pizza meal consumed two hours later. There was an increase in the intake of the pasta with added pulses compared to the control pasta, but this did not lead to a higher cumulative food intake over the two-hour period of pasta and pizza consumption. In addition, though there was no difference in total carbohydrate intake, available carbohydrate (starches and sugars) intake was reduced after the intake of pasta with added navy bean. There were also some differences observed regarding subjective appetite and physical comfort ratings. The treatments with added navy beans and yellow peas suppressed desire to eat compared to the control pasta. All participants expressed minimal (<5%) symptoms of gastrointestinal discomfort.

The added puréed pulses provided nutritional benefits to children through increased dietary fibre and protein consumption (see below). Adding puréed navy beans and yellow peas to a mixed meal results in about three to four times higher intake of dietary fibre. While fibre intake is suboptimal or low for many Canadian children, this presents a convenient way to improve nutrient intake. Adding puréed navy beans and yellow peas to a mixed meal also results in approximately 1.5 times higher intake of protein, which could potentially improve the diet of children who have high intake of carbohydrates and fat. Children with a risk of diabetes could also benefit from puréed navy beans, because it was shown to have the lowest level of available carbohydrate intake (sugar + starch).

In order to market puréed pulse products to all potential consumers, the food industry needs to formulate puréed products with acceptable palatability. Due to the success of adding puréed pulses to meals, Dr. Bohdan Luhovyy, in collaboration with Dr. Rebecca Mollard, have initiated a new study investigating the effect of cooked whole navy beans and yellow peas on short-term food intake, satiety and physical comfort in children.



PRINCIPAL INVESTIGATOR Dr. Bohdan Luhovyy, Department of Applied Human Nutrition, Mount Saint Vincent University

MPSG INVESTMENT $10,000

DURATION 2 years

Development of a Pulse-Based Protein Drink Mix for Vegan Athletes

Pea protein combined with quinoa flour creates a vegan smoothie dry mix with 20 grams of protein per serving.

VEGAN ATHLETES REQUIRE adequate amounts of protein to meet their dietary needs and maximize their athletic performance; however, this can be a challenge as their diet excludes any animal or animal-derived protein sources (e.g. meat, fish, dairy, eggs). These traditional animal sources are complete proteins, meaning they contain all essential amino acids. In contrast, plant derived-proteins are incomplete proteins and most are limited in lysine, threonine, tryptophan or sulphur-containing amino acids. To achieve a complete protein ratio, pulses and cereal proteins can be combined as they contain complementary set of amino acids. This study looked to develop a vegan dry smoothie mix that contains 15–25 grams of high-quality protein. For marketability, the mix also could not contain artificial flavours or colours and be easy to blend and drink.

Pea protein was selected as the pulse protein source due to its widespread availability, low cost and amino acid content. Pea protein contains lysine, which increases calcium absorption, and threonine, which helps with muscle and tissue strength and elasticity. Quinoa flour was chosen as the cereal protein source as it is the only cereal that is a complete protein and its taste masks the beany off-flavours of the pea protein. To increase palatability, a blend of cane sugar and stevia was added for sweetness and two flavours, vanilla-chai and chocolate were created (see below).

One serving of each of the dry mixes (42 grams for vanilla-chai and 46 grams for chocolate) contained 20 grams of protein. Allowable nutrient claims for the smoothie mix included source of energy, trans fat free, cholesterol free, a source of magnesium and molybdenum, a good source of calcium and an excellent source of iron and phosphorus.

To prepare the smoothie, sensory panels tested water, almond milk, soy milk and coconut as possible hydrating agents. All were deemed acceptable, but testers preferred almond and soy milk. The panel described the smoothies as *refreshing*, *smooth textured*, *no lumps*, *easily dissolved*, *sweet* and *appealing*.

The availability of a delicious, good quality vegan protein dry mix with a full amino acid profile that is easily incorporated in smoothies may reduce health care costs and provide a well-researched and practically conceived product for sports dietitians, nutritionists and other exercise science and medicine experts. These experts recognize the importance of nutrition for optimal performance as well as the difficulties in implementing sports nutrition for vegan athletes. The creation of a high protein dry mix can provide a value added product for Manitoba food processors and can be used to promote and market pulse ingredients to the food industry.

The project industry partner, Jordan Ciciewa of One Fit City (Winnipeg, MB) is actively marketing the product through website, radio and TV promotions. Several local juice stores and yoga, spin and Barre fitness studios have tested selling the product and are excited to retail a made-in-Manitoba product. The final product, with all the required labelling will be available in early 2017.

Vanilla chai dry protein mix (left) and chocolate protein smooth formulation (right) both made from pea protein powders.

PRINCIPAL INVESTIGATOR Meeling Nivet, Food Development Centre

MPSG INVESTMENT $20,000

CO-FUNDER $5,000 – One Fit City

DURATION 1 year

Can Increasing Pulse Consumption in Canada Reduce Healthcare Expenditures?

If 5–50% of adults increase their pulse consumption to one serving per day, healthcare costs for Type 2 diabetes and coronary heart disease would be reduced $30–$300 million annually.

TYPE 2 DIABETES (T2D) and coronary heart disease (CHD) are two major diseases that contribute greatly to Canada's social and healthcare costs. In 2014, federal healthcare costs of T2D were estimated at $2.5 billion and $13 billion for CHD. Pulses are a low glycemic index, high fibre protein source that can reduce cholesterol levels, thus reducing the risk of developing T2D and CHD. Current dietary guidelines recommend the consumption of two to three servings (175 ml or 130 grams of cooked pulses) per day. Only 13% of the adult Canadian population consume an average 113 grams of pulses per day, which is well below the recommendation. Increasing pulse consumption could lead to reduction in the development of T2D and CHD, thus reducing federal healthcare costs.

This study created an economic simulation to estimate the reduction of T2D and CHD and associated costs. Four scenarios were developed to estimate the percent of the Canadian population to adopt the recommendation of one pulse serving per day. The best, optimistic, pessimistic and worst case scenario assumed a 50%, 25%, 15% and 5% adoption rate, respectively.

To determine annual healthcare cost savings, illness costs were broken down into two categories: direct and indirect. Direct costs refer to hospital care, physician care and drug expenditures, whereas indirect costs refer to the dollar value of lost production due to illness, injury or premature death. Using recent systematic meta-analyses, the estimated effect of daily pulse consumption lead to a 7% reduction of T2D and 5% reduction of CHD. For all cost reductions, the analysis assumed a proportional reduction with disease incidence. The exception to this is the cost reduction for hospitalization. Fixed costs remain largely the same, regardless of admissions, whereas variable costs decrease with fewer admissions. As there has been no study on the breakdown of these costs in Canada, an American study found the approximately 84% of hospital costs are fixed with the remaining 16% variable. Therefore, for every 1% reduction of disease incidence, there would be a 0.16% reduction in costs.

A full overview of cost savings is outlined in the table below. In Canada, total cost savings from increased pulse consumption ranged from 7.3 to 73 million dollars for T2D and 20.8 to 208.6 million dollars for CHD, depending on the adoption scenario. In Manitoba alone, these values ranged from 0.3 to 3.0 million dollars for T2D and 1.1 to 11.3 million dollars for CHD.

Based on the economic simulations, there is potential for healthcare cost savings in Canada by increasing consumption of pulses. However, there is a need for strategies to increase pulse consumption through education and policy. The food industry must create acceptable pulse-based products for consumers so they can easily increase their consumption in a convenient and affordable way.

Potential Canadian healthcare savings in T2D and CHD costs under various recommended pulse consumption scenarios among Canadian adults (CAD $million)

	Scenario							
	Best Case		Optimistic		Pessimistic		Worst Case	
	T2D	CHD	T2D	CHD	T2D	CHD	T2D	CHD
Direct Cost Savings								
Hospital	3.0	21.7	1.5	10.8	0.9	6.5	0.3	2.2
Physician Visits	18.6	114.3	9.3	57.1	5.6	34.3	1.9	11.4
Drugs	45.8	62.9	22.9	31.5	13.8	18.9	4.6	6.3
Indirect Cost Savings								
Mortality	0.5	2.5	0.2	1.2	0.1	0.7	0.0	0.2
Morbidity	5.1	7.2	2.5	3.6	1.5	2.2	0.5	0.7
Total Cost Savings	**73.0**	**208.6**	**36.4**	**104.2**	**21.9**	**62.6**	**7.3**	**20.8**
Manitoba Cost Savings	**3.0**	**11.3**	**1.5**	**5.6**	**0.9**	**3.4**	**0.3**	**1.1**

PRINCIPAL INVESTIGATOR Dr. Jared Carlberg, Department of Agribusiness, University of Manitoba

MPSG INVESTMENT $30,000

CO-FUNDER $6,000 – University of Manitoba

DURATION 1 year

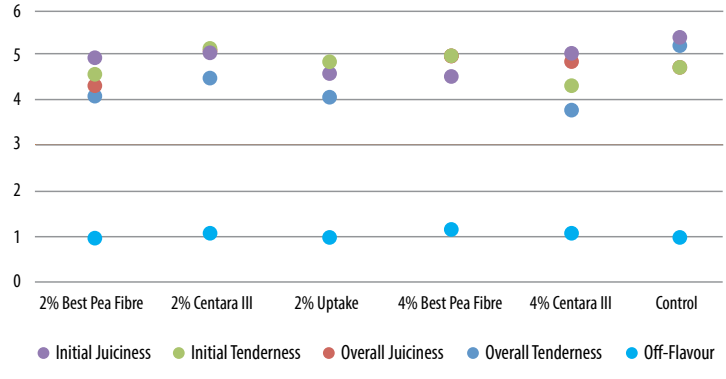
Pea Fibre Utilization in Ground Poultry, Beef and Pork

Pea fibre can be added to ground meat patties without affecting sensory attributes allowing for a nutritional fibre claim and cost reduction.

CONSUMERS ARE REQUESTING healthy versions of familiar products, but are still concerned about cost. Incorporating pea fibre into frozen ground meat patties can allow manufacturers to make a fibre nutrient content claim while reducing product cost. Previous research indicates that adding pea fibre can also aid in moisture retention and improve cooking yield. This study investigated the possibility of creating ground beef, pork and chicken patties with the addition of pea fibre while maintaining sensory acceptability. Cooking yield, colour, moisture retention and cost were also evaluated.

Different beef, pork and chicken recipes were created with the inclusion of three yellow pea fibre ingredients (Best Pea Fibre, Centara III and Uptake 80) at three different inclusion rates (2, 4 and 6%). All rates would allow for a Health Canada fibre nutrient claim. Spices were also added to the patties to mask any pea off flavours, but no additional binders or extenders were added. For the preliminary study, patties, along with a "control" from each meat type, were scored by a sensory evaluation panel. Beef, pork and chicken patties with 2 and 4% fibre from Best Pea and Centara III and 2% fibre from Uptake 80 receiving a score of three or higher and was used for the second part of the study. Patties were then evaluated for specific sensory attributes (juiciness, tenderness, off-flavour) and for cooking yield, moisture loss and colour (lightness, redness and yellowness).

All ground beef patties ("control" and fibre added) showed no significant difference in juiciness, tenderness or off-flavour (see graph below). The addition of pea fibre at both levels increased cooking yield, however the results were inconclusive for moisture loss as only one recipe had reduction moisture loss (Centara III fibre at 4%). There was no significant difference in redness and yellowness, but patties with Best Pea Fibre at 2 and 4% and Centara III fibre at 4% had a significant difference in lightness.



Ground beef patties sensory attributes.

All ground pork patties (control and fibre added) showed no significant difference in juiciness, tenderness or off flavour. All additions of pea fibre increased cooking yield with the exception of Centara III fibre at 2%. There was no significant difference in moisture loss for any of the patties. The addition of Centara III fibre at 2% had a significant effect on lightness and the station of Best Pea Fibre at 4% had a significant effect on redness.

All ground chicken patties showed no significant difference in off-flavour. However, patties with the addition of 4% fibre were rated as less juicy and patties with 4% of Centara III fibre were rated as less tender. The limited amount of water in the patties may have led to insufficient hydration of the fibre ingredients, thus accounting for the lower juiciness and tenderness ratings. There was no significant difference among cooking yield and moisture loss for any of the chicken patties, which may also be an effect of the limited amount of water. All patties with the exception of Centara III fibre at 2% had significantly different lightness. Best Pea Fibre at 4% also significantly affect redness and yellowness.

Based on the above results, adding 2 or 4% yellow pea fibre to frozen ground beef and pork patties does not have a detrimental effect on sensory or cooking properties while still allowing for a Health Canada fibre nutrient claim. The same was determined for ground chicken patties as well, except only at the 2% pea fibre level. Depending on the amount and type of fibre used, including fibre reduced the cost of the meat patties anywhere from 1–15%.

The addition of pea fibre in ground beef and pork patties has many benefits for both manufacturers and consumers. Cooking yield and fibre content are increased and cost of production is lowered. For chicken patties, fibre content is increased and production cost is lowered. Manufacturers can use the fibre claim to promote the patty as a value-added product.

PRINCIPAL INVESTIGATOR Luis Maya Desdier, Food Development Centre

MPSG INVESTMENT $24,500

CO-FUNDER $5,000 – Food Development Centre

DURATION 1 year

Extension of Shelf Life on Refrigerated Soy Spread and Utilization of Soy in Non-Dairy Yogurt

Locally produced GM soybeans can be utilized for food and beverage applications without beanie off-flavours.

Seasoned soy spread

SOY PRODUCTS SUCH as soy milk, tofu, yogurts and spreads are increasing in popularity due to the increase in lactose intolerant diets and interest in Asian foods, which commonly use soybeans. Soybeans are also an economical source of plant protein and Health Canada recently recognized that consuming 25g of soy protein per day can help lower the risk of coronary heart disease. Soybeans grown in Manitoba are typically produced for the oil crush market and these genetically modified (GM) varieties are not bred for speciality food markets. These varieties tend to have a gritty mouth feel, beanie off-flavours and other undesirable traits for food developers. Advances in processing such as fermentation, dehulling and thermal treatments have been used to limit some of the off-flavours. If flavour and quality issues can be overcome, there may be opportunities for value-added markets for both farmers and processors.

This project utilized a laboratory scale pilot plant to improve a soy yogurt beverage and soy spread prototypes, develop nutrition fact tables, conduct sensory evaluations and assess the products' shelf life to prepare products for commercialization. Manitoba-grown food grade soybeans were soaked overnight, washed and rinsed. The beans were then ground with hot water to obtain a slurry product using a blender. Soy milk was extracted and used as the base material for the production of soy spread and soy yogurt.

Soy yogurt beverage

To develop the soy spread, the milk was coagulated with a salt or lemon juice to precipitate the proteins and form curds and whey. The curd, also known as tofu, was separated from the whey by draining, filtering and pressing. To create the soy spread, the tofu was mixed thoroughly with other ingredients and seasonings. Because seasonings' composition and source of origin can compromise the microbial load and subsequently increase the rate of quality deterioration, the soy spread was developed using pre-treated seasonings. The final soy spread had negligible beanie off-flavour and an acceptable pH level. In sensory evaluations, the overall appearance, colour, mouth feel and spreadability met consumers' expectations. Based on the results of nutrient analyses, the soy spread was high in calcium, a source of fibre, low in fat, saturated fat and sodium, and free of trans fat and cholesterol.

To develop the soy yogurt, high fat soy milk was extracted from whole soybeans. Additional ingredients were added for hydration (to achieve a drinkable texture), pH adjustment (to achieve the tangy yogurt characteristic) and additional berry flavouring and masking agents (to mask the beany off-flavours). A product development challenge identified was the separation of the yogurt beverage into two phases after storage meaning that the beverage requires shaking before drinking or the use of gums and stabilizers to improve the homogenization. A 93-day shelf life study indicated acceptable microbiological safety and sensory analysis. Sensory panelists scored the beanie off-flavours as very low and liked the colour, creamy texture and fruity flavours of the yogurt. According to the Canadian Guide to Food Labelling and Advertising, the soy yogurt beverage product can be labelled as low in saturated fat and sodium, free of trans fat and cholesterol and a source of energy and iron.

This research demonstrated how Manitoba-grown GM soybeans for the oil market can also be used in food products by utilizing processing techniques, preservatives and seasonings to alter taste and functionality. These products were developed as part of the Canadian Climate Advantage Project, which promotes novel Manitoba-grown and processed foods and food ingredients with health benefits beyond basic nutrition. Industry partner, NuEats Food Innovation (managed by MAHRN), can be contacted regarding product commercialization. This research could be expanded to frozen soy desserts, kefir, energy bars or baked good applications.

PRINCIPAL INVESTIGATORS Dr. Paulyn Appah and Meeling Nivet, Food Development Centre

MPSG INVESTMENT $21,400

CO-FUNDER $5,000 – Manitoba Agri-Health Research Network (MAHRN), NuEats Food Innovation

DURATION 1 year

Photo courtesy of Rejean Picard, Manitoba Agriculture

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