BIOEXPLORATION OF WILD ALASKAN BERRIES: FROM FIELD SCREENING TO FUNCTIONAL FOOD

BY

JOSHUA J KELLOGG

THESIS

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Adviser:

Professor Mary Ann Lila

Abstract

Wild berries are fundamental components of traditional diet and medicine for Native American and Alaska Native tribes and contain a diverse array of phytochemicals, including anthocyanins and proanthocyanidins, with known efficacy against metabolic disorders. Bioexploration represents a new paradigm under which bioactive preparations are screened in coordination with indigenous communities, to prepare for subsequent in-depth chemical and biological analysis. The inclusive, participatory philosophical approach utilized in bioexploration has additional benefits that could be realized in seemingly disparate areas, such as education and economics.

Five species of wild Alaskan berries (*Vaccinium uliginosum, V. ovalifolium, Empetrum nigrum, Rubus chamaemorus*, and *R. spectabilis*) were tested using "Screens-to-Nature" (STN), a community-participatory approach to screen for potential bioactivity, in partnership with tribal members from three geographically distinct Alaskan villages: Akutan, Seldovia, and Point Hope. Berries were subsequently evaluated via HPLC and LC-MS^2, yielding significant species and location-based variation in anthocyanins (0.9-438.6 mg eq /100g fw) and proanthocyanins (73.7-625.2 mg eq /100g fw). A-type proanthocyanidin dimers through tetramers were identified in all species tested. Berries were analyzed for *in vitro* and *in vivo* activity related to diabetes and obesity. *R. spectabilis* samples increased preadipocyte-factor-1 levels by 82% over control, and proanthocyanidin-rich fractions from multiple species reduced lipid accumulation in 3T3-L1 adipocytes. Furthermore, extracts of *V. uliginosum* and *E. nigrum* (Point Hope) reduced serum glucose levels in C57bl/6j mice up to 45%.

The same precepts of bioexploration, especially the inclusion of indigenous community perspectives and knowledge, have relevance in other areas of study, such as education and economics. Studies have established the apathetic, low-motivational

environment characteristic of many introductory science laboratory classes is detrimental to student interest, learning, and continuation in scientific education. A primary means of arresting this decline and stimulating the students' attention and excitement is via engagement in hands-on experimentation and research. Using field workshops, the STN system is investigated as to its potential as a novel participatory educational tool, using assays centered around bioexploration and bioactive plant compounds that hold the potential to offset human health conditions. This evaluation of the STN system provided ample evidence as to its ability to augment and improve science education.

Furthermore, Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis was employed as a theoretical framework to review the potential benefits and hurdles associated with developing a wild Alaskan berry commodity. Synthesizing various sources of information – including logistics and harvest costs, sources of initial capital, opportunities in the current superfruit industry, and socioeconomic factors – the development of a berry commodity proves to be a complex amalgam of competing factors which would require a delicate balance before proceeding.

Acknowledgements

Graduate school often can seem a Sisyphean task, an epic struggle against an obstacle that won't allow a moment's rest. It is not an easy decision to enter into, and being successful at finally pushing the boulder to the hilltop requires a team of people who are instrumental in helping you inch forward. This is a small gesture of thanks and gratitude to those involved in making my boulder lighter.

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1 Introduction

In recent years, investigation into plants and their impacts on human health has gained increased importance, due to a renewed interest in human wellness through natural means (Barnes et al., 2004; Craig, 1999). Indigenous knowledge provides a rich source of information to help identify and explore possible botanical sources of food and medicines (Oubré et al., 1997). As defined by the UN Convention on Biological Diversity, traditional ecological knowledge (TEK):

> refers to the knowledge, innovations and practices of indigenous and local communities around the world. Developed from experience gained over the centuries and adapted to the local culture and environment, traditional knowledge is transmitted orally from generation to generation. It tends to be collectively owned and … is mainly of a practical nature, particularly in such fields as agriculture, fisheries, health, horticulture, forestry and environmental management in general (United Nations, 2007).

The research presented in this thesis was conducted in cooperation with a particular indigenous group – Alaska Natives (AN) – who 1) have a rich cultural heritage and tradition of using natural resources (including local wild berries) for subsistence food and medicines, 2) provide a wealth of TEK surrounding the resources, 3) have adapted to living in a severe, extreme climate and environment, 4) have exhibited a strong predisposition towards diabetes and obesity in modern times and 5) face additional social and economic challenges. The discovery programs described in this thesis were aimed at linking the natural botanical resources (wild berries), the TEK and the partnership with AN to address three seemingly disparate areas: human health, education, and economic development.

1.1 From Bioprospecting to Bioexploration

Bioprospecting for plants of nutritional, medicinal, or functional value has a long and complex history. Bioprospecting began in earnest with post-Renaissance exploration and subsequent colonization of the world by European nations. The newly uncovered lands of Eastern Asia, Africa, the Americas, and the Caribbean provided a plethora of plants with unique and beneficial properties not known to contemporary European scientists. The indigenous peoples of these regions had an established history of utilizing endemic plant, animal, and fungal species to effectively counteract numerous diseases and conditions, providing efficient remedies at a time when European doctors were still using bleeding, purging, and other methods that often increased – rather than relieved – stress on patients. Nicolas-Louis Bourgeois, a Frenchman living in Saint Domingue during the mid-eighteenth century, praised the multitude of novel medicines found in the Caribbean: "the most dangerous [plant] poisons can be transformed into the most salubrious remedies when prepared by a skilled hand; I have seen cures that very much surprised me." (Bourgeois, 1788).

Plant tissues and phytochemicals continue to provide valuable sources of pharmaceuticals to this day. Over 600 botanical items are referenced in various editions of the *United States Pharmacopoeia*, and more than 50% of all pharmaceuticals in clinical use today have a natural product origin (Tyler, 1993);(Balandrin et al., 1993). A comprehensive review of 847 small-molecule human health drugs introduced since 1981 showed a heavy reliance on natural products. While only 32% were direct or derived natural products, 262 of the 572 remaining chemicals were inspired by natural products or could be considered natural product analogs – the implications being that a total of 63% of all small-molecule drugs in the last 25 years had their origins in natural products (Newman and Cragg, 2007). Until fairly recently, the modern pharmaceutical industry has not fully appreciated the traditional ecological knowledge (TEK) of indigenous people – who have developed a system of plant-based traditional medicine over millennia, and continue to employ its practices today – as a launch pad for new drug discovery and exploration. One study, correlating data from the World Health Organization's Traditional Medicine Programme (WHO-TRM) with natural product research and development literature, found that botanical TEK references led to the development of 98 out of 122 identified chemicals, illustrating how ethnobotanical knowledge can play a large part in the drug discovery process (Fabricant and Farnsworth, 2001).

However, until recently, bioprospectors viewed TEK as a facile means of gleaning information to uncover possible new plant remedies. The typical bioprospecting approach has often failed to give appropriate credit or recompense to the original owners of the TEK when it leads to new drug discovery, as exemplified by the case of the San people of Southern Africa and the plants of the genus *Hoodia*. The San have used the plant to suppress hunger while traveling or hunting, knowledge which was first recorded by colonists in the 18th century (Masson, 1796), and eventually obtained by pharmaceutical corporations, who began to investigate Hoodia's potential to control obesity. Supplement and drug development in this multi billion-dollar industry continued without the San's knowledge or compensation until 2001. However, in 2003, they entered into arbitration with Council for Scientific and Industrial Research (CSIR) to share royalties associated with Hoodia-related pharmaceuticals (Wynberg, 2004). Other groups have fought for their intellectual property rights regarding endemic flora, for example, the Coordinating Body of Indigenous Organizations of the Amazon Basin (COICA)'s successful five-year struggle to annul a patent issued by the United States Patent Office to an American citizen for a variety of *Banisteriopsis caapi* (ayahuasca) that was removed from the garden of an indigenous person in Ecuador (Wiser, 2002).

These and many similar incidents have created substantial distrust and animosity among indigenous groups towards 'bioprospecting' expeditions. However, an increasing number of researchers are now taking steps to recognize the intellectual property rights of the indigenous people. Ratification of the United Nations Convention on Biological Diversity (CBD) recently brought international attention to bioprospecting issues (Baker et al., 1995), with an agreement to establish the rights of a nation and its people with respect to the biodiversity present within its borders. The CBD's protective legal framework has helped spawn more collaborative approaches to nature-based therapeutics - moving from bioprospecting to bioexploration. Unlike bioprospecting, which is primarily concerned with extracting profitable resources from nature, bioexploration works in the broader context of natural resources, their relationship to the environment, and the indigenous people who have used the resources, have acquired related TEK, and may have implied or legal ownership of the resources. Bioprospecting is viewed as a process that 'takes away' resources from the place of origin and the local people who have pioneered the use of the resources. Bioexploration, in contrast, reverses this trend by emphasizing an in-country discovery process , on-site training for local participants, and in-country building of local infrastructure for further laboratory analysis of resources. The Global Institute for BioExploration (GIBEX), borne out of a research collaboration between the University of Illinois, Rutgers University, North Carolina State University, and numerous international institutional partners, is a partnership which features the bioexploration approach, and works in active engagement with local, indigenous peoples to ensure that resources (natural, social, and intellectual) remain under the control of the local origin (http://www.gibex.org). GIBEX operates on the paradigm of partnership with local people in the biodiscovery process, so that full intellectual property rights with regards to any compounds or drugs discovered from endemic plants remain in local hands. The development of this coalition strives to develop an equitable research relationship between Western university scientists, and the people who have the closest connection with the land and plants of interest, while also providing scientific, cultural, and economic motivations for responsible management of these natural resources.

An integral component of the bioexploration approach is called Screens-to-Nature (STN), a system of qualitative bioassays that are designed to be run in the field, with a minimum of laboratory equipment and chemical input, while yielding reproducible results that give a preliminary indication of the plant's bioactive properties (Dushenkov and Raskin, 2008). These assays bring the science of discovery to the environment as opposed to traditional bioprospecting, where resources are removed to a research laboratory for initial analysis. The STN are an main tenet of GIBEX's core commitment is to keep the resources of bioexploration in the country of origin, or "reversing the flow" of traditional bioprospecting. To date, nearly a score of individual STN assays have been designed to investigate the activity of natural plant metabolites, such as anthocyanins, or evaluate bioactivity relating to human health issues including chronic and infectious disease agents, metabolic disorders, and general health maintenance. These assays form a first level of screening for potentially bioactive plants, helping to identify candidates for further evaluation and investigation.

1.2 Metabolic Syndrome within Alaska Native Populations

Type-2 diabetes mellitus (T2DM) is associated with a progressive resistance to insulin, accounting for 90-95% of all adult cases of diabetes, and is often correlated with obesity. Insulin resistance is a condition where the muscular, hepatic, and adipocyte cells do not adequately metabolize glucose in response to insulin signaling. Despite producing ever-increasing levels of insulin, the pancreas is unable to contain the serum glucose concentrations and suffers tissue damage, rendering the patient in a state of prolonged hyperglycemia (National Diabetes Information Clearinghouse). In the United States alone, the prevalence of T2DM has increased dramatically over the last two decades, rising from 5.6 million cases in 1980 to 15.8 million by 2005 (Centers for Disease Control & Prevention). NA/AN populations suffer disproportionately high rates of diabetes and obesity compared to other ethnic groups in the United States (Halpern, 2007). While incidence rates vary between tribal groups, the American Diabetes Association (ADA) reports that NA/AN populations, with a 15.1% occurrence rate of diabetes, are more than twice as likely to have diabetes as non-Hispanic whites (American Diabetes Asociation, n.d.a.). The CDC corroborated this finding, reporting a 15.3% occurrence rate of diabetes in NA/AN populations, compared to an all-race incidence of 7.3% in 2002 (Acton et al., 2003). Alaska Native populations are particularly at risk, due to changes towards a more Western lifestyle and the associated shifts in physical activity, high-calorie diet, and high fat consumption (Gahagan and Silverstein, 2003) that have coupled with the population's genetic predisposition to metabolic conditions to produce a serious socio-medical situation within the Alaska Native population (Burrows et al., 2000). The alterations in dietary intake patterns have led to a decline in native food consumption among adults under 30 years of age in favor of store bought processed food products, with the associated increase in levels of carbohydrates and fats (Murphy et al., 1995; Nobmann et al., 2005). The higher incidence of obesity and type 2 diabetes among native populations has led to higher rates of numerous other diabetes-related complications, such as kidney failure, retinopathy, cataracts, and cardiovascular disease (Ghodes, 1995). Metabolic disorders are primarily associated with dysfunctional energy balance and distribution within the human body; as such, adipocytes, which are the primary site of energy storage, play an important role in the development of metabolic syndrome.

1.2.1 Adipocytes' Role in Diabetes and Obesity

Adipocytes function to regulate metabolism and maintain glucose homeostasis by sequestering triglycerides (TGs) during feeding and releasing free fatty acids (FFAs) during fasting. Obesity involves two mechanisms. The first is the energy balance and regulation within adipocytes (Fernyhough et al., 2005). Excess caloric intake can lead to a metabolic overload of adipocyte tissue, forcing adipocytes to enlarge to accommodate the larger influx of TGs. Prolonged enlargement and hypertrophy develops within the adipocytes, attracting macrophages that secrete tumor necrosis factor-α (TNFα), beginning a chronic inflammatory condition. This results in adipocyte dysfunction, disrupting the dynamic equilibrium of TG metabolism and leading to increased lipolysis and FFAs (**Figure 1.1**).

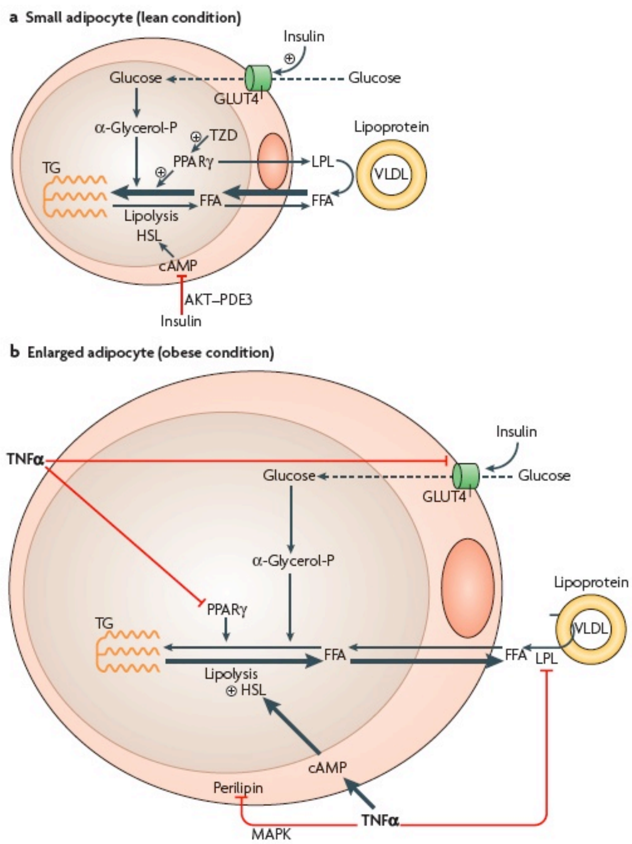


Figure 1.1: Regulation of TG Synthesis in Regular (a) and Obese (b) Adipocyte Cells. In regular adipocyte cells, insulin stimulates the up-regulation of TG synthesis via glucose uptake (GLUT4 pathway), PPARγ signaling for LPL incorporation of FFAs into adipose tissue. In obese adipocytes, inflamed tissue with high levels of TNFα decreases GLUT4, LPL, and PPARγ expressions, decreasing FFA esterification and an increase in lipolysis (from Guilherme et al., 2006)

These activated lipids accumulate in the tissue, triggering insulin resistance and beginning the onset of T2DM (Guilherme et al., 2008). Insulin resistance increases the inflammation of adipocytes, which in a vicious cyclical process further reduces their ability to regulate TG metabolism (Tsuda, 2008). Multiple studies have provided evidence of a causal relationship between obesity and insulin resistance (Guilherme et al., 2008) (Freidenberg et al., 1988).

The second process is adipocyte maturation, known as adipogenesis. This is the set of cellular transitions for a fibroblast developing into a mature adipocyte (Nakae et al., 2003). This process is controlled by the up-regulation of a number of genetic factors, such as Foxo1, which serve to couple differentiation with extracellular signals like insulin and begin the differentiation process (Nakae et al., 2003). Peroxisome proliferators-activated receptor-γ (PPARγ) (Ntambi and Kim, 2000) is a central signaling modulator in the development of adipocytes and considerable research has been investigated the ability to control genetic expression of PPARγ (Choi et al., 2006; Furuyashiki et al., 2004). The major genetic regulators studied to date have been positive signaling systems, ones which induce the differentiation process. There are few negative controls of differentiation, the most prominent being *Pref-1*.

Preadipocyte factor-1 (*Pref-1*) is a protein of 385 amino acids coded by the *pref-1/dlk1* gene (Lee et al., 2003). It belongs to the same protein family as Notch/Delta/Serrate, which are responsible for determining the fate of cells. *Pref-1* contains six epidermal growth factor (EGF)-like repeats, and exists in multiple forms, both as a transmembrane protein as well as a soluble form (**Figure 1.2**), though only the large soluble form of *Pref-1* (50 kDa in size) is active in regulating adipogenesis (Mei et al., 2002).

Pref-1 is highly expressed in preadipocytes, but drops precipitously during the differentiation process (Lee et al., 2003). Subsequent investigations have demonstrated that *pref-1* is a negative regulator of adipocyte differentiation in 3T3-L1 cells (Smas and Sul, 1993), increasing MEK/extracellular signal-regulated kinase (ERK) phosphorylation (Kim et al., 2007). Mice expressing the *pref-1/hFc* gene in adipose tissue showed a substantial decrease in total fat weight and decreased adipocyte markers (Lee et al., 2003), whereas *pref-1*-knockout mice displayed accelerated rates of adipose tissue accumulation (Moon et al., 2002). However, *pref-1* does not affect all aspects of metabolic syndrome; a recent study involving mice over-expressing *Pref-1* demonstrated a decrease in high-fat diet-induced obesity, but also heightened insulin resistance (associated with decreased insulin signaling) (Villena et al., 2008).

Adipogenesis is believed to take place primarily during the very young stages of life, from embryo to early infancy, when the body is conditioning itself for metabolic regulation (Ntambi and Kim, 2000). Thus, being able to modulate adipogenesis is especially relevant to NA/AN populations, as it has been shown that the trend to obesity begins during these early years (Halpern, 2007).

1.2.2 Potential for Botanicals to Ameliorate Symptoms of Metabolic Syndrome

Although multiple drugs have been developed to counteract deleterious effects of T2DM, none of them are without side effects. For example, sulfonylurea-based pharmaceuticals, while stimulating insulin receptors, are not able to control normal blood glucose levels by reducing the β-cells' ability to maintain consistent insulin secretion, and can also lead to increased weight gain (Pfeiffer et al., 2003). Thus, an opening exists to use botanicals to assist in controling metabolic disorders such as T2DM and obesity. Such food-based approaches have received increased attention in recent years, as Salsberg and Ludwig (2007) note: "traditionally, food is thought to influence human health through its nutrient content, whereas drugs are recognized to act through molecular pathways. However, consumption of a meal … can powerfully affect signal transduction and gene function." (Salsberg and Ludwig, 2007). Plant-based foods that demonstrate activity in regulating blood glucose levels, reduce the prevalence of obesity and fat accumulation, or induce other counter-T2DM physiological or biochemical responses could be employed as agents in combating T2DM without the side effects of synthetic pharmaceuticals. The Cree First Nation in Quebec, Canada have employed species of *Vaccinium* to treat symptoms of diabetes (Leduc et al., 2006), utilizing both the fruit as well as decoctions and infusions of blueberry leaves are used as hyperglycemic agents (Allen, 1927) (Cignarella et al., 1996). Thus, Native Americans and Alaska Natives have an opportunity to combat these degenerative diseases utilizing foods that were, at least historically, staples within native diets: wild berries.

1.3 <u>Wild Alaskan Berries</u>

1.3.1 Ethnobotany of Wild Alaskan Berries

Small berries, principally in the Ericaceae and Rosaceae families, are found across the North American continent, and provided an important component of the Native American subsistence diet, although Western lifestyles had diets have increasingly encroached on the traditional diets. The northern American landscape is home to a wide variety of berries that have great ethnobotanical importance to the Pacific Northwest's indigenous tribes. Five of those species are of particular importance to Alaska Native people.

The salmonberry (*Rubus spectabilis*), a relative of the raspberry, is found throughout the Pacific Northwest. Its coastal habitat extends from southern Alaska to northern California, along stream banks and mountain slopes, and it is rarely found east of the Cascade Range (University of Texas, 2007). Portions of the salmonberry plant have been used for such diverse health remedies as wound healing, gynecological aids, and a source of powerful vitamin-containing fruit among tribes stretching from western Washington and the Olympic Peninsula to Alaska (Moerman, 1998). A related species, *Rubus chamaemorus* (sometimes called salmonberry but more often known as cloudberry) is a circumpolar wild berry found at higher latitudes. *R. chamaemorus* has been noted as an important foodstuff for Alaska Native tribes (Heller, 1953).

Two other berries of ethnobotanical importance are found throughout the Alaskan wilderness. *Empetrum nigrum*, known alternatively as mossberry, crowberry, or blackberry across different regions of the state. The Tanaina, a tribal group located near Anchorage, have used the leaves and stems of *E. nigrum* for diarrhea, and the plant is also described as having beneficial effects countering kidney trouble (Viereck, 2007). Also found is the blueberry, which includes two species: the blue huckleberry (*Vaccinium ovalifolium,* also known as the high-bush blueberry), and the bog blueberry (*Vaccinium uliginosum*, or the low-bush blueberry). Blueberries (*Vaccinium sp.),* have been used as foodstuffs by over 40 different tribes, including Alaska Natives (Inupiaq and Aleut), Pacific Northwestern tribes (Bella Coola, Kwakiutl, and Chinnook), and nations of the eastern woodlands (Cree, Algonquin, and Ojibwa) (Moerman, 1998). The bog blueberry is found throughout Alaska, the Canadian Yukon, and as far south as the upper continental United States (United States Forest Service). In addition to eating raw berries and freezing them, the Inupiat– an Inuit tribe indigenous to Alaska's Northwest arctic region – also ferment the berries, turning them into a vinegar for cooking, and even developed berry-based relishes (Jones, 1983). Northern and Aleutian Alaska Natives also use the berries as part of agutuk, a traditional dessert made with fish or seal oil (Bersamin et al., 2006).

1.3.2 STN Assays to Investigate Wild Berry Bioactivity

Wild Alaskan berries have been used traditionally to alleviate symptoms and complications of diabetes incidence in tribal populations, and serve as a primary subsistence food for numerous tribes. Three STN assays are relevant to studying metabolic syndromes, establishing potential candidates for further laboratory investigation.

Amylase inhibitors. *In vitro* studies have demonstrated the ability of polyphenolic compounds to inhibit protein-mediated breakdown of starches into simpler monosaccharides in the mouth and upper gastrointestinal tract (GIT). Acylated anthocyanins are effective in providing inhibition of starch degrading enzymes, such as α-glucosidase present in the GIT (Matsui et al., 2001) and α-amylase in the oral cavity (Queseda et al., 1995). Inhibition of such enzymes reduces the concentration of monosaccharide available to be absorbed from the small intestine into the blood stream, thus lowering the patient's postprandial serum glucose level. Indeed, this is considered by some researchers to be the main *in vivo* action of anthocyanins against T2DM (McDougall and Stewart, 2005). The STN system contains an assay for investigating α-glucosidase and α-amylase activities, utilizing a colorimetric starch indicator to examine whether a-amylase sampled from saliva has been inactivated by the berry extract, and plant compounds that demonstrate the potential to inhibit their function are possible anti-diabetic food sources or medications (Lee et al., 2007).

Antioxidant. In persons with T2DM, radical oxygen species (ROS) and oxidative stress have a common pathway leading to such complications as vascular dysfunction, retinopathy, nephropathy, and neuropathy (Baynes and Thorpe, 1999). Insulin resistance coupled with oxidative stress acts synergistically in the emergence of different metabolic syndromes including cardiovascular complications. Postprandial hyperglycemic episodes are highly correlated with increases in oxidative stress, and figure prominently in the development of vascular complications (Jakus and Rietbrock, 2004). T2DM also reduces the synthesis of endogenous antioxidants, further exacerbating the problem (Montonen et al., 2004). The STN antioxidant assay screens the berries' activity in preventing an organic oxidant from degrading a colored structure, indicating if it is possible to prevent the accumulation of oxidants upon consumption.

Protease inhibitors. The protease screens give a preliminary indication of the sample's ability to break down (or prevent the degradation of) proteins. Proteolytic inhibitors have formed a large contingent of antiviral pharmaceuticals, including retroviral HIV drugs (Carr et al., 1998) and Hepatitis C (Sarrazin et al., 2007) as well as showing potential to combat protozoa infections from agents such as *Plasmodium falciparum*, also known as malaria (Greenbaum et al., 2004). The STN demonstrates an extract's ability to prevent the degradation of a protein-covered film by common proteases, such as trypsin.

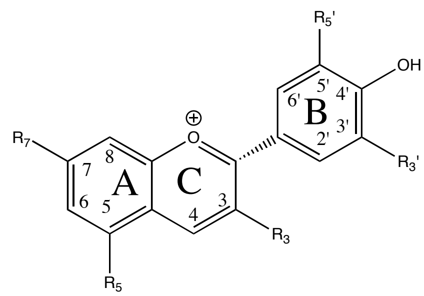
1.4 Phenolic Composition of Berries

The bioactive properties investigated by the STN assays originate from the secondary metabolites synthesized by the berries for protection against biotic and abiotic stresses. These metabolites, mostly in the form of polyphenols, have come under increasing scrutiny for their roles in human health, and in the past two decades a large diversity of protective effects have been discovered, including antioxidant, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative, anti-mutagenic, anti-microbial, anti-carcinogenic, cardiovascular protection, vascular circulation improvement, anti-diabetic, and vision augmentation (Ghosh and Konishi, 2007). This project focused on two main classes of polyphenols, anthocyanins and proanthocyanidins, as the basis for laboratory investigations.

1.4.1 Anthocyanins

Anthocyanins derive their name from the Greek *anthos*, meaning flower, and *kyanose*, meaning blue. They comprise one of the major families of flavonoids, a class of plant secondary metabolites that encompasses over 8000 distinct chemicals, with over 500 anthocyanin structures having been elucidated by the year 2000 (Pietta, 2000). Anthocyanins are widespread in the plant kingdom, occurring in nearly every plant family and in 27 different food plant families.

Anthocyanins are C_6-C_3-C_6 flavonoids, water-soluble glycosides of 2-phenylbenzopryrlium (flavylium) salts, ranging in color from red to an almost blackish purple. While over 19 anthocyanidin aglycones backbones have been identified, the six most commonly present are cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin (Crozier and Clifford, 2006). The structural differences between the aglycones are determined by the number and substitution pattern of hydroxyl and methoxy groups on the carbon backbone, as seen in **Table 1.1**. The aglycone anthocyanidins are modified to form anthocyanins via glycosylation. The addition of the sugar molecule occurs through the three-carbon bridge that links the two aromatic rings of the anthocyanidin to the 3 or 5-carbon position on the sugar moiety. An anthocyanidin can experience multiple modifications, resulting in a mono- to tri-glycosylated anthocyanin. The sugar moieties can be further modified via acylation with cinnamic or aliphatic organic acids. Extended conjugation and acylation increases stability over non-acylated anthocyanins (Mazza et al., 2004).



	R3	R5	R7	R3’	R5’
Pelargonidin	OH	OH	OH	H	H
Cyanidin	OH	OH	OH	OH	H
Peonidin	OH	OH	OH	OCH3	H
Delphinidin	OH	OH	OH	OH	OH
Petunidin	OH	OH	OH	OCH3	OH
Malvidin	OH	OH	OH	OCH3	OCH3
Capensinidin	OH	CH3	OH	OCH3	OCH3
Hirsutidin	OH	OH	OCH3	OCH3	OCH3
Apigeninidin	H	OH	OH	H	H
Luteolinidin	H	OH	OH	OH	H
Tricetinidin	H	OH	OH	OH	OH

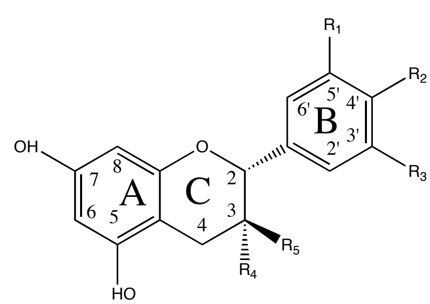
Table 1.1: Anthocyanin Aglycone Structures

Anthocyanins are widespread in vascular plants, found on all habitable continents. Cyanidin, delphinidin, and pelargonidin are the most common aglycone in plants, present in 80% of pigmented leaves, 50% of flowers, and 69% of fruits (Harbone and Williams, 2001); within fruits cyanidin dominates, followed by pelargonidin, peonidin, delphinidin, petunidin, and malvidin (Wrolstad et al., 2005). Anthocyanins are found in an extensive diversity of edible plants, including apples, soft berries, cabbage, cherries, grapes, kiwi, plums, red onions, red radishes, and sweet potatoes (Timberlake et al., 1975);(Wrolstad et al., 2005). In fact, there are 24 plant-based foods in the modern American diet that have significant anthocyanin content, and it is estimated that the average American diet contains 12.53 mg of anthocyanin per day (Wu et al., 2006).

1.4.2 Proanthocyanidins

Proanthocyanidins, or condensed tannins, represent another class of polyphenolic secondary metabolites. Proanthocyanidins are oligomeric or polymeric constructions of the basic flavanoid structure flavan-3-ol. They are named for their yielding of anthocyanins upon acid hydrolysis, e.g. proanthocyanidins that are homo-oligomeric (containing two ($C_{3'}$,$C_{4'}$)-β-ring hydroxyl groups, catechin or epicatechin units) are termed procyanidins (Xie and Dixon, 2005). Proanthocyanidins are widespread in the plant kingdom, utilized as defensive chemicals against herbivorous attack. They impart an astringent flavor to the plant as well as any potential food products (Prior and Gu, 2005).

The majority of proanthocyanidins are formed six possible monomeric constituents, with (epi)catechin dominating. The specific monomer conformation depends upon the substitution pattern of the aromatic rings and the carbon bridge (**Table 1.2**).



	R1	R2	R3	R4	R5
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH
Epigallocatechin	OH	OH	OH	OH	H

Table 1.2: Proanthocyanidin Monomer Structure

Oligomers are comprised of two or more differing monomer units, with the linkage between consecutive monomers occurring primarily between the C_4 of the ‘‘upper’’ unit and C_8 of the ‘‘lower’’ or ‘‘terminal” monomer. The link can also form with C_6 of the lower unit (Ferreira et al., 2003). The number of linkages differentiates two major types of proanthocyanidins; B-type proanthocyanidins are connected by a single bond between monomer units, whereas A-type proanthocyanidins possess a second bond, an ether linkage occurring between either C_2 or C_4 of the upper-unit’s A-ring and the oxygen at C_7 of the lower unit (Xie and Dixon, 2005). The degree of polymerization varies between plant species, and multiple degrees of polymerization can be found even within a single fruit (Schmidt et al., 2004).

Proanthocyanidins from monomer to hexamers are commonly found in plants around the globe. The diversity of foods that possess proanthocyanidins is vast, including fruits (e.g. grapes, apples, avocados, and soft berries), cereals and beans (e.g. cocoa, sorghum, and black beans), nuts (e.g. almonds, pecans, walnuts, and cashews), spices (cinnamon and curry), and two popular beverages: tea and wine (Prior and Gu, 2005). Gu et al. (2004), synthesizing data from the United Stated Department of Agriculture’s 1994–1996 Continuing Survey of Food Intakes by Individuals, calculated the mean proanthocyanidin consumption as 57.7 mg per person per day (Gu et al., 2004), higher than any other category of flavonoids (Prior and Gu, 2005).

1.5 STN as an Engaging Educational Experience

1.5.1 The Struggle for Science Classrooms

The attributes of the STN system that encourages a new approach to scientific exploration also hold the potential to impact science classroom curriculum. Teachers face an uphill battle against student apathy and complacence, especially in introductory science courses. Low motivation is the result of several overlapping factors: large class sizes, science's "required" status as part of a degree program, and the overall negative preconceptions of the course material (Kern and Carpenter, 1984; Lila and Rogers, 1998). This is compounded by the generally impersonal structure of lecture and repetitive physical techniques and calculations in the laboratory (Seymour and Hewitt, 1997). Such laboratory instruction is commonplace among introductory science courses, and is derided by Hofstein and Lunetta as:

> 'cook-book' lists of tasks for students to follow ritualistically. [Traditional labs] do not engage students in thinking about the larger purposes of their investigation and of the sequence of tasks they need to pursue to achieve those ends (Hofstein and Lunetta, 2004).

The didactic nature of traditional laboratory instruction results in a profound disconnect between the 'real-world' and the material being covered, isolating students from the learning process (Bransford et al., 1999; Diamond, 1986) while decreasing enthusiasm for the subject. This "excitement" is one of the most significant factors in influencing student performance and retention (Basey et al., 2008).

1.5.2 Active Engagement in Laboratory Curriculum

Encouraging active student participation in science courses is a main method by which teachers can positively impact students' attention and interest in the material (Freedman, 1997), and also lead to an improvement in retention and aptitude (Rissing and Cogan, 2009). To achieve this, educators can alter the structure of laboratories, moving from repetitive, pre-determined bench-top activities to include open-ended applications-based experiments. In this way, students are exposed to a broader scope of material and increased relevancy with the world outside the classroom.

Small, research-oriented projects developed in tandem between the students and faculty have become increasingly popular among science departments across the country (Lue and Losick, 2009) as a way of involving students in the scientific process. These methods improve students' critical thinking and communication skills in science and increase motivation (Sales et al., 2006), leading to a greater interest in science, a higher degree of understanding, and ultimately a larger retention of students in scientific fields (Anagnopoulos, 2006). The STN system of field-deployable bioassays possesses a number of attributes that make it ideally situated to engage students in participatory science. The STN assays are inexpensive, thoroughly tested and simple to execute by students from any scientific background. Moreover, the guiding principle of the STN method – bioexploration – allow the curriculum to be expanded in multiple directions, including plant biology, human health and disease, ecological conservation, and ethnobotany. These innovative tools envelop students in participatory science instruction focused around real-world discovery, while imparting essential scientific skills and concepts and stimulating student interest.

1.6 Evaluating a Commercial Alaskan Berry Superfruit

The growing body of research into polyphenols and bioactive phytochemicals, coupled with the rise in many degenerative and lifestyle health conditions, have brought about an emerging market in the United States: superfruit. These products represent an attractive arena for the introduction of the wild Alaskan berries as a potent health-benefiting commodity. However, careful consideration must be paid to the economic and cultural factors surrounding the potential of such a development project.

1.6.1 Superfruit

Consisting of such fruits as cranberry, açaí, goji, blueberries, papaya, lingonberry, and mango, these fruit receive greater attention due to their elevated levels of secondary metabolites (Gross, 2010). Sales of such fruit have dramatically increased, with sales surging nearly 200% year-over-year for açaí (Anonymous, 2007). This market growth is due in part to a heightened health consciousness among consumers, as well as marketing targeting the 'exotic' nature of these fruit, many of which are not endemic to North America (Suryanata, 2000). However, novel products to compliment the current repertoire of superfruit are constantly being sought, with an increase in availability and desire for traditional subsistence foods (Aranowski, 2009; Gruenwald, 2009). Alaskan berries, with their high levels of phytochemicals and evidence of bioactivity, can represent a new, domestic superfruit for the lucrative superfruit market and function as a commercial product to stimulate economic development.

1.6.2 Alaska Native Economic Structure

NA/AN communities continue to stagnate economically compared to the rest of the nation; poverty rates in indigenous communities hover near 34% (compared with a nation-wide 12.3% poverty rate) (Glasmeier, 2006; USDA, 2004). Several avenues exist for ameliorating the rampant poverty in the Alaska Native community (Vinje, 1996; Wagner, 2007), including the utilization undeveloped land under the control of the tribal governments (Anderson and Parker, 2009), representing access to the berry populations that can be marketed as the newest superfruit.

However, Alaska Natives are involved in an economic system distinct from tribes in the contiguous United States. The Alaska Native Claims Settlement Act (ANCSA) of 1971 formed 12 regional native corporations to administer economic development projects for each region (a 13th was developed representing out-of-state Alaska Navies in 1976) (ANCSA Network, 2009). It was a motion by the federal government to introduce Alaska Natives to a market economy structure, with the thought that this would foster resource and economic development. However, these corporations effectively transferred property rights from the communal governance that had been practiced for generations (and was recognized by the Indian Reorganization Act of 1934) to for-profit companies structured around modern American capitalism. The regional corporations were now in control of the in addition 44 million acres of land and $962 million granted to Alaska Natives. The end result has been mixed, with some areas experiencing increasing intratribal conflict (Berardi, 1998), while others have strived to minimize the differences in approaches and form a cohesive development plan (Thornton, 2007).

1.6.3 SWOT Analysis

To better understand the benefits and challenges associated with commercialization, an analytical framework is necessary to categorize and address each potential obstacle. All organizations operate in an interconnected multi-dimensional environment, and are faced with forces that can present benefits or challenges to their objectives. Formulating a plan begins by identifying strengths, weaknesses, opportunities, and threats (SWOT) associated with the project. The SWOT analysis is a strategic organizational planning tool first developed in the 1950s to assess comparative business strategies (Panagiotou, 2003). The internal appraisal examines the organizational strengths and weaknesses of the agency, including personnel, facilities, location, products, while the external evaluates the economic, social, and competitive arenas to uncover possible opportunities and threats (Dyson, 2004). It has proven to be a remarkably versatile tool, gaining a ubiquitous presence in organizational planning across multiple disciplines, from law enforcement (Garner, 2005) to metabolomics theory (Miller, 2007). This project will utilize a SWOT approach to investigate the factors associated with commercialization of the berries.

1.7 Climate Instability and Impact on Wild Berries

The Alaskan climate is considered one of the most stressful in the northern hemisphere, a unique environment, and these harsh conditions are hypothesized to be responsible for the potency of the wild Alaskan berries. However, as worldwide evidence for global climate change has become incontrovertible, Alaska's unique position has also made it especially vulnerable. Few places are feeling the effects of shifting climate more rapidly or more dramatically than the Arctic, which has experienced a rapid increase in temperature over the last three decades (Serreze et al., 2000); (Simpson et al., 2002). Meteorological studies have demonstrated a temperature increase averaging 0.47 °C per decade over the last 80 years, corresponding with a lengthening in the growing season by 2.6 days per decade and an earlier leaf onset date of 1.1 days per decade, with rises in primary productivity up to 20% for some species of vascular plants (Keyser et al., 2000). Visual studies confirm a marked shift in vegetation patterns in the tundra, with an increase in shrub-like plant distribution along the tundra (Sturm et al., 2001). Controlled experiments have demonstrated a marked increase in vascular plant tissue, especially shrubs, and the concurrent diminishing of other plants, such as lichen and moss (Van Wijk et al., 2003). While the projected increase in growing season could prove beneficial to berry species by promoting wider ranges for berries and larger growing seasons, the conditions also stimulate competition for the limited resources available in the area.

The Scenario Network for Alaska Planning (SNAP), a program run by the University of Alaska Fairbanks and University of Alaska Geography Program, uses the scenarios put forth by the Intergovernmental Panel on Climate Change (IPCC) to model temperatures across the state for the next century. The winter temperatures for January 2008, 2050, and 2099 are below in **Figure 1.2** (SNAP, 2009). The shift winter temperatures projected would be severely detrimental to the berries, as many species require overwintering to produce fertile flowers and fruit (Wendell and Alsanius, 2008).

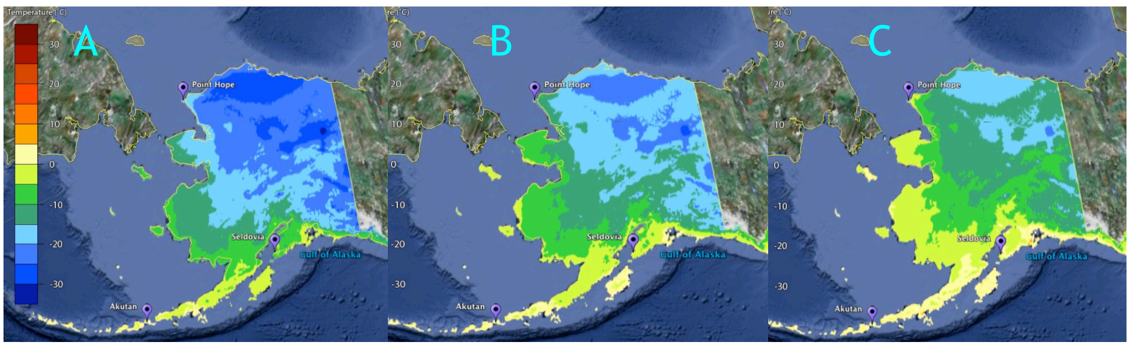


Figure 1.2: January Temperature Shifts Predicted for Alaska in the Years 2008 (A), 2050 (B), and 2099 (C). Warmer colors represent warmer average temperatures. Obtained from the Scenario Network for Alaska Planning (SNAP, 2009), in conjunction with Google Earth©.

Changes to the environment can precipitate an unintentional alteration to the berries, either in harvest quantity or quality. As the climate becomes more favorable for vegetative growth, it is possible to decrease the levels of protective polyphenols that are at the core of the health-conferring properties of wild berries. However, due to a lack of definitive scientific projections on the impacts of climate change on endemic vegetation, the ultimate effect that climate change will have on wild berries, is not currently fully understood.

1.8 Thesis Objectives

The main thrust of this research is to explore the bioactive properties of five species of wild Alaskan berries (*Vaccinium ovalifolium*, *V. uliginosum*, *Rubus chamaemorus*, *R. spectabilis*, and *Empetrum nigrum*) from three regions within Alaska

(**Figure 1.3**). The berries were first evaluated for preliminary bioactivity using the novel system of field-deployable bioassays (STNs), followed by more in-depth investigations into their chemistry and ability to potentially offset metabolic syndrome.

Figure 1.3: Collection Sites in Alaska

Beyond investigating plants for active compounds, there are other arenas that stand to benefit from an integration of traditional and modern approaches as envisioned in the STN system. The same principles of scientific discovery within the STN assays hold potential to improve science laboratory education, creating innovative, engaging classroom activities that are based upon scientific research principles. In addition, the results of the bioexploratory research demonstrate the possibility of wild Alaskan berries to form a competitive commercial product, and potentially provide economic development opportunities for Alaska Native tribes. All of these developments stem from extending the principles of collaborative bioexploration and recognizing traditional knowledge as a valid worldview.

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2 Alaskan Wild Berry Resources and Human Health Under the Cloud of Climate Change[1,2]

2.1 Abstract

Wild berries are integral dietary components for Alaska Native tribes and a rich source of polyphenolic metabolites that can ameliorate metabolic disorders such as obesity and diabetes. In this study, five species of wild Alaskan berries (*Vaccinium ovalifolium, V. uliginosum, Rubus chamaemorus, R. spectabilis,* and *Empetrum nigrum*) were screened for bioactivity through a community-participatory research method involving three geographically-distinct tribal communities. Compositional analysis by HPLC and LC-MS[2] revealed substantial site-specific variation in anthocyanins (0.01-4.39 mg/g-FW) and proanthocyanidins (0.74-6.25 mg/g-FW), and identified A-type proanthocyanidin polymers. *R. spectabilis* increased expression levels of preadipocyte-factor-1 (182%), and proanthocyanidin-enriched fractions from other species reduced lipid accumulation in 3T3-L1 adipocytes. Selected extracts reduced serum glucose levels in C57bl/6j mice by up to 45%. Local observations provided robust insights into effects of climatic fluctuations on berry abundance and quality, and preliminary site-specific compositional and bioactivity differences were noted, suggesting the need to monitor this Alaska Native resource as climate shifts impact the region.

[1] Kellogg, J., Wang, J., Flint, C., Ribnicky, D., Kuhn, P., de Mejia, E. G., Raskin, I., Lila, M. A. "Alaskan Wild Berry Resources and Human Health Under the Cloud of Climate Change" J. Agric. Food. Chem. - In Press

[2] Community interviews conducted and interpreted by Dr. Courtney Flint. *In vivo* mouse study conducted by Drs. David Ribnicky and Peter Kuhn at Rutgers University (sections 2.3.4 and 2.4.4).

Keywords: Anthocyanins, proanthocyanidins, *Vaccinium ovalifolium, Vaccinium uliginosum, Rubus spectabilis, Rubus chamaemorus, Empetrum nigrum*, Traditional Ecological Knowledge, *pref-1*, adipocytes, diabetes, obesity, metabolic syndrome

2.2 Introduction

Type 2 diabetes mellitus (T2DM) incidence rates in the United States have increased nearly 200% over the past two decades, rising from 5.6 million cases in 1980 to 15.8 million in 2005 (Centers for Disease Control & Prevention). This has coincided with a surge in overweight and obese individuals; over 2/3 of the population of the United States is classified as overweight, with 32% of the population diagnosed as obese (National Center for Health Statistics, 2009). American Indian/Alaska Native (AI/AN) populations suffer disproportionately high rates of T2DM and obesity and are twice as likely to have T2DM as non-Hispanic whites (Centers for Disease Control & Prevention). This ethnic group has the highest obesity rates in all age classes (American Diabetes Asociation, n.d.a.; National Center for Health Statistics, 2009), which is in part attributed to a shift from a traditional to a more Western lifestyle, including higher calorie and fat diets (Story et al., 2000) and lowered physical activity (Mendlein et al., 1997; Yurgalevitch et al., 1998). Alaska Natives are particularly at risk, experiencing increased glucose intolerance, T2DM, and obesity across all age groups (Acton et al., 2003; Gahagan and Silverstein, 2003; Murphy et al., 1995).

Northern America sustains a wide range of berries that are integral parts of the traditional ecological knowledge (TEK) of indigenous Arctic tribes. Salmonberries (*Rubus spectabilis* and *R. chamaemorus*), in the same genus as raspberry, have been used for such diverse health remedies as wound healing and gynecological aids (Moerman, 1998), and both species are important to tribal populations as a foodstuff (Heller, 1953; Moerman, 1998). The Tanaina, a tribal group near Anchorage, Alaska, have used the leaves and stems of *Empetrum nigrum* (alternatively known as crowberry, blackberry, or mossberry in various regions of the Arctic) to treat diarrhea, and the plant is noted for countering kidney trouble (Viereck, 2007). Highbush and bog blueberries (*Vaccinium ovalifolium* and *V. uliginosum*) are also integral dietary resources, and are used both topically and orally as medicines (Kari, 1995). In addition to eating the raw berries, the Inupiat tribe – an Inuit tribe indigenous to Alaska's Northwest arctic – also ferment them to prepare a vinegar for cooking, and make berry-based relishes (Jones, 1983). Northern and Aleutian Alaska Natives also use the berries as part of agutuk, a traditional treat made with fish or seal oil (Bersamin et al., 2006).

Although scant phytochemical analyses have been documented for circumpolar berries such as those found in Alaska, significant circumstantial evidence suggests that they may be protective against diabetes-related health complications. Related berry species have demonstrated substantial bioactivity countering a wide variety of human diseases and conditions. Berries contain complex phytochemical mixtures including anthocyanins and proanthocyanidins, powerful antioxidants capable of neutralizing free radicals responsible for an array of cardiovascular diseases including atherosclerosis and ischemic stroke (Al-Awwadi et al., 2005; Neto, 2007), DNA damage (Dulebohn et al., 2008), and neurodegeneration (Schroeter et al., 2002). Bilberry (*Vaccinium myrtillus*) extracts have been shown to improve night vision and decrease myopia symptoms (Lee et al., 2005). Berry species, including blueberry, strawberry, raspberry, and cranberry, inhibit multiple stages of carcinogenesis and stimulate apoptosis of carcinogenic cells (Kraft et al., 2005; Seeram et al., 2006). The A-type proanthocyanidins found in blueberries and cranberries have antiadhesion and antiproliferation properties that are effective in preventing bacterial infections, especially in the urinary tract (Foo et al., 2000; Howell et al., 2005; Schmidt et al., 2004).

In addition to the wide breadth of diverse health-related benefits noted above, ingestion of the significant amounts of flavonoids contained in many berries may ameliorate complications associated with T2DM (Tsuda et al., 2003). Diet is increasingly recognized as an important avenue to preventing the onset of T2DM (Hill and Peters, 2002). Members of the genus *Vaccinium* and *Rubus* have demonstrated hypoglycemic and antidiabetic activity (Jouad et al., 2002; Martineau et al., 2006), and feature in traditional anti-diabetic medicine; the Cree First Nation in Quebec, Canada used several species of fruit, including *Vaccinium sp*., to treat symptoms of diabetes (Leduc et al., 2006). Blueberry fruits and leaves were prepared as decoctions for teas used to treat diabetes in European medicine (Frohne, 1990). Strawberry, blueberry, and raspberry extracts decrease the activity of glucosidase enzymes such as α-amylase (McDougall et al., 2005), showing potential for lowering postprandial hyperglycemic episodes. Berries also stimulate glucose uptake in various cell models, including C2C12 myotubes and 3T3-L1 adipocytes (Martineau et al., 2006; Vuong et al., 2007).

In addition, the rich proanthocyanidin and anthocyanin complement in many berries is purported to offset the tendency towards obesity. Glycosylated anthocyanins actively regulate genetic markers associated with obesity (Tsuda et al., 2005). In several studies by Tsuda et al., anthocyanins prevented the onset of obesity in rats maintained on a high-fat diet, affecting adipocyte gene-expression level and adipocytokine secretion (Tsuda et al., 2003; Tsuda et al., 2004). Proanthocyanidins have been shown to regulate adipocyte function and obesity. Procyanidin-rich fractions of apples inhibited pancreatic lipase activity in *in vitro* models (McDougall et al., 2009; Sugiyama et al., 2007). Proanthocyanidins are also effective in lowering plasma triglyceride levels and cholesterol accumulation in a variety of *in vivo* studies (Sugiyama et al., 2007; Tebib et al., 1994; Zern et al., 2003).

Environmental stresses significantly modulate the phytochemical profiles of plants. Biotic and abiotic stresses present in the wild are endured by plants through defensive chemical adaptations, fostering a potentially more complex phytochemical composition in the wild fruit than in cultivated varieties (Thole et al., 2006). Wild berry fruits typically accumulate more concentrated phenolic compounds (including tannins) than their cultivated relatives (Lila, 2006; Sueiro et al., 2006), as the latter are more buffered from environmental insults due to commercial agriculture inputs, and have modified composition due to selection and breeding. A comparative study between blackberries from the Pacific Northwest and similar genotypes from Mexico demonstrated that the cooler, wetter climate of the Pacific Northwest fostered berries with higher antioxidant bioactivity, which was correlated with their polyphenol content (Reyes-Carmona et al., 2005). Controlled experiments mimicking extreme cold or drought conditions revealed that some species of berry can alter their biosynthesis of photosynthetic and adaptive secondary biochemicals in response to stress (Martel et al., 2005; Panta et al., 2007).

The Alaskan environment is one of the most stressful climates in North America. The Alaskan landscape is characterized by a short growing season, long photoperiods during the summer (approaching 24 hours above the Arctic circle), extreme shifts in temperature (as low as -50 °C in the winter and up to 27 °C in summer months), and the presence of a permafrost soil structure (Hannemann and McGinley, 2009). These combinations of climate and geochemistry extremes significantly stress indigenous plants, which prompts adaptive biochemical responses.

Alaska’s unique location has also made it especially environmentally vulnerable as worldwide evidence for global climate change has become incontrovertible. The Arctic has experienced a rapid increase in temperature over the last three decades (Serreze et al., 2000), with a sustained temperature increase averaging 0.47 °C per decade over the last 80 years (Keyser et al., 2000). In addition to shifting the weather patterns of the region (Simpson et al., 2002), the warmer temperatures translate into a visual increase in shrub-like plant distribution along the tundra (Sturm et al., 2001). The growing season has lengthened by 2.6 days per decade and the leaf onset date has shifted 1.1 days earlier per decade, yielding as much as 20% higher primary productivity for some species of vascular plants (Keyser et al., 2000). Controlled experiments have demonstrated a marked change in vegetative composition in the ecosystem; increased densities of vascular plants, especially shrubs, and diminishing populations of other plants such as lichen and moss (Van Wijk et al., 2003). By some measures, berry species may actually be favored by climatic shifts, as wider distribution ranges and longer growing seasons may be enabled. On the other hand, climate shifts could allow previously un-adapted plant species to encroach on the habitat of berries, competing for scarce resources and disadvantaging the berry populations. In addition, climate changes may attenuate the characteristic environmental stresses that trigger adaptive production of biologically-active phytochemicals in berries. In this case, plant proliferation and survival may be enhanced, but secondary product accumulation could recede. Quantitative projections of climate change-induced impacts on berries would mandate extended term data analysis, however, traditional knowledge and local observations provide robust insights into climatic factors affecting berry abundance and quality.

The objectives of this study were to characterize the phytochemical composition, especially the anthocyanin and proanthocyanidin constituents, of wild Alaskan berries from differing climatic regimes, and to evaluate their potential efficacy against symptoms of T2DM and obesity via *in vitro* bioassays (adipogenesis and lipid accumulation in 3T3-L1 cells) (Tsuda, 2008) and in an *in vivo* hyperglycemic rodent model, and to integrate the bioscience discoveries with TEK, local climate change observations, and community health concerns. The present study uses a combination of participatory field research and laboratory investigations to identify and biochemically and functionally characterize wild Alaskan berries chosen from 3 distinct geographic sites.

2.3 Materials and Methods

2.3.1 Field Work

Research Sites. Three village community sites were selected to represent significant biogeographic and climatic variation in Alaska: Akutan Island (AK) (54.1373 °N, 165.7728 °W), Seldovia (SD) (59.4112 °N, 151.6866 °W), and Point Hope (PH) (68.1737 °N, 166.9940 °W). The population in Seldovia is approximately 30% Alaska Native (AN), whereas Point Hope and Akutan villages feature 90% and 95% AN members, respectively. Annual average precipitation ranges around 28”, 34”, and 10” for Akutan, Seldovia, and Point Hope, respectively. Akutan has the narrowest yearly range of temperatures (-5.5 to 12.8 °C), whereas Point Hope demonstrates the greatest variation (-45 to 25.6 °C) (Alaska Department of Commerce). All three villages are located in coastal regions, and berry collection sites range from 3-80 m in elevation. Akutan and Point Hope are characterized by mesic graminoid herbaceous and dwarf shrub communities, while Seldovia consists of spruce-dominated coniferous forests (National Geographic and Fund, 2001).

Community Participatory Research. This study involved NA/AN people in the biodiscovery process, in a community-participatory framework that investigated and validated traditional ecological knowledge, and conducted field surveys of bioactive constituents of local medicinal plants. The research team engaged local community members to implement a set of field bioassays known as “Screens-to-Nature” (STN), an innovation developed through the Global Institute for BioExploration (www.gibex.org). The STN are field-deployable bioassays that use visual chemical indicators to reveal preliminary data on the bioactivity of plant extracts. For this study, the antioxidant potential and α-amylase inhibition assays (two potential mechanisms of antidiabetic plants (Lee et al., 2007)) were chosen to investigate the bioactivity of local wild berries, along with protease activity and inhibition. Community members were trained by the research team in the process of data collection and analysis of bioassay readings. Assays were carried out according to defined protocols described in GIBEX field manuals, provided to each community.

Based on the preliminary STN analyses, as well as recommendations from community elders, five berry species were collected for in-depth analyses: *Vaccinium ovalifolium*, *V. uliginosum*, *Empetrum nigrum*, *Rubus spectabilis*, and *R. chamaemorus*. At least 500 g of each berry fruit at each site was harvested to ensure sufficient sample for analysis. Due to climatic and other site-specific considerations, only *E. nigrum* was available and collected at all three test sites. *V. ovalifolium* and *R. spectabilis* were harvested in both Seldovia and Akutan, *V. uliginosum* from both Akutan and Point Hope, and *R. chamaemorus* only from Point Hope. Upon collection, berry fruit samples were frozen and transported on dry ice in insulated shipping containers to the University of Illinois, where samples were immediately placed in a -20 °C freezer until analysis.

Interviews with community members and surveys were conducted by local students as well as a research team investigator, to gather information regarding local berry use, community and environmental well-being, and observations of climatic influence. The interviews were conducted over a two-year period for a total of 11 interviews in Akutan, 24 in Point Hope, and 30 in Seldovia. Interviews were recorded, transcribed, and analyzed thematically. Formal interviews were supplemented by numerous conversations with community members over the course of the project.

2.3.2 Phytochemical Analysis

Reagents. Except where noted, all chemicals were purchased from Fisher Scientific (Pittsburgh, PA) and were of reagent grade. All solvents for High Performance Liquid Chromatography (HPLC) were HPLC grade.

Extraction. Procedure for frozen berry extraction was modified from Grace et al. (2009) (Grace et al., 2009). In brief, berries were homogenized with acidified methanol (99.7% methanol (MeOH): 0.3% trifluoroacetic acid (TFA) in a 2:1 v/w ratio). Homogenate was filtered and alcohol removed via rotary evaporation at temperatures ≤40 °C. Resulting aqueous suspension was partitioned with ethyl acetate (3 x 500 mL) to remove lipophilic constituents. The aqueous layer was retained, loaded on 1 L Amberlite XAD-7 resin (Sigma-Aldrich, St. Louis, MO) and polyphenols eluted with 2 L acidified methanol until all the colored residue was extracted from the column. Methanol was removed via reduced pressure, frozen, and lyophilized to yield the phenolic-rich extract (PRE). Fractions were prepared by placing 1.0 g PRE on a Sephadex LH-20 column (25 x 3 cm) (GE Life Sciences, Buckinghamshire, UK).

Anthocyanins were obtained from an isocratic elution of acidified aqueous methanol (80% H_2O: 20% methanol: 0.3% TFA), after which proanthocyanidins were eluted with 70% aqueous acetone. All fractions were individually concentrated and lyophilized to give the anthocyanin-enriched (ARF) or proanthocyanidin-enriched (PAC) fractions (**Figure** 2.1).

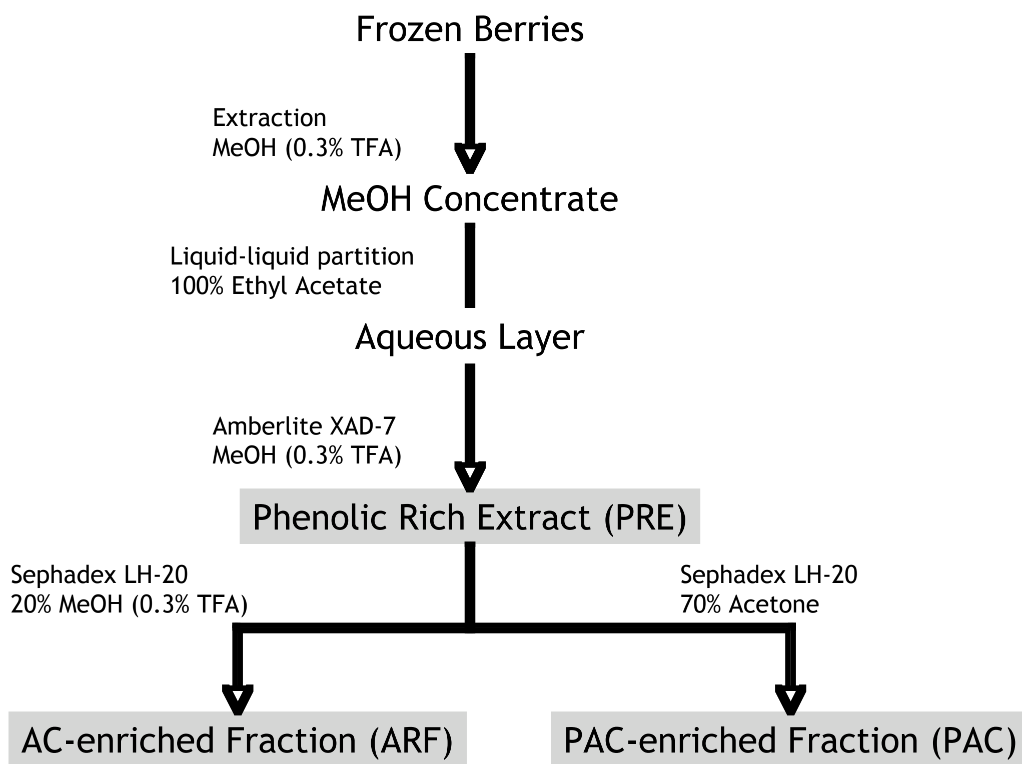

Figure 2.1: Flow Chart for Frozen Berry Extraction. Abbreviations: PRE = phenolic-rich extract (obtained after Amberlite column); ARF = anthocyanin-rich fraction (obtained after 20% methanol elution from Sephadex column); PAC = proanthocyanidin-rich fraction (obtained after 70% acetone elution from Sephadex column). Adapted from Grace et al. (2009)

Phenolic Characterization. Anthocyanin separation was conducted on an 1100 HPLC (Agilent Technologies, Santa Clara, CA) using a reverse-phase Supelcosil-LC-18 column (250 mm x 4.6 mm x 5 μm) (Supelco, Bellefonte, PA). Samples were dissolved at a concentration of 5 mg/mL in 100% methanol and filtered through 0.45 μm nylon filters (Fisher Scientific, Pittsburg, PA). The mobile phase consisted of 5% formic acid in H_2O (A) and 100% methanol (B). The flow rate was held constant at 1 ml/min with a step-wise gradient of 10%, 15%, 20%, 25%, 30%, 60%, 10%, and 10% of solvent B at 0, 5, 15, 20, 25, 45, 47, and 60 min, respectively. The same instrumentation set-up was used to separate proanthocyanidins. Mobile phases consisted of 94.9% H_2O: 5% acetonitrile: 0.1% formic acid (A) and 94.9% acetonitrile: 5% H_2O: 0.1% formic acid (B). The flow rate was held at 1 mL/min with a step gradient of 0%, 5%, 30%, 60%, 90%, 0%, and 0% of solvent B at 0, 3, 40, 45, 50, 55, and 60 min, respectively. Agilent's Chemstation software was used for both protocol control and data processing.

Proanthocyanidin LC-MS^2 analyses were conducted on a Waters Alliance 2795 (Waters Corporation, Beverly, MA) with a reverse-phase C-18 column (150mm x 2.1mm i.d., particle size 5 μm, 90 Å) (Western Analytical, Murrieta, CA). The analyses were carried out at a constant flow rate of 200 μl/min using mobile phases consisting of 94.9% H_2O: 5% acetonitrile: 0.1% formic acid (A), and 94.9% acetonitrile: 5% H_2O: 0.1% formic acid (B), with a step-wise gradient of 5%, 30%, 60%, 90%, 90%, 5% and 5% of solvent B at 0, 40, 45, 50, 55, 60, and 70 min, respectively. All samples were dissolved in 100% MeOH at a concentration of 1 mg/mL and filtered through 0.45 μm nylon before injection at a volume of 10 μL. The column was connected to a Q-TOF Ultima mass spectrometer (Waters Corporation). An ESI source working in the positive ion mode was used for all MS analyses. The mass spectrometer was tuned for maximum intensity of the parent ions of interest. Acquisition of LC-PDA-MS data was performed under Mass Lynx 4.0 (Waters Corporation) and data processing was achieved using the XCalibur Qual Browser v. 1.4 software (Thermo Electron Corporation, Waltham, MA).

2.3.3 *In vitro* Assays

Cell Culture and Treatments. The 3T3-L1 fibroblasts from Swiss albino mice (ATCC CCL-92.1) (American Type Culture Collection, Rockville, MD) were seeded at a concentration of 6 x 10^4 cells/well in 6-well plates and 5 x 10^3 cells/well in 96-well plates. All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 100 units/mL penicillin-streptomycin, 10 mM sodium pyruvate, and 10% calf serum (CS) (DMEM/CS medium) (Sigma-Aldrich). Adipocytes were continuously incubated at 37 °C in a 5% CO_2 atmosphere during culturing and treatment.

Mature and immature adipocytes were treated with the phenolic-rich extract (PRE), as well as the two post-Sephadex fractions (anthocyanin-rich (ARF) and proanthocyanidin-rich (PAC)). Samples were dissolved in dd-H_2O and applied to cells at a final concentration of 200 μg/mL.

Cytotoxicity Assay. All extracts and fractions were assayed for any increase in cell mortality before *pref-1* or lipid quantification assays. CellTiter 96AQueous One Solution was used to determine the amount of viable cells (Promega, Madison, WI). Briefly, 20 μL of CellTiter 96AQueous One Solution was charged to each well containing 100 μL of DMEM without serum, and the plates were incubated at 37 °C and 5% CO_2 atmosphere for two hours. The absorbance was measured on a 96-well plate reader (Biotek Instruments, Winooksi, VT) at 515 nm and compared against a vehicle-treated control. No samples significantly decreased cell viability (defined as cell counts >80% of control).

Preadipocyte *pref-1* Analysis. Preadipocyte cells were seeded at a concentration of 1 x 10^5 cells/well in 6-well plates and cultured with DMEM/CS medium. After 24 hours cells were treated with berry extracts enumerated above. Cells were washed with media and Dulbecco's Phosphate Buffer Saline (DPBS) and then lysed with 200 μL Laemmeli buffer (Bio-Rad Laboratories, Hercules, CA). Proteins separated via electrophoresis on 4-20% Tris-HCl ready gels (Biorad Laboratories) with Precision Plus Dual Color Protein Standard (Bio-Rad) as a control between 10 and 250 kDa. Gels were run on a PowerPac 300 (Bio-Rad) at 200 V for 30 min.

The separated proteins were transferred to polyvinylidene difluoride (PVDF) (Millipore, Billerica, MA) membranes and blocked with 5% non-fat dry milk (NFDM) (Nestle S.A., Vevey, Switzerland) in 0.1% Tris-buffered saline Tween 20 (TBST) for 1 h at 4 ºC. After blocking, membranes were incubated overnight with 10 mL anti–*pref-1* goat polyclonal IgG antibody (1:2000 in TBST with 1% NFDM) (Applied Biosystems, Foster City, CA) at 4 °C. The membranes were washed with TBST, 5 x 5 min, and then incubated with 10 mL ECL anti-goat IgG horseradish peroxidase conjugates (1:1000 in TBST with 1% NFDM) (Applied Biosystems) for 2 h at room temperature. The membranes were washed a final time in 1% NFDM in TBST, 5 x 5 min. The membranes were imaged using chemiluminescence dye (GE Life Sciences, Piscataway, NJ) on Kodak image station 440 CF (Eastman Kodak, Rochester, NY).

Lipid Quantification by Oil Red O Assay. Immature adipocytes were seeded in 6-well plates at 6 x 10^4 cell/well. Two days after reaching confluence preadipocytes were induced with DMEM containing 100 units/mL penicillin-streptomycin, 10 mM sodium pyruvate, and 10% fetal bovine serum (FBS) (DMEM/FBS medium) containing 172 nM insulin, 0.5 M isobutylmethylxanthine (IBMX), and 1.0 M dexamethasone (DXM) (Sigma-Aldrich) for 2 days. Cells were then differentiated using DMEM/FBS medium containing 1.72 μM insulin for 2 days. Medium was changed (DMEM/FBS without additional insulin) every two days for 6 days total until treatment. Mature adipocytes were treated with extracts for 48 hours before assaying using a procedure adapted from Martinez-Villaluenga et al. (2008) (Martinez-Villaluenga et al., 2008). In summary, mature adipocytes were washed with DPBS and fixed for one hour with 10% formaldehyde (v/v in DPBS) (Sigma-Aldrich). Cells were washed in 60% isopropanol and air-dried. The Oil Red O solution was prepared by dissolving 0.1 g Oil Red powder (Sigma-Aldrich) in 20 mL 100% isopropanol and diluted to a final volume of 50 mL with dd-H_2O. Each well was stained with 2 mL for 60 min, after which the cells were washed with water four times and allowed to air dry. The Oil Red O dye was eluted from the lipid droplets by adding 2 mL 100% isopropanol for 10 min. The resulting eluant was analyzed on a SpectraMax Plus spectrophotometer (Molecular Devices, Sunnyvale, CA) at 510 nm. Percent accumulation was calculated by the following equation:

$$1-((A_{control} - A_{treatment})/A_{control}) \times 100\% = \%\ \text{lipid accumulation versus control}$$

2.3.4 In vivo Assay

The bioassay for evaluation of hypoglycemic response (antidiabetic properties) due to extract administration to mice is described by Grace et al. (*55*). In summary, five-week-old male C57bl/6J mice (10–20 g) were purchased from Jackson Labs (Bar Harbor, ME) and acclimatized for 1 week in a cage with a regular ad libitum diet and water, a 12:12 h light-dark cycle, and constant 24±1 °C temperature. At 6 weeks of age, mice were randomly distributed into experimental groups fed either a low fat diet (LFD) or very high fat diet (VHFD). The LFD and VHFD (Research Diets Inc., New Brunswick, NJ) were similar in nutritional content, differing only in carbohydrates (70 kcal% vs. 20 kcal%) and fat content from lard (10 kcal% vs. 60 kcal%). Mice were maintained on assigned diet for 14 weeks, weighed weekly and blood was collected every other week for glucose analysis. Animals had food restricted for 4 h and then were gavaged with plant extracts (500 mg/kg), control vehicle (water), or metformin (positive control at 300 mg/kg). Blood glucose readings were taken at 0, 3, and 6 h after gavage (animals continued food restriction during this time), after which food was replaced until the next treatment. All protocols were approved by Rutgers University Institutional Care and Use Committee and followed state and federal laws.

2.3.5 Statistical Analysis

Data are presented as mean of triplicate runs ±SEM. Statistical significance of *in vitro* and *in vivo* assays was determined using the student's Ttest procedure of Statistical Analysis System (SAS) (version 9.2, SAS Institute Inc., Raleigh, NC). Linear correlation for lipid accumulation was achieved using Microsoft Excel (Microsoft Corporation, Redmond, WA). Mean separation of *in vivo* results were achieved through the LSD procedure of SAS (SAS Institute) with α=0.01.

2.4 Results

2.4.1 Community Participatory Research

The three STN bioassays provided a strong preliminary indication of bioactivity relevant to diabetes and obesity, and simultaneously served to engage local community members in the objectives of the research initiative. As an illustration of the outcomes, a representative data summary for the three berries assayed in Akutan is shown in **Table 2.1**. All berries demonstrated antioxidant capacity. *E. nigrum* and *V. ovalifolium* were effective α-amylase inhibitors, whereas *R. spectabilis* was not. All three berry species inhibited protease digestion of gel substrates, and correspondingly had undetectable levels of protease activity. Both ripe and unripe berries were assayed in each community, which illustrated to the community members that the secondary phytochemicals accumulated in ripe fruit were responsible for the observed bioactive properties. Berries from each of the other two sites (Seldovia and Point Hope) also demonstrated similar bioactivies and trends.

Berry	Ripe/ Unripe[1]	Protease Assay	Protease Inhibitor Assay	Amylase Assay	Amylase Inhibitor Assay	Antioxidant Assay
from Akutan, Alaska						
Rubus spectabilis	R	0	3	0	1	2
Rubus spectabilis	U	0	0	0	1	1
Vaccinium ovalifolium	R	0	3	0	2	3
Vaccinium ovalifolium	U	0	0	0	1	1
Empetrum nigrum	R	0	3	0	2	2
Empetrum nigrum	U	0	0	0	1	2

[1] Responses are evaluated on a 0-3 scale, with 3 reflecting the highest activity.

Table 2.1: Screens-to-Nature (STN) Assay Results Demonstrating Antioxidant, Protease Inhibitor, and Amylase Inhibitory Activity from Three Sampled Akutan Berry Species

Interviews and surveys with residents in all communities conducted in tandem with the field screening training sessions indicated that berries are considered a key local food resource and are valued for their nutritional contribution to local diets as well as for the traditional cultural practice of berry harvesting. In all communities, participants voiced concern about younger people moving away from local subsistence foods and consequent negative health implications. Local residents were particularly concerned about the rapid rise in diabetes and other health problems, including cancer.

Local tribal community members in each of the three test sites were keenly aware that berries are easily compromised or enhanced by fluctuations in climate. Local observations confirmed the importance of winter conditions, particularly abundant precipitation in the form of snow as crucial to developing a high yield, high quality berry harvest, with warmer, drier winters resulting in fewer berries with a decrease in taste. Moderate summer warmth, sunshine and adequate precipitation were also seen as essential to berry abundance and timing. Residents widely agreed that local climates were changing, albeit with mixed perspectives in Seldovia on the validity of anthropogenic sources of climate change. Point Hope participants reported substantial climate fluctuation with warming trends affecting sea ice, tundra conditions, wildlife migration patterns, and presence of insects. Akutan participants reported climate fluctuations, with greater uncertainty regarding weather trajectories and impacts from year to year. Seldovia participants noted local experience with climatic cycling, but rarely acknowledged a linear warming trend. In each community, climate change or fluctuation from year to year was highlighted as posing one of the greatest risks to local berry resources.

2.4.2 Phenolic Composition

Anthocyanins. The total anthocyanin content of the berries was calculated as cyanidin-3-glucoside equivalents from the total sum of peak areas as determined by HPLC. Berries showed substantial differences in anthocyanin content, ranging between 0.01 and 4.39 mg/g fresh weight of fruit (mg/g FW) (**Table 2.2**) with Point Hope's *E. nigrum* containing the highest levels of anthocyanins. Identification and peak assignment of the anthocyanins were based upon retention time comparison with previously reported values (Grace et al., 2009),(Wu and Prior, 2005), MS spectral data, and comparison to standards. The HPLC chromatograms of each berry, measured at 520 nm, revealed a complex mixture of anthocyanins (**Table 2.3**). The distribution of anthocyanin aglycones varied between locations, but some species-level trends emerged.

Berry[1]	Yield	Anthocyanin Content[2]		Proanthocyanidin Content[3]	
		PRE	ARF	PRE	PAC
	g PRE extract (500 g fruit)	mg equiv / g fruit	mg equiv	mg equiv / g fruit	mg equiv
EN-AK	4.249	2.762	238.02	0.746	21.248
EN-PH	9.768	4.386	302.96	3.741	62.005
EN-SD	4.460	2.648	154.03	2.861	42.607
RC-PH	1.960	0.010	0.09	1.256	23.367
RS-AK	2.183	0.391	29.73	0.486	16.708
RS-SD	1.323	0.086	32.09	0.737	21.372
VO-AK	6.350	3.337	154.62	2.420	38.494
VO-SD	3.644	2.364	102.73	1.732	25.363
VU-AK	5.388	3.100	130.52	2.969	56.352
VU-PH	7.308	2.063	76.57	6.252	80.41

[1]Abbreviations: AK=Akutan, SD=Seldovia, PH=Point Hope, VO=*V. ovalifolium*, VU=*V. uliginosum*, EN=*E. nigrum*, RS=*R. spectabilis*, RC=*R. chamaemorus*.

[2] measured by HPLC at 520 nm

[3] measured by HPLC at 280 nm

Table 2.2: Total Anthocyanin and Proanthocyanidin Content of Wild Alaskan Berries in the Phenolic-rich Extract (PRE) and Enriched Fractions (ARF and PAC)

HPLC analysis of the *V. ovalifolium* samples from Akutan (VO-AK) and from Seldovia (VO-SD) revealed markedly different levels of anthocyanins (**Table 2.2**). VO-AK contained 3.34 mg AC/g FW, whereas VO-SD contained 2.36 mg AC/g FW. The anthocyanin content of Seldovia's berries were comparable to the mean value of 2.65 mg AC/g FW which has been reported for cultivated *V. ovalifolium* genotypes around the Pacific Northwest (Lee et al., 2004; Moyer et al., 2002), whereas the Akutan *V. uliginosum* exceeded the 3.11 mg/g FW reported for the same species of wild blueberries from Washington (Lee et al., 2004). Both VO-AK and VO-SD contained 12 distinct anthocyanin structures (**Table 2.3**). The major species present (**Figures 2.2A,B**) were delphinidin glycosides (galactoside (peak 1), glucoside (peak 2) and arabinoside (peak 3)), cyanidin glycosides (galactoside (peak 4) and glucoside (peak 5)), and malvidin glycosides (galactoside (peak 10), glucoside (peak 11), and arabinoside (peak 12)). Also present were smaller amounts of petunidin-3-galactoside (peak 6), petunidin-3-glucoside (peak 8), and peonidin-3-galactoside (peak 7), consistent with anthocyanin profiles from previous *V. ovalifolium* analyses (Taruscio et al., 2004).

Samples of the other wild blueberry species, *V. uliginosum*, from Akutan (VU-AK) and Point Hope (VU-PH) contained anthocyanin levels of 3.10 and 2.06 mg/g FW, respectively. This was consistent with *V. uliginosum* berries found in northern Finland, which also demonstrated considerable variability in the content of anthocyanins present, ranging from 2.61-4.32 mg/g FW (Määttä-Riihinen et al., 2004). However, both samples were significantly higher than the 1.24 mg/g FW found in *V. uliginosum* samples from Wyoming (Taruscio et al., 2004). HPLC and LC/MS analysis, compared with previous studies (Andersen, 1987; Li et al., 2006) led to the identification of 12 structures (**Table 2.3**) consisting of five aglycones – delphinidin, cyanidin, malvidin, peonidin, and petunidin – congruous with the previous results (Määttä-Riihinen et al., 2004). The major anthocyanins present in VU-AK (Figs. 2C) were delphinidin-3-glucoside (peak 2), cyanidin-3-galactoside (peak 4), cyanidin-3-glucoside (peak 5), petunidin-3-glucoside (peak 7), malvidin-3-galactoside (peak 9), and malvidin-3-glucoside (peak 10). Results from VU-PH (**Figure 2.2D**) were similar, but substantial amounts of delphinidin-3-galactoside (peak 1) were also present.

E. nigrum, the only species available and collected at all three sites of this study, provided a 3-way comparison of phytochemical components. The total content of anthocyanins was similar for berries collected at Akutan (2.76 mg/g FW, EN-AK) and Seldovia (2.65 mg/g FW, EN-SD), but berries from Point Hope (EN-PH) revealed markedly higher anthocyanin concentrations (4.39 mg/g FW, the highest level of any berry tested in the study). While all three samples were higher than the reported average of 1.25 mg/g (Ogawa et al., 2008), EN-PH demonstrated higher anthocyanin content than *E. nigrum* found in northern Finland (4.08 mg/g FW) (Määttä-Riihinen et al., 2004). EN-AK and EN-PH showed similar chromatographs (Figs. 2E,F), with 10 individual anthocyanin species. EN-AK (**Figure 2.2E**) had two major species present – delphinidin-3-galactoside (peak 1) and cyanidin-3-galactoside (peak 2) – while EN-PH (**Figure 2.2F**) possessed three major species - delphinidin-3-galactoside (peak 1), cyanidin-3-galactoside (peak 2), and malvidin-3-galactoside (peak 8). These results agree with other *E. nigrum* studies (Kallio et al., 1986; Kärppä et al., 1984; Ogawa et al., 2008). The chromatograph of EN-SD (**Figure 2.2G**) contained a third delphinidin (delphinidin-3-arabinoside, peak 2), an additional petunidin residue (petunidin-3-glucoside, peak 8), and peonidin-3-arabinoside, which eluted immediately after malvidin-3-glucoside (Ogawa et al., 2008). Peaks 1 (delphinidin-3-galactoside) and 3 (cyanidin-3-galactoside) had shoulders that could not be resolved (marked as peaks 1' and 3', respectively).

Cloudberry (*R. chamaemorus*), available only at the Point Hope site (RC-PH), had the lowest concentration of anthocyanins of all berries tested, 0.01 mg/g FW. HPLC analysis revealed four structures (**Figure 2.2H**), identified as cyanidin-3-glucoside (peak 1), cyanidin-3-arabinoside (peak 2), petunidin-3-glucoside (peak 3), and malvidin-3-galactoside (peak 4) (**Table 2.3**). There has been a single report of the anthocyanins in *R. chamaemorus*, a qualitative study by Jennings and Carmichael (1979) suggesting there were cyanidin structures present (Jennings and Carmichael, 1979). Our research corroborates the previous report in 2 of the 4 identified structures, which are reported here for the first time in *R. chamaemorus*.

R. spectabilis samples from Akutan (RS-AK) and Seldovia (RS-SD) were also found to have low concentrations of anthocyanins, 0.39 and 0.09 mg/g FW, respectively. Akutan and Seldovia's *R. spectabilis* both contained two cyanidin glycosides (**Figures 2.2I,J**) (cyanidin-3-glucoside (peak 1) and cyanidin-3-arabinoside (peak 2)), with RS-AK containing an additional species, malvidin-3-arabinoside (peak 3). While *R. spectabilis* has been reported to contain anthocyanins (Jennings and Carmichael, 1979), no quantitative data has been previously reported, and these specific anthocyanin structures have also not been previously reported for *R. spectabilis*.

Table 2.3: Identification of Anthocyanins in Anthocyanin-Rich Fraction (ARF) of Wild Alaskan Berries

Peak	RT (min)	MS/MS (m/z)	Anthocyanin	Peak	RT (min)	MS/MS (m/z)	Anthocyanin
			V. ovalifolium **- Akutan (VO-AK)**				
1	19.7	465/303	Delphinidin-3-galactoside	7	27.1	463/301	Peonidin-3-galactoside
2	21.6	465/303	Delphinidin-3-glucoside	8	27.8	479/317	Petunidin-3-glucoside
3	23.0	435/303	Delphinidin-3-arabinoside	9	28.8	449/317	Petunidin-3-arabinoside
4	24.2	419/287	Cyanidin-3-galactoside	10	30.3	493/331	Malvidin-3-galactoside
5	25.0	449/287	Cyanidin-3-glucoside	11	31.5	493/331	Malvidin-3-glucoside
6	26.2	479/317	Petunidin-3-galactoside	12	33.4	463/331	Malvidin-3-arabinoside
			V. ovalifolium **- Seldovia (VO-SD)**				
1	19.6	465/303	Delphinidin-3-galactoside	7	26.9	463/301	Peonidin-3-galactoside
2	21.4	465/303	Delphinidin-3-glucoside	8	27.6	479/317	Petunidin-3-glucoside
3	22.8	435/303	Delphinidin-3-arabinoside	9	28.7	449/317	Petunidin-3-arabinoside
4	23.9	419/287	Cyanidin-3-galactoside	10	30.2	493/331	Malvidin-3-galactoside
5	24.7	449/287	Cyanidin-3-glucoside	11	31.5	493/331	Malvidin-3-glucoside
6	26.1	479/317	Petunidin-3-galactoside	12	33.3	463/331	Malvidin-3-arabinoside
			V. uliginosum **- Akutan (VU-AK)**				
1	19.8	465/303	Delphinidin-3-galactoside	7	27.4	479/317	Petunidin-3-glucoside
2	21.5	465/303	Delphinidin-3-glucoside	8	28.6	449/317	Petunidin-3-arabinoside
3	22.7	435/303	Delphinidin-3-arabinoside	9	29.9	493/331	Malvidin-3-galactoside
4	24.5	449/287	Cyanidin-3-galactoside	10	30.1	493/331	Malvidin-3-glucoside
5	25.2	449/287	Cyanidin-3-glucoside	11	31.8	443/301	Peonidin-3-arabinoside
6	26.4	463/301	Peonidin-3-galactoside	12	32.8	463/331	Malvidin-3-arainoside

Table 2.3 (con't)

			V. uliginosum **- Point Hope (VU-PH)**				
1	20.2	465/303	Delphinidin-3-galactoside	7	28.1	479/317	Petunidin-3-glucoside
2	22.1	465/303	Delphinidin-3-glucoside	8	29.4	449/317	Petunidin-3-arabinoside
3	23.5	435/303	Delphinidin-3-arabinoside	9	30.6	493/331	Malvidin-3-galactoside
4	24.6	449/287	Cyanidin-3-galactoside	10	31.9	493/331	Malvidin-3-glucoside
5	25.4	449/287	Cyanidin-3-glucoside	11	32.8	443/301	Peonidin-3-arabinoside
6	26.7	463/301	Peonidin-3-galactoside	12	33.9	463/331	Malvidin-3-arainoside
			E. nigrum **- Akutan (EN-AK)**				
1	19.3	465/303	Delphinidin-3-galactoside	6	26.9	419/287	Cyanidin-3-arabinoside
2	22.8	449/287	Cyanidin-3-galactoside	7	28.7	463/301	Peonidin-3-galactoside
3	24.1	435/303	Delphinidin-3-arabinoside	8	30.2	493/331	Malvidin-3-galactoside
4	25.2	449/287	Cyanidin-3-glucoside	9	31.9	493/331	Malvidin-3-glucoside
5	25.9	479/317	Petunidin-3-galactoside	10	33.3	463/331	Malvidin-3-arabinoside
			E. nigrum **- Point Hope (EN-PH)**				
1	19.7	465/303	Delphinidin-3-galactoside	6	27.2	419/287	Cyanidin-3-arabinoside
2	22.9	449/287	Cyanidin-3-galactoside	7	28.7	463/301	Peonidin-3-galactoside
3	24.3	435/303	Delphinidin-3-arabinoside	8	30.4	493/331	Malvidin-3-galactoside
4	25.3	449/287	Cyanidin-3-glucoside	9	31.9	493/331	Malvidin-3-glucoside
5	26.1	479/317	Petunidin-3-galactoside	10	33.3	463/331	Malvidin-3-arabinoside
			E. nigrum **- Seldovia (EN-SD)**				
1	19.4	465/303	Delphinidin-3-galactoside	6	26.8	419/287	Cyanidin-3-arabinoside
1'	20.2	465/303	Delphinidin-3-galactoside	7	27.9	449/317	Petunidin-3-glucoside
2	22.2	435/303	Delphinidin-3-arabinoside	8	29.6	463/301	Peonidin-3-galactoside
3	23.1	449/287	Cyanidin-3-galactoside	9	31.0	493/331	Malvidin-3-galactoside
3'	23.7	419/287	Cyanidin-3-galactoside	10	32.3	493/331	Malvidin-3-glucoside
4	24.9	449/287	Cyanidin-3-glucoside	11	32.9	433/301	Peonidin-3-arabinoside
5	25.9	479/317	Petunidin-3-galactoside	12	34.1	463/331	Malvidin-3-arabinoside
			R. chamaemorus **- Point Hope (RC-PH)**				
1	24.8	449/287	Cyanidin-3-glucoside	3	28.0	449/317	Petunidin-3-glucoside
2	26.7	419/287	Cyanidin-3-arabinoside	4	30.3	493/331	Malvidin-3-galactoside
			R. spectabilis **- Akutan (RS-AK)**				
1	24.9	449/287	Cyanidin-3-glucoside	3	33.8	463/331	Malvidin-3-arabinoside
2	27.5	419/287	Cyanidin-3-arabinoside				
			R. spectabilis **- Seldovia (RS-SD)**				
1	26.5	449/287	Cyanidin-3-glucoside	2	28.8	419/287	Cyanidin-3-arabinoside

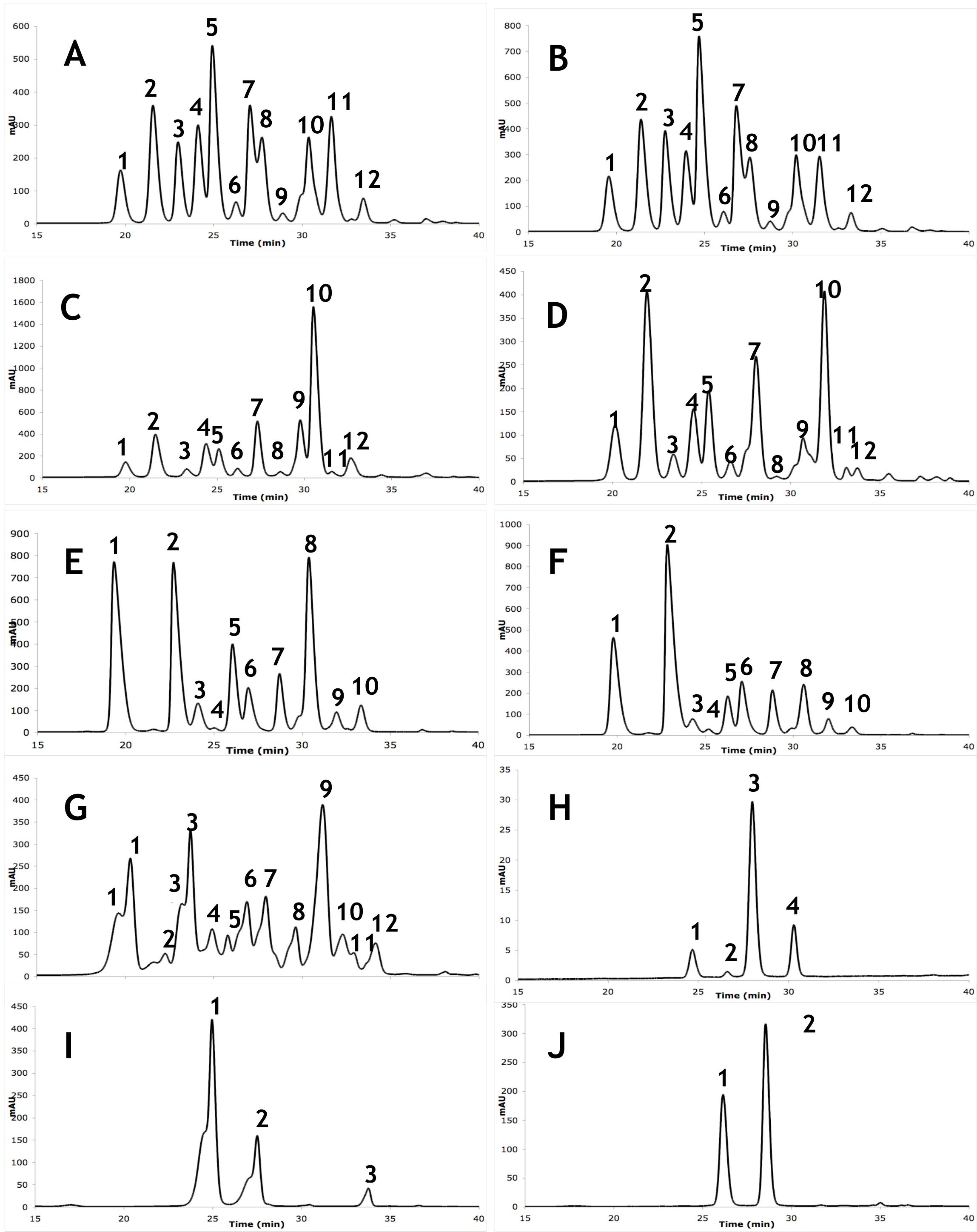

Figure 2.2: HPLC Chromatographs of Phenolic-rich Extracts (PRE) of 10 Wild Alaskan Berry Samples. Chromatograms: (A)-*V. ovalifolium* - Akutan (VO-AK), (B)-*V. ovalifolium* – Seldovia (VO-SD), (C)-*V. uliginosum* – Akutan (VU-AK), (D)-*V. uliginosum* – Point Hope (VU-PH), (E)-*E. nigrum* – Akutan (EN-AK), (F)-*E. nigrum* – Point Hope (EN-PH), (G)-*E. nigrum* – Seldovia (EN-SD), (H)-*R. chamaemorus* – Point Hope (RC-PH), (I)-*R. spectabilis* – Akutan (RS-AK), (J)-*R. spectabilis* – Seldovia (RS-SD).

Proanthocyanidins. HPLC chromatograms gave an estimate of the total proanthocyanidin (PAC) content, calculated as (epi)-catechin equivalents from the total peak area measured at 280 nm (**Table 2.2**). Concentrations ranged greatly, between 0.49 and 6.25 mg/g FW, with Point Hope's *V. uliginosum* containing the greatest concentration overall.

The most common flavan-3-ol subunits of PACs are (epi)afzelechin, (epi)catechin, and (epi)gallocatechin ('epi' refers to the epimer of the flavanoid). B-type PACs contain a single linkage, either C4 → C8' or C4 → C6'. A-types possess an additional linkage between monomers, an ether bridge from C2 → O → C7' (Prior and Gu, 2005). A-type proanthocyanidins are rarer than B-types in plants consumed as food (Gu et al., 2004; Prior and Gu, 2005), and can vary in degree of polymerization (DP), with multiple DP possible within a single fruit (Schmidt et al., 2004).

LC-MS analysis revealed a series of PAC oligomers and polymers ranging between dimers and hexamers (**Figure 2.3**). Preliminary indication of A-type PACs arose from MS spectra through the visualization of the $[M+H]^+$ ions, which are 2 Da smaller than the corresponding B-type PAC. All berry species in this study were found to possess A-type PACs. For further structural information and characterization of A-type PAC dimers through tetramers, tandem ESI-MS^2 was used to fragment the parent ions. Fragments were identified through neutral mass losses resulting from quinone methide (QM) cleavage, retro-Diels-Alder (RDA) fission, heterocyclic ring fission (HRF), benzofuran-forming (BFF) fission, and water loss (**Table 2.4**). Based upon the identity of these structural fragments and the daughter ions resulting from the fission events, a unique PAC assignment can be made, which includes the A-linkage placements in the molecule (Li and Deinzer, 2008).

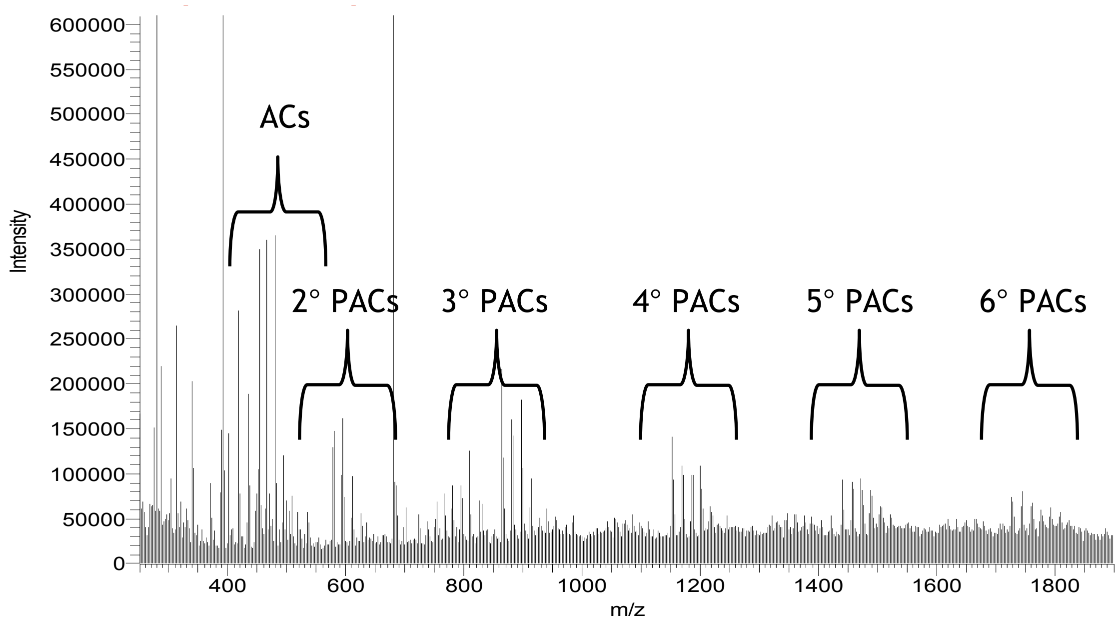

Figure 2.3: Representative LC-MS Spectrum from Phenolic-rich Extract (PRE) of *V. uliginosum* – Akutan (VU-AK) Showing ACs and PAC Dimers through Hexamers.

Monomer	Retro-Diels Alder RDA Da	Heterocyclic Ring Fission HRF Da	Benzofuran-forming Fission BFF Da
(epi)afzelechin	-136	-126	-106
(epi)catechin	-152	-126	-122
(epi)gallocatechin	-168	-126	-156

Table 2.4: Neutral Mass Losses in MS-MS Fission Events (adapted from Li and Deinzer, 2008)

Compounds **1-3** represent A-type trimers (**Figure 2.4**). The degree of A-linkage can be determined from the difference in parent ion mass compared to a B-type trimer; **1** (m/z 863) is 4 Da less than the corresponding B-type trimer and thus has 2 A-linkages, while **2** and **3** (m/z 865) are only 2 Da smaller and thus have only one A-link. The MS^2 spectrum of **1** yields a fragment peak at m/z 711, representing an RDA fission event of the upper monomer and identifying it as (epi)catechin. The peak at m/z 573 (863 Da – 290 Da) results from the loss of a terminal (epi)catechin unit via QM loss.

Difference in masses reveals the central unit to be (epi)catechin as well, giving an identification for compound **1** as (epi)catechin-A-(epi)catechin-A-(epi)catechin (with "-A-" representing an A-link between monomers). Compounds **2** and **3** are very similar in their degradation patterns. Both contain peaks at m/z 713, representing an RDA fission of the upper unit and identifying it as (epi)catechin. Structure **2** undergoes QM fission of the terminal unit to yield a peak at m/z 575, which is indicative of an A-type dimer (865 Da – 290 Da), whereas the QM fission of 3 yields a peak at m/z 577, a B-type dimer (865 Da – 288 Da). Thus, the A-linkage in each trimer is reversed; compound **2** is assigned the structure (epi)catechin-A-(epi)catechin-(epi)catechin while compound **3** is designated as (epi)catechin-(epi)catechin-A-(epi)catechin.

Compounds **4**-**6** (**Figure 2.4**) are oligomeric or polymeric trimer A-type proanthocyanidins containing (epi)gallocatechin. All three weigh 2 Da less than the corresponding B-type trimer, thus all three contain a single A-type linkage. An RDA fission event of the upper unit of compound **4** (m/z 881) yields the peak m/z 729 and identifies the upper unit as (epi)catechin. The fragment ion at m/z 577 represents an B-type dimer (881 Da – 304 Da) formed through the QM loss of (epi)gallocatechin. The corresponding peak at m/z 591 (881 Da – 290 Da) identifies the A-type dimer ion after a QM fission of (epi)catechin. Thus, the final assignment of compound **4** is (epi)catechin-(epi)catechin-A-(epi)gallocatechin. Structure **5** (m/z 897) undergoes two RDA fission events; the terminal unit yields the peak at m/z 745 and identifies the terminal unit as (epi)catechin, while the upper unit yields the peak at m/z 729, identifying the upper unit as (epi)gallocatechin. The peak at m/z 593 is an A-type unit (897 Da – 304 Da) formed from the QM fission of an (epi)gallocatechin unit. Thus, the final characterization of compound **5** is (epi)gallocatechin-(epi)gallocatechin-A-(epi)catechin. Compound **6** fragments via an RDA fission to form the daughter ion m/z

745, yielding an (epi)gallocatechin terminal unit. The peak at m/z 609 (913 Da – 304 Da) is an A-type dimer formed from a QM fission of the terminal unit. The upper unit of this dimer, also (epi)gallocatechin, undergoes another RDA fission, yielding the peak at m/z 441, and thus the final assignment of compound **6** is (epi)gallocatechin-A-(epi)gallocatechin-(epi)gallocatechin.

Compounds **7** and **8** are A-type tetramer proanthocyanidins comprised of all (epi)catechin units. Structure **7** (m/z 1151) is 4 Da less than the corresponding B-type tetramer, indicating the presence of 2 A-linkages. The peak at m/z 863 (1151 Da – 288 Da) is an A-type trimer containing 2 A-links formed from the QM fission of the upper unit ((epi)catechin), while m/z 861 (1151 Da – 290 Da) is an A-type trimer containing a single A-link formed from the QM fission of the terminal unit, identified as (epi)catechin. The trimer at m/z 863 immediately undergoes a second QM fission, yielding the peak m/z 573, which represents an A-type dimer (863 Da – 290 Da). The central unit must be (epi)catechin by mass difference, and the final assignment of structure **7** is (epi)catechin-(epi)catechin-A-(epi)catechin-A-(epi)catechin. Compound **8** is only 2 Da smaller than a B-type tetramer proanthocyanidin, and thus has a single A-link. The fragment ion at m/z 863 (1153 Da – 290 Da) represents a QM fission of the terminal unit, identifying it as (epi)catechin. The upper unit undergoes QM fission (1153 Da – 288 Da), yielding an A-type trimer at m/z 865. The trimer further fragments to give peak m/z 579 (1153 Da – 574 Da), a B-type dimer, indicating the A-link lies between the 2^{nd} and 3^{rd} units. Final assignment of compound **8** is given as (epi)catechin-(epi)catechin-A-(epi)catechin-(epi)catechin.

Compounds **9** and **10** are oligomeric A-type tetramer proanthocyanidins consisting of both (epi)catechin and (epi)gallocatechin monomers. Compound **9** is 2 Da smaller than the corresponding B-type tetramer, having a single A-type link. The upper unit undergoes QM fission, yielding the A-type trimer m/z 879 (1169 Da – 290 Da), identifying it as (epi)catechin. This trimer then fragments again, giving a B-type (epi)catechin-(epi)catechin dimer, m/z 579 (1169 Da – 590 Da), demonstrating the 2^{nd} as (epi)gallocatechin by mass difference and yielding the final structure of compound **9** as (epi)catechin-(epi)gallocatechin-A-(epi)catechin-(epi)catechin. Structure **10** is 4 Da less than it's corresponding B-type proanthocyanidin, and thus contains 2 A-links. The parent ion undergoes two RDA fission events, yielding peaks at m/z 1031 and 1015 and identifying the upper and terminal units as (epi)catechin and (epi)gallocatechin, respectively. The fragment ion with m/z 895 (1183 Da – 288 Da) arises from the QM fission of the upper (epi)catechin, yielding a trimer with 2 A-links. This trimer undergoes a second QM fission at m/z 605 (1183 Da – 578 Da), identifying the second unit as an A-type (epi)catechin, and leaving an A-type (epi)gallocatechin dimer. The final structure of compound **10** is given as (epi)catechin-(epi)catechin-A-(epi)gallocatechin-A-(epi)gallocatechin.

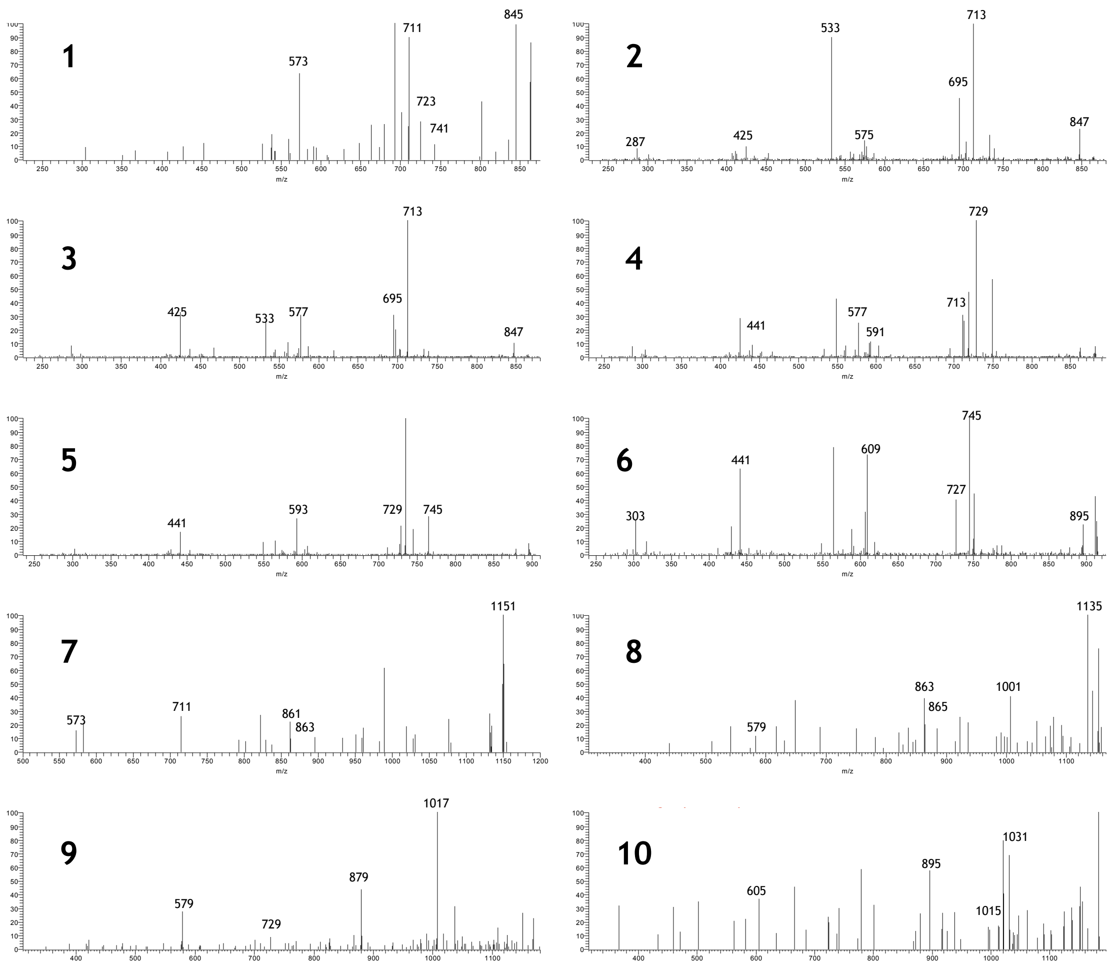

Figure 2.4: LC-MS^2 spectra of A-type proanthocyanidin trimers and tetramers from proanthocyanidin-rich fraction (PAC) of wild Alaskan berries. **1** – m/z 863, **2** – m/z 865, **3** – m/z 865, **4** – m/z 881, **5** – m/z 897, **6** – m/z 913, **7** – m/z 1151, **8** – m/z 1153, **9** – m/z 1169, **10** – m/z 1183.

Table 2.5 shows the results of the tandem ESI-MS^2 analysis. Every berry studied contained multiple proanthocyanidin structures, most possessing A-type proanthocyanidins of varying DP. All berries save Seldovia's *R. spectabilis* contained A-type dimers, and Point Hope's *R. chamaemorus* was lacking in trimer A-type proanthocyanidins. There was considerable variation between samples from different locations, and several A-type proanthocyanidin trimers (compounds **1**,**2**, and **5**) were found to be species-specific.

Proanthocyanidin	m/z	VO AK	VO SD	EN AK	EN PH	EN SD	VU AK	VU PH	RS AK	RS SD	RC PH
Dimers											
(e)C-A-(e)C	577,425,287		•	•	•	•	•	•	•		
(e)C-A-(e)Gc	593,303,287	•	•	•	•	•	•				•
(e)Gc-A-(e)Gc	609,303,287	•	•				•				•
Trimers											
(e)C-A-(e)C-A-(e)C	863,573								•	•	
(e)C-(e)C-A-(e)C	865,577			•	•	•	•	•	•	•	
(e)C-A-(e)C-(e)C	865, 575	•	•								
(e)C-(e)C-A-(e)Gc	881,729,577		•	•	•	•		•			
(e)Gc-(e)Gc-A-(e)C	897,593,441			•	•	•					
(e)Gc-A-(e)Gc-(e)Gc	913,609			•							
Tetramers											
(e)C-(e)C-A-(e)C-A-(e)C	1151,863, 573	•									
(e)C-(e)C-A-(e)C-(e)C	1153,865, 575		•						•		
(e)C-(e)Gc-A-(e)C-(e)C	1169,881, 729,577				•			•			
(e)C-(e)C-A-(e)Gc-A-(e)Gc	1183,1021, 895,605									•	

[1]Abbreviations: (e)C=(epi)catechin, (e)Gc=(epi)gallocatechin, -A- =A-linkage, AK=Akutan, SD=Seldovia, PH=Point Hope, VO=*V. ovalifolium*, VU=*V. uliginosum*, EN=*E. nigrum*, RS=*R. spectabilis*, RC=*R. chamaemorus*.

Table 2.5: A-type Proanthocyanidins Polymers in Proanthocyanidin-rich (PAC) Fraction of Alaskan Berries

HPLC analysis of *V. ovalifolium* revealed proanthocyanidin concentrations of 1.73 mg/g FW (VO-SD) and 2.42 mg/g FW (VO-AK) (**Table 2.2**). Both samples of *V. ovalifolium* contained A-type dimers (epi)catechin-A-(epi)gallocatechin and (epi)gallocatechin-A-(epi)gallocatechin, and VO-SD contained the additional dimer (epi)catechin-A-(epi)catechin. Compound **2** ((epi)catechin-A-(epi)catechin-(epi)catechin) was found exclusively in VO-AK and VO-SD, and VO-SD was found to possess compound **4** as well. The two *V. ovalifolium* samples contained A-type tetramers, albeit different structures; VO-AK was the only sample to incorporate compound **7**, whereas VO-SD contained compound **8**.

The other *Vaccinium* species, *V. uliginosum*, contained some of the highest levels of proanthocyanidins. VU-AK was found to contain 2.97 mg/g FW of proanthocyanidins, while VU-PH contained 6.25 mg/g FW; the highest level measured from all samples (**Table 2.2**). The two *V. uliginosum* samples also differed in their A-type dimer composition. VU-AK contained all three A-type dimers, but VU-PH only possessed (epi)catechin-A-(epi)catechin. Both VU-AK and VU-PH contained compound **3**, and VU-PH also contained compound **4**. The presence of compound **3** in VU-PH was previously reported in a study by Määttä-Riihinen et al. (2005) (Määttä-Riihinen et al., 2005). Only Point Hope's *V. uliginosum* sample contained an A-type tetramer, compound **9**.

The *E. nigrum* samples differed considerably in their proanthocyanidin content. The samples from Seldovia and Point Hope contained fairly large amounts of proanthocyanidins (2.86 mg/g FW and 3.74 mg/g FW, respectively), while EN-AK contained only 0.75 mg/g FW (**Table 2.2**). All three contained the same two A-type dimers, (epi)catechin-A-(epi)catechin and (epi)catechin-A-(epi)gallocatechin. They also possessed three of the same trimers: compounds **3**, **4**, and **5**. Compound **5** ((epi)gallocatechin-(epi)gallocatechin-A-(epi)catechin) was unique to *E. nigrum*, not found in any other samples. Only EN-PH contained an A-type tetramer, compound **9**.

R. spectabilis contained the lowest levels of proanthocyanidins of all berries sampled, possessing 0.49 and 0.74 mg/g FW for RS-AK and RS-SD, respectively. RS-AK contained a single A-type dimer, (epi)catechin-A-(epi)catechin, while RS-SD was found to contain no A-type proanthocyanidin dimers. RS-AK and RS-SD both contained an A-type trimer with two A-links, compound **1**, as well as compound **3**. The presence of **1** was unique to the *R. spectabilis* samples, not seen in any other berry. Both samples contained A-type tetramers, albeit different structures; RS-AK having compound **8** while RS-SD contained a double A-linked tetramer, **10**.

Point Hope's *R. chamaemorus* contained modest amounts of proanthocyanidins, with HPLC analyses indicating 1.26 mg/g FW. Two A-type dimers were identified; (epi)catechin-A-(epi)gallocatechin and (epi)gallocatechin-A-(epi)gallocatechin. While B-type dimers have previously been described in this species (Puupponen-Pimiä et al., 2005), this marks the first time that A-type dimers have been identified in *R. chamaemorus*.

2.4.3 *In Vitro* Assays

Preadipocyte *pref-1* Analysis. *Pref-1* is an inhibitory protein which is responsible for preventing the maturation of adipocyte cells. The ability of an extract to increase or maintain high *pref-1* levels is a marker of potential anti-obesity activity. The phenolic-rich extracts of Alaskan berries were assessed as to their capacity to increase the expression of *pref-1*. Total *pref-1* expression was obtained via chemiluminescent Western blot imaging (**Figure 2.5**), and calculated as the ratio between *pref-1* levels and β-actin levels, compared against control. **Figure 2.6** shows the effect of PRE extracts on *pref-1* expression levels in immature 3T3-L1 adipocytes. Of the ten Alaskan berry samples, most demonstrated no substantial increase in expression levels; 4 samples possessed moderate increases of *pref-1*, and of those only two were found to significantly increase the amount of *pref-1* in immature adipocytes (**Figure 2.6**). RS-AK and RS-SD heightened expression levels 82% and 69%, respectively.

Figure 2.5: Western Blot Analysis of Several Representative Wild Alaskan Phenolic-rich extracts (PRE). (a) – *pref-1* expression levels, (b)- β-actin expression levels. Overall *pref-1* expression is calculated as the ratio between the two for each sample, compared against control. Abbreviations: AK=Akutan, SD=Seldovia, VO=*V. ovalifolium*, EN=*E. nigrum*, RS=*R. spectabilis*.

In addition to the PRE extracts, the anthocyanin-rich (ARF) and proanthocyanidin-rich (PAC) fractions of the two active samples (RS-AK and RS-SD) were evaluated in order to further investigate which group of berry phytochemicals affect the activity of the adipogenesis inhibitor *pref-1* (**Figure 2.7**). The two enriched fractions did not significantly increase *pref-1* expression compared to the control, while the PRE from each continued to demonstrate consistently high levels of *pref-1* expression.

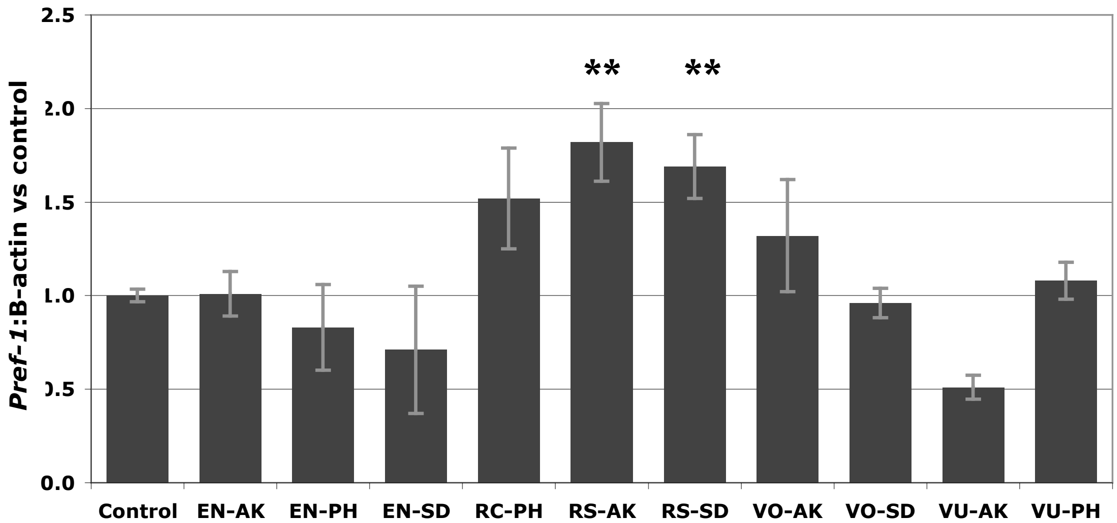

Figure 2.6: Effect of Wild Alaska Berry Phenolic-rich Extracts (PRE) on *pref-1* Expression Levels of Immature 3T3-L1 Adipocytes. Run in triplicate, figure represents mean response ± SE. Asterisks denote significant activity, ** $p \leq 0.05$. Abbreviations: PRE = phenolic-rich extract, ARF = anthocyanin-rich fraction, PAC = proanthocyanidin-rich fraction, AK=Akutan, SD=Seldovia, PH=Point Hope, VO=*V. ovalifolium*, VU=*V. uliginosum*, EN=*E. nigrum*, RS=*R. spectabilis*, RC=*R. chamaemorus*.

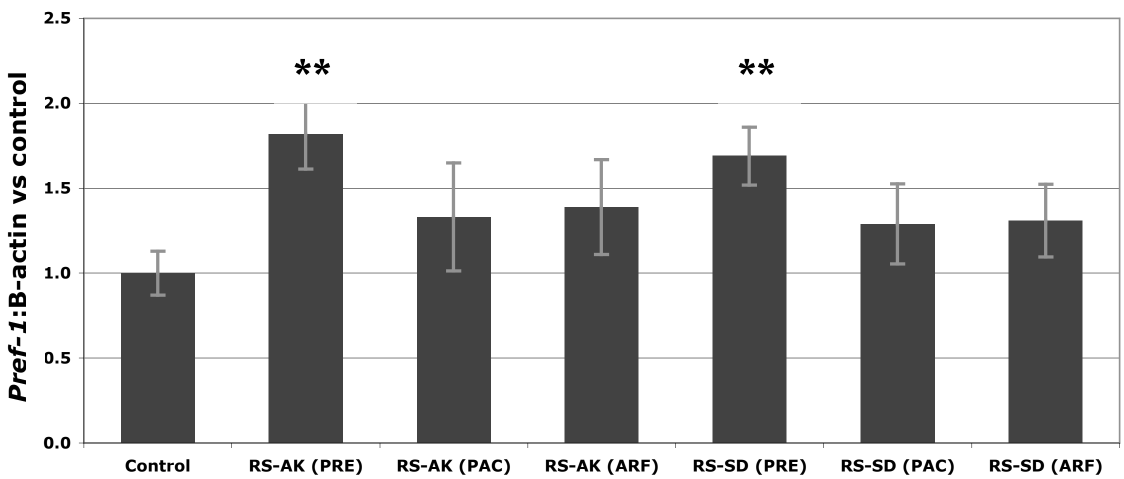

Figure 2.7: Effects of RS-AK and RS-SD Fractions on *pref-1* Expression Levels in Immature 3T3-L1 Adipocytes. Run in triplicate, figure represents mean response ± SE. Asterisks denote significant activity, ** $p \leq 0.05$. Abbreviations: PRE = phenolic-rich extract, ARF = anthocyanin-rich fraction, PAC = proanthocyanidin-rich fraction, AK=Akutan, SD=Seldovia, RS=*R. spectabilis*.

Oil Red O Assay. Phenolic-rich extract (PRE) of the ten wild Alaskan berry samples were assayed as to their ability to attenuate lipid accumulation in mature 3T3-L1 adipocytes (**Figure 2.8**). Accumulation levels ranged from 80.43% to 100.7% of the control. Four of the ten samples exhibited significant inhibition of aggregation of intracellular lipids; EN-AK (80.43% of control lipid levels), EN-SD (82.67%), VU-PH (89.85%), and VO-SD (92.33%).

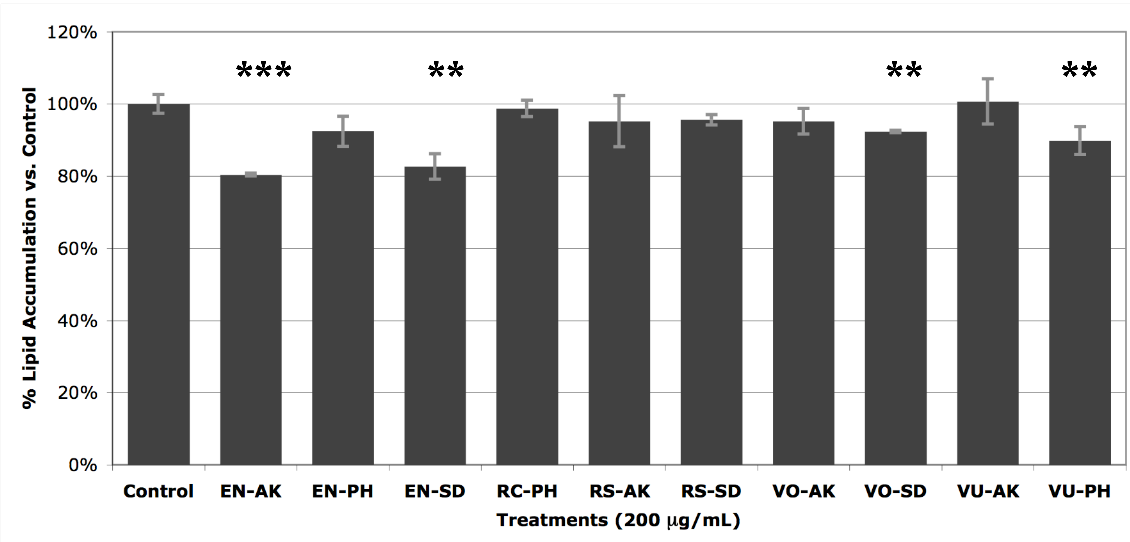

Figure 2.8: Effect of Phenolic-Rich Extracts (PRE) of Alaskan Berries on Lipid Accumulation in Mature 3T3-L1 Adipocytes. Results from Red Oil O Assay given as percent lipid accumulation versus control. Run in triplicate, figure represents mean response ± SE. Asterisks denote significant activity, ** $p \leq 0.05$, *** $p \leq 0.01$. Abbreviations: PRE = phenolic-rich extract, ARF = anthocyanin-rich fraction, PAC = proanthocyanidin-rich fraction, AK=Akutan, SD=Seldovia, PH=Point Hope, VO=*V. ovalifolium*, VU=*V. uliginosum*, EN=*E. nigrum*, RS=*R. spectabilis*, RC=*R. chamaemorus*.

The enriched fractions (ARF and PAC) of the four active berry samples were also investigated for their effects on lipid accumulation (**Figure 2.9**). While most of the PRE extracts were active, only one of the ARF fractions (EN-SD) significantly reduced lipid levels in the cells, while all four PAC fractions significantly inhibited the aggregation of cellular lipids. As the PAC fractions possessed the highest activity, the inhibition levels of the active PAC fractions were compared on the basis of their respective proanthocyanidin content, revealing a linear response (**Figure 2.10**). The proanthocyanidin content (as mg/g fraction) was positively correlated with lipid accumulation inhibition, with a correlation coefficient (R^2) of 0.896.

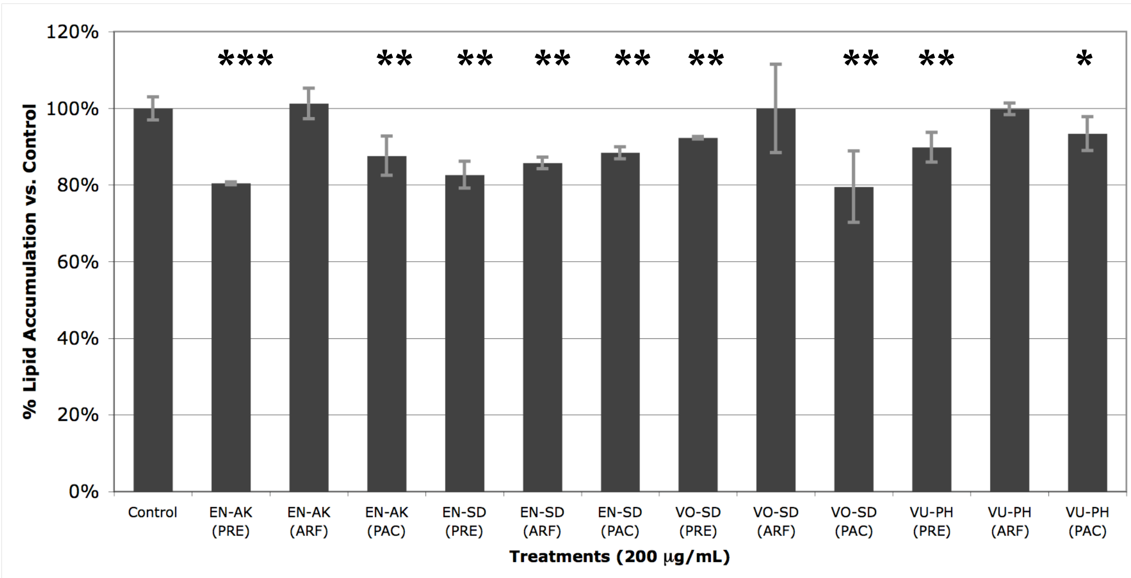

Figure 2.9: Effect of Enriched Fractions from Alaskan Berries on Lipid Accumulation in Mature 3T3-L1 Adipocytes. Results from Red Oil O Assay given as percent lipid accumulation versus control. Run in triplicate, figure represents mean response ± SE. Asterisks denote significant activity, * $p \leq 0.1$, ** $p \leq 0.05$, *** $p \leq 0.01$. Abbreviations: PRE = phenolic-rich extract, ARF = anthocyanin-rich fraction, PAC = proanthocyanidin-rich fraction, AK=Akutan, SD=Seldovia, PH=Point Hope, VO=*V. ovalifolium*, VU=*V. uliginosum*, EN=*E. nigrum*.

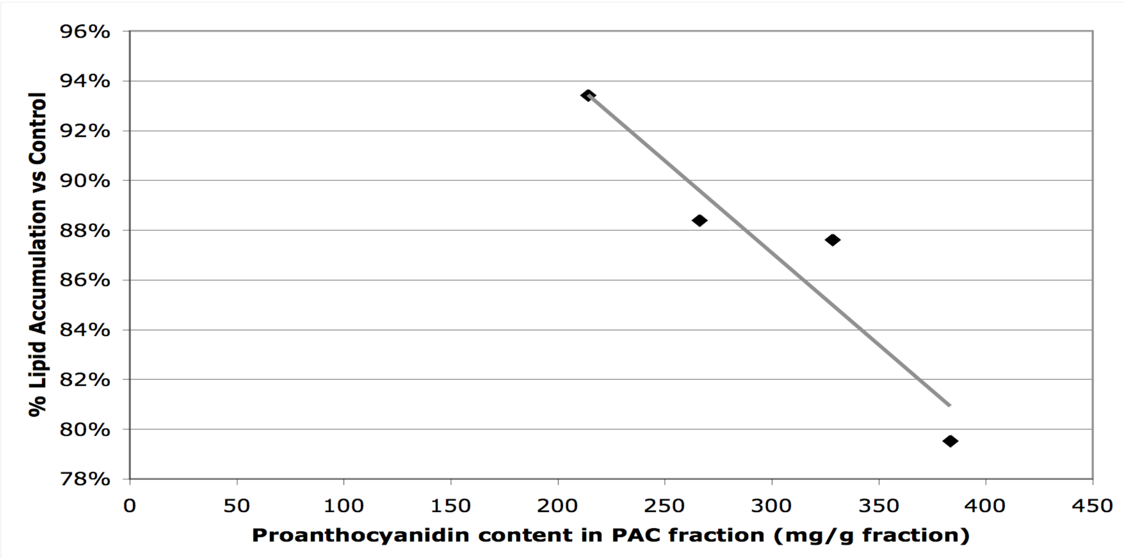

Figure 2.10: Response of Proanthocyanidin-rich fractions (PACs) on Lipid Accumulation in Red Oil O Assay Correlated with Proanthocyanidin Content (in mg/g fraction). Linear correlation coefficient R^2=0.896.

2.4.4 *In vivo* Assay

The PRE and ARF extracts of VU-PH and EN-PH were tested for potential hypoglycemic activity using an acute model of T2DM. Insulin resistance was induced after maintaining the mice on the VHF diet for 14 weeks. The berry extracts were administered to the mice orally following a 4 hr food restriction. Both the ARF and PRE preparations from VU or EN effectively lowered blood glucose levels in the hyperglycemic C57Bl/6J mice (**Figure 2.11**). The PRE extract exhibited greater hypoglycemic activity (45% decrease in blood glucose level) relative to the ARF extract from *V. uliginosum* (23% decrease in blood glucose level). The VU-PH PRE extract showed greater absolute hypoglycemic activity than the drug metformin (24% decrease in blood glucose level), which was included as a positive control. The animals treated with the water vehicle only showed a slight increase in blood glucose concentrations.

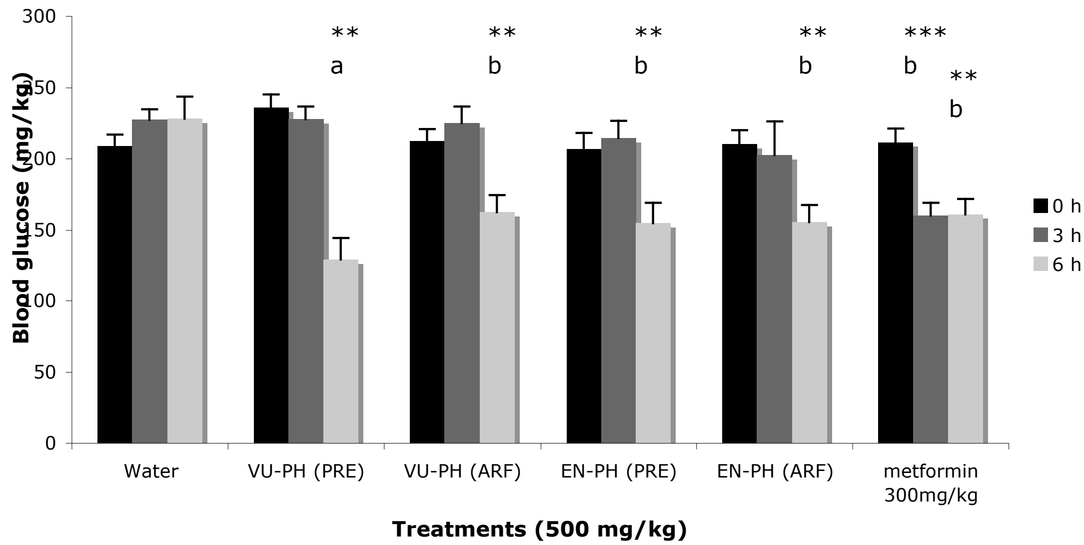

Figure 2.11: Glucose levels in dietary-induced obese C57BL/6 mice at 3 and 6 h post-treatment. The experiment was repeated with similar results, figure represents mean response ± SE. Abbreviations: VU-PH=*Vaccinium uliginosum* from Point Hope, EN-PH= *Empetrum nigrum* from Point Hope, PRE=phenolic-rich extract, ARF=anthocyanin-rich fraction. Asterisks denote significant activity, ** $p \leq 0.01$ vs initial, *** $p \leq 0.001$ vs initial; letters designate significantly different treatment means at α=0.01 (n=5).

2.5 Discussion

Wild Alaskan berry species continue to play a strong role in traditional tribal culture and diet, recognized by AN tribes as a contributing factor towards a healthy lifestyle. Not only are tribal members aware of the health contribution of the berries within the diet, but berry harvesting also provides a constructive means of exercise, which has been shown to help lower incidence rates of T2DM (Laaksonen et al., 2005). The positive results from the STN assays conducted in the tribal communities gave first-hand scientific evidence as to the health properties of the berries, thereby providing a validation of the traditional perceptions of these fruits. They also generated additional interest in the berries as a part of tradition and source of nutrition, especially with the younger members participating in the berry screening; one high school student commented that “learning if our berries were healthy” was the most interesting part of the STN project.

Wild Alaskan berries contain a complex mixture of bioactive flavonoids, including anthocyanins and proanthocyanidins. The presence of such chemicals may play an important role in health promotion and metabolic disease prevention, including T2DM and obesity. The anthocyanin profile of the berries revealed a varied number of potentially beneficial anthocyanin compounds. All berries tested were found to possess A-type proanthocyanidin dimers, trimers, and/or tetramers. However, the phytochemical content and characterization was different for each berry, dependant on both species and biogeographic location. The berries from Point Hope yielded the highest concentrations of anthocyanins (EN-PH) and proanthocyanidins (VU-PH); concentrations larger than other circumpolar berries (Määttä-Riihinen et al., 2004; Ogawa et al., 2008). Point Hope is the site with the most extreme annual climate, and thus the severity of the environment could be a contributing factor to the overall composition of these particular fruits. However, additional years of collection are needed to analyze year-to-year variation in these adaptive chemicals and better correlate these values with climate metrics such as precipitation levels and type, temperature, and sunlight availability.

Adipocyte differentiation is controlled by multiple feedbacks (Fernyhough et al., 2005; Nakae et al., 2003; Ntambi and Kim, 2000), one of which is the negative control exerted by preadipocyte factor-1 (*pref-1*) (Smas and Sul, 1993). Levels of *pref-1* remain high in immature adipocyte cells, decreasing to nearly zero as differentiation begins. Mice with decreased levels of *pref-1* demonstrated increased adipose tissue formation (Moon et al., 2002). While numerous studies have demonstrated that various proteins and small organic compounds actively inhibit adipogenesis, only one study to date (on grape-seed procyanidins with varying degrees of polymerization) has investigated the effect of flavonoids on *pref-1* expression levels (Pinent et al., 2005b). In the current study, the mixture of phytochemical compounds in the phenolic-rich extract of *R. spectabilis* increased *pref-1* expression. Activity was lost when the extract was fractionated and component phytochemicals were administered separately. This result suggests that interactions between phytochemicals in the mixed PRE extract are required to potentiate biological activity. Both *Rubus* berries, which possessed the highest *pref-1* activity, contained the lowest levels of anthocyanins and proanthocyanidins. This could indicate that minor compounds not quantified in this study are responsible for *pref-1* activity. For example, *Rubus* fruits are known to have incredibly high levels of ellagic acid conjugates such as ellagitannins (Mullen et al., 2003; Vasco et al., 2009).

Lipid accumulation is one mechanism of energy balance and regulation within adipocytes (Fernyhough et al., 2005). Excess caloric intake can lead to a metabolic overload of adipocyte tissue, forcing adipocytes to enlarge to accommodate the larger influx of triglycerides. Prolonged enlargement and hypertrophy develops within the adipocytes, initiating a chronic inflammatory condition. This adipocyte dysfunction results in a disruption of the dynamic equilibrium of triglyceride metabolism within the cells, thereby causing obesity and insulin resistance (Guilherme et al., 2008). Multiple phenolic-rich extracts of Alaskan berries were able to decrease the lipid levels within mature adipocytes, but this activity was not conserved across all locations or species. Proanthocyanidin-enriched fractions were the preparations that most actively decreased lipid accumulation. The effect was reinforced by the linearity of the response between proanthocyanidin content of the enriched fractions and the lipid accumulation observed in the treated cells. Proanthocyanidins, especially procyanidins, from apples and grape seed have demonstrated similar activity against tissue lipid absorption in both cellular (Del Bas et al., 2009; Pinent et al., 2005a; Sugiyama et al., 2007) and organismal (Tebib et al., 1994; Zern et al., 2003) models.

In addition, both *V. uliginosum* and *E. nigrum* preparations from Point Hope (VU-PH and EN-PH) exhibited hypoglycemic activity in the acute *in vivo* T2DM model. These two samples were chosen as they represented the highest levels of the two most intensively investigated phenols: anthocyanins (EN-PH) and proanthocyanidins (VU-PH). Both samples possessed activity comparable to metformin, the anti-diabetic drug used as the positive control for the study. Both the PRE and ARF extracts of these hypoglycemic berries showed similar activities despite the dramatic differences in their phytochemical content. The robust hypoglycemic activity observed from treatments of Alaskan berry preparations was also observed when presented to animals with a vehicle of water. In previous work with comparable lowbush blueberry (*V. angustifolium*) extracts, hypoglycemic activity was pronounced only when provided in a formulation containing Labrasol®, an emulsifing agent that enhances absorption in the gastrointestinal tract (Grace et al., 2009).

The current study investigated the diverse phytochemical composition of wild Alaskan berries, with a focus on the identification of anthocyanin and proanthocyanidin compounds. The berry extracts demonstrated the ability to influence specific cellular targets related to metabolic disorders, with clear differences between the various fractions used in each of the assays. The two berries studied in the acute *in vivo* T2DM model exhibited significant hypoglycemic activity. This evidence exhibiting the hypoglycemic activity of wild Alaskan berries is particularly relevant in light of the high incidence of T2DM in Alaska Native populations. More detailed studies, including activity-based fractionation of the extracts, mechanistic studies in animals and investigations into the bioavailability and metabolism of the active compounds will further validate this biological activity.

Local observations support the hypothesis that climate fluctuations affect both berry quantity and quality, and thus the impacts of continued climate change on these berries and their bioactive health properties could be significant. Negative impacts to wild Alaskan berries could be possible if winter precipitation decreases or if summer temperatures substantially increase and affect the sun/precipitation balance. However, changing berry abundance and quality from year to year was considered a longstanding pattern. The Arctic's growing fluctuations in meteorological, hydrological, and biological processes (Hinzman et al., 2005) cast an uncertain pall questioning the continued availability and potency of the berry populations, and their implications for community health.

2.6 Acknowledgements

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3 Simple, predictive field bioassays lead students in the quest for biodiscovery

3.1 Challenges for Science Educators

Teachers have increasingly struggled to sustain students' attention and interest in science courses. Richard Feynman, renown scientist and professor of physics, illustrated these challenges when he said, "It's impossible to learn very much simply by sitting in a lecture, or even by simply doing the problems that are assigned. But in our modern times we have so many students to teach that we have to try and find some substitute for the ideal [teaching situation]" (Feynman et al., 1963). Introductory science courses such as those found in high school and undergraduate curricula are challenged to motivate students for several reasons: they are usually 'required' rather than elective courses, class sizes can be large, and students tend to have negative preconceptions (Kern and Carpenter, 1984) (Lila and Rogers, 1998). Students frequently criticize the impersonal lecture style in these courses, which discourages interaction between the students and professor (Seymour and Hewitt, 1997). Often there is a perceived disconnect between the material being taught in class and the 'real-world,' further isolating students from learning (Bransford et al., 1999). This decline in student attitude has an impact on the retention and application of knowledge transferred in class (Henderleiter and Pringle, 1999), as students are only motivated to spend the necessary time to learn and to analyze problems they find interesting (Bransford et al., 1999). These results are all the more disturbing for university level courses, as the undergraduate years have been found to be a "filter point" in mathematics, science, and engineering classes, a critical time when negative experiences in classes can lead students to alter their career paths (Seymour and Hewitt, 1997).

As an added complication, the rapid advances of science that have occurred in the latter half of the 20^{th} century have resulted in an eruption of interest in interdisciplinary research. Both life and physical sciences are becoming more dependent on each other (NRC 2003), and this intermingling of disciplinary tools is essential to identifying and understanding the mechanisms of the most pressing problems of the day (Jacobson and Robinson, 1990). The marked shift in real world research requirements necessitates a parallel shift in teaching strategy in order to ensure future scientists and members of society are able to participate in a broader, collective scientific discovery process (Godwin and Davis, 2005). While a transdisciplinary research approach is essential to future scientific discovery, individual disciplines have moved in the opposite direction for decades, increasingly emphasizing the value of specialization, and developing very different philosophical outlooks and underlying paradigms (Jacobson and Robinson, 1990). Thus, modern teachers face a plethora of hazards and obstacles in their quest to develop educational programs that reflect the current and future trends of scientific discovery.

While laboratory sessions are usually an integral part of science courses, many curricula tend to take a pedantic approach involving 'cook-book' lists of tasks for students to follow ritualistically. Students are not engaged in thinking about the larger purposes of their investigation and of the sequence of tasks they need to pursue to achieve those ends (Hofstein and Lunetta, 2004). Traditional laboratory experiments provide a great deal of control and reproducibility (Diamond, 1986), but can be reduced to a mechanistic abstraction, in stark contrast with everyday environments where contextual reasoning is often required. By limiting the hands-on education experience only to traditional laboratory exercises, students are given little chance to translate their knowledge into real world situations (Resnick, 1987).

In this manuscript, an innovative "Screens-to-Nature" (STN) system is introduced as a conduit to direct, participatory science instruction, with the added advantage that students are able to make novel, undocumented discoveries with real-world applicability using resources that have cultural significance. The STN system, described below, centers around a central topic: bioexploration and its applications to plant biology, human health, biodiversity conservation, community frameworks and traditional ecological knowledge (TEK).

3.2 <u>The STN System</u>

The "Screens-to-Nature" (STN) system, originally developed through collaborations between Rutgers University, North Carolina State University, and the University of Illinois, is a portfolio of field-deployable assays that allow students to explore the bioactivity, and potential human health ramifications, of natural plant extracts, while mastering basic biological and chemical principles. Currently, a score of individual STN assays have been designed to investigate the pharmaceutically-relevant activity of natural plant chemicals (such as alkaloids, or anthocyanin pigments) for human health protection. Relevant health targets include chronic and infectious disease agents (parasitic worms, fungi, and bacteria), metabolic disorders (diabetes and obesity), and general health maintenance (via the antioxidant potential or anti-inflammatory properties of phytochemical constituents). The STN system engages students in 1) plant identification and field collections, 2) study of traditional, historic natural product use and ethnobotany, 3) vouchering and archiving, 4) computer-based data entry 5) extraction tactics, and 6) screening plant samples using biologically-relevant bioassays based on recognized, diagnostic chemical reactions or responses. All STN bioassays have been lab-validated, and are presented in tandem with a comprehensive field training manual which explains the set up, execution, and significance of each bioassay in the kit.

3.2.1 Illustration of an STN Assay

Roundworm species, including parasitic pinworms, hookworms, and whipworms, are a serious health hazard worldwide. The World Health Organization estimates that 400 million school-age children are infected with worms, which can lead to malnutrition, anemia, or even retarded growth (World Health Organization, 2008). One pertinent bioassay in the STN portfolio uses a (non-parasitic) nematode in a simple model system to gauge roundworm lethality when exposed to a plant's bioactive extracts. The nematode is easy to grow and maintain, and poses no threat to human health, yet still provides a good indicator to screen for natural extracts that would be lethal to parasites, and would therefore provide effective cures for diseases caused by roundworms.

The Roundworm Lethality STN assay process begins in the field, where students identify and collect plants in the wild. Both traditional ecological knowledge (provided by elders in the local community) and/or ethnobotanical reference books can be used to zero in on prospective candidate plant species which might have efficacy in this bioassay. Each plant's location is recorded (using a portable GPS unit) and two small samples are taken: one for extraction and one for positive taxonomic identification and retention as a herbarium specimen. An extract can be prepared from any and all parts of the plant that may have medicinal value, including the leaves, bark, fruit, or inflorescences. Extraction may be done in ways that mimic a traditional method of preparation (e.g. a poultice, tea infusion, or masticant), or pulverized in alcohol, which is a laboratory standard that extracts multiple compounds from the plant and creates a stable extract. Depending on the availability of candidate samples and the time allotted, several different plant extracts can be screened in a single assay run. The assay includes positive and negative controls. Extracts are used within 24-48 hours because the active principles may be sensitive to degradation.

The non-pathogenic model nematode species is cultivated quickly on readily-available media (oatmeal and yeast) and is large enough to observe with a magnifying lens. The screening procedure involves plating a small sample of worms into each well of a 96-well plate, after which the plant extract is added to the worm culture. The worm lethality is then observed after several hours (**Figure 3.1**), ranked on a scale of 0 (worms remain alive after 4 hours treatment with the plant extract) to 3 (all worms are dead in the treatment), and data on the effectiveness of each plant extract is recorded in a computer-based database. Student teams are subsequently provided with potential strategies for further evaluation of plant extract bioactive potential, through laboratory-based bioassays, if warranted.

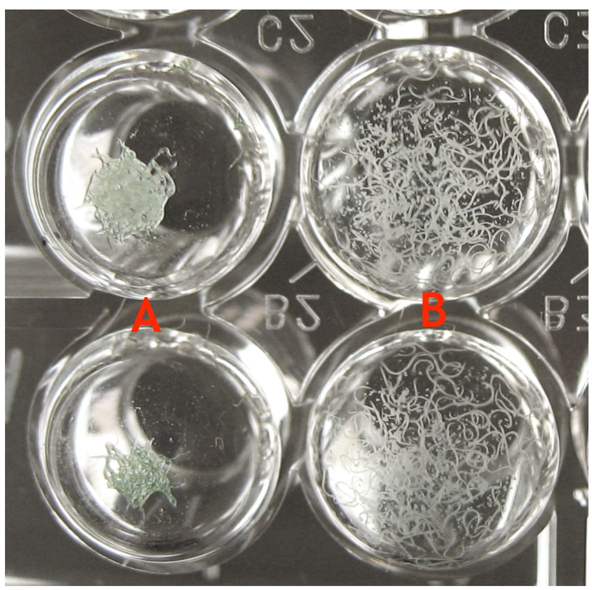

Figure 3.1: Dead (A) and Live (B) Nematode *Panagrellus redivivus* Cultures After Treatment by Plant Extracts in a STN Assay. The Roundworm Lethality STN assay uses *Panagrellus* as a model for roundworm parasites.

Other bioassays in the STN portfolio specifically evaluate the ability of plant extracts to regulate blood sugar levels in diabetic patients (by inhibiting key human enzymes that degrade starches into sugar), to inhibit microbial infections (by inhibiting fungal or bacterial organisms), to bolster immunity (through antioxidant action), or to inhibit viral infections (by breaking down proteins involved in viral replication), for example.

3.2.2 Advantages of the STN System as an Educational Tool

The assays that make up the STN system are designed to be simple and efficient, using a rigorously-tested, guided step-by-step approach to each experiment. In-field work is kept manageable by pre-aliquoting all main reagents to ensure reproducibility and standardization of the results. The tests rely upon visual indicators to qualitatively determine the bioactive potency (or, alternatively, the inactivity) of each extract. For example, viability, after exposure to a plant extract, of a model organism like a nematode is gauged by visually evaluating movement and appearance under magnification; in other cases, or colorimetric chemical reactions in other bioassays mark the efficiency of the plant components to inhibit critical enzymes or disease pathogens. These design elements ensure that a broad spectrum of students can be engaged in the laboratory exercise, even when they lack previous laboratory experience. The bioassays are functional on a miniature scale, requiring as little as 2 grams of material for analysis and utilizing multi-well plates to increase efficiency, minimize costs, and allow multiple samples to be evaluated in a reasonable time frame.

The materials required for the extraction of plants and the set up and implementation of assays are generally inexpensive and readily-available, such as the oatmeal and yeast used as growing media for worms. Solvents used for bioassays are non-toxic, affordable, and easily accessible on a global scale. Students are engaged in a hands-on discovery process from the beginning to the conclusion of each STN experiment, actively collecting, extracting, assaying, and analyzing medicinal plants. Through directed study, students are introduced to modern research techniques such as pipetting, use of positive and negative controls, replication of experiments, preparing and using growth media, and analysis of experimental results. The hands-on attributes of the STN place the student in direct control of the research discovery process, conducting tests which have no predetermined outcome, although are based on previous research with plant extracts. Many of the candidate plants can be expected to demonstrate biological activity in some screens. Moreover, STN bioassays create a richer more complete educational experience than the didactic exercises of many traditional labs by combining two differing styles of experimentation: the rigor and reproducibility of bench-top laboratory experiments and the larger context and applicability of fieldwork (Diamond, 1986).

The multi-disciplinary approach to the STN system blends several fields of science into a single educational experience, including such diverse topics as biochemistry, plant biology, organic chemistry, ecology, and medicine and human health. This web of interrelated science leads the students beyond the results to formulate more complicated questions and explorations, facilitating critical thinking and discussion from a single bioexploration lab experiment. The incorporation of fieldwork with the laboratory assays places the STN results in a real-world context, incorporating scientific theory into a contextual environment that is relevant and applicable to the students' life. This inquiry-based approach is essential for implementing state and federal science curriculum standards (Llewellyn, 2002; National Research Council, 1996).

The STN system is particularly compelling for science instruction with students who are members of tribal indigenous communities because the medicinal use of local plant-based extracts is often embedded into the cultural history of the community. The STN methods use local indigenous wild plant species as the subjects for experimentation, include traditional extraction methods, and rely on the expertise of engaged local guides to facilitate field collections. These features infuse these educational science labs with a cultural context, incorporating the students' traditional ecological knowledge into the curriculum. Typically, the results of the STN allow science to reinforce the traditional medicinal uses for local plants. STN methods incorporate tribal histories and cultural practices into the classroom and field instruction, which helps students realize the relevance of science principles (Medina-Jerez, 2008), while bridging the gap between the classroom and the real world (Anagnopoulos, 2006). As reported by Long et al. (2008), students who study botanical identification – a key portion of the STN system – had greater engagement with community elders and traditional medicinal plants. The students who cultivated this background knowledge demonstrated an increased ability to balance modern scientific principles with their traditional ecological knowledge (Long et al., 2008). This cross-over between science and culture has received greater attention in recent years, with several states incorporating the idea that cultural observations and traditional knowledge can play a part in scientific investigation and discovery into their educational rubrics (NDDPI 2006) (AKDEED).

3.3 Case Studies

To date, Illinois, Rutgers and NCSU faculty and graduate students have conducted six training courses over the past three years with participating communities in Africa, South America, and the United States. The author has served as a principal instructor in several of these workshops, teaching and ascertaining overall effectiveness of the implementation. In each case, the bioexploratory research experiences have proven to be invaluable learning tools for both educators and students.

Training sessions in Africa were conducted in Botswana and South Africa (funded through a grant from the Key International Science Capacity (KISC) initiative of the South African National Research Foundation), and in Tanzania (funded through a grant from the National Collegiate Inventors and Innovators Alliance, NCIIA). Local university professors, students, technicians, traditional healers, and local community members all participated in the training workshops. Each group entered into the workshop with different preconceived ideas as to how plant-based medicinal knowledge could be utilized effectively. Traditional medicine accounts for 80% of health care administered in Africa, 90% of which is plant-based (Kasilo et al., 2005). Traditional healers, confident in their remedies, were initially skeptical as to how science could add to their considerable practical knowledge, while some participants from the university questioned the benefits of utilizing the TEK of local communities as the basis of investigative research.

The STN approach provided an excellent means to familiarize African students with science methods and to encourage receptivity of local people to the potential benefits of science-based examination of indigenous wild species. Despite their initial opinions, both healers and university members grew to acknowledge the strengths of a system combining STN assays with traditional knowledge. Moreover, the community members and all 23 traditional healers taking part in the Botswana workshops felt renewed pride as the results of the STN assays substantiated their original health claims. All participants indicated that their experience with the STN assays increased their motivation to learn more about plant active chemistry and bioactivity, conservation of traditional knowledge, and seek higher cooperation between traditional and modern healers. Levels of participation and interest grew substantially, and by the end of the seminar, many local people were bringing extra plants from their own backyards to be tested, merely curious to see if they “worked.” One local resident exclaimed, “That day you tested our plants I was very, very happy.” Following the on site training session in Gabarone, Botswana, new trainers from the University of Botswana’s Centre for Scientific Research Indigenous Knowledge and Innovation (CesrIKi) conducted follow-up workshops and focus group discussions in four geographically separated village sites in eastern and western Botswana. Although traditional medicine is still widely practiced in the country, many younger people are reticent to admit that they practice ‘the old ways’, and it is not held in the same high regard as western medicine. However, following an STN training workshop which revealed potent antibacterial properties in an extract from local wild medicinal plants (that is, the bioassay validated the traditional knowledge), the traditional healer who had donated the plant materials exclaimed, “Now, I heal with pride!”

For implementation of STN in South America, Ecuador was selected as the initial test site. Funding for introducing the concepts in Ecuador was provided through the College of ACES, University of Illinois, and the BioTech Center, Rutgers University, in support of joint projects in bioexploration. The project in Ecuador enjoyed a great degree of synergy by engaging government (Ministry of Environment), non-governmental organizations (NGOs), the University of San Francisco Quito (USFQ), and local ecotourism guides. The course was conducted in the Maquipucuna Foundation’s ecological reserve north of Quito and incorporated two groups of students; one comprised of students and professors from USFQ and one made up of local guides, farmers, and park rangers.

The cloud forest environment provided the project in Ecuador the ability to explore a region with very high biodiversity; the guides identified over a dozen plants with medicinal properties within the first half-mile of hiking. The STN system provided an excellent method for engaging students who had no scientific background (**Figure 3.2**). By combining a detailed manual with lectures into a guided lab approach empowered the students in their own learning experience, giving them a feeling of accomplishment to independently carry out these assays that was evident in their reaction to the teaching seminar. One participant remarked, “a brilliant project, very well done for being clear and simple … so that people without any scientific background can do it without [the training instructors].” Another elaborated on that sentiment, saying that, by using the STN assays, “[the guides] realized they were capable to do some research … the assays and demonstrations … and visual aids and hands-on really helped re-enforce the assay.”

Figure 3.2: In Ecuador, Dr. Gili Joseph Works with Local Guides on Pipetting Technique while Screening Plants for Roundworm Lethality. Photo © Josh Kellogg.

Finally, the STN system has been conducted with American Indian/Alaska Native partners in both Alaska (funded through the EPA STAR program, National Center for Environmental Research) and in North Dakota (funded through the USDA Tribal Colleges Research Grants Program). High school and elementary school teachers and students, and local residents from three distinct Alaska Native villages – Point Hope, Seldovia, and Akutan – participated, with additional involvement from the Alaska Native Tribal Health Consortium. United Tribes Technical College (UTTC) in Bismarck, North Dakota served as the hub for the STN training with participants from 5 tribes in the Dakotas. In these teaching sessions, both tribal elders and youth were engaged simultaneously in learning and applying the STN assay system to locally-important subsistence foods and indigenous herbs.

By incorporating both tribal elders and youth in the laboratory exercises, the STN approach successfully incorporated the traditional knowledge of the tribe and modern scientific practices into a cohesive educational experience. In the Alaska cases, prior to working with STN materials, students conducted interviews with elders and other adults to gather local information on the use and importance of berries and other subsistence foods in the community past and present. Interaction between elders and younger community members generated enthusiasm for passing along traditions to the next generation, helping mitigate a trend in Native American tribes in which successive generations rapidly lose their traditional culture and knowledge (Tsuji, 1996). Elders in North Dakota led the plant collection and identification fieldwork, sharing their knowledge about the plants' medicinal value and place in local tribal culture with the students. The students had the opportunity to utilize the STN assay system to develop scientific support for their elders' traditional health claims for the plants. The students responded positively to this interdisciplinary scientific approach, saying the best parts of the project were “learning about traditional plants and the different uses for them,” and “learn[ing] the properties of the berries.”

3.4 Conclusion

Active student participation in science courses can greatly enhance the connection between the laboratory environment and the real world. Active learning scenarios enable instructors to substantially impact the attitude and interest of the students, and thus enhance their retention of material. The goal of these multidisciplinary laboratories is to heighten student engagement with the scientific material, translating into a feeling of “excitement” by the students. This is the single largest factor in improving student attitude towards labs (Basey et al., 2008), as well as towards science (Freedman, 1997). Students report higher feelings of confidence, interest, and enjoyment with the laboratory when they are participants (Henderleiter and Pringle, 1999; Kern and Carpenter, 1984). The advantages of applicable, hands-on laboratories go beyond a higher enjoyment of the subject matter; they are more effective in transmitting information to the students (Freedman, 1997), and catalyze significant improvement in student achievement on test scores compared to a standard laboratory (Rissing and Cogan, 2009). Studies utilizing the Relevance of Science Education (ROSE) questionnaire system have demonstrated that incorporating nature into autonomous learning projects, which reflect the students’ natural scientific interests outside the classroom, could be a powerful tool in increasing student interest and generating more inquisition in science education (Lavonen et al., 2008).

Using the STN system, teachers engage students by adding real-world context to the labs. Using a multidisciplinary, interactive approach, the STN assays expand scientific skills and concepts into directed field experiments that activate student attention and interest. The STN approach provides substantial benefits to students and school programs as compared to traditional lab exercises. The STN assays are inexpensive, readily deployable to the field or class laboratory, and implement modern laboratory techniques while encouraging a synergistic relationship with the traditional culture and knowledge of the students.

Students within the workshops have thus far found this to be a great learning experience, saying, "I learn best when I get involved with hands on learning." "The screens were totally ingenious, sensible, and useful … [providing a] strong connection between local guides' knowledge and scientific tests," asserted another participant. In perhaps the best demonstration of the effectiveness of the STN system and its ability to engage students in science, two students from the Ecuador training course have initiated post-graduate research at the University of Illinois and Rutgers University, based upon traditional plants examined in the initial STN screening. Teachers have found it a useful resource, saying it provides, "so many great ideas for my classes," and, as one college instructor commented at the end of a STN workshop session, "I am excited to go further."

For further information on the STN training opportunities and the global research and development network that has fostered this approach, the reader is referred to www.GIBEX.org.

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4 Prospects for Commercialization of an Alaska Native Wild Resource as a Commodity Crop

4.1 Introduction

4.1.1 Alaska's Berry Resources

Largely undeveloped wilderness, Alaska's unique environment harbors multiple species of wild berry that are fundamental to the traditional ecological knowledge (TEK) of Native American/Alaska Native (NA/AN) tribes. These indigenous berries, including blueberries (*Vaccinium uliginosum* and *V. ovalifolium*), salmonberries (*Rubus spectabilis* and *R. chamaemorus*), and mossberries (*Empetrum nigrum*, also known as crowberries and blackberries), are species largely unknown to consumers in the lower 48 states. NA/AN communities harvest the berries in the wild as a subsistence food, and rely on their medicinal properties to counteract kidney trouble (Viereck, 2007), promote wound healing, aid in gynecological problems, and treat diarrhea (Heller, 1953; Moerman, 1998).

This northern American landscape is defined by its extremes: wide temperature fluctuations, short but intense growing seasons, near-24 hour summer photoperiod duration, and the presence of a permafrost soil structure (Alaska Department of Commerce). Endemic flora, and berry species in particular, evolve numerous physical, ecological, and biochemical methods to ensure survival against biotic and abiotic stresses (Bliss, 1960; Chapin et al., 1987), including the accumulation of protective phenolic phytochemicals (anthocyanins, proanthocyanidins, etc.) that can be more highly expressed as the environment grows more hostile (Boyko and Kovalchuk, 2008; Grant-Downton and Dickinson, 2005; Reyes-Carmona et al., 2005; Satoe et al., 2001). Phenolic compounds not only help protect the berry species in the face of environmental adversity, but also act as powerful antioxidants capable of offsetting multiple human health concerns: cardiovascular disease, (Neto, 2007), DNA oxidative damage (Dulebohn et al., 2008), neurodegeneration (Schroeter et al., 2002), multiple stages of carcinogenesis (Seeram et al., 2006), bacterial infections, especially in the urinary tract (Howell et al., 2005), and components of the metabolic syndrome such as Type 2 Diabetes and obesity (Martineau et al., 2006; Tsuda, 2008). The established link between the austere Alaskan climate and the enhanced accumulation of novel health-protective wild berry constituents (Kellogg et al., 2010), may represent a novel opportunity for development of Alaskan berries as a potential new 'superfruit' commodity for distribution to the broader functional foods segment of American consumers.

4.1.2 Incentives for Developing a New Alaska Berry Commodity

Economic development is a pressing issue for the majority of North American indigenous tribes (Duffy and Stubben, 1998). Despite fluctuating levels of government support throughout the 20th century, the socioeconomic status of the indigenous people has stagnated (Vinje, 1996), culminating in a state of chronic economic underdevelopment; poverty rates in NA/AN communities are approaching 34% (compared with 12.3% poverty rate overall in the United States) (Glasmeier, 2006; USDA, 2004). Many Alaska Native communities continue to rely on transfer payments from the government, which is dependent on state revenue sources. As Alaska derives up to 85% of its revenue from oil production, this is an inherently unsustainable situation (Berman et al., 1992). High rates of poverty and unemployment have exacerbated social problems, including domestic violence, alcoholism and substance abuse, health issues, and depression (Bohn, 2003; IHS, 2006; Tann et al., 2007). In contrast, efforts to create culturally congruent, low-impact economic development projects (such as marketing local floral commodities, crafts, ecotourism, and indigenous wild food products) can bolster economic development within an underdeveloped natural area, while simultaneously maintaining its value as wilderness and preserving biodiversity (Ballard et al., 2002; Colton, 2005; Emery, 1999; Vaughan, 2000).

Issues aside from economic concerns may also weigh into local decision-making regarding the potential to develop the local berry resources as a commodity crop. Indigenous people across North America have cited a growing chasm between the elders and the Western-oriented youth within tribes, as tribal youth have increasingly adopted diet, clothing, and cultural attitudes in alignment with a more pro-Western worldview, but at odds with traditional values (Greer, 1992; Story et al., 2000; Tsuji, 1996). The shift from traditional diets to Western commodity diets has precipitated a dramatic rise in lifestyle-related diseases (such as obesity and Type 2 diabetes) (Acton et al., 2003; Burrows et al., 2000), and has exacerbated a profound disconnect from core Native values such as respect, reciprocity, and empowerment (Frideres, 1993). However, creation of a new economic development project, which validates the traditional beliefs about wild berry health benefits and unites generations in the harvest and processing, has the potential to strengthen the community. When tribal youth and elders work together on the traditional lands, a renewed forum for communicating traditional ecological knowledge and traditions to successive generations is fostered (Colton, 2005). Culturally-sensitive development projects are better positioned to succeed and address multiple goals for the tribe (Middleton and Kusel, 2007). Overall, utilizing traditional natural products as an instrument for development has the possibility of providing a unique opportunity for the communities, both economically and culturally, as it grows a novel, financially viable market centered on traditional plants and activities.

4.1.3 SWOT Analysis

A thorough evaluation of pertinent strengths, weaknesses, opportunities, and threats (SWOT analysis) (Panagiotou, 2003) can serve as a useful framework for evaluating the opportunity for development, under the direction of NA/AN tribal communities, of the unique Alaskan berry resource as an external commodity.

In this manuscript, we consider the ramifications for development of wild Alaskan berries as a commercial enterprise that could present an exclusive opportunity for Alaska Native communities. SWOT analysis will be applied to better assess the potential barriers and determine the practicality of this commercialization project.

4.2 <u>Internal Strengths</u>

4.2.1 Potential New "Superfruit"

The demonstrated health benefits of Alaskan berry resources are positive indicators for a potential commercial product. The 'superfruits' on the market today - cranberries, blueberries, pomegranates, tart cherries, açaí berries, black currants, lingonberries, mangosteen, goji berries and others– all are marketed on the basis of high levels of endogenous health-enhancing phytochemicals (Facenda, 2007). In comparison, some of the Alaskan berries contains up to 4.39 mg anthocyanins per g fruit, greater than most current superfruits (**Table 4.1**). As an additional bonus, the Alaskan berries feature multiple anthocyanin aglycone structures (in contrast to açaí, which contains predominantly cyanidin) (de Rosso et al., 2008) along with proanthocyanidins, phenolic acids, and other polyphenol compounds, creating a phytochemical cocktail potentially capable of providing a greater degree biological protection *in vivo* (Grace et al., 2009; Kellogg et al., 2010).

Fruit	Anthocyanin Content [mg/g fruit]	Source
Açaí	3.03	(de Rosso et al., 2008)
Blueberry	3.27	(Grace et al., 2009)
Cranberry	3.60	(Prior et al., 2001)
Lingonberry	1.74	(Andersen, 1985)
Alaska Mossberry	4.39	(Kellogg et al., 2010)
Alaska Blueberry	3.34	

Table 4.1: Anthocyanin Content of Selected Superfruit Compared to Wild Alaskan Berries.

Certain Alaskan berry species demonstrated remarkable potential to offset aspects of metabolic syndrome; mossberry and blueberry lower triglyceride accumulation in mature 3T3-L1 adipocytes up to 20%, while salmonberry was found to offset adipocyte generation by up-regulating *pref-1* expression 82% over control levels. This translates into a strong potential to counteract obesity by preventing fat cell growth and triglyceride uptake in the cells. Furthermore, selected Alaskan berries lowered postprandial serum glucose levels by 26-45% in an *in vivo* model, on the same magnitude of the anti-diabetic pharmaceutical Metformin© (Kellogg et al., 2010). The powerful bioactivity demonstrated by Alaskan berries places them in line with other superfruits on the market today, and is one potential favorable condition towards their future development as an agricultural commodity.

4.2.2 Land and Berry Resource Access

Wild berries are distributed throughout Alaska, on both public and private land. Alaska Native tribes hold title to 44 million acres (10% of total landmass in Alaska) (Alaska DNR, 2000) distributed throughout the state (**Figure 4.2**). NA/AN tribes' access to reserve land translates into a supply advantage (Anderson and Parker, 2009) via the property rights conferred surrounding the local natural resources. A synergy between economic development and land rights exists; as an advisor to the Woodland Cree First Nation (WCFN) in Canada stated: "By controlling commercial activities … the WCFN are in a better position to gain even greater control of their land, especially if at some point they make the decision to make a land claim" (Colton, 2005). Alaska Native tribes are able to manage local resources independently, and thus, access to reserve land confers the potential to harvest berries across the state.

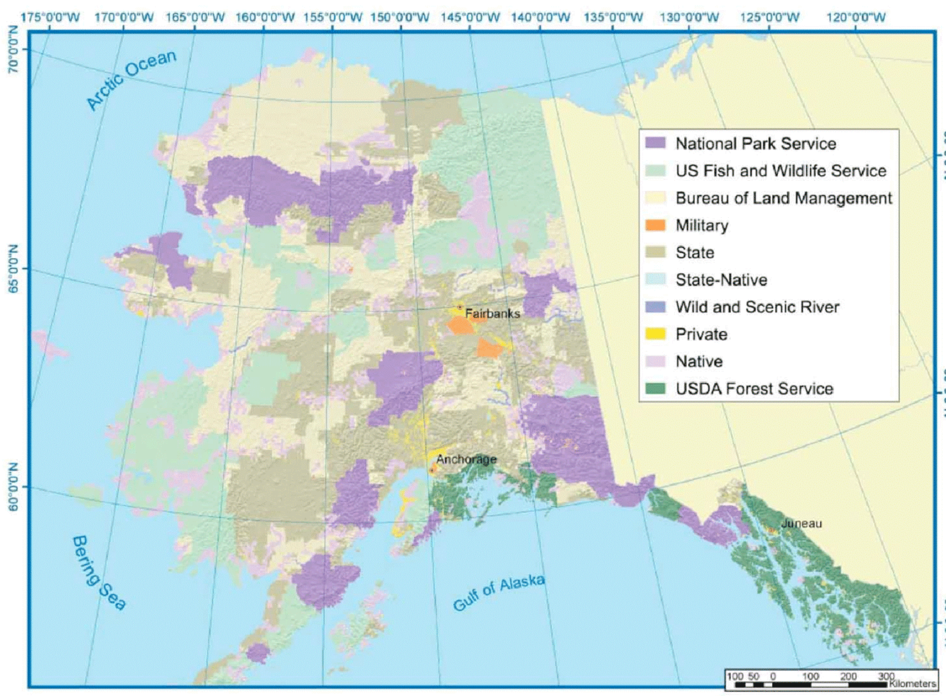

Figure 4.1: Land Ownership Distribution in Alaska (from http://www.wri.org).

4.2.3 Alaska Berries as a Wild Commodity

Maintaining the berries as a wild population is essential to the integrity of any potential berry commodity. Wild fruit that is routinely exposed to environmental stress demonstrated a marked increase in phytochemical content compared to cultivated varieties, which translates into improved health benefits to consumers (Deighton et al., 2000). The stress inherent in wild habitats fosters the unique health beneficial composition of the wild berry fruits. In addition, wild berry stands require no agriculture maintenance, which is a cost benefit to the local tribal communities. In comparison, wild blueberries of New England are often treated as a semi-regulated agricultural product, and are managed with minimal inputs – including weed management, herbicides, irrigation, pollination, fertilization, and pruning – which in turn increases yield of the crops (Yarborough, 2004). Having fewer requirements for the physical upkeep of the crop dramatically reduces the overhead costs of production. Wild blueberry production in Maine carries annual maintenance costs (i.e. not including harvesting or processing) between $400-500 per acre (DeGomez et al., 2001), whereas growing cultivated blueberries elsewhere (such as blueberry production in California's San Joaquin Valley) can cost $1,395 per acre per year (Bervejillo et al., 2002). Both estimates do not include the capital costs of field equipment. In the case of Alaskan berries, it is not clear whether agricultural inputs could even improve on the abundance of the arctic crop. Mossberries increased vegetative growth in response to nitrogen and phosphorus fertilization, however no correlation with fruit production was determined (Holloway, 2006), and the necessity of subsurface fertilizer application (due to permafrost) for the Alaska barrens would be cost-prohibitive. It is evident that maintaining Alaskan berries in their native wild stands is a cost effective production regime, but it is also the strategy best suited to preserving the unique health-protective composition of the berry fruits.

4.3 Internal Weaknesses

4.3.1 Commodification of Subsistence Resources

Potential negative consequences of Alaskan berry commercialization must also be considered, as berries risk exploitation and degradation if they came to be viewed as short-term profit-generators rather than valued tribal resources. Alaska Native communities do not generally consider subsistence products as private resources, possessing social and cultural importance beyond their function as a food stuff (Parlee et al., 2005). Consequently, there are complex rules interrelating ecological conditions and harvesting practices, governing resource access, information sharing, and harvest sharing within the tribe (Ostrom, 1990; Parlee et al., 2006). A prime example is the Yakutat village in the Gulf of Alaska, where moose were harvested by 22 percent of households but used by 70 percent of the population; shrimp were collected by just 18 percent of households, but 86 percent of the community shared in the harvest (Mills and Firman, 1986).

This attitude of communal property extends to berries as well; the Gwich'in in northern Canada frequently share their harvest within the village, but would "never" consider selling their berries for economic gain (Parlee et al., 2005). Thus, there is a disconnect between the traditional uses of subsistence products, which are inherently noncommercial, and a possible commodity developed from these resources. While a balance could be struck between subsistence needs and economic development, undoubtedly a portion of Alaska Native tribes would be against such development, and decline participation in any venture to commercialize wild berries.

A berry production scheme regulated by tribal communities and maintained as a communal resource represents a stronger buffer against the threat of over-harvesting. The community would, using both cultural guidelines towards harvest and incorporating other research (such as the recommendation by the Alaska Department of Natural Resources of harvesting no more than 1/3 of berries in a specific area, to allow natural regeneration to occur (Alaska DNR, 2008) to set specific harvest quantities. Communal resource management has been shown to discourage over-extraction and poaching of the resource from both natives and non-natives (Ballard et al., 2002), to prevent instances like British Columbia, where non-native harvesters came onto the land of the Ktunaxa – an indigenous people in the Pacific Northwest - to collect huckleberries for export to commercial markets in Canada and the United States. Their harvesting activity went un-checked until the huckleberry bushes were decimated by over-harvest, leading to a decreased subsistence harvest for the Ktunaxa (and subsequent food shortages), and widespread ecological damage (Turner, 2001).

4.3.2 Economic Structure of Alaska Native Communities

Furthermore, communal decision-making process has been complicated by the recent corporation structure on Alaska Native communities. The twelve regional native corporations organized under the Alaska Native Claims Settlement Act (ANCSA) were designed for pro-profit tribal economic development (ANCSA Network, 2009). While this was ostensibly geared towards independent income generation for the tribes without government oversight and control, the corporations were implemented on top of pre-existing political structures that had been in place since the 1930s, which were organized along more traditional concepts of communal property and consensus-based policy. This bifurcated the control of tribal activities: the political organization of the tribes no longer held title to the land (Berardi, 1998).

The resulting dichotomy precipitated multiple conflicts between regional corporations and tribal members over extraction of resources and development; for example, timber rights sold by a corporation was viewed by some community members as disrupting subsistence resources and damaging the local ecosystem (Bristol, 1996). The ultimate structure of the corporations, and its role in directing Alaska Native subsistence economies, is yet to be determined, with multiple directions being sought to procure an optimal balance (Thornton, 2007). Some communities could perceive the commercialization of wild berries as an undesired avenue of economic development forced upon them by the corporations, ultimately leading to more environmental degradation and compromising the traditional values of the resources, including sharing among community members. It is essential that the entire tribal community is involved in the decision process of whether to produce berries as a commodity and consensus is achieved over the decision to move forward with such a development project.

4.3.3 Inconsistent Production

Other internal weaknesses could undermine any attempt for tribal communities to commercialize the wild berry resource, including erratic availability of local harvesters and unpredictable fruit production levels. Indigenous individuals may occasionally sell subsistence products to supplement their income, but it is not usually a full-time occupation (Emery, 2002). Denali BioTechnologies, a non-native commercial supplement company located in Alaska, had to investigate the option of hiring harvest crews from the lower 48 United States to supplement local harvesters, as the labor supply from local communities was viewed to be erratic and a possible production obstacle (Bauman, 2005). For a tribally owned commercial enterprise, such outsourcing may or may not be viewed as a tenable solution to labor needs.

Maintaining the berries as a wild crop exposes them to the Alaskan environment, and berry development can fluctuate wildly year-over-year due to alterations in seasonal temperatures and precipitation (Flint et al., 2010). The mossberry is the most reliable of the Alaskan berries in producing year-to-year yields (Holloway, 2006), and considering the range of berries in Alaska it is conceivable that decreases in one region could be offset by increases elsewhere, keeping the overall harvest levels stable. Wild blueberries (*V. angustifolium*) from Maine and maritime Canadian provinces report an average yield of 2,500 lb/acre (DeGomez et al., 2001), yet reliable production yields for Alaskan berries have not been determined.

4.3.4 Cost Structures

In addition, the costs associated with the harvest of the commercial wild blueberry crop in Main and Canada represent the greatest annual input for crop production, yet is highly variable, costing producers between $375 and $900 an acre by raking (DeGomez et al., 2001), while hand-picking can raise those costs up to $2,300 per acre (Bervejillo et al., 2002) for hourly workers. Alaska Native tribes managing the harvesting process could potentially negotiate their own costs of harvest. The Alaska Tribal Cache, a berry-based enterprise operated by the Seldovia tribe in Southwest Alaska, uses a voluntary harvest model where individuals are paid according to the volume collected, as opposed to hourly rates. In 2009, the Cache paid $4.00 per pound for blueberries and $2.50 a pound for salmonberries, which would have to be taken into account when determining a final price structure for the product. In addition, if the Alaskan berries were promoted as a commodity, these berries would need to be transported from wild lands, which are geographically isolated, to large processing facilities, which would incur both transport costs as well as potential product loss from spoilage and physical damage.

4.4 External Opportunities

4.4.1 The Superfruit Marketplace

The superfruit industry has enjoyed substantial growth over the last several years, and may present an opportunity for Alaska berries as a niche commodity. Sales of the top three superfruit – açaí, pomegranate, and goji berry – have experienced year-over-year sales exceeding 140% from 2006 to 2007 (**Table 4.2**), with açaí nearly tripling 2006 sales receipts ($9.8 million to $29.3 million) (Anonymous, 2007b).

Superfruit	2007 Sales (USD)	2006 Sales (USD)	% Change
Açaí	$29,331,200	$9,878,068	+ 197%
Pomegranate	$23,120,946	$9,223,004	+ 151%
Goji Berry	$9,611,345	$3,906,986	+ 146%

Table 4.2: Sales from Three Superfruit-based Products: Açaí, Pomegranate, and Goji Berry. From Anonymous (2007b).

The 'exotic' nature of some of these fruits – previously unknown and unavailable to American consumers – also contributes to the new interest exhibited by consumers. The American consumer is increasingly in tune with the benefits of functional, health protective foods, which has created an escalating sales potential for superfruits with benefits that can be backed by credible science-based evidence. Also contributing to this market expansion is the ubiquity of food products that can feature superfruits. In 2003, 50 superfruit-themed pomegranate products first hit the market. Since then pomegranate has become a mainstream superfruit, and in 2007 over 400 unique products contained pomegranate, an annual growth surge averaging over 100% (Aranowski, 2009). Superfruits have become ingrained in nearly every major food category, including baby food, confectioneries, even pet food (Facenda, 2007). Manufacturers are continually developing novel ways of incorporating fruit into food products. Ocean Spray has developed a line of superfruit fusions, which can be utilized in baking applications (Rigik, 2009), and Jelly Belly recently announced a new line of jellybeans named the Superfruit Mix, which includes the açaí berry, Barbados cherry, cranberry, blueberry, and pomegranate (Anonymous, 2009). Beverages are the largest segment of new superfruit product development, and superfruits have attracted interest from a number of companies, with Coca-Cola's Minute Maid, Pepsi's Tropicana, Apple & Eve, Blue Bunny, POM, and Anheuser-Busch all releasing products that are marketed based on superfruit content (Anonymous, 2007b). The new market is so lucrative that, in order to establish itself in the superfruit arena, Welch's is spending $20 million to re-brand its purple Concord grape juice as the "original" superfruit (Hein, 2008).

4.4.2 New Superfruit Directions

There is an ongoing hunt for new fruit and botanicals to widen the superfruit arena, a trend that is a reflection of evolving consumer preferences and scientific research, which has subsequently been translated into the popular press. Ethnic and traditionally-used products are gaining more popularity in the superfruit category, leading to the inclusion of noni, Cat's claw, and baobab as the newest superfruit entries to hit supermarket shelves (Aranowski, 2009; Gruenwald, 2009). Researchers are recognizing the importance of indigenous peoples' traditional knowledge in identifying novel superfruits, utilizing subsistence foods and medicinal plants with potentially powerful health properties as superfruit candidates (Netzel et al., 2007). The status of Alaskan wild berries as a tribal resource, backed by traditional ecological knowledge (TEK) and a long history of human use, could have a strong appeal in the marketplace.

Another trend gaining traction is localized food production, including superfruits. Consumers have become increasingly aware of issues related to where food is produced and how far it must travel before reaching the market shelves (Aranowski, 2009). The superfruits currently at the top of the sales charts are mostly exotic, tropical plants, and are not produced within the United States (Oliver, 2008), leaving consumers lacking in a local alternative to goji, açaí and mangosteen. The Alaskan berries are domestically produced; yet still evoke an image of exoticism and mystique due to their distant, extreme arctic origin.

4.4.3 Product Marketing Considerations

Consumer preferences and tastes continue to dictate whether a food commodity will succeed, and research has focused on how advertising and scientific claims impact these choices. Consumers must have confidence in the validity of science-based claims and university research before they will be compelled to purchase superfruit products. Wansink et al. (2005) demonstrated that purchasing is more likely to occur when the consumer is fully aware of the *outcomes* (health benefits) of consumption, rather than just aware of the *content* of the food. For example, consequence-based knowledge (e.g. soy contributes to a lowering the risk of heart disease) will contribute to sales more than knowledge of the attributes of the food itself (e.g. this product contains soy, which is low in cholesterol) (Wansink et al., 2005).

The appeal of nature and the exotic also resonates with consumers who are attracted to purchase superfruits. Many health-conscious consumers prefer 'natural' products and may be less inclined to purchase functional foods which they think have been adulterated with additives and extra processing (Urala, 2005). A Dutch study found that consumers prefer natural functional foods over supplements with the same phytochemical content (de Jong et al., 2003). Exoticism plays to the imagination of the consumer, evoking images of pristine, far away places. Hawaii has embraced exoticism as a marketing mechanism to develop a niche agricultural market for its sugarcane and pineapple crops in the face of increasing global competition (Suryanata, 2000). Alaska has a wild, unspoiled character which is ingrained on the collective psyche for decades (the state's nickname is "The Last Frontier"), and has been utilized effectively by Alaska Natives in marketing other cultural pieces, namely art and crafts (Moore, 2008), and to a more minor extent, the marketing of Alaska Tribal

Cache wild plant products from the Seldovia Village Tribe, which claims, "On the sunny slopes above pristine Seldovia Bay, in Alaska's south-central region, grow the wildest of the wild berries. Nourished by clean winter snows, fresh spring rains and the long sunshine filled days of Alaska's summer" (http://www.alaskatribalcache.com/).

However, even the most attractive marketing cannot override the role that sensory attributes play in consumer decision making (Urala, 2005). A majority of consumers will not significantly compromise taste for additional health benefits (Verbecke, 2006), though certain segments of the population, most notably health-conscious consumers exhibiting less food neophobia, are slightly more willing to accept a product with inferior taste (Sabbe et al., 2009). To develop a comprehensive marketing plan for Alaskan berry products, all product features must be considered, including health claims and functions, lack of adulteration, exotic status, texture, and taste.

4.4.4 Government Partnerships

Federal and state governmental programs could assist in structuring a development plan for wild berries as a commercial product involving Alaska Native communities. The federal government has advisory programs available to NA/AN tribes, specifically designed to help adapt indigenous commodities to the marketplace. The United States Department of Agriculture (USDA) has partnered with the Intertribal Bison Cooperative (ITBC) to develop production and market strategies for traditionally maintained bison herds (Schofer, 2008), using business tools to exploit the best methods possible for introducing bison products into the marketplace. Government projects centered around environmental protection and research are another potential target for partnership. Alaska Native tribes possess a strong sense of stewardship towards the land, because land has cultural and spiritual significance and provides a source of physical subsistence (Whiting, 2004). Given this strong connection with the land, Alaska Native tribal communities may coordinate with government programs to promote sustainable land use and develop land-based commercial activities which protect biodiversity (Watson et al., 2003). For example, the Northwest Research and Harvester Association, which assists floral green producers in the Pacific Northwest, enacted an agreement with the State of Washington Department of Natural Resources and Washington State University to manage the floral greens and understory species in the Hood Canal and Green Mountain State Forests (>40,000 acres). This aided the government against unauthorized harvesting (poaching), maintained sustainable harvesting measures while ensuring the harvesters had recurring access to large tracts of land (Ballard et al., 2002).

4.5 External Threats

4.5.1 Small Producers in a Large Market

As with many small-scale agricultural producers, tribes involved in commercializing wild Alaskan berries face multiple obstacles in interacting with the larger agricultural market, including a lack of current industry information, little control over prices and labor regulations, and variable buyer regulations regarding product quality and standardization (Gulati et al., 2007). Small producers are chronically excluded from management decisions, leaving them without a voice in the market and exacerbating their economic disadvantage (Ballard et al., 2002; Dubey, 2007). As a result, aboriginal producers are more likely to divest themselves from a controlling interest in market structures and rely on non-native businessmen to conduct operations (Cleary et al., 2008). Despite this disparity in power, small producers are integral to many areas of national and global agriculture; over 70% of the milk produced in India is generated by households with only one or two animals, which are linked together by a nationwide cooperative network of dairies (Food and Agricultural Organization, 2004). Collective agricultural organizations are able to provide members with essential assistance by strengthening the role of small agricultural producers (Hellin et al., 2009). These associations are able to utilize economies of scale from aggregating the interests of multiple small producers, empowering them to engage larger marketplaces, negotiate more equitable price contracts, and compete fairly in a global economy (Stockbridge et al., 2003). Another potential model would link the Alaskan tribal communities with University partners, who take on the commercialization project as a research and development project borne out of health-related industry research. Frequently, federal and foundational grants to University researchers mandate an economic development component in a research program – these partnerships could provide the leverage to help the small-scale community enterprise to reach broader markets by taking advantage of well-established university linkages.

4.5.2 An Ongoing Subsistence Debate

Subsistence rights in Alaska have been a long-standing source of political and social debate, having only recently been crystallized under federal law, with the passage of the Alaska National Interest Lands Conservation Act (ANILCA) in 1980 (Thériault et al., 2005). ANILCA defines "subsistence uses":

> the customary and traditional uses by rural Alaskan residents of wild, renewable resources for direct personal or family consumption as food, shelter, fuel, clothing, tools, or transportation; for the making and selling of handicraft articles out of nonedible byproducts of fish and wildlife resources taken for personal or family consumption; for barter, or sharing for personal or family consumption; and for customary trade (USFWS, 2009).

Subsistence rights have now been granted to all Alaskan residents, both tribal and nontribal persons, urban and rural, though numerous regulatory hurdles still remain in implementing this policy across the state. This labyrinth of policy has led to conflict along those divides, with nonnative Alaskans asserting their rights for the same resources Alaska Natives utilize (State vs. Morry, 1992). It is an unanswered question of how the promotion of a native project to commercially develop berries would be received by nonnatives, either as a threat to their own subsistence livelihoods or due to the fact that there has been other interest expressed to harvest berries for commercial projects (Pilz et al., 2006).

4.5.3 Changes in Consumer Taste

Consumer taste patterns are governed by a wide variety of socioeconomic stimuli, which result in a large number of short-term food fads that rise in popularity and then decrease precipitously. This is partially attributed to species survival psychology; humans possess multiple biological and psychological mechanisms that promote variety in the diet and can cause our tastes to wander (Mela, 1999), such as sensory-specific satiety, in which there is a marked decrease in desire for a specific food after consumption (Lyman, 1988). The United States has a long history of food fads, many which have long faded from prominence (Lovegren, 2005). Industry publications monitor such trends, attempting to predict future shifts in consumer preference and taste (Aranowski, 2009). As superfruits are a relatively new industry, there is little indication of whether the popularity of superfruit is a limited consumer fad or a more permanent alteration in consumption patterns; however, substantial declines in shopper preference for superfruits would represent a substantial negative effect on the continued viability of a wild Alaskan berry commodity.

4.5.4 Climate Change and Future Viability

As discussed earlier, the Alaskan berries are heavily reliant on seasonal temperature and precipitation for proper development and maturation of the fruit.

Disruptions to berry growth would be a significant supply concern for any commercial Alaska berry commodity, and thus concerns over climate change represent a large external threat to wild berries. Alaska's unique environmental position has made it especially vulnerable to the effects of shifting climate, with Arctic temperatures increasing substantially over the last three decades (Serreze et al., 2000; Simpson et al., 2002). This has triggered a prolonged growing season for Arctic plants, and an upsurge in vascular vegetation by as much as 20% (Sturm et al., 2001). A shorter and warmer winter, as predicted by the Scenario Network for Alaska Planning (SNAP, 2009), could prove severely detrimental to the berry crop, as many species require overwintering to produce fertile flowers and fruit (Wendell and Alsanius, 2008).

However, the ultimate effects of climate change upon the berries, and thus the berry commodity, are unclear at the present time. While the projected increase in growing season could prove beneficial to berry species by promoting wider ranges for berries and larger growing seasons, the conditions also stimulate competition for the limited resources available in the area. There also exists the possibility for a decrease in the phytochemical content as the atmosphere becomes less hostile towards fruit development and the plant down-regulates biosynthesis of protective chemicals. Decreases in polyphenols would lower the fruits' attractiveness as a health-benefiting dietary product. However, the full extent of climate change on the berries' chemistry has yet to be determined.

4.6 Prospects for Start-up Financing

Commercializing Alaskan tribal berries as a super fruit will require considerable financial resources to manage harvesting, channel partner distribution, and implement marketing and branding. There are many options when considering the capitalization of a new business venture, which can range from traditional debt financing to the more “hands on” venture capital investment. In the specific case of wild berry resources on Alaska Native lands, there is an additional consideration in that any commercialization would require full endorsement of the associated tribal council(s), and their buy-in as to how financial arrangements would ultimately benefit the community. It may in fact be difficult to identify a single representative or entity that would be able to speak on behalf of the tribal community to set up the terms of capitalization.

Although venture capital firms invested over $28 billion into approximately 3800 companies in 2008 (PriceWaterhouseCoopers, 2008), starting a new business using venture capital financing is generally perceived as difficult to attain compared to starting a company with traditional bank loans or supplier investments. Less than three percent of entrepreneurs that solicit venture funding actually receive funding (Casparie, 2006). Venture capitalists usually invest in high growth potential companies that can show significant cash return in 3-6 years. High profile examples of successful, venture-initiated companies include Google, Genentech, Facebook, and AOL. Other important factors in a venture capital deal include the presence of an experienced management team, a built-in unfair advantage via intellectual property protection or a technically unique capability, and geographic proximity to the venture capitalist’s headquarters. The proximity factor appears to be the most significant barrier for venture capital investment in Alaskan wild berries, as the berry harvest sites are for the most part in remote, difficult to reach locations. Griffith et al. suggest that most venture capital investors typically fund companies that are within a one hour drive of their location because of the efficiencies it creates regarding skilled labor pools and service providers (Griffith et al., 2007).

Outside of producing a monetary return, some venture capital backed companies also produce economic-development and positive social good outcomes. Typically companies funded by venture firms have two pathways to maturity, IPO (initial public offering) or acquisition by a larger firm (e.g. Time Warner acquiring AOL). If a company is not acquired and reaches maturity via the public markets, often that maturation process includes significant job creation for a specific locale. For example, in 1999, Google had 18 employees (Vise and Malseed, 2005) and today they tout over 20,000 employees according to their website. Lenoir, a small town in North Carolina that was devastated by the decline of the furniture and textile industry, was selected by Google in 2009 as the location for its $600 million data center. For Lenoir, this means over 200 high paying jobs where wages are almost double the average wage in the region (Anonymous, 2007a). Venture capital portfolio companies account for over 12 million jobs in the US (National Venture Capital Association, 2009) and have introduced valuable products like Avastin, the first FDA (Food and Drug Administration) approved cancer drug designed to slow down angiogenesis, the cell process by which new blood vessels develop and carry important nutrients to a tumor for its growth and survival.

With the many benefits that venture capital facilitates, it is still not a wide spread financing opportunity for most entrepreneurs, especially ones in a locale that is isolated from traditional venture funding pools. Tribal berries commercialization most likely will have to rely on alternative investment models such as Small Business Innovation and Research (SBIR) contracts, economic development loans, and grants from foundations.

The SBIR program is a $2 billion federal funding source that started in 1982 (Wessner, 2008). The goal of the program is to tap the small and disadvantaged business community for assistance in meeting the research and development needs of the eleven federal agencies. Five federal agencies (Department of Defense, Department of Health and Human Services, Department of Energy, NASA and the National Science Foundation) make up over 90% of the SBIR funding (Wessner, 2008). The USDA's SBIR program is may be the most appropriate funding source to help commercialize tribal berries. Solicitations include requests for novel ways to develop specialty crops and genomic tools that assist with the development of new agricultural products that generate wealth. Within the USDA's SBIR program, the section of Economic & Community Development would be the best avenue, as the program assists developers to:

> promote increased prosperity and economic security for individuals and families, farmers and ranchers, entrepreneurs, and consumers across the nation. Working together with land-grant university partners and a host of public and private collaborators, EC staff provide national leadership for research, education, and extension activities that help people incorporate sound financial management strategies in their daily lives, discover new economic opportunities, develop successful agricultural and nonagricultural enterprises, take advantage of new and consumer-driven markets at both the local and international levels, and understand the implications of public policy on these and other activities (USDA, 2004).

Venture philanthropy is a relatively new term that describes a more aggressive means to achieving social goals in the non-profit sector by employing tools used in the business sector. For example, Fast Forward is an organization that funds promising, early-stage research that might lead to new treatments for multiple sclerosis. According to the National Institutes of Health, only 250,000-350,000 people in the United States have been diagnosed with MS (NINDS, 1996). With such a relatively small patient population, typical pharmaceutical companies find it difficult to invest in a disease with a risky financial return. Fast Forward, a wholly owned subsidiary of the National Multiple Sclerosis Society, is charged with addressing a risky part of MS research so that new discoveries are more attractive to pharmaceutical companies who will subsequently invest in additional research to get drugs to the market place. Venture philanthropy funds and organizations that have an interest in facilitating economic development could be a logical resource for tribal communities searching for start up funds.

Independent of venture capital funds, multiple funding options are available (federal grant funds, venture philanthropy funds) to tribal communities that wish to start a new business venture. However, the unique characteristics of Alaskan tribal communities could require a commercialization partner (e.g. university collaborator) to help reach the multiple milestones required by the funding organization.

4.7 Conclusion

Wild Alaskan berries form an integral part of Alaska Native traditional ecological knowledge and subsistence lifestyle. The superfruit agricultural industry has demonstrated the marketability of exotic, nutrient-rich fruit as a viable commodity, providing a potential opening for wild Alaskan berries to emerge into a niche product within the marketplace. However, careful consideration of the opportunities and challenges is essential in approaching such an enterprise, and this analysis has provided insight into some of the most critical aspects of development.

There are several factors in favor of a commercial enterprise by the Alaska Native tribes, notably the remarkable bioactive potency of the berries, facile access to their natural habitat, and reduced need for inputs into production and harvesting costs. The tribes could benefit from government partnerships that assist in developing marketing strategies and business opportunities. However, cultural perceptions of Alaska berries, and their prominent position in native lifestyle, could heighten latent fears of a disconnect from that tradition with the pursuit of a commercial commodity.

In addition, other obstacles facing the tribes related to commercialization of a wild berry product relate to the source of initial financing for the business and the absence of current logistical cost data, which could be substantial considering the remoteness of some Alaska Native communities. Conflicts with nonnative Alaskans and policy regulations, shifting consumer preferences, and the ever-looming specter of climate change also pose concerns for an Alaska berry commodity.

While a wild berry commodity could be instrumental in arresting the chronic underdevelopment experienced by Alaska Native tribes, and possibly contributing to the continued propagation of traditional culture and values to successive generations, the possibility of commercializing a novel superfruit product would require a strategic balance between the tribes and the marketplace, extraction and preservation of resources, traditional activities and modern business practices, and more investigation into the interplay of these various factors is required.

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5 Summary

Bioprospecting, or biopiracy, is an issue that has only in recent years begun to be addressed, with growing attention paid to the intellectual, social, and financial rights of traditional knowledge and resources. However, this shift in consideration has occurred at a glacial pace, and decades of natural resource exploitation and relative indifference to traditional ecological knowledge has engendered feelings of suspicion and distrust among some indigenous people towards western institutions, including science and economics. Education policies that favored modern science at the expense of traditional beliefs have only deepened the chasm between the two cultures.

A main tenet of the work presented here has been the benefits associated with a more inclusive methodology approaching traditional ecological knowledge and a greater degree of engagement with indigenous people at the interface between traditional culture and western society. With a majority of modern medicines owing their origin to botanical sources, continued pharmaceutical and nutraceutical discovery relies on the opportunities and leads provided by traditional healers' knowledge. However, to ensure an integrated approach, with the assistance and input from native populations, an awareness and respect of their beliefs and customs is a part of current trends in bioexploration. The philosophy behind the Screens-to-Nature (STN) system represents a step towards a more balanced perspective of medicinal plants and the value of traditional ecological knowledge. The STN assays are the initial tools for this enhanced interaction, a bioexploratory methodology that establishes a more equitable research relationship. By partnering with indigenous people, making them active participants in the discovery process, the STN system supports their perceptions and traditions as perfectly legitimate avenues for investigation. The assay results frequently provide corroborating scientific observations that affirm the validity of traditional ecological knowledge.

There is also an ideological schism between single molecule pharmaceuticals - the approach currently favored by the medical establishment - and complimentary mixtures of active agents. Research is emerging that chemical composites, even isomers, can be more effective than any single species. Traditional medicine is centered on preparing concoctions for medicinal use, utilizing a select portion of the plant with the inherent diversity of biochemical composition. The multi-compound theory is slowly gaining among health care professionals, recognizing that therapies with multiple components, such as berries, can provide multiple areas of protection. The STN system uses a similar perspective in its methodology, grinding an entire plant or plant organ to be assayed and thus eliciting a general response from a mixture of several compounds simultaneously. The benefits of multi-component treatments are still being discovered, but the STN are a step forward in how disease treatment and prevention is approached.

While there are a diverse array of STN already developed to target multiple human health concerns, the arenas of communicable diseases, degenerative disorders, and wellness issues provide new avenues for novel assay development. Viral infections (including HIV), anticancer leads, anti-plasmodium agents, advanced radical oxygen species scavengers, and steroids or steroidal mimics are examples of areas that warrant further exploration. The World Health Organization maintains a list of infectious conditions that remain an issue throughout 3rd world nations, called Neglected Tropical Diseases, which represent a prime opportunity for STN research and fabrication. Expanding the STN arsenal is beneficial to increase not only the scope of potential plants and treatments, but also allowing this system of bioexploration to reach new regions of the world. As a participatory research approach to biodiscovery, indigenous people are able to utilize their unique health concerns as a basis to formulate the main direction of discovery. Having a larger portfolio of bioexploratory assays opens new doors for collaboration and engagement with local populations.

The STN provide a novel approach to teaching science, utilizing a similar active participation method with investigations centered around traditional plant-based medicines to excite students in scientific laboratory instruction. These innovative assays achieve this engagement by using the interdisciplinary nature of modern scientific research to reach out to students marginalized by repetitive science labs. The diversity of STN assays compliments numerous educational fields and integrates them into a single package with substantial applicability to real-life situations, reflecting changes in educational policy that have occurred over recent years. The use of ecological knowledge as a foundation allows indigenous students to reconcile their traditions with modern scientific educational principles, bridging a gap that tribal youth often face today. In order to make STN more effective as a teaching tool, detailed study is required to evaluate how the STNs are received by students, which portions are most applicable and which need to be modified to have a greater impact. This involves surveying students' attitudes and perceptions of science before and after STN-based curricula, analyzing how the STN contributes to a classroom education, and to what degree it improves the students' attention, interest, and aptitude.

Economic development is a challenge for many NA/AN people, as the most visible projects within the United States – casinos – have failed to emerge as the economic panacea originally envisioned. Subsistence resources have been peripheral concerns for forest managers, who until recently were primarily focused on non-renewable timber extraction. However, closer attention to traditional forest products has demonstrated their potential value in a larger marketplace, and a careful reassessment of forest policy is underway across the United States to better represent and utilize these resources. This represents an area where Native American and Alaska Native tribes have an opportunity establish a traditional subsistence product as a commercial good while remaining within traditional culture. The financial incentives can also improve socioeconomic conditions for NA/AN tribes, arrest emigration from native lands and help perpetuate culture between generations. Partnerships between tribes and other organizations, such as government agencies or universities, are requisite aspects of successful commercialization, and require reciprocity to generate profitable relationships.

Alaskan berries are diverse and rich subjects of study, with numerous paths upon which one could expand on the work presented here to better characterize the berries and their bioactivity. The berries are hypothesized to possess other phytochemical families, such as carotenoids, phenolic acids, and ellagic acids (which are hypothesized to underlie the *pref-1* activity seen in *R. spectabilis*). This thesis has just begun investigating how berries impact the complex biochemical signals and responses of metabolic syndrome. A more comprehensive mechanistic understanding of how the berries are functioning in *in vitro* and *in vivo* systems is crucial to lend stronger support to Alaskan berries as a viable commercial product, as this strengthens the health perceptions upon which superfruit marketing is based.

All of the arenas discussed in this body of work demonstrate that traditional resources can impact multiple areas of society. They hold promise for health promotion for other persons across the country; the assays serve as a hook to interest students in scientific exploration; and they are a potential viable commercial products for the market at a national or global scale. It is from the traditional knowledge of Native Alaskan tribes that we are aware of the multifaceted potential of this resource. Ultimately, this work is part of a movement beginning to alter the attitudes underpinning bioprospecting projects of years past, which held traditional ecological knowledge as an archaic worldview incompatible with modernity. In actuality, these two vantage points are not so alien to one another, and great synergy is possible when the two are considered together through an inclusive, balanced approach.

Appendix A: Collection Data

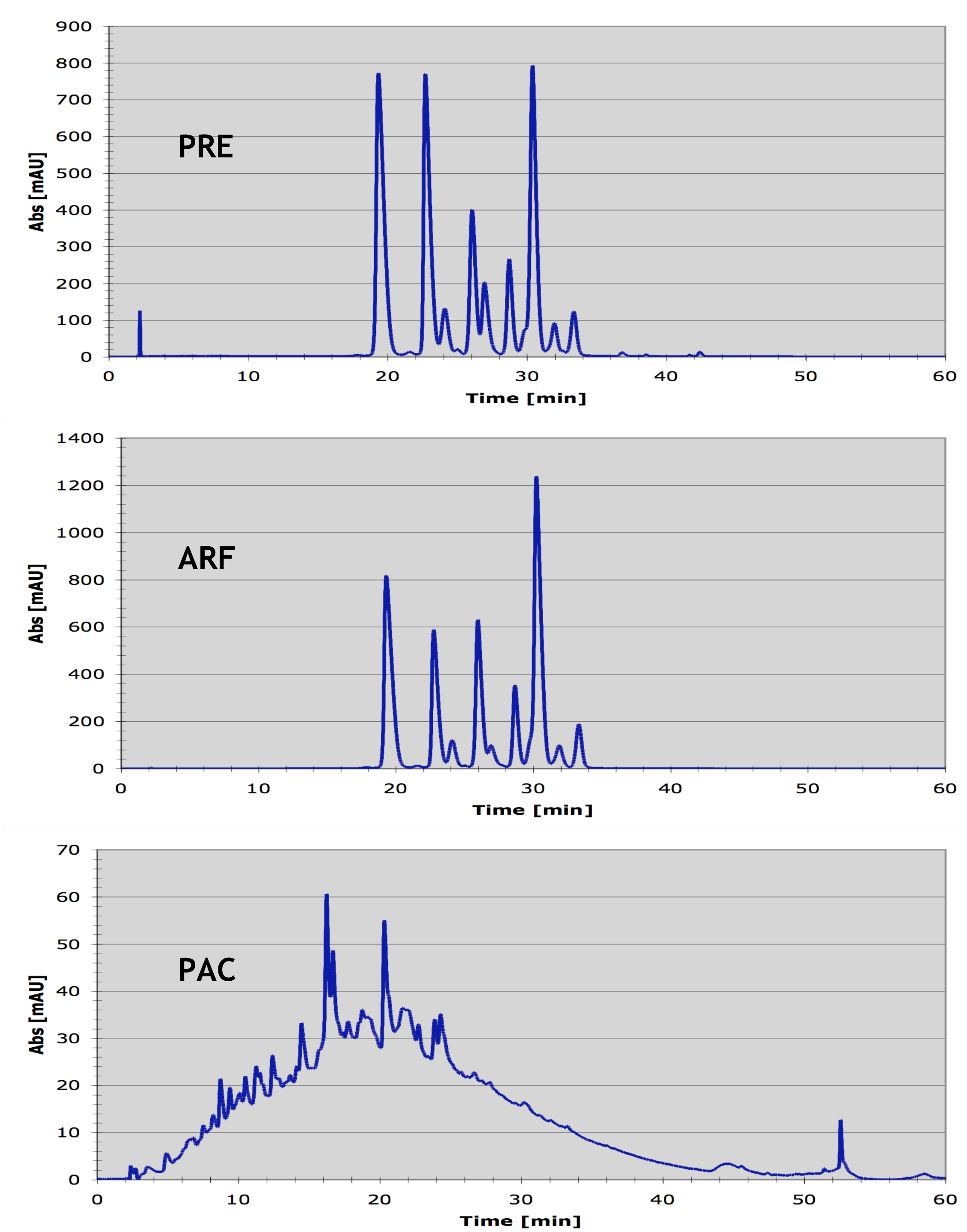
Seldovia Berry Collection			
Date Collected	7/28/2008	7/29/2008	September 2008
Scientific Name	*Vaccinium ovalifolium*	*Rubus spectabilis*	*Empetrum nigrum* [2]
Common Name	High-bush blueberry	Salmonberry	Crowberry
Laboratory ID	VO-SD	RS-SD	EN-SD
Latitude	59.4° N	59.4° N	n/a
Longitude	151.8° W	151.7° W	n/a
Elevation	152'	370'	n/a
Herbarium Voucher	JK0001	JK0002	n/a
Accession # [1]	242786	242787	n/a
Amount Collected	2L	2L	n/a
Habitat	Mixed forest	Mixed woodland	n/a
Face	NW	E	n/a
Temperature	54°F	60°F	n/a
Herbivory/Fungus	None	none	n/a

Point Hope Berry Collection			
Date Collected	8/30/2008	8/29/2008	8/30/2008
Scientific Name	*Vaccinium uliginosum*	*Rubus chamaemorus*	*Empetrum nigrum*
Common Name	Blueberry	Salmonberry	Blackberry
Laboratory ID	VU-PH	RC-PH	EN-PH
Latitude	68.1° N	68.1° N	68.2° N
Longitude	160.9° W	160.5° W	160.9° W
Elevation	104'	24'	50'
Herbarium Voucher	JK0003	n/a	JK0004
Accession # [1]	242788	n/a	242789
Amount Collected	1L	1.5L	1L
Habitat	Dwarf scrubland	Dwarf scrubland	Dwarf scrubland
Face	SW	n/a	SW
Temperature	41°F	n/a	42°F
Herbivory/Fungus	none	n/a	none

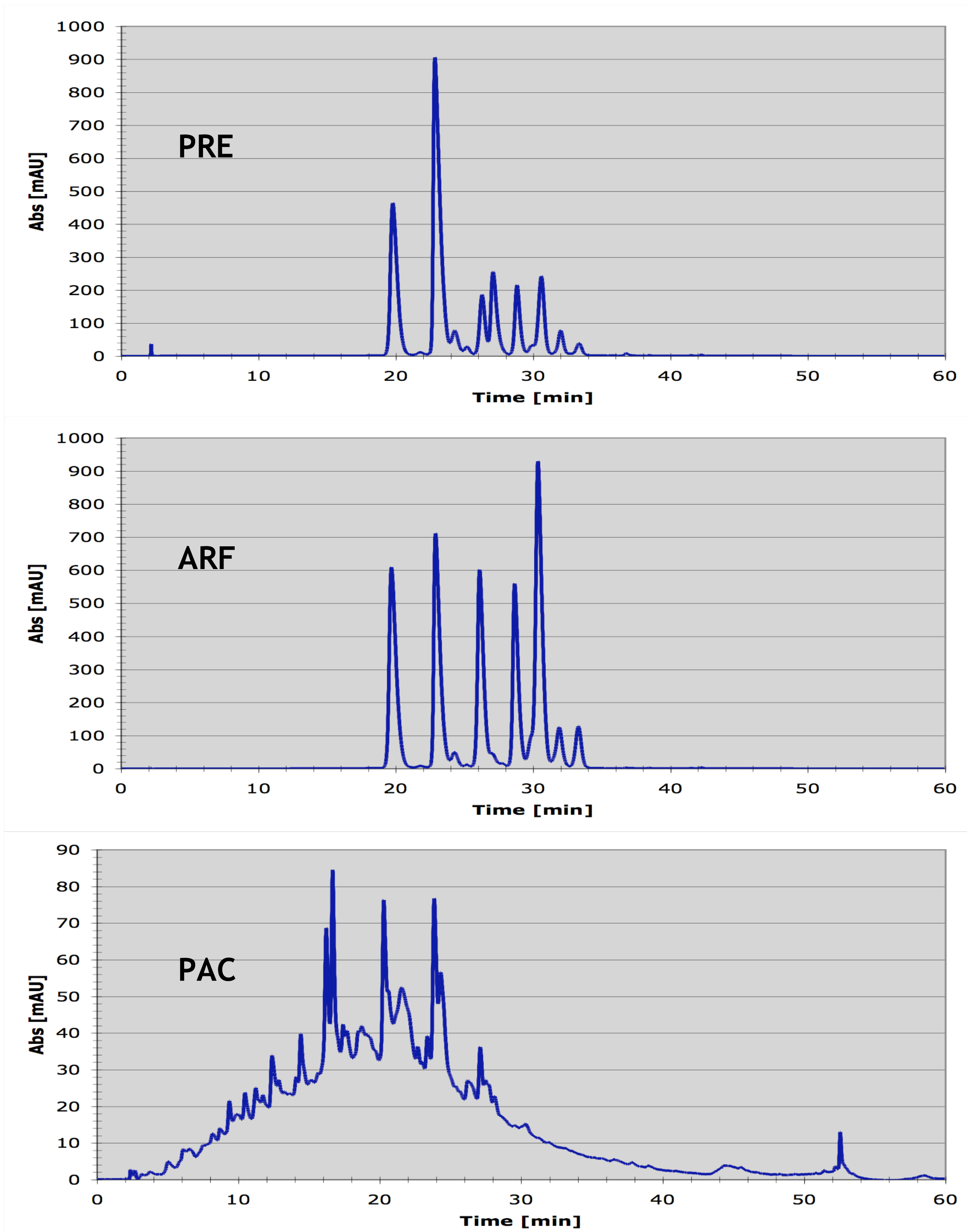
Akutan Berry Collection				
Date Collected	9/3/2008	9/3/2008	9/3/2008	September 2008
Scientific Name	*Vaccinium ovalifolium*	*Rubus spectabilis*	*Empetrum nigrum*	*Vaccinium uliginosum*
Common Name	High-bush blueberry	Salmonberry	Crowberry	Low-bush blueberry
Laboratory ID	VO-AK	RS-AK	EN-AK	VU-AK
Latitude	54.1° N	54.2° N	54.1° N	54.1° N
Longitude	165.8° W	165.8° W	165.8° W	165.8° W
Elevation	120’	261’	261’	261’
Herbarium	JK0005	JK0008	JK0006	JK0007
Accession # [1]	242790	242785	242791	242792
Amount	1L	2L	1L	2L
Habitat	Low scrubland	Low scrubland	Low scrubland	Low scrubland
Face	E	S	S	S
Temperature	57°F	60°F	60°F	60°F
Herbivory/Fungus	Insect/Low	none	none	none

[1] Herbarium samples submitted to Illinois Natural History Survey (http://ellipse.inhs.uiuc.edu:591/INHSCollections/plantsearch.html)
[2] EN-SD was obtained by community members post-trip; exact geographic and botanical data was not gathered, no herbarium voucher submitted.

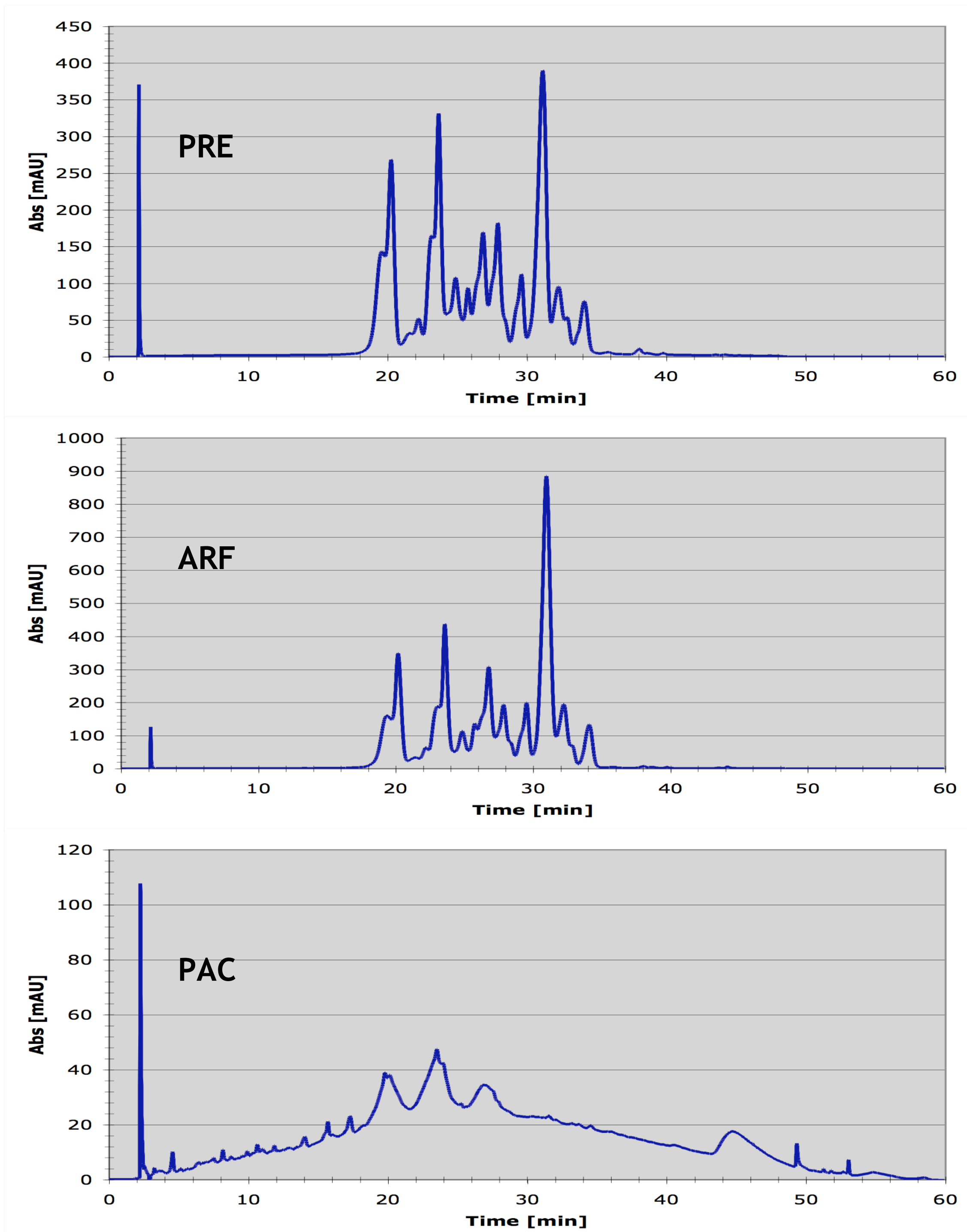
Appendix B: HPLC Chromatograms



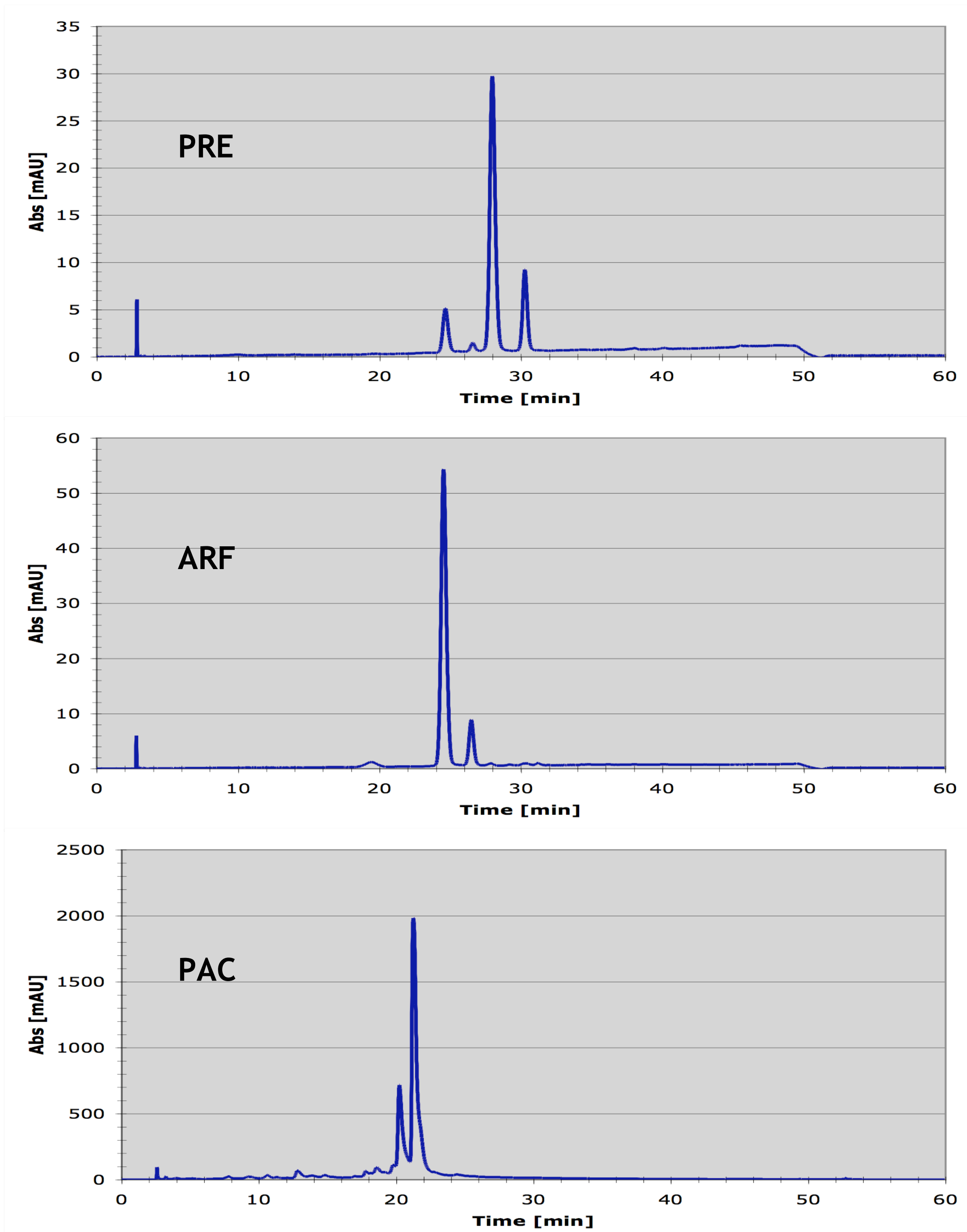
HPLC chromatograms of *E. nigrum* – Akutan; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm. PAC at 280nm.



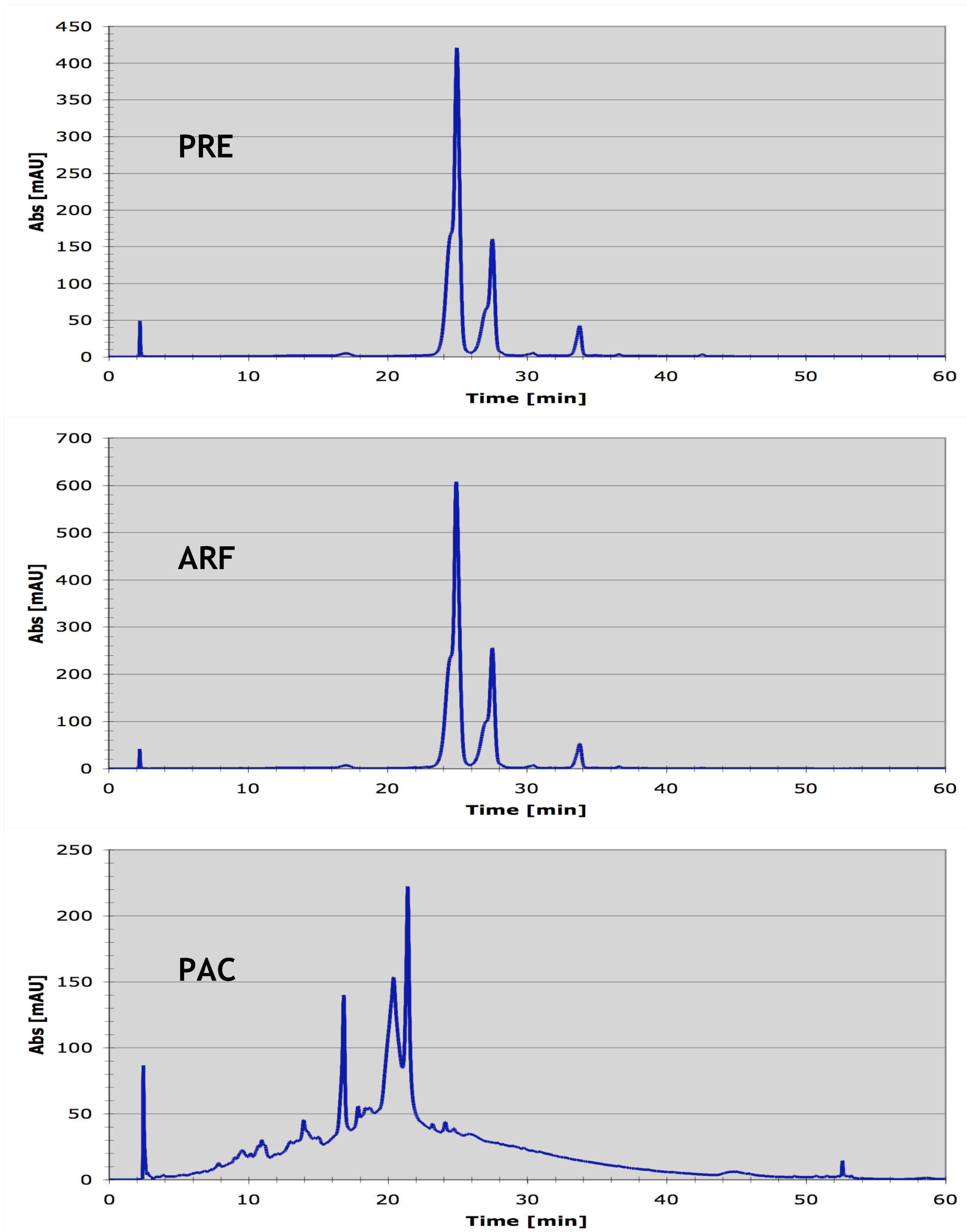
HPLC chromatograms of *E. nigrum* – Point Hope; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



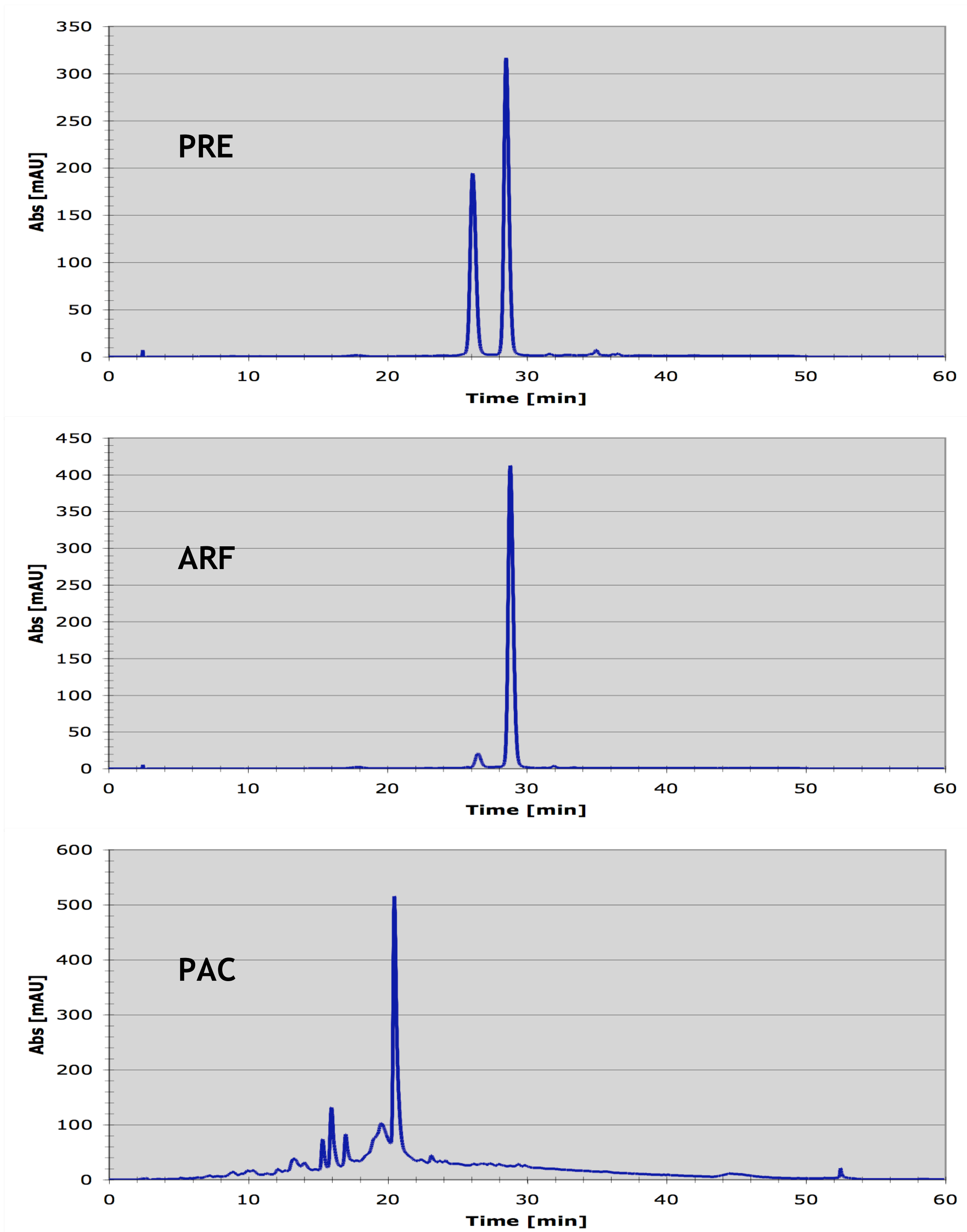
HPLC chromatograms of *E. nigrum* – Seldovia; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



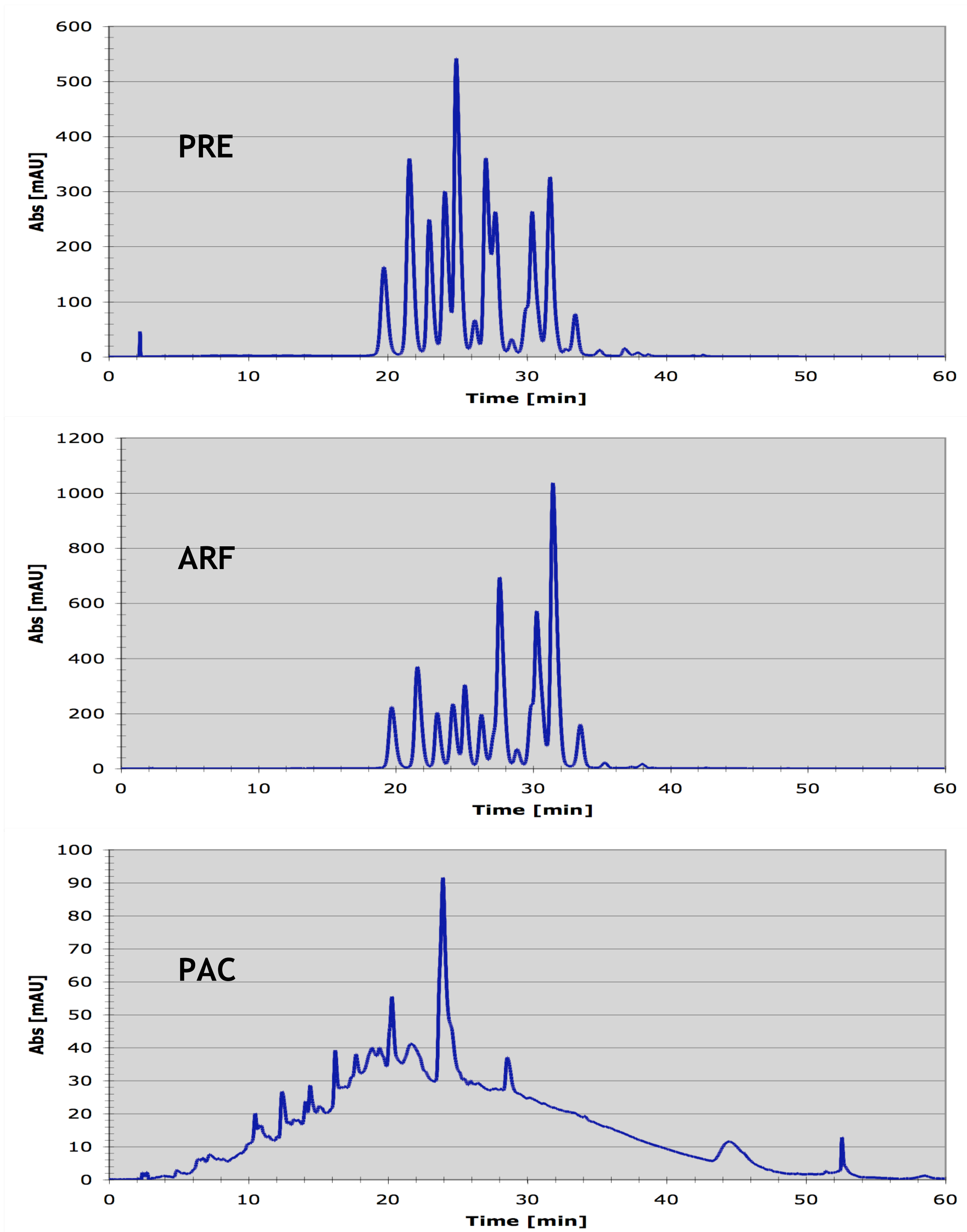
HPLC chromatograms of *R. chamaemorus* –Point Hope; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



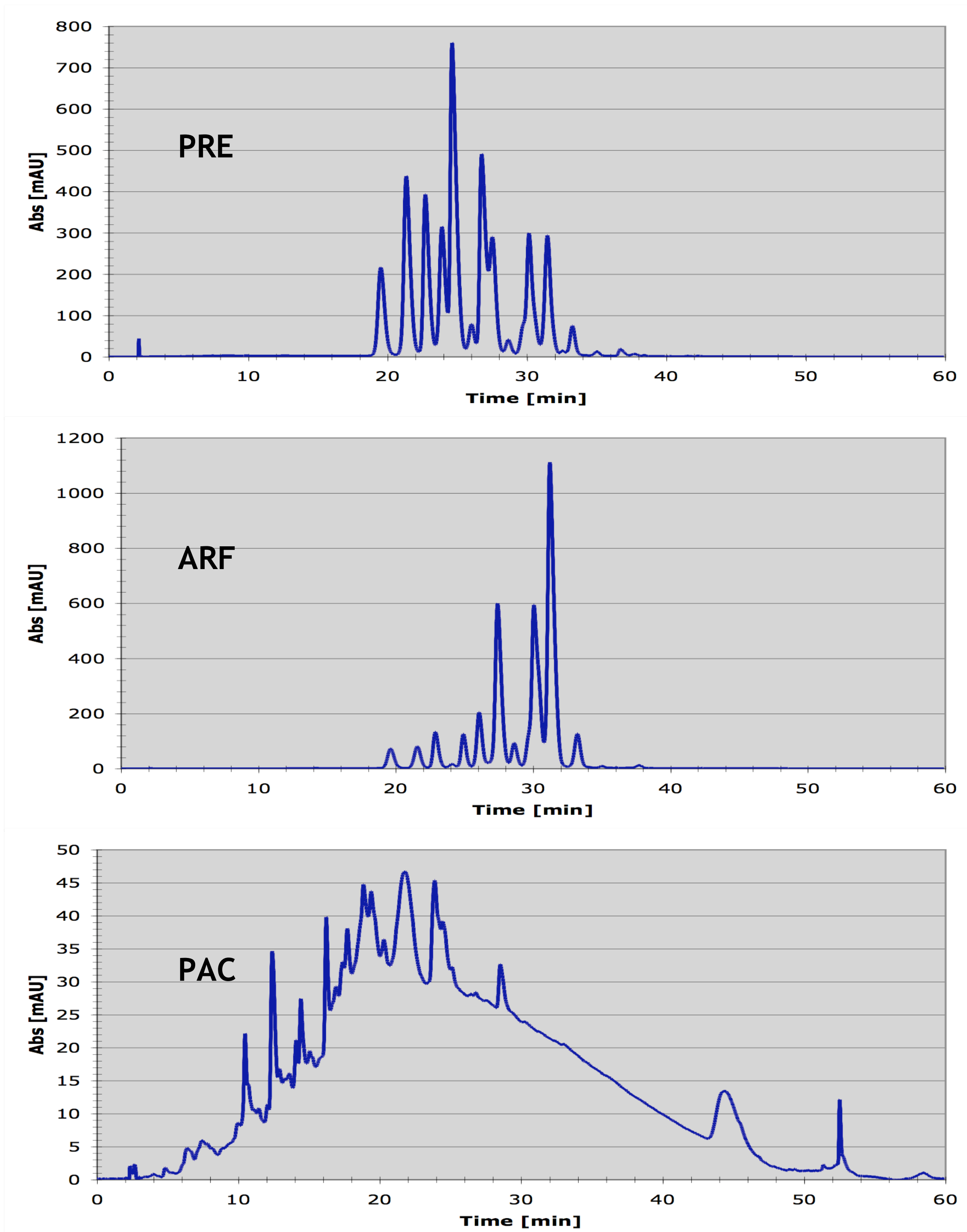
HPLC chromatograms of *R. spectabilis* –Akutan; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



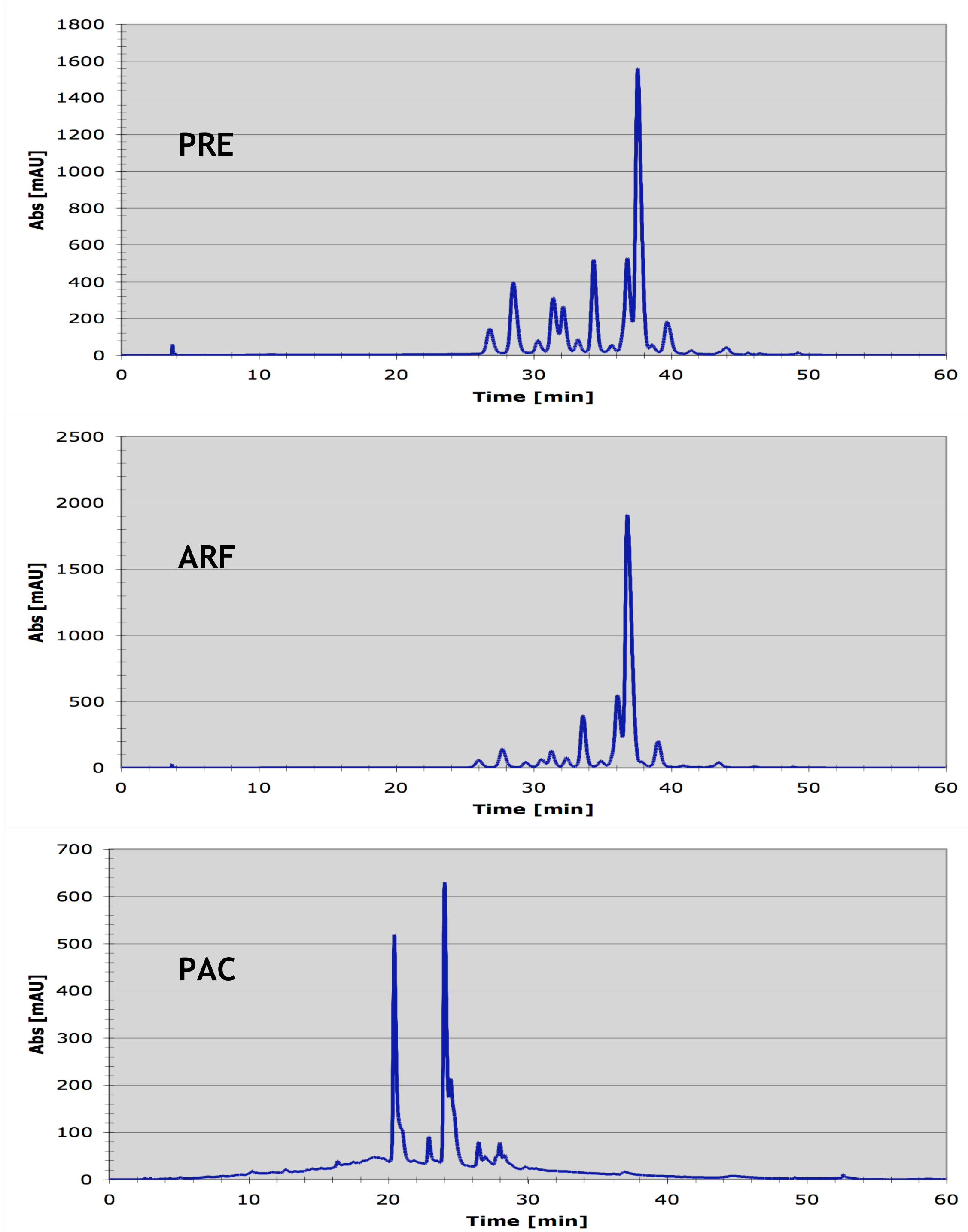
HPLC chromatograms of *R. spectabilis* –Seldovia; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



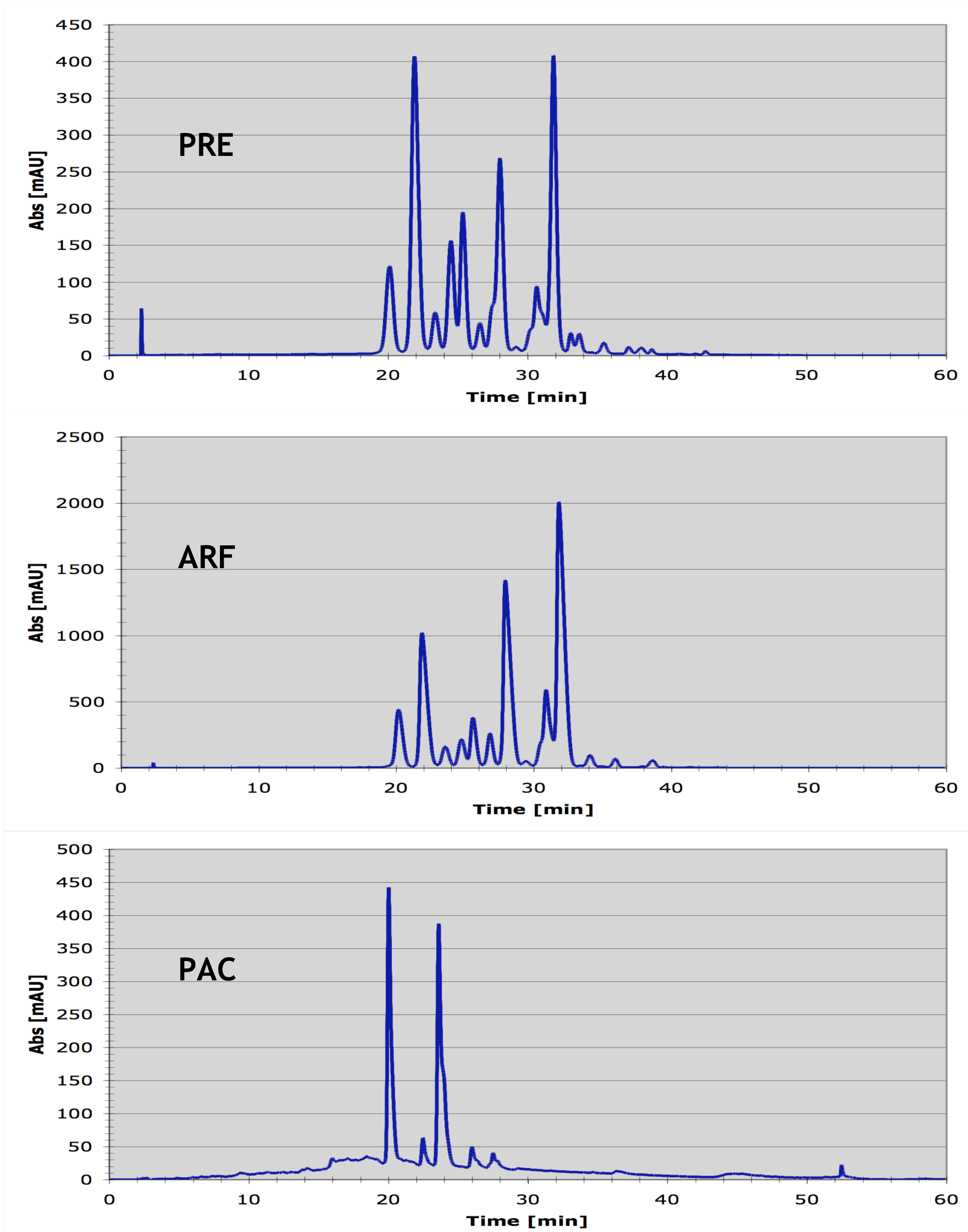
HPLC chromatograms of *V. ovalifolium* –Akutan; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



HPLC chromatograms of *V. ovalifolium* –Seldovia; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.

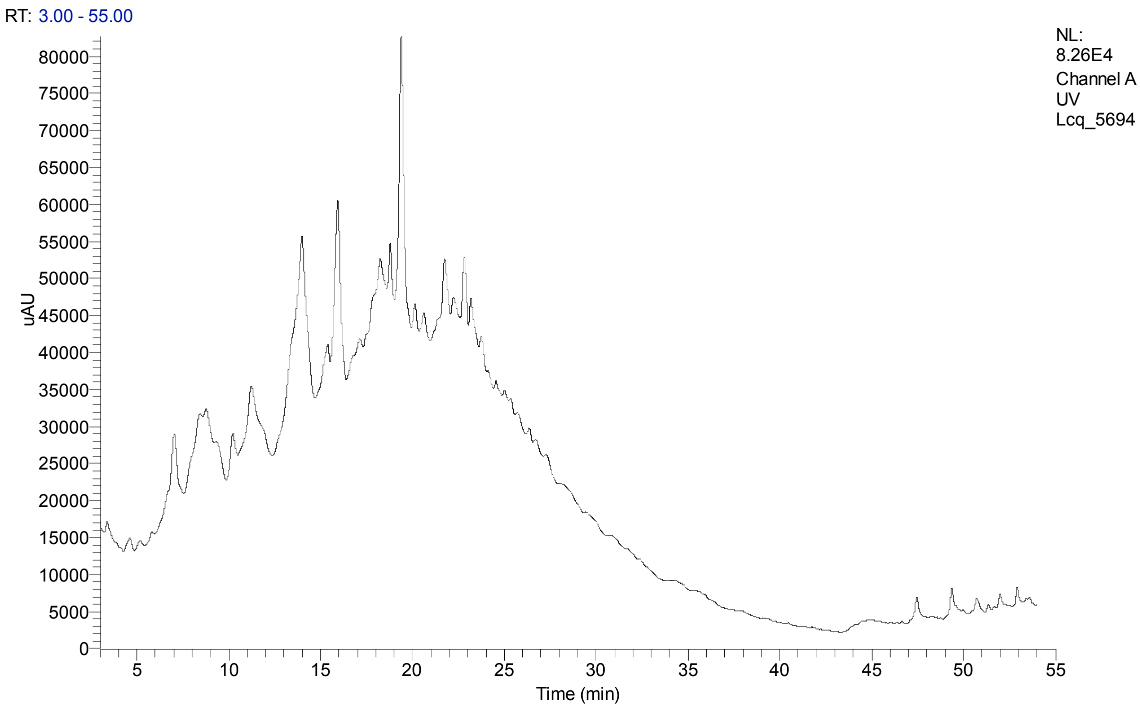


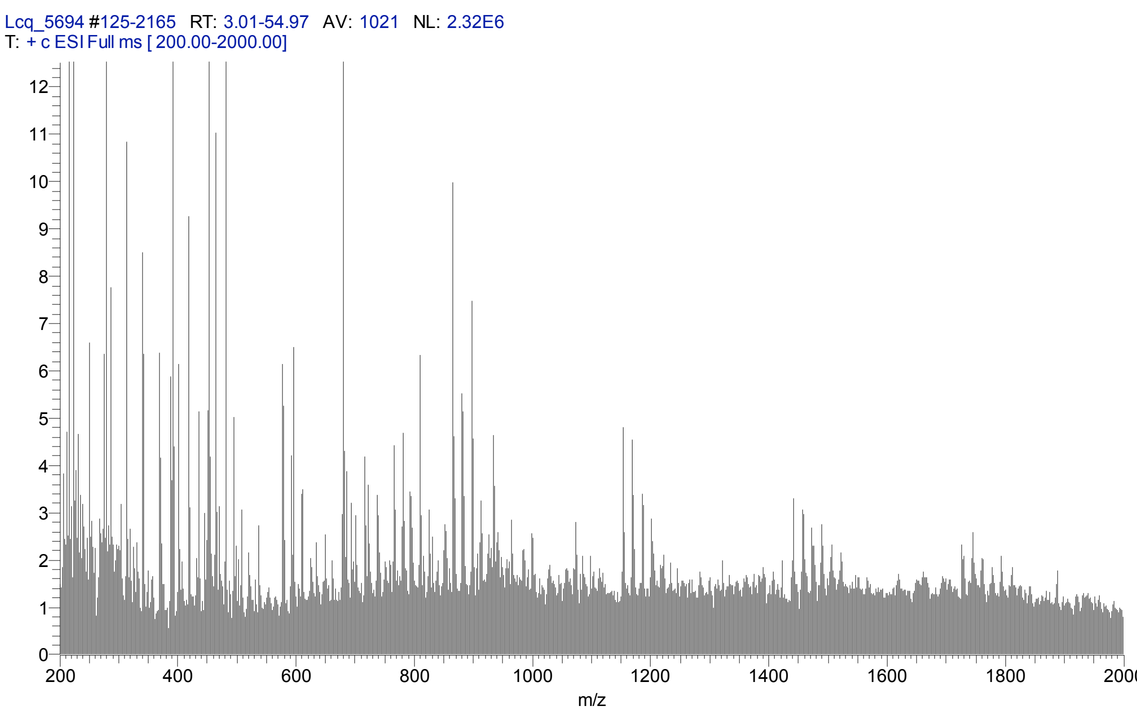
HPLC chromatograms of *V.uliginosum*–Akutan; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.



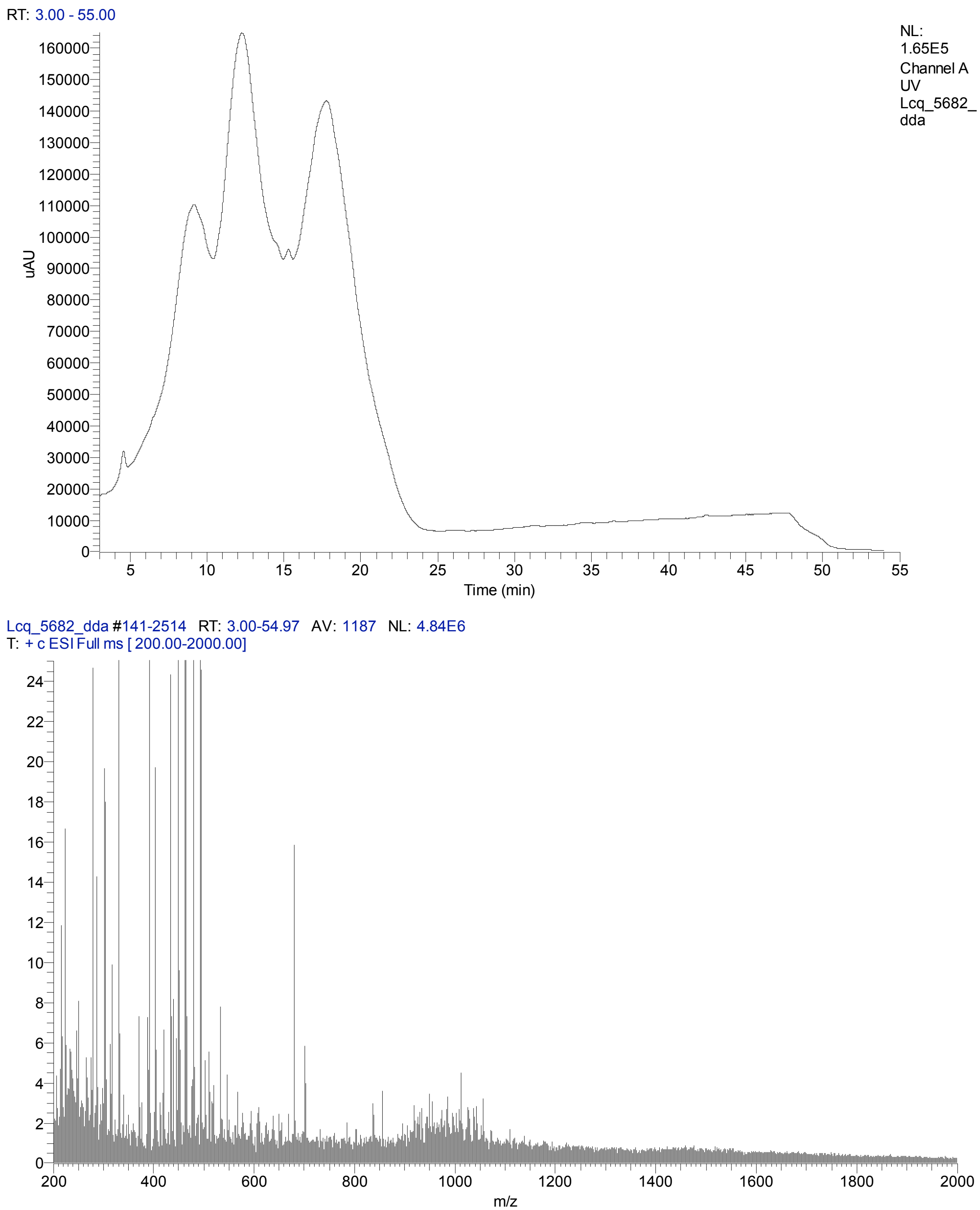
HPLC chromatograms of *V.uliginosum*–Point Hope; Phenolic-rich Extract (PRE), Anthocyanin-rich Fraction (ARF), Proanthocyanidin-rich Fraction (PAC). PRE and ARF run at 520nm, PAC at 280nm.

Appendix C: Mass Spectra

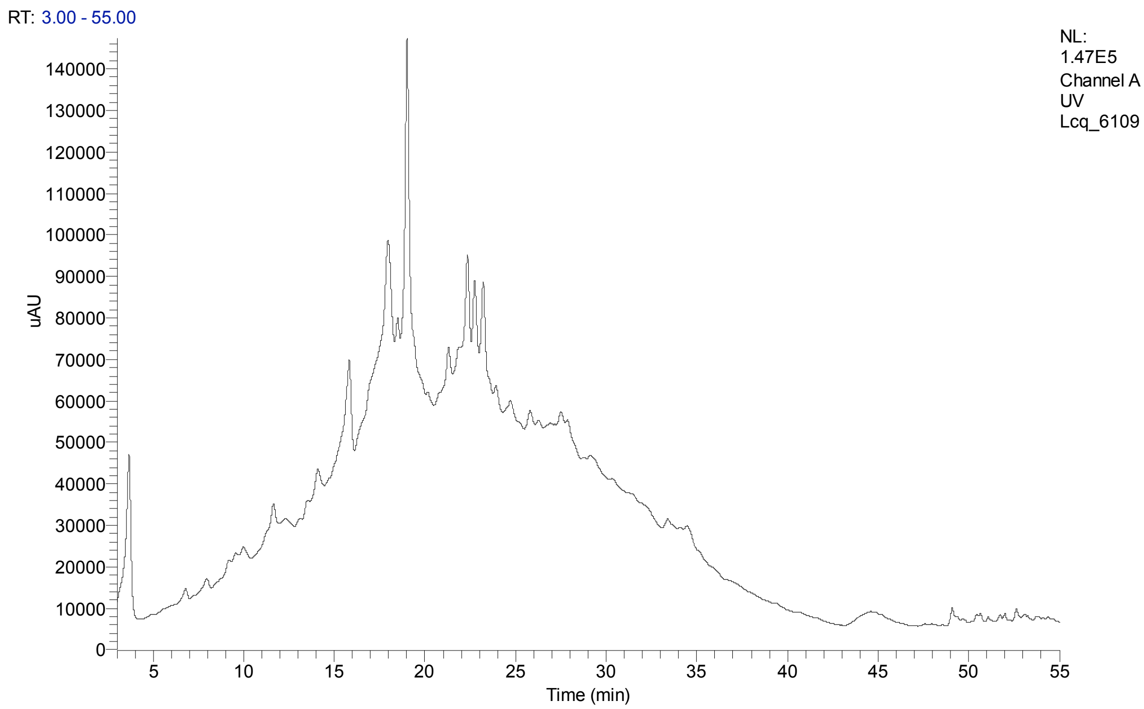


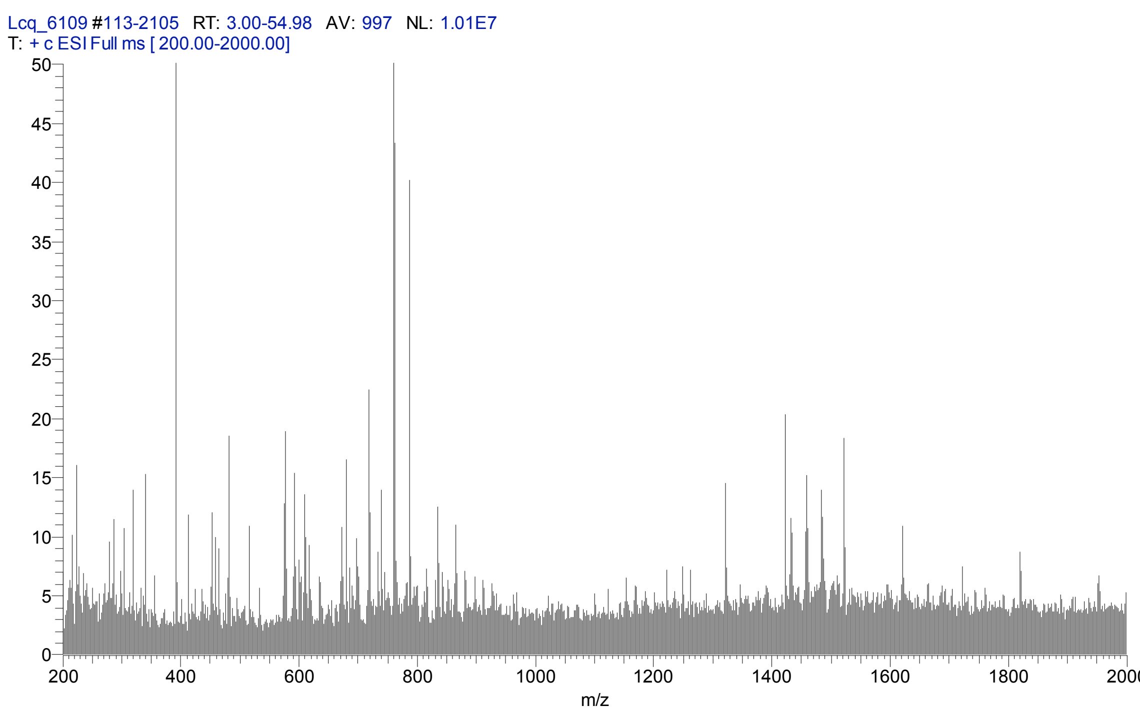


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *E. nigrum* – Akutan.

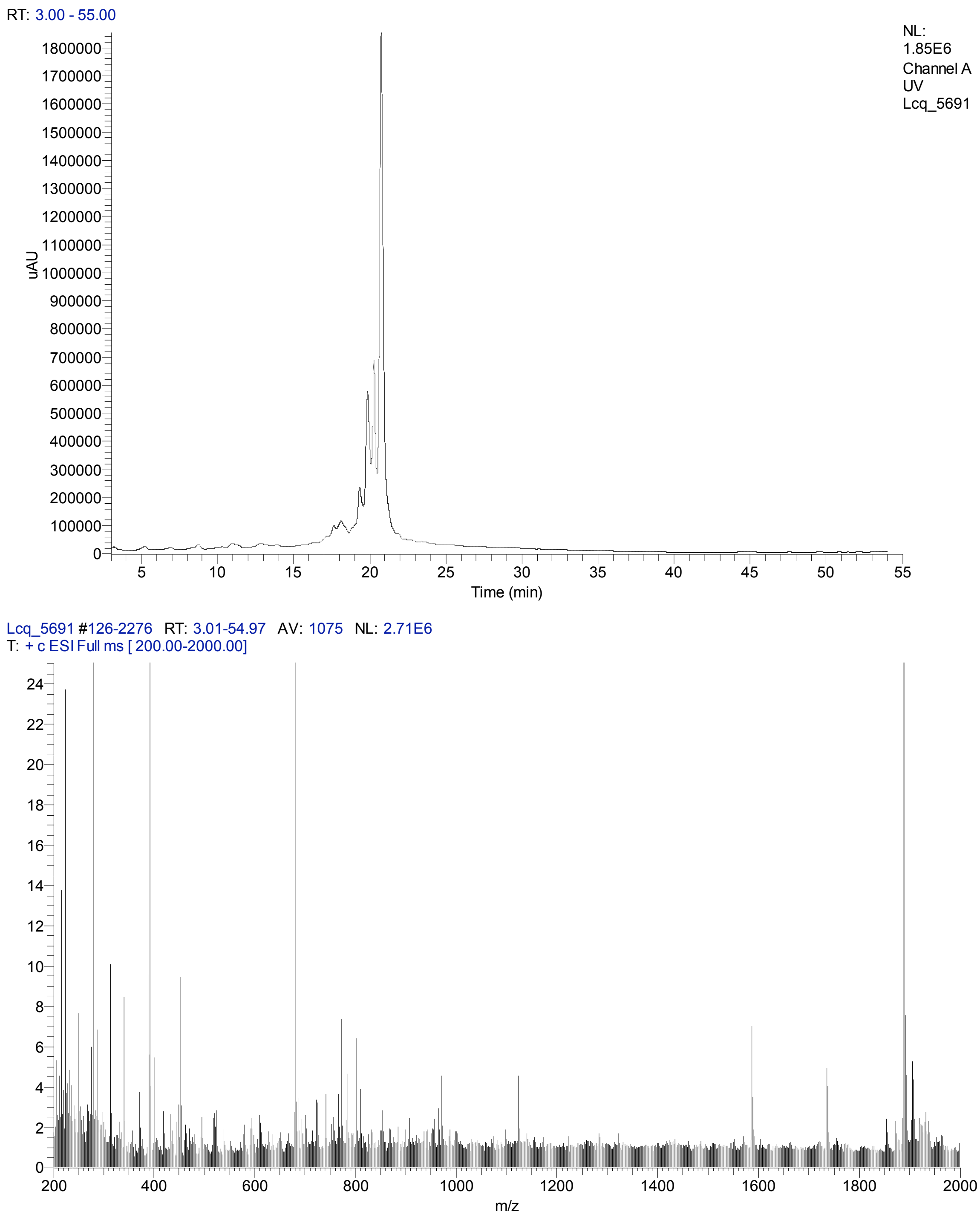


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *E. nigrum* – Point Hope.

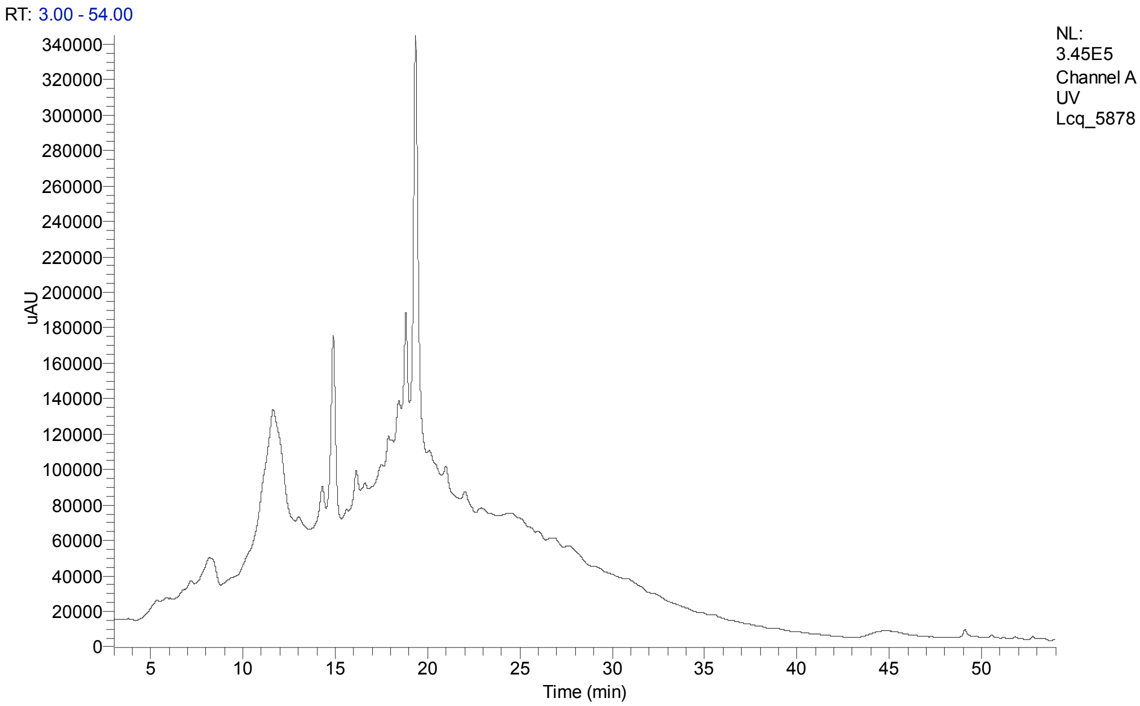


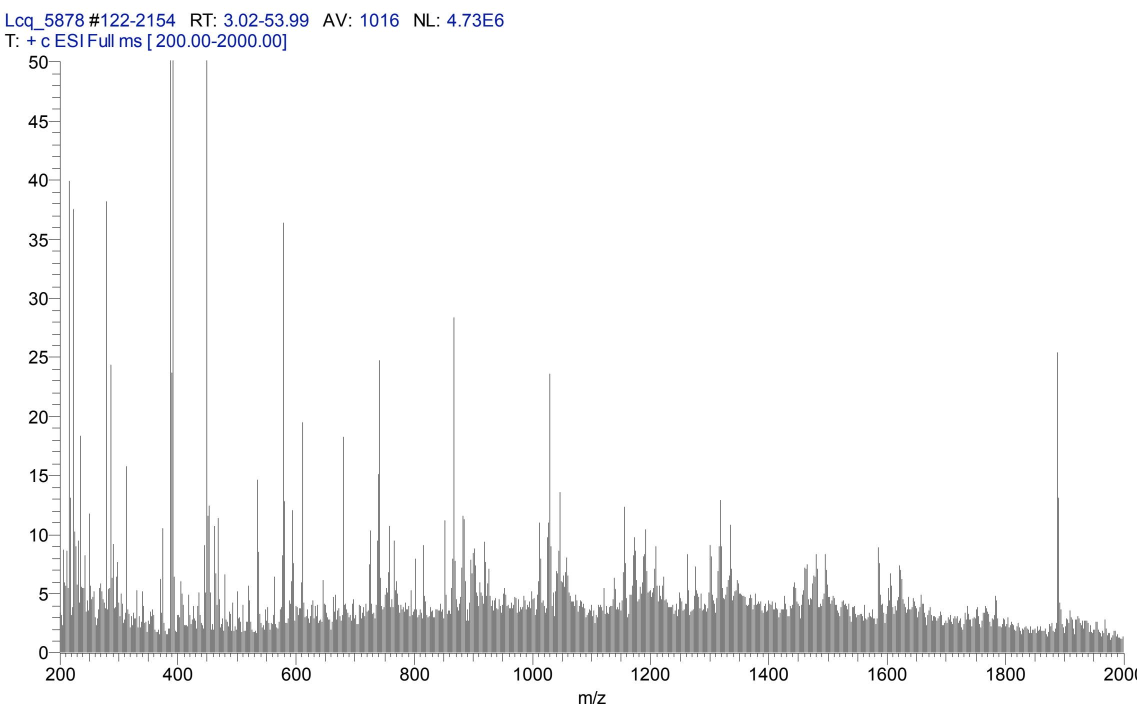


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *E. nigrum* – Seldovia.

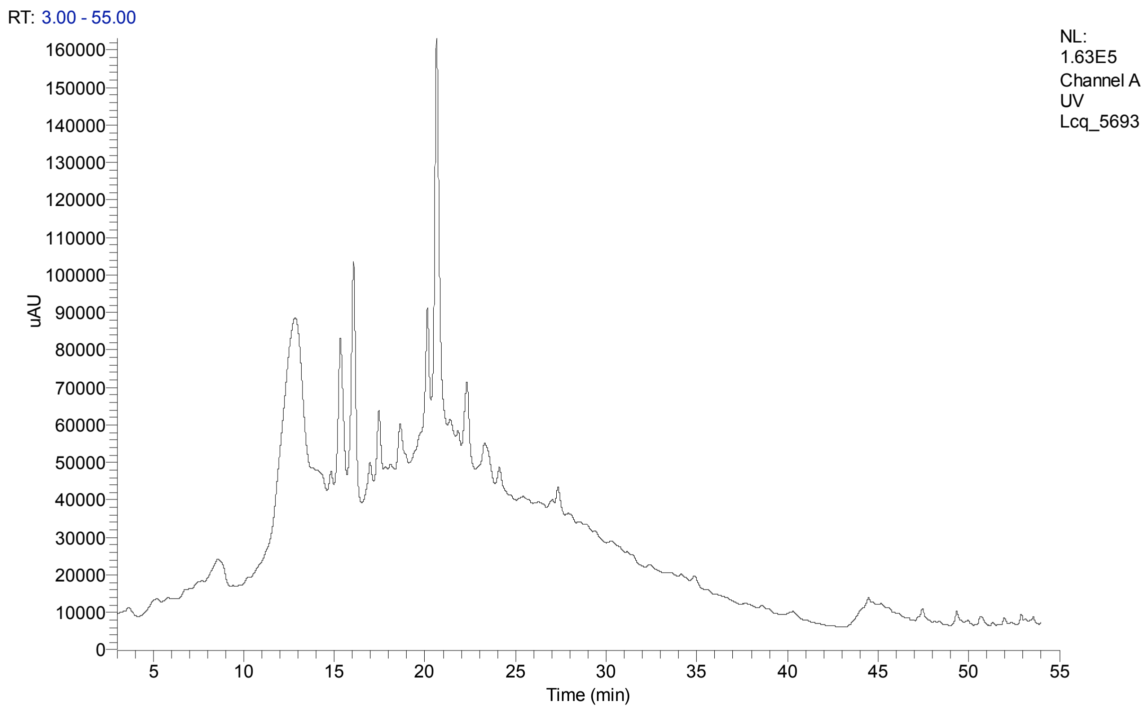


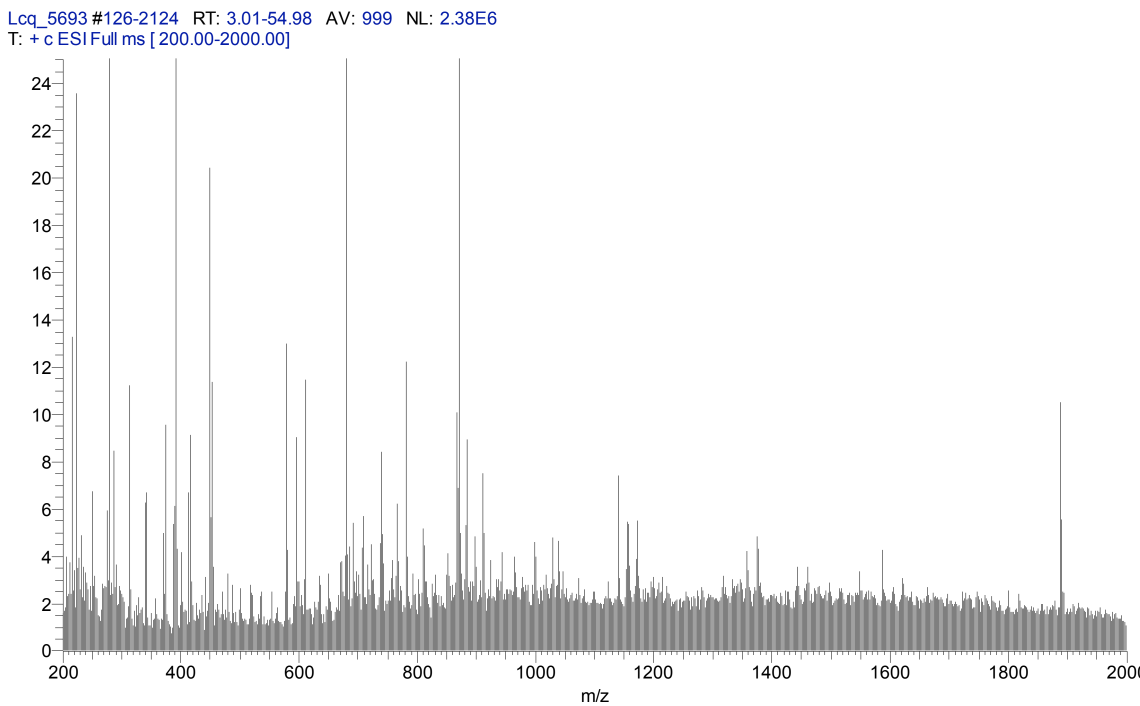
Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *R. chamaemorus* – Point Hope.



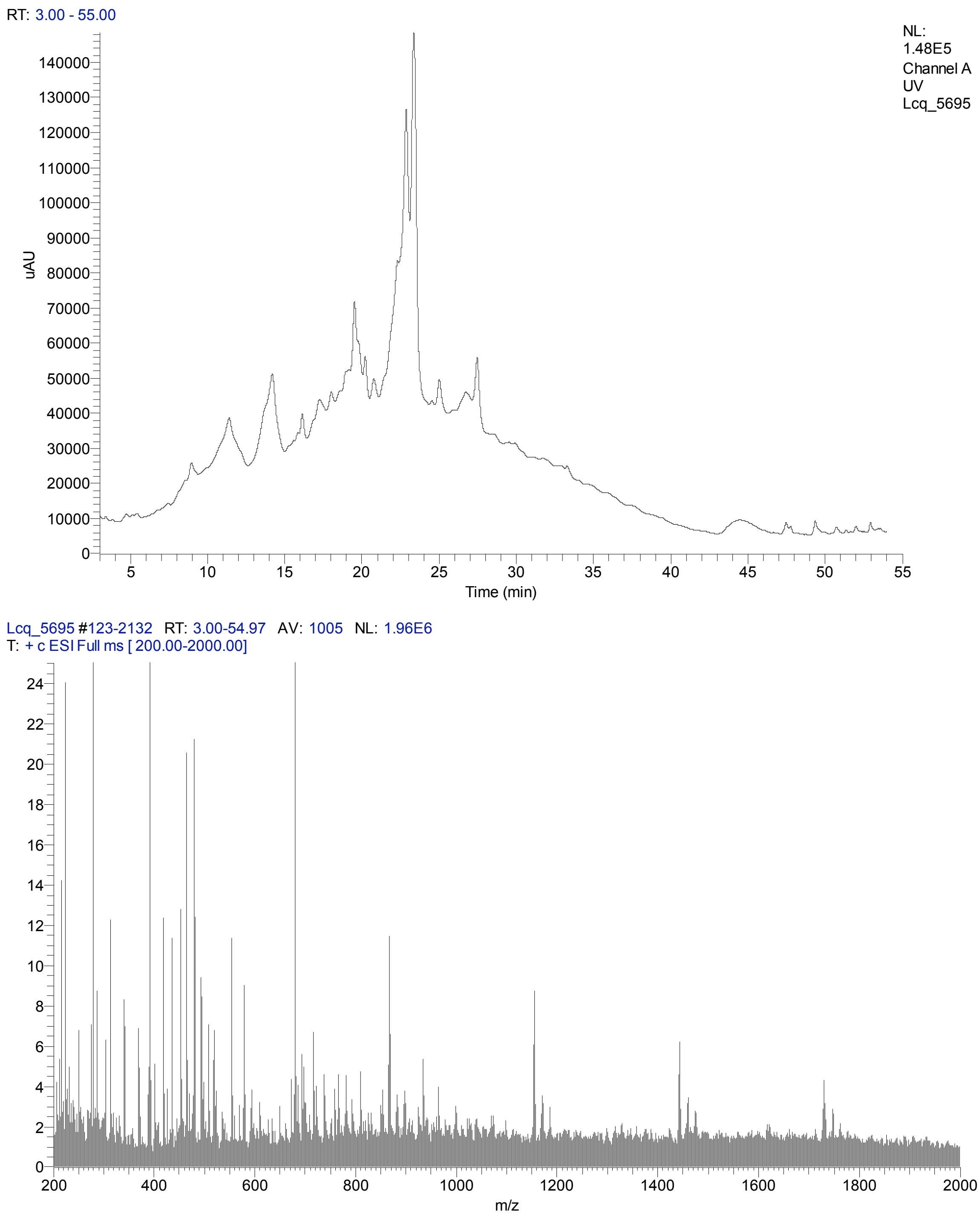


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *R. spectabilis* – Akutan.

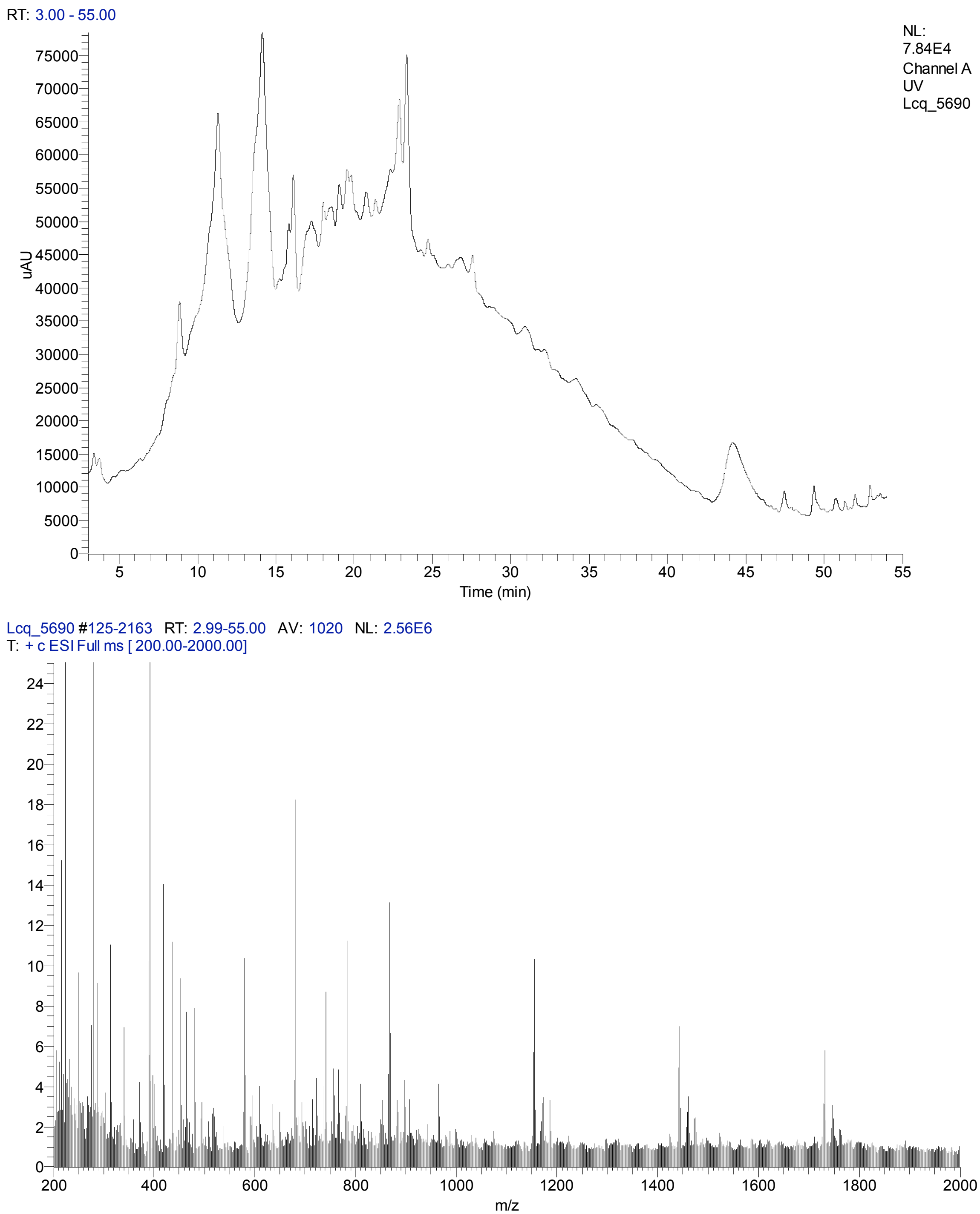




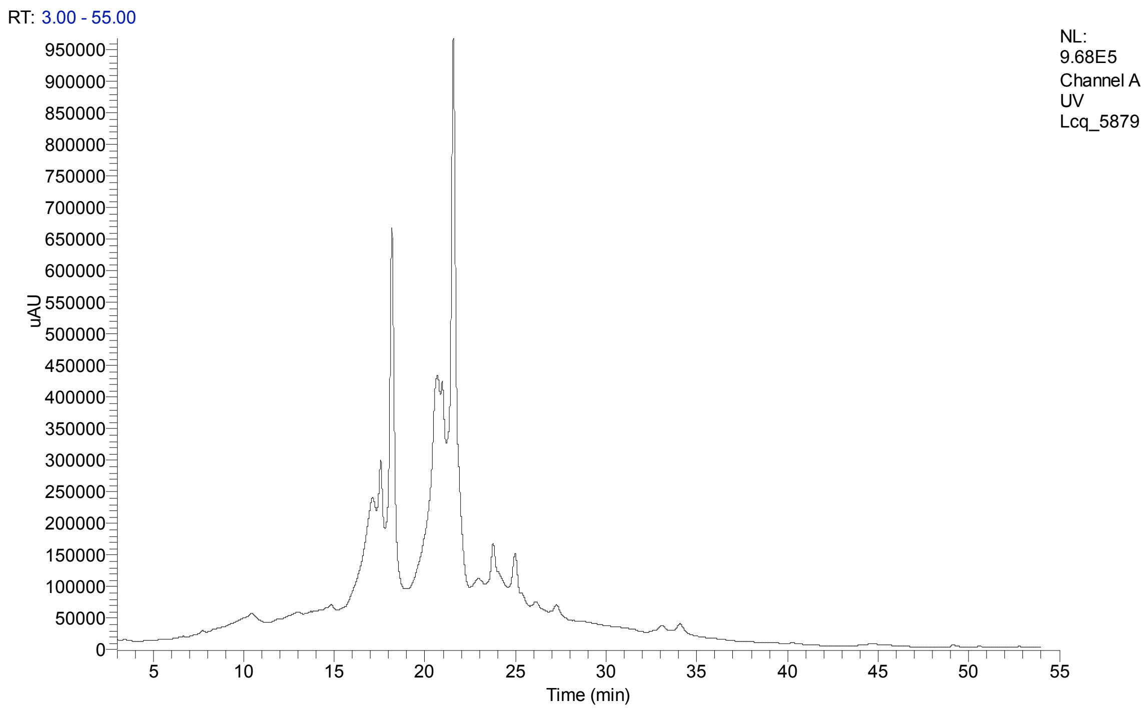
Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *R. spectabilis* – Seldovia.

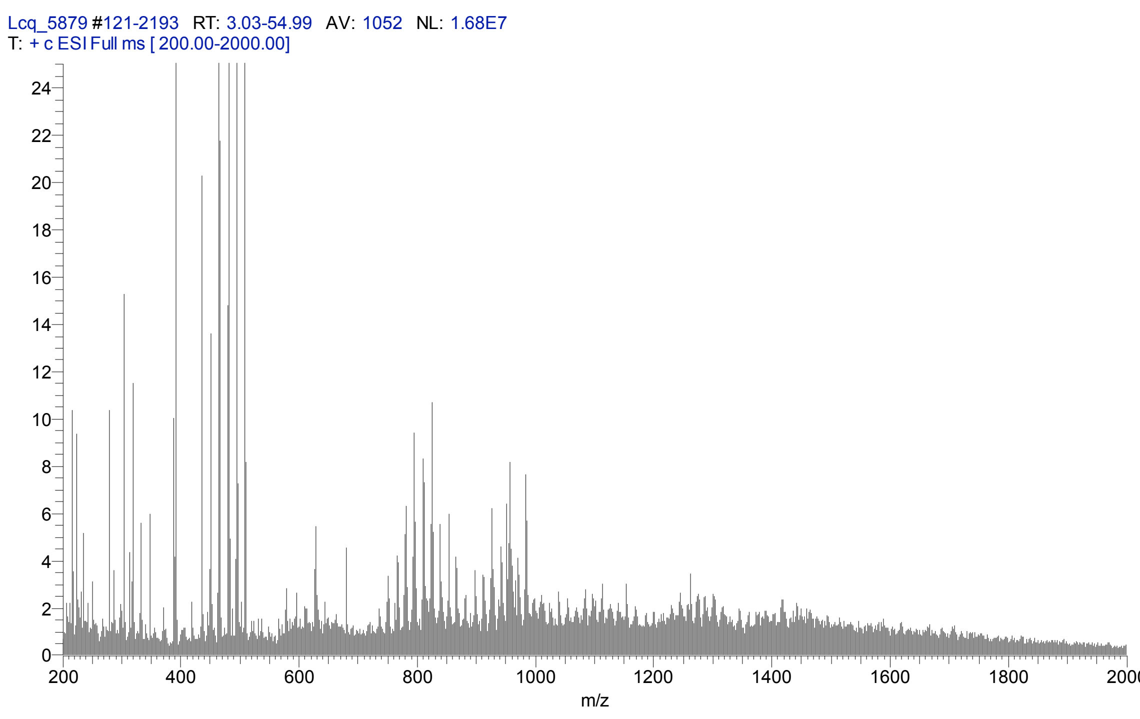


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *V. ovalifolium* – Akutan.

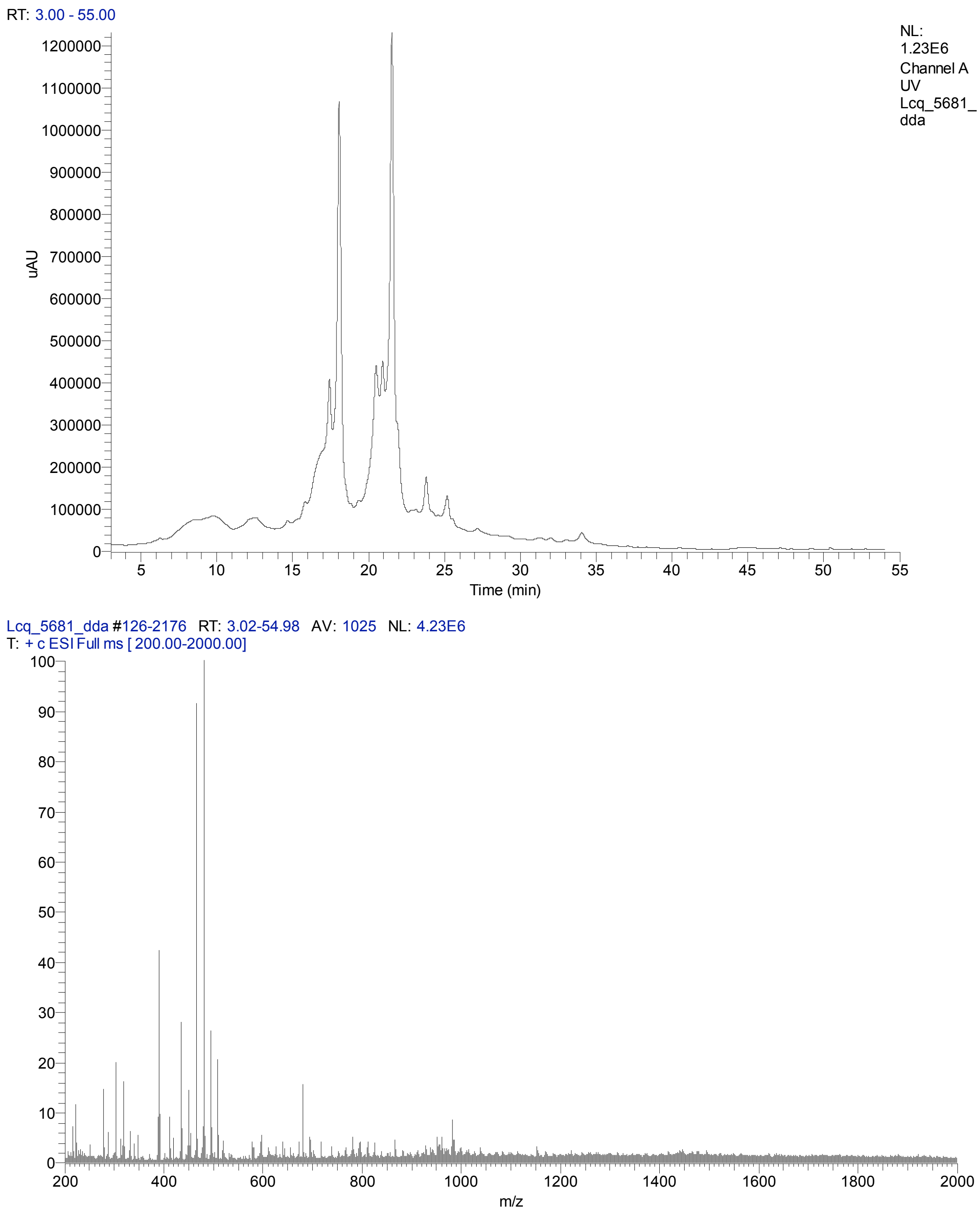


Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *V. ovalifolium* – Seldovia.





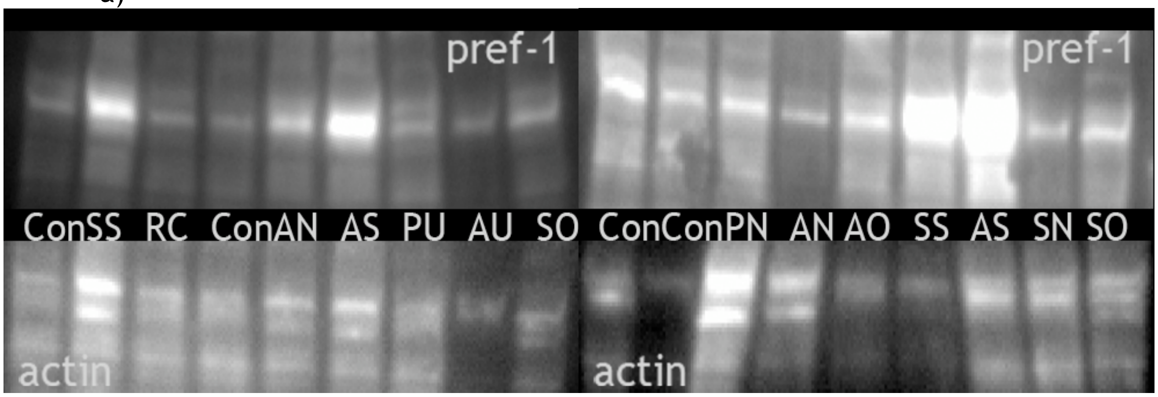
Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *V. uliginosum* – Akutan.



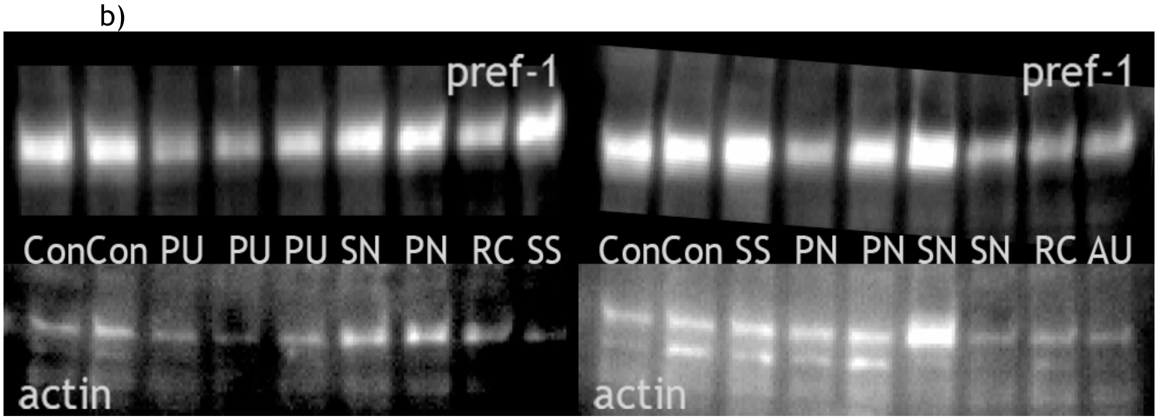
Graphs showing UV chromatograph (A) and MS spectra (B) of PAC fraction from *V. uliginosum* – Point Hope.

Appendix D: Western Blot Data

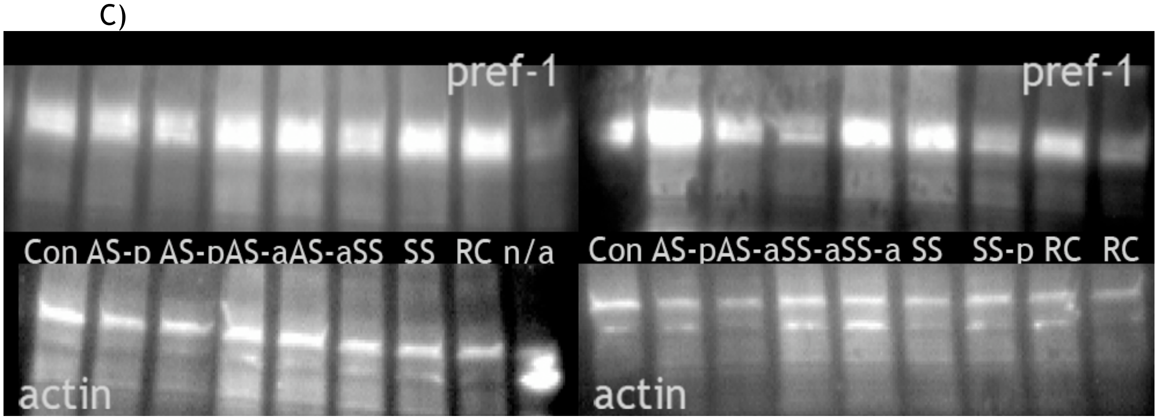
a)

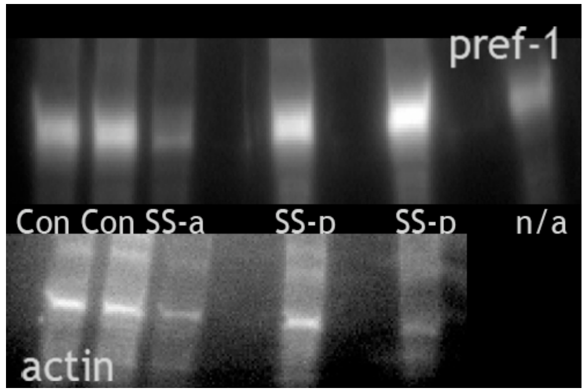


b)



C)





Abbreviations: Con = Control; AN = EN-AK; SN = EN-SD; PN = EN-PH; RC = RC-PH; AS = RS-AK; AS-a = RS-AK (ARF); AS-p = RS-AK (PAC); SS = RS-SD; SS-a = RS-SD (ARF); SS-p = RS-SD (PAC); AO = VO-AK; SO = VO-SD; AU = VU-AK; PU = VU-PH.

Sample[1]	**Actin/*pref-1* Ratio**			**Vs. Control**	**St Dev**
	6.29	6.34		100%	3.3%
Control[a]	6.71	6.68			
Control[b]	10.67	7.28		100%	13%
Control[b]	9.55	9.21			
Control[c]	2.78	3.25		100%	34%
Control[c]	4.28	2.89			
EN-AK[a]	7.07	6.94	5.66	101%	12%
EN-SD[b]	4.34	8.75	7.12	71%	34%
EN-PH[b]	9.18	6.14	7.24	83%	23%
RC-PH[c]	4.22	3.67	6.79	150%	36%
RS-AK[a]	12.80	11.69	10.89	182%	20%
RS-AK (ARF)[c]	3.34	5.38	4.57	143%	32%
RS-AK (PAC)[c]	3.32	5.54	4.10	139%	28%
RS-SD[c]	4.35	5.87	5.41	169%	17%
RS-SD (ARF)[c]	3.76	6.02	4.25	140%	26%
RS-SD (PAC)[c]	3.52	3.24	8.26	137%	24%
VO-AK[a]	7.20	9.96	8.46	132%	30%
VO-SD[a]	5.91	6.63	6.22	96%	7.8%
VU-AK[a]	3.60	3.01	3.44	51%	6.3%
VU-PH[b]	9.23	10.53	9.77	108%	10%

[1]-letter designates specific control used for calculations

Appendix E: Red Oil Data

Sample[1]	Absorbance			Avg Lipid Level	St Dev
Control[a]	0.333	0.336	0.270	100.00%	8.03
Control[b]	0.357	0.400	0.381	100.00%	10.1
Control[c]	0.205	0.199	0.201	100.00%	2.10
Control[d]	0.176	0.178	0.185	100.00%	9.87
EN-AK[d]	0.144	0.145	0.143	80.43%	0.39
EN-AK (PAC)[a]	0.268	0.291	0.278	87.62%	5.09
EN-AK (ARF)[a]	0.332	0.314	0.320	101.25%	3.99
EN-SD[c]	0.162	0.172	0.165	82.67%	3.50
EN-SD (PAC)[a]	0.275	0.289	0.281	88.40%	3.10
EN-SD (ARF)[a]	0.277	0.270	0.274	85.74%	1.55
EN-PH[b]	0.357	0.380	0.305	92.45%	4.20
RC-PH[a]	0.315	0.324	0.310	98.75%	2.31
RS-AK[d]	0.180	0.162	0.175	95.18%	7.08
RS-SD[b]	0.361	0.363	0.363	95.64%	1.41
VO-AK[b]	0.351	0.370	0.362	95.24%	3.54
VO-SD[c]	0.187	0.186	0.188	92.33%	0.37
VO-SD (PAC)[b]	0.276	0.326	0.302	79.52%	9.34
VO-SD (ARF)[a]	0.345	0.293	0.319	100.00%	11.53
VU-AK[d]	0.189	0.173	0.178	100.74%	6.29
VU-PH[c]	0.187	0.176	0.181	89.85%	3.85
VU-PH (PAC)[a]	0.288	0.308	0.299	93.42%	4.43
VU-PH (ARF)[b]	0.382	0.374	0.377	99.87%	1.49

[1]-letter designates specific control used for calculations

Curriculum Vitae

RESEARCH INTERESTS

My research interests center around the discovery and development of novel botanical therapeutics in the form of drugs, nutraceuticals, or functional foods. My interests are focused on a variety of afflictions, including metabolic disorders as well as infectious agents. The discovery process combines field collection and preliminary screening with more detailed structural and biological laboratory analysis. It has an applied component as it addresses these research questions in a participatory research model, a collaborative effort with local peoples around the world, whose intimate knowledge of plants, their ethnobotanical uses and effectiveness is key in developing novel pharmaceuticals. By forging partnerships with local people, this approach creates a unique synergistic research relationship, in which their traditional ecological knowledge is supported by modern science while promoting sustainable and equitable scientific practices.

Based upon the traditional ecological knowledge of indigenous people and the results of field assays, target plants are subjected to in-depth analysis in the laboratory. Natural product research is conducted using modern chemical structural identification technology such as HPLC, LC/MS, and NMR, which allows for the isolation and accurate characterization of phytochemical compounds of interest. This chemistry work is coupled with pharmaceutical biology assays, principally cellular and animal studies, to establish mechanisms of action to finely detail the interactions between secondary metabolites and organisms.

EDUCATION

University of Illinois, Urbana-Champaign

Master's of Science
Thesis Project: "Bioexploration of Wild Alaskan Berries: From Field Screening to Functional Fruit"
Thesis Advisor: Dr. Mary Ann Lila
GPA: 3.9/4.0

University of California, Berkeley

Bachelor's of Science (with Honors), 2004

Major: Chemistry
GPA: 3.6/4.0
Additional Education

ACS Synthetic Organic Chemistry: Modern Methods and Strategies seminar 2006
Mercury Porosimetry Analysis Certification 2005

AWARDS

Recipient, James Baldwin Turner Fellowship 2007 - 2008

Recipient, AYRE Travel Fellowship 2007

Recipient, California Masonic Scholarship 2000 – 2003

EMPLOYMENT HISTORY

Bio-Rad Labs Inc. October 2004 – August 2007

Chemist II

Worked to optimize polymerization and functionalization conditions for chromatographic products. Utilized multiple testing procedures to characterize and evaluate batch performance, including protein separation chromatography, mercury porosimetry, scanning electron microscopy (SEM), and particle size distribution analysis. Completed research for new protein separation chromatography resins, including the polymerization of a base matrix, functionalization to an active moiety, and subsequent bio-conjugation with various bioorganic ligands. Utilized Design of Experiment techniques and software to optimize coupling conditions, worked with various proteins and evaluation methods to analyze product. In addition, created tabular and graphical representation of results, and worked to interpret these results in context of project goals.

College of Chemistry, University of California at Berkeley Summer 2001, 2002

Laboratory Researcher

In Professor John Arnold's organometallic chemistry laboratory, assisted graduate students in research as well as conducted individual chemical research. Responsible for setting up and running specific air- and moisture-sensitive reactions that formed the groundwork for other research projects. Also worked on an individual research project, which involved designing and executing various synthetic reactions. Organic and organometallic synthetic methods were used to achieve desired molecule, and worked with various structural elucidation techniques to determine final target and presented the products and results of these investigations.

PRESENTATIONS/PUBLICATIONS

Kellogg, Joshua, Wang, Jinzhi, Flint, Courtney, Ribnicky, David, Kuhn, Peter, de Mejia, Elvira Gonzales, Raskin, Ilya, Lila, Mary Ann. Alaskan wild berry resources and human health under the cloud of climate change. J Ag Food Chem (In press 12-2009)

Kellogg, Joshua, Yousef, Gad G., Grace, Mary H., Flint, Courtney, Raskin, Ilya, Lila, Mary Ann (2009) Partnering with Alaskan communities to examine health benefits of traditional wild berries. Poster presented at the Experimental Biology Conference.

Kellogg, Joshua (2006) UNOsphere Characterization of porous polymers by mercury porosimetry and scanning electron microscopy. Poster presented at the Bio-Rad Laboratories Science Exchange Poster Session

Wang, Danni, Kellogg, J (2006) UNOsphere-Based Protein A Resins: Feasibility Studies. Poster presented at the *Bio*-Rad Laboratories Science Exchange Poster Session.

EXPERIENCE

Academic Tutor, University of Illinois Irwin Academic Services Center, 2008-2009

Instructor, Global Institute of BioExploration (GIBEX) – UTTC Training Seminar, North Dakota, July 2009

Instructor, EPASTAR Berry Seminars, Alaska, July-September 2008

Assistant Instructor, Global Institute of BioExploration (GIBEX) – Maquipucuna Field Training Seminar, Ecuador, January 2008

TECHNICAL AND SPECIALIZED SKILLS

Chemical Structural Techniques:

Nuclear Magnetic Resonance (NMR)
Ultraviolet-Visible Spectroscopy (UV-VIS)
Mass Spectrometry (MS)
High-Performance Liquid Chromatography (HPLC)
Scanning Electron Microscopy (SEM)

Biological Techniques:

Animal Cell Culturing
Gel electrophoresis / Western Blot Analysis

Research Software:

Design of Experiment methodology (UMetrics MODDE)
Statistical Analysis (SAS)
Geographical Informational Systems (Arc-GIS)
Biodiversity and Collection Database (NAPIS)

LANGUAGES

French: Fluent in spoken, written

Spanish: Proficient in spoken, written