FINAL PROJECT REPORT

Project Title: Health benefits of Oregon & Washington pears

PI: Kalidas Shetty
Organization: University of Massachusetts at Amherst
Telephone: 413-545-1022
Email: kalidas@foodsci.umass.edu
Address: Chenoweth Laboratory
Address 2: 102 Holdsworth Way
City: Amherst
State/Zip: MA 01003

Co-PI: Duane W. Greene
Organization: University of Massachusetts at Amherst
Telephone: 413-545-5219
Email: dgreene@pssci.umass.edu
Address: Bowditch Hall
Address 2: Holdsworth Way
City: Amherst
State/Zip: MA 01003

Cooperators: NA

Other funding sources:
UMass supported Teaching Assistant also worked on this project.
Agency Name: University of Massachusetts at Amherst

Amount awarded: Year 1: $ 47,050 Year 2: $ 51,400

Total Project Funding: $ $98,450 for 2 years

Budget History:

Item	**(Year 1)**	**(Year 2)**
Salaries: (50% Research Associate)	$ 23,000	$ 24,000
Benefits (35% of Salary)	$ 8,050	$ 8,400
Wages	NA	NA
Benefits	NA	NA
Equipment (HPLC Service Maintenance and Columns)	$ 3,000	$ 4,000
Supplies (Reagents, Biochemicals & Enzyme)	$ 7,000	$ 8,000
Travel	NA	NA
Miscellaneous :(Orchard needs-preparation & extraction of samples)	$ 6,000	$ 7,000
Total	$ 47,050	$ 51,400

RECAP OF OBJECTIVES:

1) Determination of effect of whole pear phenolic and oligosaccharide bioactives from fresh and long-term CA stored pear fruit on stimulating growth of select lactic acid bacteria relevant for better digestive and gut health.

2) Determination of pear and lactic acid bacteria fermented combinations to enhance bioactives that have antioxidant activity and which can slow enzyme activity relevant for carbohydrate metabolism-linked oxidation (anti-diabetes potential) and inhibit ulcer bacteria (*Helicobacter pylori*) for better digestive and gut health.

The objectives were carried out over 2 growing seasons with fresh pear varieties and one growing season with stored and commercial pear varieties following storage from markets in the East coast

SIGNIFICANT FINDINGS:

A) Fresh Variety Analysis: (Manuscript 1 being written for publication in J. Medicinal Food)

1) Important pear varieties Bartlett, Comice, Bosc, Starkrimson, Concorde, Red Anjou and Anjou have significant total soluble phenolic antioxidants distributed between pulp and peel and on an average 250 gram fresh pear have between 40 to 70 mg of total soluble phenolics. At the higher end for Concorde the content was about 75 mg from one 250 gram serving of fruit.

2) There is positive correlation between total soluble phenolic content and free radical scavenging-linked antioxidant activity. The peel extracts of Starkrimson and Concorde especially stand out in terms of antioxidant activity. Overall all varieties do have significant soluble phenolics-linked antioxidant activity.

3) High α-glucosidase and α-amylase inhibitory activity (reflection of *in vitro* anti-diabetes potential through the ability to inhibit key enzyme involved in carbohydrate digestion and uptake) was observed in all pear cultivars during both years. Pulp extraction showed higher inhibitory activity of these enzymes compared to peel extracts.

4) A positive correlation was observed between α-glucosidase inhibitory activity and total soluble phenolic content in peel and pulp extractions of majority of pear cultivars. Significant dose-dependent response (a key factor for pharmaceutical relevance of drugs like acarbose) in the enzyme inhibitory activity was also observed in all pear cultivars. Red pear, Red Anjou, Comice, Starkrimson and Concorde showed higher α-glucosidase inhibitory activity in both peel and pulp extracts. This is significant since the entire fruit has high anti-diabetic potential.

5) The major phenolics found in fresh and stored varieties were protecatechuic acid, quercetin dervitives (Rurin), gallic acid and small levels of resveratrol in peel and protocatechuic acid and gallic acid in pulp extracts.

B) Fermentation and Probiotic Benefits of Pears: (Manuscript 2 on fermented anti-diabetic potential being written for Journal of Food Biochemistry and Manuscript 3 on anti-ulcer and probiotic benefits being written for publication in International Journal of Food Microbiology)

1) Probiotic bacteria, *Lactobacillus acidophilus* (LA) can grow effectively on juice extracts of all pear varieties and mobilize the soluble phenolics to higher antioxidant function over 72 hours.

2) The growth of probiotic LA on all pear varieties also enhances bioactives that inhibit carbohydrate metabolizing enzyme alpha-gluosidase which has relevance for management of early stages of type 2 diabetes similar to the drug acarbose.

3) The growth of probiotic LA on all pear varieties enhances bioactives that inhibit ulcer bacteria, *Helicobacter pylori.*

4) The growth of probiotic LA on all pear varieties enhances bioactives that enhance the growth of other beneficial probiotic bacteria such as *Bifidobacterium longum.*

5) Epicatechin and quercetin derivatives were the major phenolic compounds detected in the fermented samples.

6) Juice extraction and concentration increases ACE (angiotenin converting enzyme) inhibitory activity, which is a target for reducing high blood pressure, which a macro vascular complication of type 2 diabetes.

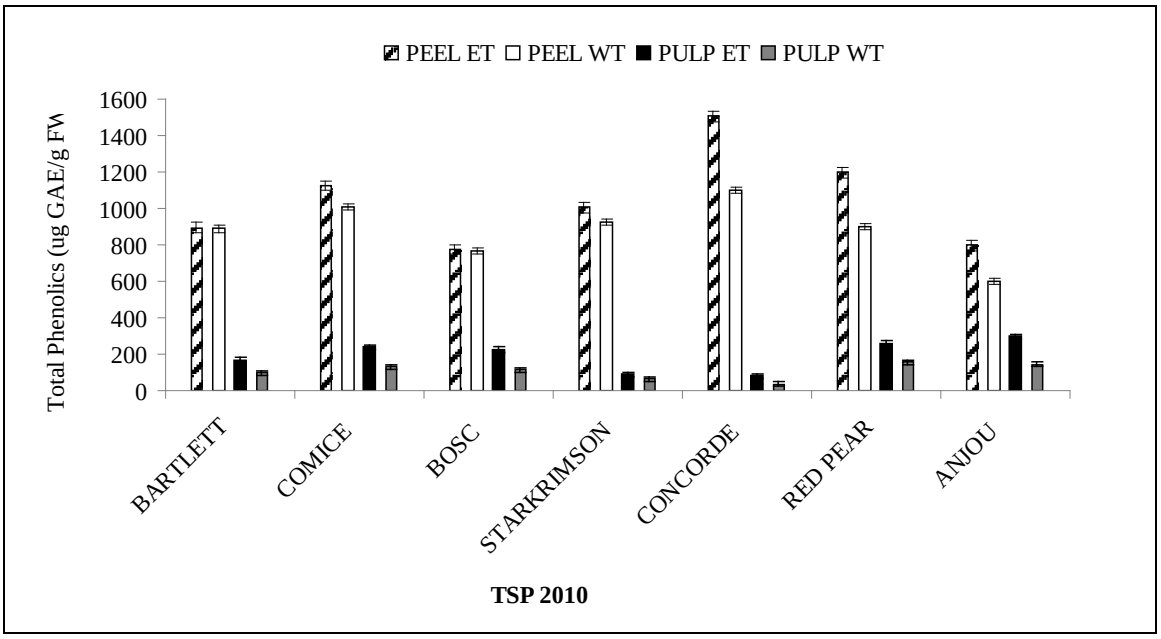
RESULTS & DISCUSSION:

Six pear cultivars (Bartlett, Comice, Bosc, Starkrimson, Red Anjou and Anjou) form Northwest obtained through US Pear were evaluated in 2009 whereas seven different pear cultivars (Bartlett, Comice, Bosc, Starkrimson, Concorde, Red pear and Anjou) were evaluated in 2010. Pears were first peeled then cut and weighed. Peel and pulp of each variety were extracted using water and 12% ethanol (20 g of peel in 50 mL of water and 5 g of peel in 15 mL of 12% ethanol and 100 g of pulp in 50 mL of water and 10 g of pulp in 20 mL of 12% ethanol). All the samples were homogenized using a Waring blender for 3 min. All samples were adjusted to pH 6.00 to reflect the enzyme contents of pancreatic alpha-amylase and intestinal alpha-glucosidase. The samples were centrifuged at 15,000 g for 1 min. Supernatant were collected and kept at -20˚C during the period of study.

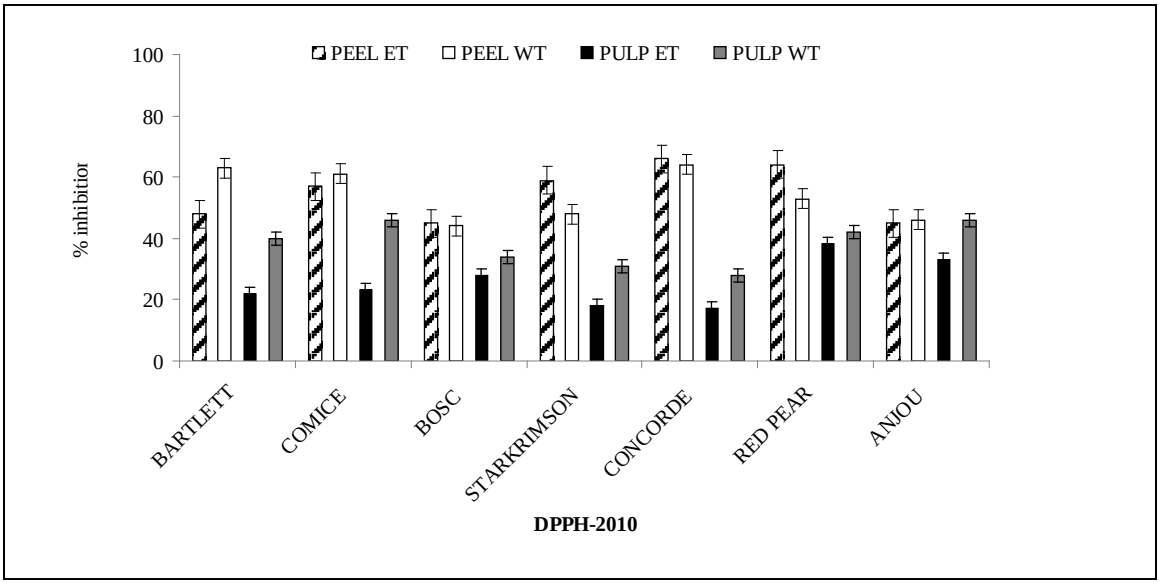
Total Soluble Phenolic and DPPH Free Radical Scavenging-Linked Antioxidant Activity:

Total soluble phenolic content of peel varied between 577- 1270 ug GAE/ g F.W. The higher total soluble phenolic content was observed in ethanol extraction of Starkrimson followed by Bosc, Bartlett, Red Anjou, Comice and Anjou. Whereas total soluble phenolic content of pulp varied between 64-204 ug GAE/ g F.W. and higher soluble phenolic was observed in ethanol extraction. High soluble phenolic content of pulp was observed in Red Anjou followed by Anjou, Bosc, Comice, Bartlett and Starkrimson. Similarly, in 2010 out of all seven cultivars highest total soluble phenolic was observed in ethanol extraction of Concorde peel (1505 ug GAE/ g F.W.) followed by Red pear, Comice, Starkrimson, Bartlett, Bosc and Anjou. The higher soluble phenolic in pulp was observed in Anjou followed by Red Anjou, Bosc, Comice, Bartlett, Concorde and Starkrimson. Results from both years showed similar general trend. Only Comice showed significantly higher soluble phenolic content in peel in 2010 compared to 2009.

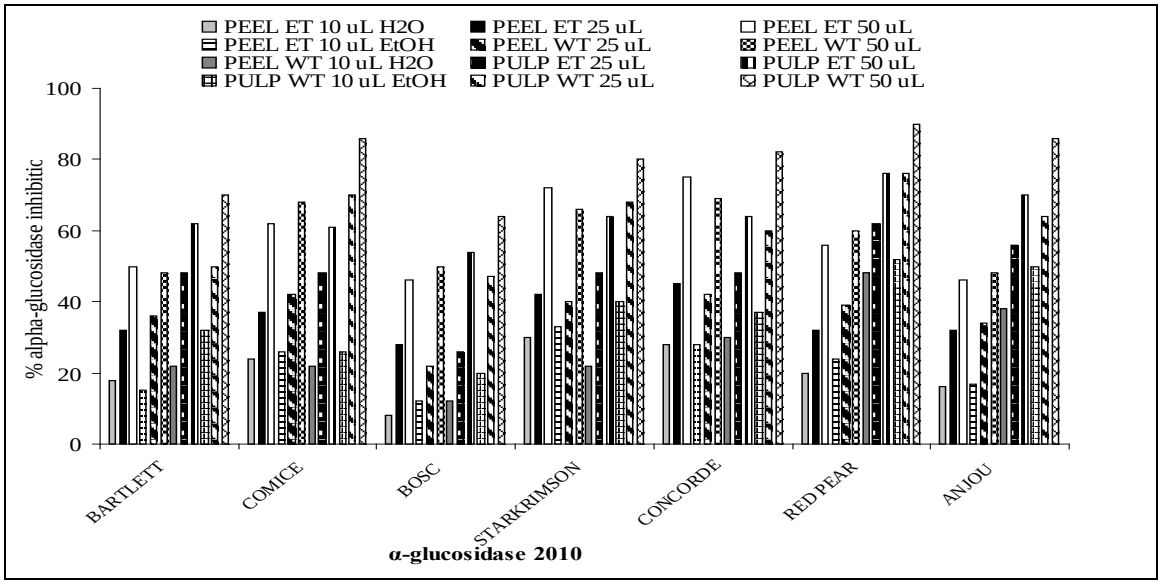
An average 250 gram fresh pear has between 40 to 70 mg of total soluble phenolics. At the higher end for Concorde the content was about 75 mg from one 250 gram serving of fruit.

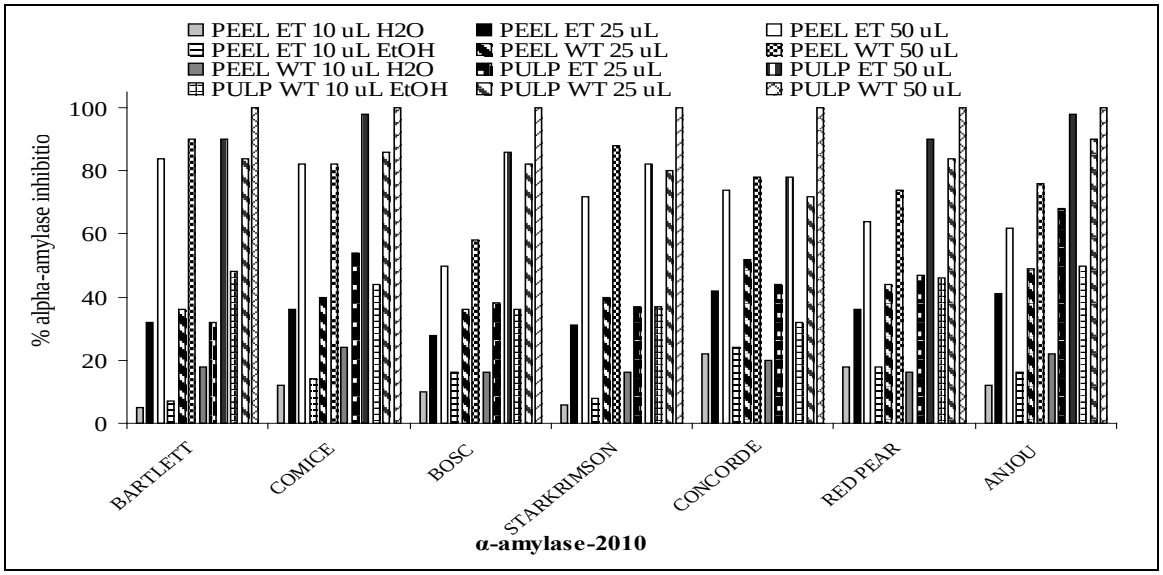


Like total soluble phenolics, total antioxidant activity (DPPH radical inhibition) of pear cultivars also showed similar trend in both years (2009-2010). Significant positive correlations were observed between total phenolic content and total antioxidant activity in peel and pulp extractions of all pear cultivars. High total antioxidant activity was observed in peel extraction of Starkrimson (74% inhibition of DPPH oxidation) and pulp extraction of Red Anjou (38%) similar to total soluble phenolic content in 2009. During 2010, high total antioxidant activity was observed in Concorde peel extracts (66%) and pulp extracts of Anjou (46%) and Comice (46%). High phenolic content and high total antioxidant activity of these pear cultivars particularly in peel clearly show promise for using whole pear fruit for health benefits and for enhancing overall fruit and vegetable intake in the United States from less than 1 serving per day to the needed 7-8 serving per day. **Pear due to it ease delivery as whole fruit combined with food such as yogurts can at least meet the needs of 2 servings per day per person of total fruit and vegetable in take..**



Alpha- glucosidase and Alpha- amylase activity: High α-glucosidase and α-amylase inhibitory activity are reflections of anti-diabetic potential through inhibitions of these key enzymes involved in carbohydrate breakdown and uptake. The inhibitory activity was observed in all pear cultivars during both years. Pulp extraction showed higher inhibitory activity of these enzymes compared to peel extracts. A positive correlation was observed between α-glucosidase inhibitory activity and total soluble phenolic content in peel and pulp extractions of majority of pear cultivars. Significant dose response (reflection of structure-function pharmaceutical potential) in the enzyme inhibitory activity was also observed in all pear cultivars. Red pear, Red Anjou, Comice, Starkrimson and Concorde showed higher α-glucosidase inhibitory activity in both peel and pulp extracts.





Significant α-amylase activity was observed in all pear cultivars and particularly in pulp extractions. Cultivars like Starkrimson, Red pear, Red Anjou and Concorde showed comparatively higher α-glucosidase inhibitory and low α-amylase inhibitory activity with higher total soluble phenolic content. These observations showed higher promise for better type 2 diabetes management

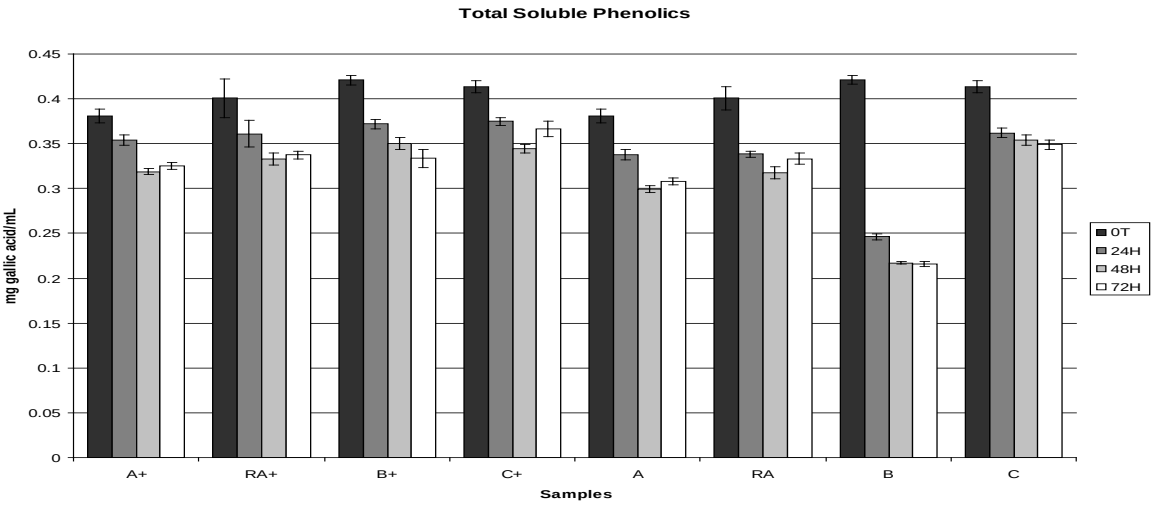
in these cultivars since these can potentially overcome the side effects undigested starch observed in currently used pharmaceutical drugs.

No to negligible ACE (Angiotensin-I converting enzyme) inhibitory activity was observed in all pear cultivars. The activity of found is important as we could use fruits to help long term management of blood pressure, which is a macro vascular complications of type 2 diabetes.

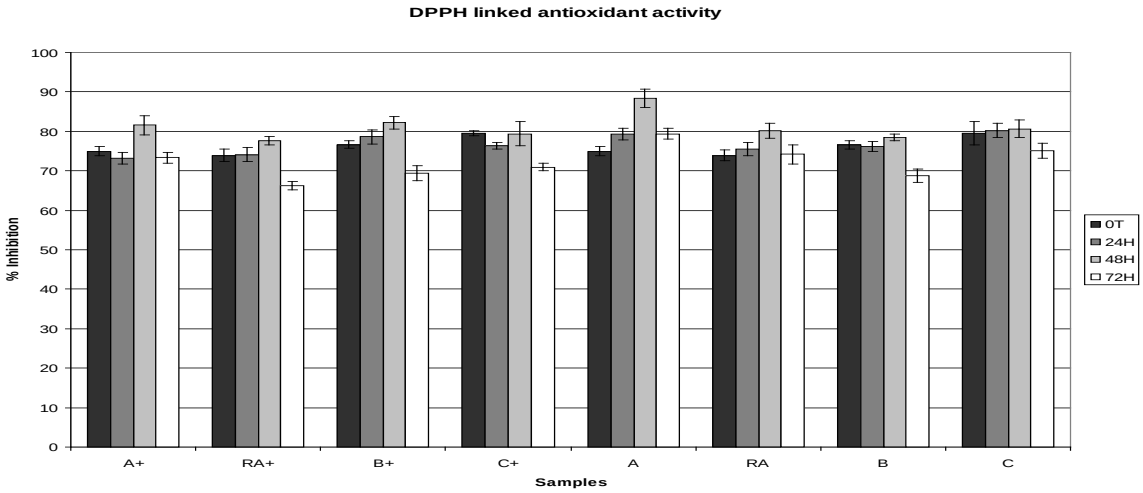
B) Anti-Diabetic, Probiotic and Ulcer Bacteria Inhibiting Benefits of Fermented Pears:

Type 2 diabetes and other chronic diseases are oxidation-linked diseases that are emerging challenges globally and fruits have been shown to have good potential in managing oxidation linked diseases. Four cultivars (Anjou, Red Anjou, Bartlett and Comice) of pear were homogenized to extract their juice, fermented using *Lactobacillus acidophilus* for 0, 24, 48 and 72 h juice and changes in bioactive functionality related to total phenolics, antioxidant potential, inhibition of key enzymes in carbohydrate metabolism and hypertension linked ACE were evaluated. The pH of the juices was adjusted to 6.0-7.0 before fermentation and assays at each time point were carried out at fermented acidic pH and by adjusting the pH to 6.0-7.0. Total phenolics decreased with fermentation and DPPH-linked antioxidant activity increased until 48 h and then it decreased for most samples at 72 H. α-Glucosidase inhibitory activity did not change significantly for pH adjusted samples whereas for pH not adjusted samples there was a significant increase in inhibition with fermentation for most samples. α-Amylase inhibition was not observed since the sample was diluted due to high sugar content. ACE inhibitory potential was high at 0 H and decreased with fermentation for all samples. Protein content decreased or remained constant for all samples. Epicatechin and quercetin derivatives were the major phenolic compounds detected in the fermented samples This suggests that fermentation of pear juice is a good strategy to enhance antioxidant potential and α-glucosidase inhibitory potential to reduce post-prandial rise in blood glucose linked to type 2 diabetes.

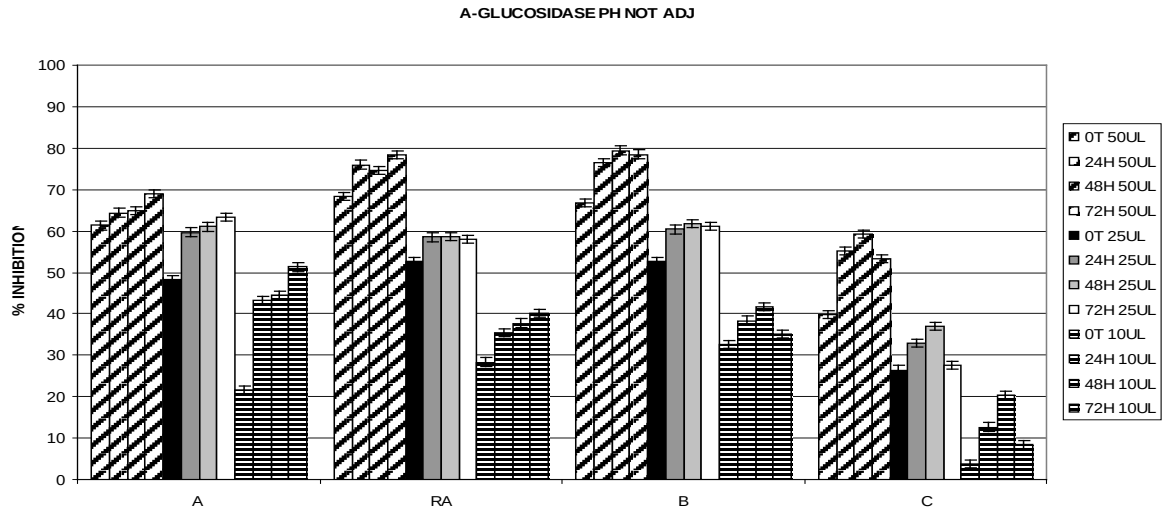
Soluble Phenolics: Soluble phenolics were readily utilized by probiotic *Lactobacillus acidophilus* without its growth being inhibited. Over a 72 H growth period the level of phenolics falls and type of phenolics also vary. With pH adjustment the phenolic profiles change in acidic environment (analogous to the stomach) to environment closer to pH 6.0 as in parts of the gut. Phenolic mobilization in Bosc is novel with rapid utilization and mobilization changes.



Free Radical Scavenging-Linked Antioxidant Response: Following phenolic mobilization in the initial 48 H the antioxidant activity increases before falling at 72 H. This indicates the potential of polymeric antioxidant phenolics being generated from pear by probiotic LA that is more bioactive. This indicates that pear following probiotic mobilization has excellent potential for supporting host antioxidant protective response.



Alpha-Glucosidase Inhibition Response: *Lactobacillus acidophilus* fermentation of all pear varieties in this study enhanced alpha-glucosidase inhibitory activity over 72 H of growth. Further the activity was enhanced in a dose-dependent manner indicating a structure-function relationship with pharmacological potential. This indicates the probiotic bacteria either externally or when in the gut/intestine can enhance biological activity related to control of critical enzyme such as alpha-glucosidase activity involved with uptake of glucose from soluble sources in the diet. This critical enzyme is also the target of anti-diabetic drug acarbose prescribed for glucose control or hyperglycemia in early stages of type 2 diabetes. This study indicates that pear phenolic bioactives and beneficial probiotic bacteria can work synergistically to enhance bioactive function. Such synergistic combinations are needed for better efficacy and overall health benefits from the rapidly growing probiotics market (e.g. Goodbelly) globally. Such synergies are also essential for recovery of stomach, gut and intestinal health after repeated antibiotic therapy that reduces beneficial bacteria. **Consumption of pears can not only help re-populating good beneficial bacteria but the enhanced antioxidant function has the potential to stimulate host cellular response to injury. Currently several fruit synergy products with probiotics are entering the market and pear is much superior to many of these choices. Pear combinations with yogurts would be of great interest. This will be further investigated in community school lunch program in Massachusetts.**

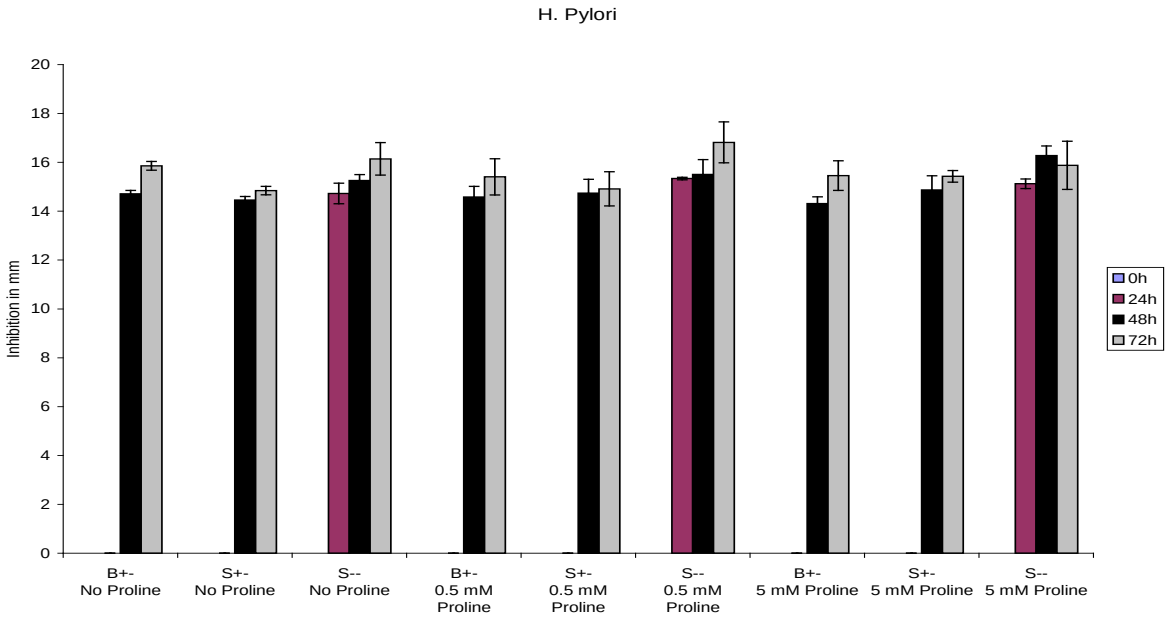


Extracted juice at 0 hours had ACE (angiotensin converting enzyme) inhibitory activity, which is target of reducing high blood pressure.

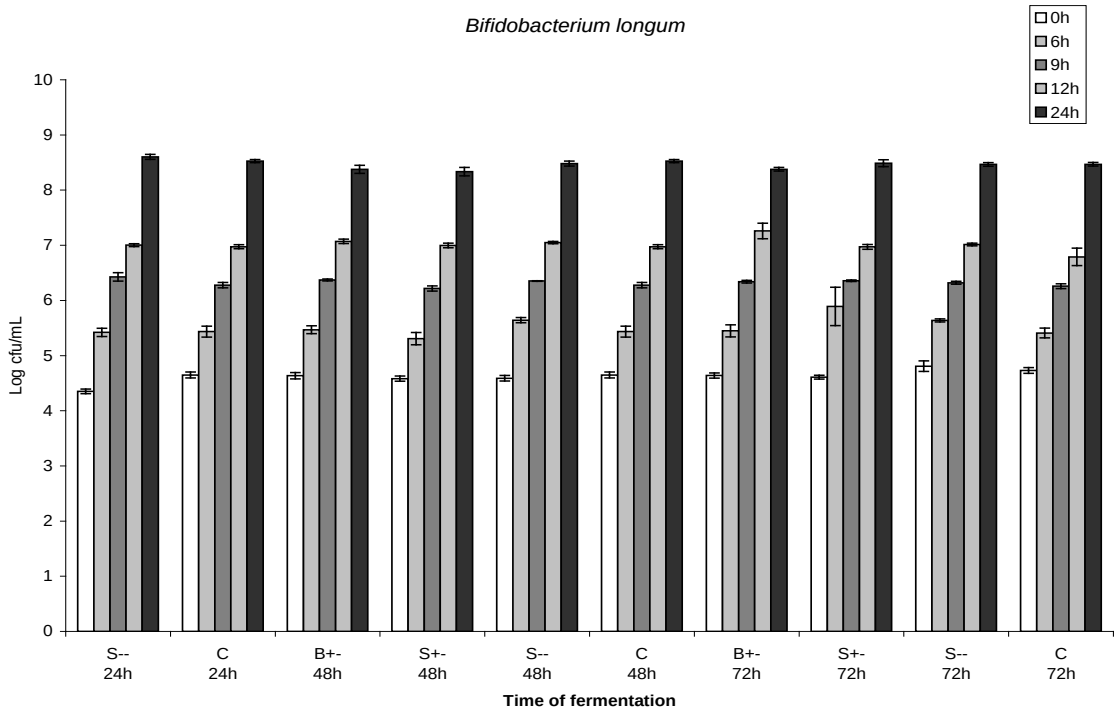
Ulcer Bacteria Inhibition: *Helicobacter pylori* has been identified as an etiological agent in the development of gastric ulcer, peptic ulcer, gastritis and many other stomach related diseases. Recent research has shown that fermented extracts of lactic acid bacteria has potential in inhibiting the stomach ulcer causing pathogen. Two cultivars of pear (Bartlett: B; Starkimson: S) were homogenized to extract their juice and then fermented using *Lactobacillus acidophilus* by adjusting the pH before and after fermentation. *H. pylori* inhibition was observed for all samples (B+-, S+-, S--) at 48 and 72 H where final pH was not adjusted except Bartlett where both initial and final pH were not adjusted (B--). In case of Starkimson where initial and final pH was not adjusted (S--) inhibition was observed at 24 H. Samples which showed *H. pylori* inhibition were further tested to evaluate their effects on lactic acid bacteria with probiotic potential. No inhibition and in some cases proliferation was observed in case *Bifidobacterium longum* which indicates fermented pear juice may be able to inhibit *H. pylori* without affecting or in some cases stimulating the probiotic lactic acid bacteria. **Fermented pear juice therefore would offer a low cost dietary support food system in the management of *H. pylori* without affecting the beneficial lactic acid bacteria in the colon. Therefore a health-based yogurt or probiotic-based pear whole fruits or juice products can be developed for multiple bioactive health functions.**

Overall *Lactobacillus acidophilus* fermentation of pear varieties resulting in phenolic enrichment also translates into inhibition of ulcer bacteria. These pathogenic bacteria need some level of oxygen and therefore the metabolic pathway to using oxygen is blocked by pear phenolics, while leaving lactic acid bacteria unharmed as they do not need oxygen for energy production. These differences in oxygen requirements between pathogenic bacteria and beneficial lactic acid probiotic bacteria can be exploited by pear phenolics to help manage pathogens. Further with the antioxidant function of pears it enhances protective functions of host higher cellular form (eukaryotic cells like human and animal cells) to fight the bacterial pathogens better, while at the same time living harmoniously with probiotic beneficial bacteria. This is an exciting direction for phenolics from the family Rosaceae and especially pear. Many healthy food design and product application strategies can be developed from this insight and provides rationale for enhancing healthy fruit intake in the

American diet. In particular range of yogurt and probiotic beverage-based products using pear and pear products are feasible for school lunch and community health programs where obesity is high.



Beneficial Bifidobacterium stimulation: When 48 H or 72 H *Lactobacillus acidophilus* fermented pear extracts are used to grow highly anaerobic and well established probiotic beneficial bacteria, *Bifidobacterium longum* its proliferation was stimulated. Therefore pear improves product stability.



EXECUTIVE SUMMARY:

Potential Health Benefits of pear has been defined at many levels using sound biochemical rationale and this has significant impact on diverse use and applications of Pacific Northwest grown pear as a part of a healthy diet in the US and in the global market place. Results clearly indicated the pears have significant soluble phenolic content in range of close to 40 to 70 mg soluble phenolics per 250 gram fruit. This also translates into high antioxidant capacity across all cultivars. There are multiple bioactive health application of the pear phenolics both in whole fruit form and also processed fruit form in form of canned fruits or juice concentrates since phenolic bioactives are spread well across peel and pulp.

An exciting discovery from this study is that overall all pear fresh varieties have phenolic bioactive factors which indicate anti-diabetic potential by inhibiting key enzyme such as alpha-glucosidase and alpha-amylase associated with hyperglycemia (high sugar in the blood). The phenolic bioactives which when fermented by lactic acid bacteria increase the bioactive function to potentially inhibit glucose uptake and therefore a pear-based probiotic drink could also have health benefits. The high antioxidant potential of phenolics both in fresh fruits and fermented juice have relevance for managing cellular oxidative stress related to management of diabetes complications from higher soluble sugars and control of glucose uptake (hyperglycemia).

A third exciting discovery is that the increased pear phenolic bioactive factors from lactic acid fermentation inhibit ulcer bacteria *Helicobacter pylori* while leaving good beneficial bacterial such *Bifidobacterium longum* unaffected or slightly stimulated in their growth. This offers further evidence of multiple bioactive health potential from managing negative effects of high glucoses to inhibiting pathogenic bacteria while supporting beneficial probiotic bacteria.

All the results of this study point to exciting ***health benefits of pear with phenolic-linked antioxidant protective functions*** that can influence positively the management of oxidative stress and management of infections. Therefore use of pear products can have impact on design of better diet to manage gut health and associated infections in combination with probiotics in food delivery systems such as fresh fruits, fruit smoothies or yogurts in dry or semi-solid form. In the immediate next phase we are exploring the use of pear and pear products to increase the fruit intake in poor urban school and elderly communities where fruit intake is low. A yogurt-based pear design has the potential to not only increase healthy fruit intake but in the long run we want design experiments whether we can reduce the level of general infections and colds in children and elderly. Likewise in specific communities where obesity and associated type 2 diabetes is high we want to initially increase fruit intake using pears and apples and bring it to a range of 3-5 servings per day per person. We are further exploring the use of pear in military use for combating stress-related and excess antibiotic use-linked bacterial infections (*Clostridium difficile*), where recovery of good bacteria and inhibition of infections are important. These pear-based food designs along with apple and cherry also have implications for endurance management in sports activities and exercise due to high levels of relevant bioactive phenolics.

From these studies the health benefits of pears are better defined and this advances the wider use of pears as a part of healthy diet with enhanced fruits and vegetables. The bioactive potential indicates that pears particularly have relevance for not only managing oxidative stress but also stimulating beneficial bacteria, while at the same time being a hurdle to some form of bacterial infections. Pear along with other important species in the family Rosaceae are essential to increase the per capta fruit intake for a healthy diet from the current US levels of 1 serving per day per person to close to 7-9 servings per day per person. This study provides clear new biochemical rationale for inclusion of *pear in a everyday healthy diet* for the American and global consumer.

Additionally the phenolic-linked antioxidant regulation in pears has implications for innovative strategies for *post-harvest preservation based on natural phenolic regulation* using natural elicitors as pre-harvest sprays or combining with post-harvest treatments. We are exploring the use of oligosaccharides to enhance phenolics for both health benefits and better preservation.