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## **Title: Relationship Between Global Protein Content Claim Regulatory Frameworks and Nutrient Intakes of Canadians**

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**Background:** Consumers rely on food labels to identify “source of protein” foods. Whereas Australia and New Zealand (AZ) and European Union (EU) consider only protein quantity of the food, Canada and the United States (US) are stricter and consider both protein quantity and quality of the food. Thus, these regulatory framework differences may deter the identification and, hence, consumption of healthy plant-based protein foods by Canadians. Although protein from plant-based sources may have a lower quality compared to animal sources, these foods may still be valuable protein sources and contribute to a healthy diet.

**Objectives:** To compare protein intake, protein quality, energy, and nutrient intakes among Canadian adults identified as consumers of plant-based protein foods meeting the criteria for a protein content claim (PCC) according to the regulatory frameworks in both, either, or neither used in Canada compared to each of the US, EU, and AZ frameworks.

**Methods:** The first 24-hour dietary recalls of adults (n=11,817) from the Canadian Community Health Survey-Nutrition 2015 (CCHS-2015) were used to identify consumers of plant protein foods meeting PCC criteria within each regulatory framework. The Canadian Nutrient File was used to determine which plant-based food groups in CCHS-2015 could meet a PCC under each framework. Consumers of  $\geq 1$  plant-based protein food(s) in a food group meeting a PCC according to the regulatory frameworks in both, either, or neither used in Canada and each of the US, EU, and AZ frameworks were each compared using ANOVA, adjusting for misreporting and covariates.

**Results:** Among the 74 food groups in CCHS-2015 categorized as plant-based, 5 in Canada, 3 in US, 28 in EU, and 12 in AZ contained a food that met a PCC. Consumers of food groups meeting a PCC under the US framework were consumers under the Canadian framework. Protein intake and quality were not significantly different among consumers in any of the regulatory frameworks. There were significant differences in dietary fibre, calcium, magnesium, and potassium intakes

( $p < 0.05$ ) in Canada-EU and Canada-AZ comparisons, where generally consumers in both frameworks and AZ/EU alone had significantly higher intakes compared to non-consumers.

Conclusions: Consumers of plant-based protein food groups in frameworks with broader PCC definitions did not differ in protein intake or quality compared to consumers eating food groups under the stricter Canadian framework and demonstrated greater nutrient density compared to non-consumers. Therefore, the present work highlights potential benefits to modifying the current Canadian PCC regulatory framework.

**Statement on how this science or technology supports or advances public health:**

This project is designed to provide evidence-based information to support the national protein regulatory and/or policy modernization. It was developed in consultation with Protein Industries Canada (PIC), one of Canada's 5 Global Innovation Clusters, and advice was provided by Health Canada. Broadening the ability for plant protein-based foods to meet a protein content claim will allow for greater communication to consumers supporting food choices that align with Canadian food policies such as Canada's Food Guide, which encourages the selection of plant-based protein foods.

## **Title: Assessment of Anti-Nutritional Factors and *in vitro* Protein Quality in Yellow Peas and Their Derivatives under High-Hydrostatic Pressure**

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**Background:** Pea seeds contain excellent amounts of proteins, carbohydrates, dietary fibers, essential vitamins and minerals, while being low in fat. They offer a sustainable source of plant-based proteins, with potential benefits relative to soy and animal-based proteins in terms of allergenicity, cost, nutrition, animal welfare, health, and environmental impact. However, similar to other pulse crops, yellow peas inherently possess anti-nutritional factors (ANFs) such as phytic acid, trypsin/chymotrypsin inhibitors, hemagglutinins, condensed tannins, and polyphenols. These substances, which are a major concern in pulse sources, could decrease their nutritional value by impacting nutrient bioavailability and protein digestibility, thus limiting the protein quality of pulses compared to animal sources. High-hydrostatic pressure (HHP) is a novel non-thermal technology that could mitigate these issues while preserving thermosensitive beneficial compounds.

**Objectives:** This study examined the effects of HHP treatment on the ANFs and *in vitro* protein quality of yellow pea flour, protein concentrate, and protein isolates.

**Materials and Methods:** Yellow pea flour, protein concentrate, and isolates (both low (80%) and high (90%) protein contents) were subjected to HHP treatment at 600MPa for a 10-minute holding time at room temperature. Both unprocessed and processed yellow pea samples underwent analyses for dry matter, crude protein, trypsin inhibitors, phytic acid, total polyphenols, condensed tannins, amino acid composition, *in vitro* protein digestibility (IVPD), and *in vitro* Protein Digestibility Corrected Amino Acid Score (IVPDCAAS).

**Results:** The application of HHP demonstrated significant efficacy in reducing trypsin inhibitors and phytic acids in yellow pea flour and protein concentrate. Polyphenol levels decreased in all samples post-treatment, while condensed tannins increased notably. *In vitro* protein quality showed a slight enhancement, with an approximately 5% increase observed in the low protein isolate.

**Conclusion:** HHP treatment applied in the yellow pea products have reduced ANFs, with the exception of condensed tannins. However, there was no significant improvement of *in vitro* protein quality in the yellow pea samples after HHP processing.

**Significance:** This research aimed to overcome the constraints posed by ANFs, aiming to enhance the utilization of pea proteins and promote the adoption of sustainable plant-based protein ingredients. It also provides some practical insights for the application of HHP treatment for researchers and food processors.

**Statement on how this science or technology supports or advances public health:**

Upon optimizing yellow pea samples and processing methods, the research is intended to develop healthier and more sustainable food options, aligning with public health goals and promoting the overall well-being of individuals and the environment.

## Title: Genetic Modification of the Association of the Portfolio Diet Score and its Components with LDL-C in a Population of Young Adults

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**Background:** The Portfolio Diet, a plant-based dietary pattern of cholesterol-lowering foods, has demonstrated clinically meaningful reductions in LDL-C. Variations in genes including *ABCA1*, *ABCG8*, *APOA5*, *ANGPTL3* and *APOC1* have also been associated with blood lipids. However, the interaction between these genes and the Portfolio Diet on LDL-C has not been explored.

**Objective:** We examined the genetic modification of the association of the Portfolio Diet Score (PDS) and its components with LDL-C.

**Methods:** This cross-sectional analysis included 1,509 young adults from the Toronto Nutrigenomics and Health Study. Adherence to the Portfolio Diet was assessed using the validated PDS based on six components (sources of nuts, plant protein, viscous fibre, plant sterols, monounsaturated fat and saturated fat & cholesterol) derived from a validated Toronto-modified Harvard food frequency questionnaire. DNA was obtained from blood and genotyped for SNPs in *ABCA1* (rs1883025), *ABCG8* (rs6544713), *APOA5* (rs662799), *ANGPTL3* (rs10889353) and *APOC1* (rs4420638). Data were analyzed using multiple linear regressions, adjusted for potential confounders, to examine the association of (1) the Portfolio Diet with LDL-C and (2) gene-diet interactions with LDL-C.

**Results:** Participants had a mean age of 22.7±2.5 years and LDL-C of 2.3±0.6mmol/L. A 1-point increase in PDS was significantly associated with lower LDL-C (p=0.02). *ABCA1* genotype modified this association (p=0.01). A 1-point increase in PDS was significantly associated with lower LDL-C, among *ABCA1* CC (β [95% CI]: -0.015mmol/L [-0.025, -0.006], p<0.01) and TT (-0.036mmol/L [-0.067, -0.005], p=0.02) homozygotes but not among those with the CT genotype. A 1-serving increase in plant protein, plant sterols and decrease in saturated fat & cholesterol sources were also significantly associated with lower LDL-C (p<0.05). *ABCG8* genotype modified the association of plant protein (p=0.01) and plant sterols (p=0.01) on LDL-C. Among *ABCG8* TT homozygotes, a 1-serving increase in plant protein was significantly associated with lower LDL-C (-0.298mmol/L [-0.575, -0.021], p=0.04) and a 1-serving increase in plant sterols trended towards lower LDL-C (-0.048mmol/L [-0.096, 0.000], p=0.05).

**Conclusions:** In young adults, greater adherence to the Portfolio Diet and its components showed significant favourable associations with LDL-C. Our findings suggest that *ABCA1* and *ABCG8*

genotypes modify the association of PDS, plant protein and plant sterols with LDL-C. These results will inform personalization of dietary advice to encourage adherence to the Portfolio Diet.

**Statement on how this science or technology supports or advances public health:**

This work will support a large clinical trial, the Coronary Heart Effectiveness Assessment of the Portfolio Diet in Primary Care (CHEAP) program, of 1,100 high-risk patients in primary care, to assess the clinical impact of our validated PortfolioDiet.app, a digitally-enabled health services tool translating guidelines through education and supporting the patient-provider relationship, on LDL-C (at 1-year) and cardiovascular events (at 7-year). The results of the current project will be directly applied in the PortfolioDiet.app to personalize the advice/messaging to encourage adherence to the Portfolio Diet. The app will be freely available on the Canadian Cardiovascular Society website.

**Title: Development and Mechanisms of Oil-Based Antimicrobial Delivery for Dry Cleaning and Sanitization in the Processing Environments of Low-Moisture Foods**

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The inherent safety of low-moisture foods (LMF) is a faulty assumption frequently made based upon the low-water activity ( $a_w$ ) nature of these products. Contamination with *Salmonella* spp. is of particular concern in LMFs and across their processing environments due to the survival of the organism under dry conditions and the subsequently induced cross-tolerance. It is of general principle to avoid aqueous-based sanitation systems within dry food processing environments as moisture promotes the proliferation of microorganisms and gives rise to the need for equipment dry-out leading to production downtime. We discovered that food-grade oils mixed with organic acids is an effective means of tackling desiccated *Salmonella enterica*. Lawn-based inoculum was desiccated on stainless-steel coupons at controlled relative humidities for 20 h prior to treatment. The acidified oils resulted in structural integrity of the treated cells, such as membrane disruption, periplasmic irregularity, and cytoplasmic granularity, as investigated with SYTO 9/Propidium Iodide staining (Live/Dead) and Transmission Electron Microscopy (TEM). Furthermore, it was found that dispersal of a controlled, low level of water (0.3% by volume) within the acidified oil, termed acidified water-in-oil (W/O) emulsion, enhanced the antimicrobial efficacy by a pronounced margin. The dispersion of water allows partitioning of organic acids from the continuous oil phase to the dispersed water phase which functions as another way of entry into the bacterial cytoplasm. A 20-min contact at room temperature reduced desiccated *Salmonella* (a four-strain cocktail) and *Listeria monocytogenes* (a three-strain cocktail) by greater than 6.5 log MPN/coupon. Such microbial killing was attenuated as glycerol was used to reduce the emulsion  $a_w$  while maintaining a constant water concentration, indicating that the antimicrobial enhancement from water was due to differential osmotic pressure. This was confirmed by Scanning Electron Microscopy (SEM) revealing cell remnants alongside a significant cell density reduction after treatment with acidified W/O emulsion. Additionally, the developed oil-based antimicrobials exhibited increased efficacy with temperature elevation (45°C), which was linked to a reduction of *Salmonella* membrane viscosity at elevated temperatures, as studied with Fluorescence Lifetime Imaging Microscopy (FLIM). In sum, we believed that the antimicrobial mechanisms of the developed oil-based system were due to the membrane permeabilization caused by the organic acids loaded in oil, coupled with the hypoosmotic stress created by the water dispersion, facilitating the transport of organic acids and water influx, eventually lysing the cells with damaged membrane, which also worked in synergy with mild heat treatment.

**Statement on how this science or technology supports or advances public health:**

Adaptation of the developed oil-based system would allow dry sanitation of food-processing equipment without applications of traditional wet cleaning and flammable alcohol-based sanitizers.



## **Title: Quantification of Total Glutathione in Mushrooms with LC-MS**

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Mushrooms are renowned for their rich nutrient content, including vitamins, minerals, and dietary fiber, and are identified as a significant source of the antioxidant tripeptide, glutathione. This naturally occurring compound plays a vital role in protecting against oxidative damage and is recognized for its beneficial effects on the immune system, among other functions. This study aimed to establish a robust analytical method for quantifying total glutathione in edible mushrooms, employing liquid chromatography-triple quadrupole mass spectrometry (LC-QqQ MS). Glutathione exists in various forms in mushrooms, including reduced (GSH), oxidized (GSSG), and protein-bound (GSSP) forms. To circumvent the potential alteration in the ratios of GSH, GSSG, and GSSP during sample preparation, which could lead to measurement artifacts, we used dithiothreitol (DTT) to uniformly convert all glutathione forms to GSH. Ethanol and protease inhibitors were added to the extraction solvent to prevent GSH degradation by mushrooms' endogenous enzymes. The extraction parameters, including temperature, time, and reducing agent concentration, were optimized to maximize the GSH extraction rate. Notably, different types of mushrooms required varying DTT concentrations for optimal GSH extraction; for example, crimini mushrooms required 60 mM DTT, whereas shiitake mushrooms needed only 5 mM. GSH quantification was conducted using LC-QqQ MS in the multiple reaction monitoring (MRM) mode, alongside a calibration curve and the isotopically labeled internal standard, GSH ( $^{13}\text{C}_2$ ,  $^{15}\text{N}$ -glycine), to enhance measurement accuracy. The method was validated through a spike-and-recovery assay, yielding an accuracy range of 78–101%, which varied with spiking levels. Finally, we applied this method to analyze glutathione levels in eleven types of mushrooms, thereby broadening our understanding of this antioxidant peptide's availability in these key dietary sources. Based on their average glutathione concentrations (fresh weight basis), white button, portabella, crimini, and beech fell in the lower range (4–10 mg/100 g), shiitake, king oyster, lion's mane, and oyster exhibited medium levels (10–20 mg/100 g), whereas pioppini, enoki, and maitake had higher levels (20–30 mg/100 g). Additionally, considerable variability was observed within the same types of mushrooms sourced differently, as indicated by coefficients of variation ranging from 16% to 45%.

### **Statement on how this science or technology supports or advances public health:**

The analytical method was applied to quantify total glutathione content in various mushrooms, revealing variability across and within mushroom types and contributing new data to FoodData Central, the U.S. Department of Agriculture's publicly accessible food composition database. These results are instrumental for estimating the dietary intake of antioxidants and glutathione's constituent amino acids in clinical studies and consumers' diets and also set the stage for further public health research in pertinent areas.

## **Title: Probiotics Development and CRISPR-Cas System**

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Probiotics, defined as live microorganisms with beneficial properties, were drew interest for their immune-modulating and gut restoration potential, but interest by food and pharmaceutical industries has expanded to include a wider array of benefits. The fast-growing potential of probiotics is mostly attributed to their versatile nature, remarkable tolerance, production of antibacterial substances, and therapeutic value. Currently, genetic engineering techniques, especially the CRISPR (Cluster Regularly Interspaced Short Palindromic Repeats) method, are used extensively in different applications. The use of the CRISPR-Cas system in microorganism modulations is well-documented. The flexibility, specificity, and accuracy of this system enable the researcher to use it for the development of engineered probiotics. This system is also used to enhance the intrinsic properties of microorganisms, treat genetic diseases, enhance food production, and for use in different biotechnological processes. The widely used *Lactobacillus* probiotics have a CRISPR-Cas system in more than 40% of their genome. The natural presence of CRISPR-Cas genes in probiotics aids to their safety as it is known that more CRISPR genes indicates the absence of virulence and antibiotics resistance genes. Moreover, this system can also be used to modify the probiotics' genome and develop engineered probiotics with advanced applications and more potential.

### **Statement on how this science or technology supports or advances public health:**

Probiotics may be beneficial in disease treatment and may have a role in food science. Manipulation of the probiotic's genome can facilitate understanding of the potential of probiotics for therapeutic use and other benefits.

## **Title: Development of a Food-Based Score (clinical-Portfolio Diet Score) and a Health Application (PortfolioDiet.app) to Translate a Nutrition Therapy for Cardiovascular Risk Reduction**

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**Background:** The Portfolio Diet combines cholesterol-lowering foods for the management of cardiovascular disease (CVD). To enhance implementation of this nutrition therapy into clinical practice, our objectives were to:

- 1) Develop a score that facilitates self-tracking of the Portfolio Diet (clinical-Portfolio Diet Score [c-PDS]) and assess the score's validity.
- 2) Develop a health app (PortfolioDiet.app) utilizing the c-PDS and test the app's acceptability in its intended population.

**Methods:** For objective 1, we developed the food-based c-PDS (range, 0 to 25-points) and assessed its validity in a secondary analysis of a 24-week completed trial in participants with hyperlipidemia. Validity of the c-PDS was assessed against weighed 7-day diet records [7DDR] and concomitant changes in a biomarker of adherence to the diet, low-density lipoprotein-cholesterol (LDL-C), from baseline to 24-weeks. For objective 2, we developed the PortfolioDiet.app, a multicomponent, patient-facing engagement and educational tool, which incorporated the c-PDS to facilitate self-tracking. The PortfolioDiet.app was evaluated in adults at high CVD risk from an ongoing study (ClinicalTrials.gov Identifier: NCT02481466). Participants were invited to join an ancillary study and be randomized to the PortfolioDiet.app or a control (no app) for 12-weeks. Acceptability was assessed through a multifaceted approach, including dietary adherence using 7DDRs, app usage, usability through the System Usability Scale (SUS), with a score higher than 70 being considered acceptable, and a qualitative analysis using Nvivo 12.

**Results:** The c-PDS was positively correlated with dietary adherence from 7DDRs ( $r=0.94$ ,  $P<0.001$ ) and negatively correlated with change in LDL-C ( $r=-0.43$ ,  $P<0.001$ ), where a 1-point increase was associated with an LDL-C reduction of  $-0.04$  mmol/L (CI:  $-0.06, -0.03$ ;  $P<0.001$ ). In the ancillary study, 14 participants (8 female, 6 male) were randomized (8 intervention, 6 control) and completed the study. There was a tendency for an increase in adherence to the Portfolio Diet by  $1.25\pm 2.8$  (5.0%) and  $0.19\pm 4.4$  (0.8%) points in the app and control groups, respectively, with no difference between groups ( $P>0.05$ ). Participants used the app on average for  $18\pm 14$  days per month and rated the app as usable (SUS of  $80.9\pm 17.3$ ). Qualitative analyses complemented the quantitative data.

**Conclusion:** These results indicate good validity of the c-PDS and allow estimation of a clinically meaningful reduction in LDL-C of  $0.53$  mmol/L (13.1%) with an achievable 12-point increase in

the c-PDS, informing our messaging within the PortfolioDiet.app. Adults at high CVD risk consider the PortfolioDiet.app acceptable. This research supports an upcoming large cardiovascular outcome trial in primary care using the PortfolioDiet.app.

**Statement on how this science or technology supports or advances public health:**

As CVD continues to be a leading cause of mortality globally, prioritizing lifestyle behaviour interventions for chronic disease prevention and management is pivotal. Among these interventions, the Portfolio Diet is an effective therapy for managing dyslipidemia and reducing the risk of CVD. As a tool for disseminating this nutrition therapy, the PortfolioDiet.app serves to increase adoption of the Portfolio Diet in both clinical practice and among the general population.

**Title: The Potential of Using *in vitro* Digestion Models for the Determination of Protein and Amino Acid Digestibility in Assessing Protein Quality****Author(s)** and affiliation(s): Nguyen Bui<sup>1</sup>, Jason Neufeld<sup>1</sup>, Adam Franczyk<sup>1</sup>, Jiayi Chen<sup>1</sup>, Zhongyang Wan<sup>1</sup>, James House<sup>2</sup><sup>1</sup> Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada<sup>2</sup> Richardson Centre for Food Technology and Research, University of Manitoba, Winnipeg, Manitoba, Canada

This study evaluated the ability of two *in vitro* static digestion models, namely the pH-drop and the INFOGEST, to determine the *in vitro* protein quality of casein and seven protein samples, including a nut, legumes, oilseeds, and single-cell protein sources. The pH-drop model was able to measure *in vitro* protein digestibility directly for the subsequent calculation of *in vitro* Protein Digestibility Corrected Amino Acid Score (IV-PDCAAS) values. The INFOGEST model digesta, however, were analyzed by three different methods i) OPA derivatization, ii) total nitrogen via Kjeldahl, and iii) individual amino acid analysis to determine both *in vitro* protein digestibility and *in vitro* amino acid digestibility. The latter data was then used to calculate both IV-PDCAAS and *in vitro* Digestible Indispensable Amino Acid Score (IV-DIAAS). All samples displayed high digestibility results with low variation across all four measurement methods. The limiting amino acids were generally tryptophan or sulfur amino acids (for legumes and single-cell proteins), and lysine (for the nut and oilseeds). Strong correlations were observed between in-house *in vivo* PDCAAS data and IV-PDCAAS results from all *in vitro* methods. Between the four assessment methods, the OPA assay ( $R^2 = 0.942$ ) and total amino acid analysis ( $R^2=0.944$ ) from the INFOGEST digestion products demonstrated closer associations with *in vivo* PDCAAS compared to the pH-drop model ( $R^2=0.912$ ) and the Kjeldahl analysis ( $R^2=0.904$ ) from the INFOGEST model. On the other hand, the pH-drop model exhibited better repeatability across measurements. Aiming to investigate the impact of using different protein quality assessments, all samples were evaluated by the PDCAAS – the official method in the USA/Canada, the DIAAS – the recommendation method by the FAO/WHO, and the Protein Efficiency Ratio (PER) – the official method in Canada, then categorized to respective protein content claims of each method. It was observed that there was no difference in terms of protein content claims determined by *in vivo* or the four *in vitro* methodologies. Additionally, six out of eight samples were ranked with different protein content claims when assessed by three protein quality evaluation assays. The PDCAAS generally offered higher protein permitted claims than the DIAAS and the PER methods and their respective protein content claims. The present data demonstrated the potential of both the static *in vitro* pH-drop and INFOGEST digestion models in determining protein digestibility and PDCAAS, with the INFOGEST model having the added benefit of being used for measuring amino acid digestibility and DIAAS.

**Statement on how this science or technology supports or advances public health:**

Both the static *in vitro* pH-drop and INFOGEST digestion models can be valuable screening tools for researchers and industries interested in protein quality assessment. Further development of these *in vitro* methodologies can provide research and regulatory bodies with effective and sustainable non-animal testing alternatives for protein quality.

**Title: Structural and Elemental Analysis of Waraka and Wala Jackfruit Seed Flour Samples by SEM-EDS Method**

**Author(s)** and affiliation(s): Yenisha T.Senaweera and Prof BD Rohitha Prasantha, Sabaragamuwa University of Sri Lanka

Jackfruit seeds are well known for desirable functional and physiochemical properties. It has at least two types of varieties based on the habitat, climate and other properties. Jackfruit diversification is dependent upon phenotypic and organoleptic properties. Seed flour is a good source of starch, dietary fiber and minerals. Scanning electron microscopy-energy dispersive X-ray spectroscopy analysis (SEM-EDS) provides a quick non-destructive determination of the elemental composition of the sample, readily identifying some elements present in the biological material. The objectives of this study were to identify the structural morphological characteristics and elements present in the seed flour samples of two jackfruit varieties, waraka and wala. Jackfruit seeds flour samples of waraka and wala were prepared by using de-coted, dried seeds. The SEM-EDS method was used to identify the element profile within the ultra-structure. Scanning electron microscopic analysis showed that the starch granule has bell and spherical shapes and average granules size is respectively  $7.99083 \pm 0.78 \mu\text{m}$  for waraka seed and  $8.9335 \pm 0.31 \mu\text{m}$  for wala seed flour. Wala flour showed comparatively higher macro and micro element content including 47% oxygen, 41% carbon, 8% nitrogen, 2% sodium and 0.7% potassium. Waraka seed flour contains 53% oxygen, 41% carbon, and 0.5% potassium. Wala seed flour contains sodium which was not present in the waraka seed flour. Waraka seed flour did not contain nitrogen as a macro element.

**Statement on how this science or technology supports or advances public health:**

These two varieties of Jackfruit seeds contain valuable nutrients that may have immunomodulatory and anti-diabetes health benefits.

**Title: Effects of 2'-Fucosyllactose and Bifidobacterial Species on Yogurt During Fermentation and Cold storage**

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2'-Fucosyllactose (2'-FL) is postulated to provide health benefits and promote the growth of beneficial bacteria. Meanwhile, bifidobacteria are well-known probiotic microorganisms. This study was undertaken to evaluate the effects of 2'-FL and bifidobacteria on properties of yogurt during fermentation and refrigerated storage. Yogurts were produced containing 2'-FL (0, 2 g/L), and bifidobacterial strains (*Bifidobacterium longum* BB536 and *Bifidobacterium longum* ssp. *infantis* ATCC 15697) at a concentration of at least 10<sup>9</sup> CFU/mL. All yogurts were stored at 4 °C for 5 weeks. Bifidobacteria and yogurt cultures, *Streptococcus thermophilus* and *Lactobacillus delbureckii* subsp. *bulgaricus*, were enumerated from undisturbed aliquots before fermentation, and after fermentation (once a week for 5 wk). In addition, metabolites of yogurt samples during cold storage were measured using high-performance liquid chromatography (HPLC). Results showed that 2'-FL was stable in yogurt for more than 5 wk of storage, and addition of 2'-FL does not affect yogurt properties and viability of mixed yogurt cultures. The addition of bifidobacteria had a negative impact ( $P < 0.01$ ) on the survival rate of *S. thermophilus* and *L. bulgaricus*, because of competition effect. Bifidobacterial strains are sensitive to environmental factors. *B. longum* BB536 survived at a level higher than 10<sup>6</sup> CFU/g for 28 days, while only 7 days for *B. longum* ssp. *infantis* ATCC15697. In summary, this study has shown the impact of 2'-FL and bifidobacterial species on yogurt properties and viability of mixed yogurt cultures. It is promising to use 2'-FL in yogurt products as a prebiotic.

**Statement on how this science or technology supports or advances public health:**

Yogurt is known for its beneficial effects on human health and nutrition. This study reported the production of symbiotic yogurt as a functional food for specified health uses containing bifidobacteria and 2'-fucosyllactose (2'-FL).