Alkylresorcinol in *Portulaca Oleracea* (Purslane) Seeds as a New Source of Dietary Phenolic Lipid

by

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in

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ABSTRACT

As individuals in today’s society become more informed and considerate about what they consume, the food industry is shifting towards identifying and incorporating natural, healthy and safe ingredients in manufactured foods. This study identified, quantified and optimized extraction yields of alkylresorcinol from seeds of the *Portulaca oleracea* (purslane) plant. Supercritical fluid extraction using CO$_2$ and 10% ethanol as a co-solvent yielded the highest amount of total alkylresorcinols at 577.9 mg/100 g of dry material. Oxygen radical absorbance capacity, total phenolic content and 2,2 diphenyl-1-picrylhydrazyl inhibition assays were performed to assess the antioxidant capacity of purslane seeds resulting in values of 136.1 ± 8.72 μmol TE/g, 7.67 ± 0.64 mg GAE/g and 14.5 ± 1.02 %, respectively. Purslane’s aptitude to contribute to oxidative stability of margarine evaluated with Rancimat resulted in an increase of induction time from a baseline 1.18h to 2.00h. In a separate study, alkylresorcinol homologues C15, C17, C19, C21, C23 and C25 demonstrated high ability to delay the onset of rancidification in margarine to a range of 10.98 - 12.80h, with C15 performing best amongst the six homologues. Alkylresorcinols were shown to possess the ability to incorporate within and permeate through a polycarbonate membrane, similar to a biological membrane. Homologue C17 exhibited the highest percent at 23.2% and C19 exhibited the highest permeability at 95.8%. Scanning electron microscopy revealed the formation of crystal structures in margarine and chocolate as a result of alkylresorcinol and purslane seed extract. The findings in this study present results that confer alkylresorcinol and purslane seeds as valuable compounds that may be used as a nutraceutical, natural preservative and source of crystalline structure in the enhancement of manufactured food.
ACKNOWLEDGEMENTS

This project is a function of my research in synergy with my collaborators, professors, educators, peers, guiding figures and mentors.

My first mention is dedicated to my academic supervisor, Dr. Farah Hosseinian, for her unwavering support, patience and guidance throughout my research. I am privileged to have had the opportunity to work with someone who inspires and empowers me to work past adversity and my perceived limitations. Her innovative approach to research is the most valuable knowledge I have learned and I will continue to carry this with me forward in order to apply what I have studied in novel and interesting ways.

My work could not have been completed without the generous knowledge and equipment that was made available to me by Dr. Owen Rowland and Dr. Ian Pulsifier. Their expertise and guidance allowed me to perform in the lab meticulously. Dr. Jianqun Wang’s knowledge and skill along with the help of Hengguang Xu are accredited to the detailed microscopy photos I was able to take. A thank you is also due to Dr. Aynur Gunenc, who has not only helped me but served as an example of academic excellence in our lab. As well, I express my appreciation to Dr. Richard Nahas, who encourages me to perform at my maximum potential and gives me the tools and insights to reach these new heights. A special thank you is also dedicated towards Ms. Chantelle Gravelle. She made my first and every consecutive experience at Carleton University a warm, supportive and welcoming one - this kindness does not go unnoticed.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AF</td>
<td>2-aminofluorene</td>
</tr>
<tr>
<td>AAPH</td>
<td>2’2’-axobis(2-methylpropionamidine)dihydrochloride</td>
</tr>
<tr>
<td>ABPR</td>
<td>Automated back pressure regulator</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-linolenic acid</td>
</tr>
<tr>
<td>AR</td>
<td>Alkylresorcinol</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>B[a]P</td>
<td>Benzo[a]pyrene</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>BSTFA</td>
<td>N,O-Bistrifluoracetamide</td>
</tr>
<tr>
<td>CL</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>DDPH</td>
<td>2,2-diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Double distilled water</td>
</tr>
<tr>
<td>DM</td>
<td>Dry material</td>
</tr>
<tr>
<td>DRC</td>
<td>Daunorubicin</td>
</tr>
<tr>
<td>ERK2</td>
<td>Extracellular signal-regulated protein kinase 2</td>
</tr>
<tr>
<td>FC</td>
<td>Folin-Ciocalteu reagent</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally recognized as safe</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>MMS</td>
<td>Methyl methanesulfonate</td>
</tr>
<tr>
<td>ORAC</td>
<td>Oxygen radical absorbance capacity</td>
</tr>
<tr>
<td>PA</td>
<td>Purslane</td>
</tr>
<tr>
<td>PG</td>
<td>Propyl gallate</td>
</tr>
<tr>
<td>RPM</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>SC-CO₂</td>
<td>Supercritical carbon dioxide</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SFE</td>
<td>Supercritical fluid extraction</td>
</tr>
<tr>
<td>TB</td>
<td>Triticale bran</td>
</tr>
<tr>
<td>TBHQ</td>
<td>Tert-butyl hydroquinone</td>
</tr>
<tr>
<td>TE</td>
<td>Trolox equivalent</td>
</tr>
<tr>
<td>TPC</td>
<td>Total phenolics content</td>
</tr>
<tr>
<td>Trolox</td>
<td>6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION

1.1 *Portulaca oleracea*

1.1.1 A Brief Overview

As a descendent of Portulacaceae family, the *Portulaca oleracea* L. species (Ocampo & Columbus, 2012) is noted for its use in traditional medicine as well as an edible source of nutrients (Naeem & Khan, 2013). Wild purslane is comprised of smooth and fleshy small leaves in alternate formation on a cylindrical stem contained close to the ground (Figure 1.1) (Egea-Gilabert, Ruiz-Hernández, Parra, & Fernández, 2014).

![Figure 1.1 Portulaca oleracea L. steam and leaves (A) and seeds within pods (B)](image)

As one of the most prevalent weeds in the world, purslane is specifically deemed as a cosmopolitan weed characterized by its ability to withstand harsh climactic conditions in various regions (Uddin et al., 2014). As such, purslane (PA) has been reported on individual counts as originating in South America, North Africa, Eastern Asia and Europe (Hoyle & Mcelroy, 2012). In Mediterranean and tropical Asian countries the leaves of the purslane plant are consumed in soups and salads while Americans and native Australians utilize the seeds to create flour (Zhou et al., 2015). On the other hand, purslane’s role in traditional medicine includes, but is not
limited to, its use as an astringent, anti-inflammatory, remedy for gastrointestinal diseases and skin irritations, diuretic, and for its anti-hypertensive properties (Al-Asmari et al., 2014; Cakilcioglu & Turkoglu, 2010; Chen et al., 2009; Lans, 2006; Mosaddegh, Naghibi, Moazzeni, Pirani, & Esmaeili, 2012). This unique plant has established itself in many different cultures and countries given its multimodal properties that heal or maintain the health of an individual.

1.1.2 The Pharmacology of Purslane

Modern studies have demonstrated the merits to which purslane has been used for its pharmacological abilities and the recognition as the ‘global panacea’ (Alam et al., 2014). Chan and colleagues studied the anti-inflammatory effect of purslane in rats induced with inflammation in the hind paw (Chan, Islam, Kamil, & Radhakrishnan, 2000). Findings revealed that a 10% ethanolic extract of the leaves and stem of *P. oleracea* when applied topically reduced inflammation significantly due to the postulated active organic and polar components of the extract. The same study also revealed that purslane’s analgesic potential was comparable to that of sodium diclofenac, a common pain killer for musculoskeletal complaints (Ulubay, Yurt, Kaplan, & Atilla, 2016).

Furthermore, recent studies on the metabolic effects of purslane have also been conclusive. Purslane seeds at a dose of 500 mg twice daily were given to obese adolescents with abnormally high blood lipid content, formally known as dyslipidemia. After 4 weeks participants’ levels of low density lipoprotein (LDL) cholesterol and triglycerides (TGs) were significantly reduced (Sabzghabaee et al., 2014). In another study, diabetic patients given 5 g of purslane seeds twice daily demonstrated reduced blood glucose levels equal in effect to metformin, the classic drug for the treatment of type 2 diabetes (El-Sayed, 2011). Overall this
study derived that the metabolic outcomes of purslane seeds in diet provide anti-hyperglycemic and anti-hyperlipidemic capability that prove to be beneficial in humans, in particular those diagnosed with diabetes.

The neuroprotective effects of *P. oleracea* are also notable amongst the many pharmacological abilities of this plant. A study by Moneim and colleagues exhibited the plant’s potential to inhibit dopamine metabolism and apoptosis induction in rats administered with rotenone (Abdel Moneim, Dkhil, & Al-Quraishy, 2013). Rotenone is a classic drug used to induce a Parkinson’s disease model in rat neurons for experimental study (Ravenstijn et al., 2008). The administration of this isoflavone causes the inhibition of mitochondrial complex I which consequently produces O$_2$ free radicals. The resulting oxidative stress is what causes apoptosis induction. It is this mitochondrial impairment and oxidative stress that is the major cause of degeneration of dopaminergic neurons that result in the neurodegenerative disease of Parkinson’s (Meiser, Weindl, & Hiller, 2013). The ability of purslane juice to significantly decrease these negative occurrences hints at its neuroprotective ability as a function of its antioxidant activity, although the exact constituents that may be responsible for such mechanism of action were not identified in this study.

The anti-microbial ability of *P. oleracea* is also uniquely noted given the various pathogens this plant has been demonstrated to inhibit. The prevention of bacterial growth such as *Neisseria gonorrhea, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were illustrated in a 2008 study with a 70% ethanolic extract of purslane (Elkhayat, Ibrahim, & Aziz, 2008). In a separate study purslane was shown to inhibit the growth of *Penicillium sp.*, *Rhizopus sp.*, *Mucor racemosus* and *Aspergillus niger* (Dan, 2006). The growth hindrance of these food-
borne pathogens becomes specifically of interest and relevance in industry uses to prevent the spoilage of food.

The neuroprotective, anti-microbial, analgesic and antiseptic properties of this powerful plant are only a few mentioned among its many medicinal uses. The pharmacological ability of *P. oleracea* is just one attribute of this plant that adds value to its multipurpose capacity.

### 1.1.3 The Phytochemistry of Purslane

The phytochemicals present in purslane are not only diverse in nature but also variable in quantity dependent on growing conditions, harvest time, and location of growth and origin including factors such as soil conditions and environment (Uddin, Juraimi, Anwar, Hossain, & Alam, 2012). Despite the variable content of bioactive compounds present in PA, there are common phytochemicals that underlie the foundation of this plant’s makeup. Among its chemical composition, the most notable composite is its content of flavonoids. The most prominent of these flavonoids include kaempferol, apigenin, myricetin, quercetin and luteolin that are mainly found in the root, stem and leaves of the plant (Xu, Yu, & Chen, 2006). In addition, the presence of different types of alkaloids and terpenoids have been identified as other significant components of PA (Petropoulos, Karkanis, Martins, & Ferreira, 2016; Xin et al., 2008).

The health benefits of purslane are also derived from the many vitamins present in this species. For example, the vitamin E class of tocopherols, vitamin C and vitamin B-12 complexes are all prominent nutrients found in this plant (Oliveira, Wobeto, Zanuzo, & Severgnini, 2013; Rinaldi, Amodio, & Colelli, 2010; Uddin et al., 2014).

A major component that makes up the phytochemical background of purslane are fatty acids. In comparison to more commonly consumed greens such as spinach and buttercrunch
lettuce, purslane has a total fatty acid content of 8.50 mg/g of wet weight in contrast to 1.70 and 0.601 mg/g, respectively (Simopoulos & Salem, 1986). Among green leafy vegetables purslane has been identified as the richest source of omega-3 fatty acids (Simopoulos, 2004). In particular to note, the major constituent of purslane oil with 300-400 mg/100g of sample is alpha-linolenic acid (ALA) (Figure 1.2) (Uddin et al., 2014). This essential fatty acid is one amongst the many present in PA, including palmitoleic, palmitic, linoleic, oleic, and stearic acids (Gonnella & Aldo, 2010).

![Figure 1.2 Chemical structure of alpha-linolenic acid](image)

In addition to these nutritive products, PA is also a rich source of phenolic compounds (Alam et al., 2014). Supplementing the numerous types of flavonoids mentioned earlier, antioxidants also comprise a major part of the family of phenolics that are readily available in plants and in this case purslane. By scavenging for free radicals that cause oxidative stress, antioxidants are capable of reducing the damage caused by these harmful agents (Catherine, 1997). In the leaves and stem portion of *P. oleracea* the antioxidant activity of the phenolics present were analyzed to be between 121±11 and 132±11 mg GAE/100g based on the cultivars tested in one study (Lim & Quah, 2006). Among these phenolic compounds are chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, rosmarinic acid and quercetin as identified by Erkan (Erkan, 2012).
Given the diverse bioactive compounds that contribute to the beneficial effects of purslane, it is clear as to why this unique plant has been incorporated in diet and healthcare for many years and amongst many cultures. Based on previous and future research PA has strong potential as a source for nutritional, pharmacological and industrial purposes.

1.2 Alkylresorcinol

1.2.1 Structure and function

Among the various phytochemicals present in higher-order plants, alkylresorcinol is of particular interest given its biological and biopharmacological effects. 5-Alkylresorcinols, or ARs, are characterized by their hydrophilic benzene ring with two hydroxyl functional groups (resorcinol) at the meta position of one and three. The accompanying hydrophobic alkyl chain at position five ranges only in odd numbers from 13 to 27 carbons and can be present in both saturated or unsaturated forms (Arkadiusz Kozubek & Tyman, 1999). In short, these resorcinolic lipids take structure as 1,3-dihydroxy-5-alkyl (Figure 1.3).

![Figure 1.3 Chemical structure of alkylresorcinol](image)
Table 1.1 Structure and nomenclature of common 5-n-alkylresorcinols in cereal grains, adapted from Ross et al., 2003.

<table>
<thead>
<tr>
<th>R</th>
<th>Common Name</th>
<th>IUPAC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{15}H_{31}</td>
<td>5-n-Pentadecylresorcinol</td>
<td>5-Pentadecyl-1,3-benzendiol</td>
</tr>
<tr>
<td>C_{17}H_{35}</td>
<td>5-n-Heptadecylresorcinol</td>
<td>5-Heptadecyl-1,3-benzendiol</td>
</tr>
<tr>
<td>C_{19}H_{39}</td>
<td>5-n-Nonadecylresorcinol</td>
<td>5-Nonadecyl-1,3-benzendiol</td>
</tr>
<tr>
<td>C_{21}H_{43}</td>
<td>5-n-Heneicosylresorcinol</td>
<td>5-Heneicosyl-1,3-benzendiol</td>
</tr>
<tr>
<td>C_{23}H_{47}</td>
<td>5-n-Tricosylresorcinol</td>
<td>5-Tricosyl-1,3-benzendiol</td>
</tr>
<tr>
<td>C_{25}H_{51}</td>
<td>5-n-Pentacosylresorcinol</td>
<td>5-Pentacosyl-1,3-benzendiol</td>
</tr>
</tbody>
</table>

Although also present in animals, bacteria and fungi, the most significant source of alkylresorcinol is found in major cereal grains such as rye, wheat, barley and millet with the most common forms outlined in Table 1.1 (Ross et al., 2003). Amongst these AR-dominant plants, the main analogues that constitute their phytochemical background vary by species with the most notable quantity present in rye, triticale and wheat (Table 1.2). These phenolic lipids are localized in the outer layer of cereal grains as characterized by microscopic and gas chromatography (GC) analysis (Landberg, Kamal-eldin, Salmenkallio-marttila, & Inge, 2008).

Table 1.2 Presence and total quantity of alkylresorcinols identified in cereal grains, adapted from Ross et al., 2003.

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Common Name</th>
<th>Range (μg/g of DM\textsuperscript{a})</th>
<th>Main Homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secale cereale</td>
<td>Rye</td>
<td>360-3200</td>
<td>C17, C19, C21</td>
</tr>
<tr>
<td>Triticosecale</td>
<td>Triticale</td>
<td>580-1630</td>
<td>C19, C21, C23</td>
</tr>
<tr>
<td>Triticum durum</td>
<td>Durum wheat</td>
<td>460-1080</td>
<td>Not determined</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Wheat</td>
<td>317-1010</td>
<td>C19, C21</td>
</tr>
<tr>
<td>Triticum spelta</td>
<td>Spelt wheat</td>
<td>337-494</td>
<td>Not determined</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>Barley</td>
<td>44-500</td>
<td>C19, C21, C25</td>
</tr>
<tr>
<td>Pennisetum americanum</td>
<td>Millet</td>
<td>100</td>
<td>C17, C19, C21</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Dry Matter
As a result of their presence in cereal grains, ARs can be utilized as markers for wholegrains and bran fractions and biomarkers for their respective intake (Alastair et al., 2004). In addition to the six main alkylresorcinol homologues present in cereals there has also been identification of minor AR derivatives. Amongst these constituents are ARs with unsaturated alkyl chains such as C15:1 and C17:1 and those with additional functional groups like 5-(2’oxo)alkylresorcinol, 5-(4’hydroxy)alkylresorcinol and 5,5’-(alkadiyl)diresorcinoi (Suzuki, Esumi, & Yamaguchi, 1999; Suzuki, Saitoh, Hyakutake, & Kono, 2014).

Given their unique structure, alkylresorcinols take an amphipathic form. Their lipophilic, and thus hydrophobic, alkyl chain paired with a hydrophilic phenol group results in this notable characteristic (Kozubek et al., 2001). Within the resorcinol group exists an interaction between delocalized electrons in the benzene ring and a lone pair of electrons from the oxygen atom of the hydroxyl group. The oxygen’s ability to donate this lone pair of electrons to the ring increases the electron density within the structure. This in turn results in the ring being more reactive. The electrons within the benzene ring become more prone to attack by positive ion or other compounds with slightly positive parts (Levitt & Perutz, 1988). With an increase in the electron density within the ring there is an increase likelihood of attracting electrophiles. As a result, this portion of the compound is subject to electrophilic substitution. In addition, the delocalization of oxygen’s lone pair of electrons causes the hydrogen belonging to the hydroxyl group to become more acidic than what is characteristic of a hydrogen within a simple alcohol. By examining the sp^3 hybridization of the carbon within the benzene ring attached directly to the hydroxyl group from the perspective of the functional group explains this. The decrease of electron density on the oxygen molecule allows it to readily break away from the hydroxyl group.
and transfer to a base. As a consequence, the hydroxyl group associated with the resorcinol structure is slightly acidic (Taft et al., 1990).

The overall chemical structure of alkylresorcinol reveals insight on its ability as a bioactive molecule and overall applied function.

1.2.2 Intake and Absorption

As a major component in wheat and rye, alkylresorcinol is a significant phytochemical in food and diet. The estimated intake of ARs in a normal diet is approximately 11.9 mg/day, based on food supply data and food consumption data collected in Sweden (Ross, Becker, Chen, Kamal-Eldin, & Man, n.d.). Of course, a certain individual may consume above or below this value of alkylresorcinol given the varying content of ARs in cereal grains and distinct diet (Gunenc, HadiNezhad, Tamburic-Ilinic, Mayer, & Hosseinian, 2013).

The amount of alkylresorcinol that is absorbed upon consumption has been studied in rat, pig and human models. The ileal digestion of ARs in rats was determined by 34% recovery of radioactive-labelled AR, implying that the remainder was absorbed and metabolized by the specimens (Ross, Shepherd, et al., n.d.). The absorption of ARs in human was indicated by their exclusive recovery in ileostomy samples during an AR-enriched diet (Ross, Kamal-Eldin, et al., n.d.). The quantification of ~40% remaining ARs in the small intestine suggests the ileal digestibility of 60% in this model, similar to and substantiating the rat model. The study more closely determined that there was slightly less uptake in longer-chain AR homologs as oppose to smaller-chain ones. The absorption of alkylresorcinol from diet was also confirmed by Linko and colleagues by identifying intact AR homologs in human plasma (Linko, Parikka, & Adlercreutz, n.d.)
The capacity of humans to absorb ARs through diet suggests that the beneficial abilities of this compound may equally be translated to exert such activities in vivo.

1.2.3 The Health Benefits

As secondary metabolites, phenolic compounds have been demonstrated as having notable physiological properties such as cardioprotective, vasodilatory and anti-inflammatory ability, amongst others (Middleton, Kandaswami, & Theoharides, 2000). A number of studies have validated the ways in which ARs can directly or indirectly contribute to the maintenance of health and prevention of disease.

A notable study highlighting the potential of ARs in health examined their effect on levels of \( \gamma \)-tocopherol in liver and lungs of rats (Ross, Chen, et al., 2004). Specimens given AR-fortified diet at a concentration between 1-4 g/kg demonstrated a significant increase of \( \gamma \)-tocopherol in liver and lungs, which is potentially explained by their ability to inhibit the tocopherol-\( \omega \)-hydroxylase pathway that would normally convert it into hydroxynoraphyromen. As a low level of \( \gamma \)-tocopherol is associated with increased coronary heart disease, the resulting increased levels \( \gamma \)-tocopherol sustained by ARs demonstrates its cardioprotective capability.

In another study by Kozubek and colleagues, the impact of alkylresorcinol on mutagenic factors that may lead to mutagenesis and carcinogenesis were assessed (Gąsiorowski, Szyba, Brokos, & Kozubek, 1996). The phenolic lipid was tested against direct-acting genotoxic compounds, methyl methanesulfonate (MMS) and daunorubicin (DRC), and indirect-acting promutagens benzo[\( a \)]pyrene (B[\( a \)]P) and 2-aminofluorene (2-AF). AR content isolated from rye bran milling fraction were tested against the activity of four mutagens using the standard Ames test. At a concentration of 100\( \mu \)g/plate, alkylresorcinol demonstrated a marked decrease in
mutagenic activity of the promutagens in a dose-dependent manner. There was a 70% and 60% decrease of mutagenic activity of B[a]P and 2-AF, respectively. Although the effects of ARs on direct-acting mutagens were not as extreme, the decrease in mutagen activity of DRC was still considerable at 43% and 20% against MMS. The significant findings of this study indicate the capacity of ARs to decrease genotoxic effects caused by mutagenic or carcinogenic factors, a favorable nature of activity in preventing health and disease.

The onset of many diseases has been linked to free radical and reactive oxygen species that are capable of causing damage to cellular structures from DNA to protein (Poprac et al., 2017). With growing interest in finding methods to modulate oxidative stress associated with disease, the potential of dietary antioxidants to prevent or delay the consequences of oxidative damage is eminent.

1.3 Oxidative Stress and Antioxidants

1.3.1 Oxidative Damage

Reactive oxygen species (ROS), including superoxide, \( \text{O}_2^- \), and hydroxyl radical, \( \text{OH}^- \), are cause of oxidative damage. The interaction between oxygen and other molecules often leads this element with an odd number of electrons. This results in a chemically reactive compound with potential to cause harmful events at the cellular level or to other macromolecules (Cadet & Davies, 2017)

1.3.1.1 Oxidative Stress in Health and Disease

In aerobic species, the production of ROS is inevitable as it is predominantly a by-product of mitochondrial electron transport, amongst other external influences. The body possesses its
own methods to combat radicals, for example through the activity of active enzymes that are capable of degrading $O_2^-$ and $OH^-$ (Zelko et Mariana, 2002). Despite this natural defense system and other mechanisms of action, the number of reactive oxygen species may exceed amounts that make an environment manageable or functional for a state of biological activity (Spector, 2000). The resulting oxidative stress caused by ROS has been linked to a number of human diseases. The formation, promotion and growth of many types of cancer has been tightly linked to oxidative stress (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). Reactive oxygen species has been demonstrated in contributing to hypertension in the brain, kidney and vasculature resulting in a number of hypertensive diseases (Loperena & Harrison, 2017). Amongst these, oxidative stress can cause inflammation leading to depression, emphysema and neurologic disorders (Lanzetti et al., 2012; Lindqvist et al., 2017; Patel, 2016). Although the underlying foundation of how reactive oxygen species exert their deleterious effects is the same, their capacity to influence normal biological function is wide range and undeniable in the contribution to disease.

1.3.1.2 Oxidation in Foods and Cosmeceuticals

The role of oxidation in food and cosmetics has a profound impact on the stability of these products in their respective industries. The oxidative damage that occurs in the aqueous and lipid phase of products is what leads to their ultimate deterioration and shortened shelf life (Skibsted, n.d.). While the aqueous phase is the site of oxidoreductase activity which produces both reactive oxygen species and reactive nitrogen species (RNS), lipid oxidation is the major cause of deterioration in foods.

Specifically, the most common oxidation that takes effect in foods is autoxidation. The initiation of lipid oxidation can be the result of exposure to light, enzymes, or metal catalysis, all
involving a free radical chain reaction (Carlsen, Møller, & Skibsted, 2005). Regardless of how the autoxidation of polyunsaturated lipids is initiated, the result is the production of polar and polymeric products. However, generally the spoilage of food products is due to the spontaneous oxidation of fats which are more susceptible to air oxidation (Angelo, 1996). The autoxidation of polyunsaturated lipids follows the general scheme outlined in Figure 1.4. The initiation step involves the event of free radical formation. Many compounds such as nitric oxide, nitrogen dioxide and ozone can pose as the source of a radical species that abstracts the hydrogen atom from the RH complex.

![Reaction mechanism for the autoxidation of polyunsaturated lipids](image)

**Figure 1.4** Reaction mechanism for the autoxidation of polyunsaturated lipids, adapted from Shahidi, 2015.

These are the most common environmental pollutants that are capable of initiating autoxidation by hydrogen atom abstraction. Propagation starts with the addition of molecular oxygen to the radical, which in solution is the peroxo radical ROO. In turn, this ROO complex abstracts a hydrogen atom from RH and as a result generates more free radicals. If by chance free
radicals collide, their lone electrons form a new bond and there is termination of the pathway. Alternatively, the free radicals from the initiation step result in the oxidation of pigments, flavours and vitamins, which alter the taste and colour of food. More prominently, the resulting hydroperoxides can be broken down into a variety of decomposition products, develop polymerization products that result in dark colours and toxicity, or produce insolubilization of proteins that alter functionality and texture. The resulting oxidation that occurs in foods translates into rancidity of the product. Aside from putting into question the microbiological safety of the food or beverage, this oxidation also affects the product’s sensory quality. The resulting microbiological, chemical or physical changes affect the product’s shelf life and sensory properties, including colour, texture, viscosity, and flavour (Skibsted, 2017). Controlling the oxidative damage in regard to these characteristics is of particular interest in the food and cosmetic industry in order to maintain product integrity. Although the oxidation of food components is inevitable, depending on the type of food and formulation, additives to these products are used in order to delay this degeneration. Amongst the array of methods used to prolong shelf life, the most prevalent is the use of antioxidants in manufactured products.

1.3.2 Antioxidants

1.3.1.1 General Overview

Antioxidants are capable of donating electrons to free radicals in order to neutralize them and inhibit the associated oxidation of compounds (Figure 1.5) (Brewer, 2011). The mechanisms through which antioxidants exert their effects include terminating peroxidation-initiating species, quenching *O2* radicals, hindering the autoxidation reaction, and reducing the concentration of O2.
Figure 1.5 Schematic of antioxidant activity.

Their specific mode of action is dependent on the physical and chemical characteristics of the antioxidant and the type of radical they are working on. Within these characteristics, the effectiveness of an antioxidant is related to factors such as its activation energy and oxidation-reduction potential (Brewer, 2011). Although many elements determine what makes a ‘good’ antioxidant, universally the best antioxidants are compounds that consist of an aromatic or phenolic ring which are capable of stabilizing their radical state once donating an electron through resonance structure of their ring (Shahidi et al., 1992). Utilizing this foundational mode of action antioxidants have the ability to contribute positively to biological systems and disease prevention as well as serve as preservative agent in foods and cosmetics.

1.3.1.2 Use as a Preservative Agent

Antioxidants in the food industry are capable of delaying, controlling or inhibiting the oxidation and thus deterioration of manufactured food. The antioxidants used in foods can be classified into two groups based on their nature: primary and secondary (Lim, Lim, & Tee, 2007). Primary antioxidants donate a hydrogen atom or single electron to neutralize free radicals. This is known as hydrogen atom transfer and single electron transfer, respectively. Moreover, secondary antioxidants work by neutralizing prooxidant catalysts, such as metal ions of iron and
copper. These two types of antioxidant activity can be implemented by both synthetic and natural antioxidants.

In today’s food industry sector, synthetic antioxidants can help protect oil compounds from oxidation degradation in order to increase shelf life (Roginsky & Lissi, 2005). Since these synthetics are inexpensive to manufacture and possess moderate to high antioxidant activity levels they can be applied to many food products. Since their introduction as food preservatives in the 1940s, synthetic antioxidants have been derived primarily from petroleum-based products (Berdahl et al., 2010). Within the group of synthetic antioxidants, the most frequently used compounds to preserve food include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) (Figure 1.6) (Hossain, Brunton, Barry-Ryan, Martin-Diana, & Wilkinson, 2008).

![Chemical structure of common synthetic antioxidants](image)

**Figure 1.6** Chemical structure of common synthetic antioxidants used in food and cosmeceutical preservation. (A) BHT, (B) TBHQ, (C) BHA, and (D) PG.
Although synthetic antioxidants are conveniently used in the food industry for their availability, research in this field has revealed the threat that they pose to human health in regards to toxicity and food safety. BHA as a food preservative has potential to break the reaction chain of autoxidation of lipids, but contrast to this positive characteristic it also has a widespread range of deleterious biological activities. BHA has been demonstrated to promote the formation of tumors through many different postulated mechanisms (Vandghanooni et al., 2013). A study by Yu and colleagues identified the ability of BHA to activate significant signaling kinases such as extracellular signal-regulated protein kinase 2 (ERK2) (Yu et al., 1997). In addition to ERKs in general being associated with cell growth, proliferation or transformation, the specific ERK2 pathway induce multiple other protein kinase activity which are postulated oncogenic factors (Boulikas, 1995). Another study published in the toxicology journal by Witschi outlines another effect of synthetic antioxidants, BHT (Witschi, 1986). In this series of experiments, mice were subjected to a single dose of urethane to induce tumour formation and fed food pellets with 0.75% BHT, BHA or ethoxyquin, another synthetic antioxidant. Subjects fed BHT-enhanced pellets developed 10.4 tumours per lung as opposed to 7.4 in mice with an additive-free diet. The same trend of increased tumour formation was observed in a group of mice given 8 weekly injections of 300mg/kg BHT compared to a control group injected with corn oil. This specific study demonstrates the increased tumour multiplicity in subjects with cancer, which is evidence that BHT is a promoting agent for tumours.

Additionally, there are some general concerns associated with the use of synthetic antioxidants as well. The ‘plastic’ group of antioxidants generally have low water solubility, which can make their incorporation in some food products difficult (Pokorný, 2007). Since they
are deemed as food additives because of their synthetic nature they are subject to be regulated by policies and legislation which increases the lead time and effort dedicated to getting a product on the market.

Despite that the FDA recognizes these synthetic antioxidants as generally recognized as safe (GRAS) there are still associated uncertainties regarding their safety that have been flagged for future assessment. This further reveals the decreasing interest towards synthetic antioxidants and an increasing interest of the value of natural antioxidants for safety and quality of use (Razalee, 2012). The notable benefits of natural antioxidants include their wide range of antioxidant activity, positive health implications and broad range of solubility in oil and water which make their incorporation in food products amenable. This strongly emerging field in food chemistry has led to many studies on identified plants with safe and natural sources of antioxidants that can be applied in the food industry, with the leading group of compounds being polyphenols. An article in the journal of agriculture and food chemistry was among the first to demonstrate the relationship between phenolic content in several plants and associated antioxidant activity (Velioglu, Y.S., Mazza, G., Gao, L., Oomah, 1998). The antioxidant activity and total phenolics of 28 plants including wheat, seeds, fruits, berries and roots were quantified with some species demonstrating measurable antioxidant value compared to BHA and BHT. Primary literature such as this one has opened the door to identify specific phytochemical compounds that may be responsible for antioxidant activity and can contribute in a positive manner towards the preservation of food.

1.3.1.2 Impact on Human Health

Antioxidants are more commonly known for their beneficial health effects that are the result of the same underlying foundation of scavenging for free radicals. Exposure to external
influences such as chemicals and pollutants can contribute to a broad range of harmful events *in vivo*. Antioxidants in our diet play a tremendously important role in combatting these deleterious effects. For one, oxidative stress is tightly linked to the pathogenesis of hypertensive disorders as indicated by the reduction of activity in oxidative-combatting enzymes such as superoxide dismutase and glutathione peroxidase (Mistry, Wilson, Ramsay, Symonds, & Pipkin, 2008). Antioxidant regimens have been shown in a number of animal studies to reduce oxidative stress and thus associated hypertension in subjects (Vaziri, 2004). Even in human studies like the one carried out by Duffy and colleagues have shown a decrease in blood pressure in hypertensive patients as a result of treatment with absorbic acid (Duffy et al., 1999). A more detailed study, in regard to cardiovascular health, demonstrated a dietary intake of antioxidants as a potential mode of treatment for hyperlipidemic children. A major contribution to this disease, symptomatic of increased blood lipid content, is endothelial dysfunction as a result of increased vascular oxidative stress (Heitzer, Schlinzig, Krohn, Meinertz, & Munzel, 2001). In response to antioxidant intervention, the flow-mediated dilation of brachial arteries which would normally be hindered as a result of this disease were elevated from baseline measurements (Engler et al., 2003).

Antioxidants have also been linked by a number of studies to a potential capacity to inhibit or prevent cancer. Since internal and external sources of radicals are what cause DNA damage that inadvertently is associated with carcinogenesis, antioxidants get their postulated anti-cancerous characteristic by combatting these radicals (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). For example, the journal of nutrition and cancer published a study that indicated the ability of polyphenols in wine to inhibit cell growth in both a dose- and time-dependent manner (Damianaki *et al*., 2000). More specifically, resveratrol was the most potent
antiproliferative agent in one of the chosen prostate cancer cell lines. Further investigation revealed the polyphenols to demonstrate a significant decrease of ROS production that indicates the method through which they may exert their effects – free radical scavenging. Through these underlying mechanisms is how antioxidants may oppose the influx of oxidants associated with disease and maintain optimal physiological conditions.

1.3.3 AR’s Role as an Antioxidant

The chemical structure of alkylresorcinol alone suggests its ability to work as a stable antioxidant in living and non-living systems. A study that examined ARs extracted from rye bran demonstrated their potential as a natural antioxidant with two methods: 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay and chemiluminescence (CL) method. The results demonstrated the ability of AR analogues C:15-C25 reduce the DPPH radical from a varying range of 10%-60% within the first 60 minutes of the assay depending on the concentration of the AR but not the considerably on the exact analogue itself (Korycińska, Czelna, Jaromin, & Kozubek, 2009). In order to compare this data with positive controls of tocopherol, which are known as the gold standard of natural antioxidants, EC\textsubscript{50} values were calculated. The best AR analogue gave an EC\textsubscript{50} value of 157\(\mu\)M in comparison to \(\alpha\)-tocopherol at 16\(\mu\)M. The ability of AR analogues to reduce luminescence in the CL method was notable with \(~\text{61-70\%}\) compared to tocopherols at \(~\text{82-89\%}\). This demonstrates the antioxidant properties of alkylresorcinol to be lower than that of tocopherols, however still present. Another antioxidant test carried out by Parikka and colleagues exhibited C:15 and C17 AR analogues’ ability to significantly inhibit copper-mediated oxidation in human low-density lipoprotein (LDL) comparable to a control (Parikka, Rowland, Welch, & Wähälä, 2006). The importance of these results is not only significant because it demonstrates
the antioxidant potential but even further exemplifies the ability of this antioxidant to exert their effects in biological systems.

The antioxidant activity of compounds can be measured in many different ways and each of value and significance dependent on how and in what system the antioxidant is applied.

1.4 Antioxidant Activity Assays

There are many different analytical methods for evaluating the efficiency of an antioxidant. With each test, we are able to better profile the antioxidants available for use (Pisoschi & Negulescu, 2012). Among the various methods of evaluation, the most common are the oxygen radical absorbance capacity assay (ORAC), the total phenolics content (TPC) assay and the 2,2-diphenyl-1-picrylhydrazyl assay.

1.4.1 Oxygen Radical Absorbance Capacity Assay

The ORAC method is the most widely recognized parameter for measuring antioxidant activity. This system uses 2,2’-axobis(2-methylpropionamidine) dihydrochloride (AAPH) as a model oxidant to generate peroxyl radicals under thermal decomposition (Bisby, Brooke, & Navaratnam, 2008). The assay detects oxidative damage to a target protein, fluorescein, by measuring the decrease of fluorescence when the compound loses its structural integrity upon destruction (Zulueta, Esteve, & Frígola, 2009). The peroxyl radicals decrease the fluorescence of fluorescein in zero-order kinetics, which is linear with time. The conclusive results are derived from the ability of the compound of interest to increase the lag phase and/or decrease the rate constant (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). In simple terms, the antioxidant
effectiveness is measured by its ability to protect the protein of degradation from oxidative damage.

1.4.2 Total Phenolics Content Assay

The TPC assay is a spectrophotometric method that is also commonly known as the Folin-Ciocalteu method. This test exposes compounds of interest to Folin-Ciocalteu reagent in alkaline medium. When the phenolic compounds transfer electrons to the phomolybdic and phosphotungstic acid complexes in the reagent a blue chromophore is formed (Ainsworth & Gillespie, 2007). At a wavelength of 760nm this value can be measured for quantification of the total concentration of phenolic hydroxyl groups that are present in a sample (Blainski, Lopes, & De Mello, 2013).

1.4.3 2,2- diphenyl-1-picrylhydrazyl Assay

The DPPH assay measures the antioxidant reaction with the organic radical 2,2-diphenyl-1-picrylhydrazyl. The DPPH• radical hosts a delocalizing nature for its spare electron throughout the chemical compound that is demonstrated by its purple colour. When the radical is reduced by an antioxidant’s ability to donate a hydrogen atom, the purple colour of the compound disappears (Molyneux, 2004). So, by spectrophotometric measurement of the disappearance of violet colour in solution, an antioxidant’s efficiency can be determined. This value is compared to the results of Trolox under the same parameters, which is an analog of Vitamin E and thus benchmark for exemplary antioxidant capacity (Al-Attar, 2011)
With the variety of methods available to test antioxidant capacity of compounds and compare their potential with each other, this creates opportunity to identify the best source and extraction method for antioxidants, which in our specific interest is phenolic lipids.

1.5 Supercritical Extraction

Conventionally, organic solvents are involved in traditional methods for extraction of plant compounds. With increased research and incentive towards efficient and safe methods of extraction, there has been growing interest towards supercritical fluid extraction (SFE). This type of extraction employs a compound that replaces a traditional solvent at a temperature and pressure above its critical point. In this state, the substance is neither explicitly a gas or liquid but possesses the properties of both in its hybrid form. The supercritical fluid is capable of diffusing through and dissolving solids, which is one reason that contributes to its efficiency. Of the supercritical fluids available, the most common type for this extraction process is supercritical carbon dioxide (SC-CO₂), taking on its supercritical form at 1100 psi and 31.7°C (Reverchon & De Marco, 2006).
Figure 1.7 Supercritical fluid extractor including (A) extraction vessel and two (B and C) collection vessels.

The sample of interest is placed in the extraction vessel (Figure 1.7.A) which is pressurized with CO$_2$. The SC-CO$_2$ then travels to collection vessels (Figure 1.7.B and 1.7.C) where the extract is accumulated. The temperature, pressure and flow rate of the SC-CO$_2$ when passing through a collection chamber can be set in a particular way in order to solubilize a specific fraction of the extract. Each collection vessel can be set to different parameters in order to divide or maximize the extracted material. When the CO$_2$ is depressurized in its respective vessel, the extract precipitates from the solvent and the CO$_2$ is ventilated away (Hadinezhad, Rowland, & Hosseinian, 2015).

The use of SFE has many major advantages over traditional solvent extraction techniques. As previously mentioned, SFE is efficient due to its solvating form as a supercritical fluid that possess a diffusivity of $\sim 10^{-4}$ cm$^2$ s$^{-1}$ in comparison to $\sim 10^{-5}$ cm$^2$ s$^{-1}$ for liquid solvents.
This physical characteristic allows it to better penetrate through the sample of interest, also leading to faster extraction times. The efficacy of SFE is also in part due to fresh supercritical fluid being passed through the sample throughout the entire extraction process (Zougagh, Valcárcel, & Ríos, 2004). As a result, this never allows for maximum solvating concentration to be reached for a set amount of fluid but since there is always new fluid entering the system this ensures that the extraction is complete. The ability of adjusting temperature and pressure of the collection vessels allows for selective extraction of complex materials (Reverchon et al., 1993).

The use of little to no organic solvent for SFE yields a number of positive outcomes. For one, this method is environmentally friendly as no large quantity of organic solvent is used, and thus needs to be disposed of. In addition, since CO₂ used is separated from the extract when it is depressurized, this eliminates the step of concentrating the extract from the solvent, which is an additional step required when using traditional solvent-based methods for extraction (Henning et al., 1993). The elimination of organic solvents associated with conventional extractions yields for purer, low toxicity products. This is of particular interest in the food industry where products of extractions are being consumed and subject to review for toxicity and thermal stability.

Supercritical fluid extraction provides an alternative method for extraction of compounds in an efficient, safe, economical and environmentally friendly manner.

1.6 Crystal Structure of Fats and Lipids

Fats and lipids are able to take shape in different crystalline forms depending on the environment during crystallization and the structure of the fat itself (Sato and Ueno, 2011). This characteristic of polymorphism is of special interest to food scientists, who take advantage of
specific types of crystals to achieve certain qualities in food material. One of the most desirable crystalline structures in regard to food is the beta-crystal, having the highest melting point of any form (Kulkarni, 2012). If food is processed in a way in which it is melted and then cooled, the crystal fats in the product are able to re-grow using the remaining beta-crystals as a template. This results in a product that is uniformly composed of beta-crystals. When applied to specific food products such as chocolate and margarine this yields a product with an overall higher melting point.

Other characteristics of fats that lead to a higher melting point include a longer fatty acid chain, which increases the overall molecular weight of the structure. As well, the degree of saturation and unsaturation is influential as saturated fatty acids take on a straighter form and thus able to be in closer proximity to each other which increases their intra-molecular attraction.

Cocoa butter is conventionally the fat component used in chocolate, however it is characteristic of melting easily. The incorporation of vegetable fats, such as *Garcinia indica* for example, has been demonstrated to increase the solid fat content of chocolate, and thus increase the melting point (Brewer, 2011). Not only does this provide an opportunity for enhancing the physical state of foods such as chocolate and margarine for manufacturers and consumers but also the potential to identify a compound which can contribute positive outcomes not only in this way, but also serve as a functional food ingredient.
CHAPTER 2: EXTRACTION AND QUANTIFICATION OF ALKYLRESORCINOL IN PURSLANE SEEDS

2.1 Abstract

Alkylresorcinols, C15:0, C17:0, C19:0, C21:0, C23:0 and C25:0, were identified and extracted from *Portulaca oleracea* using three traditional solvent-based extraction methods, with acetone, ethyl acetate and ethanol, as well as an alternative method of extraction using supercritical CO₂. Each AR homologue as well as total AR content was quantified in purslane and in comparison, with triticale bran using HPLC analysis and GC-FID. HPLC analysis revealed solvent-based extractions to yield 31.9-45.3mg/100g of dry material (DM) in purslane and 220.7-517.5mg/100g of DM in triticale bran. Supercritical fluid extraction of purslane yielded 181.1-532.2mg/100g of DM and 506.6-947.6mg/100g of DM in triticale bran. GC-FID analysis presented slightly different analysis of ARs than HPLC analysis. Solvent extracts of purslane contained 34.2-61.1mg/100g of DM and 251.8-1423.2mg/100g of DM in triticale bran by GC-FID analysis. Supercritical CO₂ fractions of sample contained ARs in amounts of 201.0-577.9mg/100g of DM in purslane and 727.5-1220.1mg/100g of DM in triticale bran. This study was conclusive in revealing the identification and quantification of AR homologues in purslane and identifying SFE as an efficient method of extraction of these compounds.

2.2 Introduction

Phenolic lipids have the potential to serve many beneficial effects to human health including antioxidative properties, cancer prevention, anti-inflammation and antimutagenic capabilities (Huang, Ho, & Lee, 1992). These valuable phytochemicals are present in *Portulaca*
oleracea however the exact constituents that fall under this category of compounds have not yet been identified in this plant (Erkan, 2012). Studies performed to present date have been limited to the leaves, stem and root of the purslane plant. Therefore, this study was chosen to evaluate the presence and quantity, if applicable, of a certain phenolic lipid of interest: alkylresorcinol.

Alkylresorcinols are characterized by their phenolic ring and long fatty acid chain that can range from 13 to 27 carbons at every odd number. In general, they possess many of the same properties of phenolic lipids, which serves as an indication that ARs may be part of the phytochemical profile of purslane. Since the presence of ARs are more strictly limited to grain cereals, their occurrence in any other family of plant would serve as an alternative source for a rarely natural occurring compound. It is hypothesized that since purslane is comprised of phenolic compounds that alkylresorcinol may constitute as one of these valuable phytochemicals.

The primary objectives of this study were to a) extract, identify and quantify ARs from purslane, b) determine the method of extraction that yields the highest quantity of AR homologues and total AR content, and c) compare traditional solvent-based methods of extraction to environmentally friendly supercritical fluid extraction.

2.3 Materials and Methods

2.3.1 Purslane seed sample

The purslane seed sample was provided by Agriculture Research Institute in Karaj, Alborz, Iran. No alterations were made to these samples for our research purposes.

2.3.2 Triticale bran sample

The triticale bran sample was provided by Agriculture Research Institute in Lethbridge, Alberta, Canada. No alterations were made to these samples for our research purposes.
2.3.3 Traditional solvent-based extraction of ARs from purslane and triticale bran

Alkylresorcinols from purslane and triticale bran (TB) were extracted with three different solvents traditionally used to yield extracts from plant material. The samples of interest were placed in solvent of ethanol, ethyl acetate or acetone in a 1:40 w/v ratio for 24 hours (Mullin, 1992; Ross, Kamal-Eldin, Jung, Shepherd, & Åman, 2001). The extraction process was done under constant stirring (Stirrer-VWR, Corning®, VMS-C4) at 150 rotations per minute (rpm) in room temperature. Subsequently, each sample was filtered under vacuum filtration using Whatman double filter paper (number 9) three consecutive times. The remaining solution was placed in a Rotavapor (Buchi-Brinkman, R100 Switzerland) and the solvent was evaporated and discarded, leaving only the extract. The extract was then re-dissolved in the solvent that was originally used for extraction and transferred into a storage container. The small quantity of solvent used for transfer was evaporated under a gentle flow of nitrogen gas. The remaining extract was weighed and stored at 4°C for further analysis. All extracts prepared for analysis were conducted in triplicates.

2.3.4 Supercritical fluid extraction of ARs from purslane seeds and triticale bran

PA and TB samples were extracted using a SFE-1000-2-FMC50 system using an identical method for the extraction process for both. The supercritical system, purchased from Thar Technology Inc., Pittsburg, USA, is comprised of the following major components: an extraction vessel, which is supplied by supercritical fluid and co-solvent, if applicable, two collector vessels, and an automated back pressure regulator (ABPR). The parameters for the extraction were set according to the paper published by the Hosseinian lab (Hadinezhad et al., 2015). The extraction vessel, of 500mL size, under a pressure of 250 bar and temperature 50°C
was supplied with SC-CO$_2$ at a rate of 15 g/min. The first and second collection vessels were set to a pressure of 120 bar and 50 bar, respectively, at a temperature of 40°C. Extraction without co-solvent was processed for a period of 6 hours total: 5 hours under flow of SC-CO$_2$ and 1 hour of static extraction without supercritical fluid. Extraction with co-solvent was under the same supercritical fluid condition for four hours and subsequently for another two hours with 10% ethanol and adjusted CO$_2$ flow to 13.5 g/min in this time period. Samples with ethanol from co-solvent extractions were evaporated under nitrogen gas. All samples were stored in 4°C until used for analysis.

### 2.3.5 High performance liquid chromatography

Quantification of AR concentration in purslane and triticale bran samples were determined by reverse-phase high performance liquid chromatography (HPLC) using a Waters Alliance® HPLC system e2695 Separation Module in combination with a 2998 Photodiode Array Detector (Milford, Massachusetts). The software used to control the system was Empower 3. In order to generate a standard curve and determine retention times for AR homologues of interest, AR standard mixtures were prepared. Homologues C15:0, C17:0, C19:0, C21:0, C23:0 and C25:0 (ReseaChem GmbH, Burgdorf, Switzerland) were prepared in methanol at concentrations of 0.05, 0.0625, 0.20, 0.25, 0.30 and 0.35 g/mL. Samples of interest were also suspended in methanol solution for HPLC analysis.

Solvents A and B were prepared to mobilize compounds through a C18 column (4.6 x 150mm, inner diameter 5μm) at 1% acetic acid in methanol and 2% acetic acid in double distilled water (ddH$_2$O), respectively. The different homologues of ARs were separated by a gradient program which ran at a flow rate of 1mL/min for a total of 50 minutes (Table 2.1). The
gradient commenced with 10% solvent A and 90% solvent B, switched to 0% solvent A and 100% solvent B at 10 minutes, and completed the remainder of the run at 40-50 minutes with 10% solvent A and 90% solvent B. The compounds were detected using the UV detector measuring samples at 280nm wavelength. The area under the curve of peaks detected by the software were quantified in conjunct with the standard curves prepared in order to determine the concentration of ARs in test samples.

**Table 2.1** The gradient program for HPLC analysis of ARs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (1 mL/min)</th>
<th>Solvent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>00.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>10.00</td>
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<td>40.00</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>50.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**2.3.6 Gas chromatography with flame ionization detector**

Samples and standards analyzed with gas chromatography with flame ionization detector (GC-FID) were first derivatized in order to produce amenable properties for better detection through the analytic method used. To formulate a standard curve of AR homologues, the same standard mix solutions prepared for HPLC analysis were used. Ten μL of each standard mix were transferred into glass tubes where the solvent was evaporated under a gentle flow of N₂. The samples were then resuspended in 50μL of N,O-bistrifluoroacetamide (BSTFA) and 50μL of pyridine and vortexed at a speed of 10 with a 1.9.302.539 Fisher Scientific vortex mixer (Waltham, Massachusetts, USA). Consequently, the solutions were heated to 110°C for 15
minutes and then the solvent was evaporated under N\textsubscript{2} gas. The samples were resuspended in 100\textmu L of ethyl acetate, vortexed and transferred to GC vials for analysis. The same protocol was carried through with PA and TB samples but with varying amounts of extract given preliminary HPLC results of AR concentration in order to prevent overloading the GC-FID with high concentration of compounds.

Standards and samples were analyzed using a Varian GC-450 system equipped with an autosampler, FID detector and polar column DB23 of 30m x 0.25mm dimension and 0.25\textmu m film (J&W Scientific, Folsom, California, USA). The instrument was configured using the software Galaxie Chromatography Data System (Varian Inc., Palo Alto, California, USA) following the method of Mazza and colleagues (Athukorala, Hosseinian, & Mazza, 2010). The carrier gas of helium was set to a flow rate of 1.3 mL/min with the temperature of the injector at 300\textdegree C. The temperature oven was initiated at 50\textdegree C and held stable for 1 minute. Afterward, the temperature was increased by 10\textdegree C/min to 280\textdegree C. At this temperature, a following increase of 350\textdegree C at a rate of 5\textdegree C/min was maintained for 10 minutes. The peaks of compounds of interest were characterized using the Galaxie software.

2.3.7 Statistical Analysis

The three trials for each purslane seed and triticale bran sample were performed. Results are summarized as mean values ± SEM. The one-way ANOVA test was applied to the results in order to determine statistical significance of the values. P values less than 0.05 are conclusive of significant results.
2.4 Results

2.4.1 AR content of purslane seeds by HPLC analysis

Alkylresorcinols were extracted from purslane and triticale bran using three different organic solvents. Figure 2.1 displays the distinct peak separation of the AR homologues in a mixture of standards. The HPLC method of analysis yielded retention times of 7.68 minutes (C15:0), 9.69 minutes (C17:0), 11.68 minutes (C19:0), 13.67 minutes (C21:0), 16.15 minutes (C23:0), and 19.52 (C25:0) as summarized in Table 2.2. These retention times along with standard curves for each homologue were used as reference to accurately quantify the concentration of alkylresorcinols in each sample.

Acetone extract of purslane yielded the highest quantity of AR homologue C25 with 8.7mg/100g of DM, while with triticale bran the extraction method resulted in 168.4mg/100g of DM (Figure 2.2). Traditional solvent extraction performed with ethyl acetate demonstrated no classification of AR homologues C21, C23 or C25 in either purslane or triticale bran samples. In both PA and TB samples, this extraction method yielded the highest amount of AR C19 with 23.6mg/100g of DM and 143.2mg/100g of DM, respectively (Figure 2.3).
Figure 2.1 HPLC Chromatogram of AR homologue standards in a mixture solution identified with ultraviolet detection

Table 2.2 AR monologue standards used for HPLC analysis and their respective retention time in minutes.

<table>
<thead>
<tr>
<th>AR Monologue</th>
<th>Average Retention Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C15</td>
<td>7.68</td>
</tr>
<tr>
<td>C17</td>
<td>9.69</td>
</tr>
<tr>
<td>C19</td>
<td>11.68</td>
</tr>
<tr>
<td>C21</td>
<td>13.67</td>
</tr>
<tr>
<td>C23</td>
<td>16.15</td>
</tr>
<tr>
<td>C25</td>
<td>19.52</td>
</tr>
</tbody>
</table>
Figure 2.2 HPLC quantification of AR homologues in purslane and triticale bran extracted with acetone. Results are expressed as mean values ± SEM.

Figure 2.3 HPLC quantification of AR homologues in purslane and triticale bran extracted with ethyl acetate. Results are expressed as mean values ± SEM.
The ethanol extraction of purslane and triticale bran extracted the greatest quantity of AR homologue C19 at a concentration of 17.1 and 79.2 mg/100g of DM, respectively (Figure 2.4). The extraction was not able to isolate homologues C15 and C25 in PA or C23 in TB. Supercritical CO₂ extractions were undertaken for both samples with and without the addition of 10% ethanol as the co-solvent (Figure 2.5). These extractions were only performed on a one-trial basis as the quantity of sample needed for extraction with supercritical fluid is a high amount that varies depending on sample density. Since the quantity of purslane available was limited, it was
not possible to undergo these extractions in triplicates. Amongst the extractions that were performed, supercritical fluid was able to extract the highest quantity of homologue C21 in both conditions of PA and TB. The integration of co-solvent ethanol at 10% significantly increased the extraction yield of homologue C21 in PA and TB from 78.6 to 202.3mg/100g of DM and 205.4 to 504.7mg/100g of DM. Within all homologues, with the exception of C15, the use of co-solvent was able to increase the yield of that specific homologue.

**Figure 2.5** HPLC quantification of AR homologues in purslane and triticale bran extracted with supercritical CO2. Samples marked ‘w/co-s’ represent the supercritical extraction process with a 10% ethanol co-solvent.
Figure 2.6 outlines total AR content that was extracted by each extraction method. Purslane and triticale bran SF extractions with co-solvent yielded the highest measure of total ARs in sample at 532.2 and 947.6mg/100g of DM. This extraction of PA performed approximately equally comparable to the traditional acetone extraction of TB.

**Figure 2.6** Total concentration of ARs in purslane and triticale bran as determined by HPLC analysis by extraction methods: acetone, ethyl acetate, ethanol and supercritical extraction.

Table 2.5 displays the exact quantities of each AR homologue that was extracted with the 5 extraction methods. Amongst the traditional solvent-based extractions, ethyl acetate extracted the greatest amount of total ARs in purslane and acetone was the most effective extraction of ARs in triticale bran.
Table 2.3 Summary of AR homologues and total AR content of purslane and bran based on traditional extraction methods with acetone, ethyl acetate and ethanol and supercritical fluid extraction method. Quantification performed by HPLC analysis. Results expressed in units of mg/100g of DM.

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Sample</th>
<th>C15</th>
<th>C17</th>
<th>C19</th>
<th>C21</th>
<th>C23</th>
<th>C25</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purslane</td>
<td>Acetone</td>
<td>5.3</td>
<td>3.2</td>
<td>5.6</td>
<td>6.8</td>
<td>5.3</td>
<td>8.7</td>
<td>34.9</td>
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<tr>
<td></td>
<td>Ethyl acetate</td>
<td>12.2</td>
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<td>0.0</td>
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<td>8.1</td>
<td>0</td>
<td>31.9</td>
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<tr>
<td></td>
<td>Supercritical CO₂ without co-solvent</td>
<td>28.6</td>
<td>5.6</td>
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<td>17.6</td>
<td>5.0</td>
<td>181.1</td>
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<td>Supercritical CO₂ with co-solvent</td>
<td>19.4</td>
<td>23.8</td>
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<td>144.5</td>
<td>73.7</td>
<td>532.2</td>
</tr>
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<td>42.4</td>
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<td>129.5</td>
<td>168.4</td>
<td>65.7</td>
<td>50.3</td>
<td>517.5</td>
</tr>
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<td>Ethyl acetate</td>
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<td>0.0</td>
<td>0.0</td>
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<td>Ethanol</td>
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<td>Supercritical CO₂ without co-solvent</td>
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<td>153.9</td>
<td>205.4</td>
<td>85.6</td>
<td>35.6</td>
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<td>Supercritical CO₂ with co-solvent</td>
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<td>198.6</td>
<td>504.7</td>
<td>114.6</td>
<td>60.7</td>
<td>947.6</td>
</tr>
</tbody>
</table>
2.4.2 AR content of purslane seeds by GC-FID analysis

Purslane and triticale bran samples were extracted with acetone, ethyl acetate, ethanol and supercritical fluid with and without 10% co-solvent. These samples were analyzed with gas chromatography with flame ionization detector. Retention times and standard curve data of AR homologues were used as reference to quantify quantities in sample were determined by mix of standards. As outlined in Table 2.4 and Figure 2.7, retention times were identified for C15 (15.36 minutes), C17 (16.70 minutes), C19 (17.96 minutes), C21 (19.15 minutes), C23 (20.51 minutes) and C25 (22.28 minutes).

By GC-FID analysis, acetone demonstrated the ability to extract the most of AR homologue C25 in purslane at a concentration of 12.1mg/100g of DM and homologue C21 in triticale bran at 217.2mg/100g of DM (Figure 2.8). Figure 2.9 represents data from ethyl acetate extraction of PA and TB. Similar to the acetone extraction, C25 was extracted in high quantity in purslane at 20.1mg/100g of DM and C21 in triticale bran at 483.2mg/100g of DM.
Table 2.4 AR monologue standards used for GC-FID analysis and their respective retention time in minutes.

<table>
<thead>
<tr>
<th>AR Homologue</th>
<th>Average Retention Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C15</td>
<td>15.36</td>
</tr>
<tr>
<td>C17</td>
<td>16.70</td>
</tr>
<tr>
<td>C19</td>
<td>17.96</td>
</tr>
<tr>
<td>C21</td>
<td>19.15</td>
</tr>
<tr>
<td>C23</td>
<td>20.51</td>
</tr>
<tr>
<td>C25</td>
<td>22.28</td>
</tr>
</tbody>
</table>

Figure 2.7 Gas chromatography chromatogram of A) AR homologue standards in a mixture solution and B) purslane seed extract characterized with ultraviolet detection.
**Figure 2.8** GC-FID quantification of AR homologues in purslane and triticale bran extracted with acetone. Results are expressed as mean values ± SEM.

**Figure 2.9** GC-FID quantification of AR homologues in purslane and triticale bran extracted with ethyl acetate. Results are expressed as mean values ± SEM.
Figure 2.10 GC-FID quantification of AR homologues in purslane and triticale bran extracted with ethanol. Results are expressed as mean values ± SEM.

The ethanol extract of purslane isolated the largest number of homologue C15 at 21.5mg/100g of DM. In regard to triticale bran, C21 was the AR found in highest composition at 82.3mg/100g of DM (Figure 2.10). The extraction with supercritical CO₂ yielded greater amounts of five out of six of the AR homologues in both PA and TB (Figure 2.11). It was most successful in extracting homologue C21 in PA (577.0mg/100g of DM) and TB (1220.1mg/100g of DM). Figure 2.12 displays total AR content as a function of method of extraction. Amongst the traditional solvent-based methods of extraction, ethyl acetate was the most successful at extracting the highest amount of total ARs in purslane and triticale bran with a concentration of 61.1 and 1423.3mg/100g of DM, respectively (Table 2.5).
Figure 2.11 GC-FID quantification of AR homologues in purslane and triticale bran extracted with supercritical CO₂. Samples marked ‘w/co-s’ represent the supercritical extraction process with a 10% ethanol co-solvent.

Figure 2.12 Total concentration of ARs in purslane and triticale bran as determined by GC-FID analysis by extraction methods: acetone, ethyl acetate, ethanol and supercritical extraction. Samples marked ‘w/co-s’ represent the supercritical extraction process with a 10% ethanol co-solvent.
Table 2.5 Summary of AR homologues and total AR content of purslane and bran based on traditional extraction methods with acetone, ethyl acetate and ethanol and supercritical fluid extraction method. Quantification performed by GC-FID analysis Results expressed in units of mg/100g of DM.

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Sample</th>
<th>C15</th>
<th>C17</th>
<th>C19</th>
<th>C21</th>
<th>C23</th>
<th>C25</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purslane</td>
<td>Acetone</td>
<td>5.7</td>
<td>3.0</td>
<td>4.9</td>
<td>5.6</td>
<td>3.0</td>
<td>12.1</td>
<td>34.2</td>
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<td></td>
<td>Ethyl acetate</td>
<td>2.1</td>
<td>11.9</td>
<td>6.5</td>
<td>12.9</td>
<td>7.6</td>
<td>20.1</td>
<td>61.1</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>21.5</td>
<td>2.7</td>
<td>3.1</td>
<td>3.3</td>
<td>8.5</td>
<td>3.6</td>
<td>42.7</td>
</tr>
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<td></td>
<td>Supercritical CO₂ without co-solvent</td>
<td>32.2</td>
<td>7.9</td>
<td>46.9</td>
<td>80.6</td>
<td>24.3</td>
<td>9.1</td>
<td>201.0</td>
</tr>
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<td>Supercritical CO₂ with co-solvent</td>
<td>18.3</td>
<td>21.1</td>
<td>74.1</td>
<td>236.6</td>
<td>161.6</td>
<td>66.1</td>
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<td>Triticale Bran</td>
<td>Acetone</td>
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<td>Ethanol</td>
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<td>81.1</td>
<td>10.5</td>
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<td>Supercritical CO₂ without co-solvent</td>
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<td>176.7</td>
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<td>115.6</td>
<td>34.5</td>
<td>727.5</td>
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<tr>
<td></td>
<td>Supercritical CO₂ with co-solvent</td>
<td>7.5</td>
<td>119.8</td>
<td>276.5</td>
<td>544.3</td>
<td>216.7</td>
<td>55.5</td>
<td>1220.1</td>
</tr>
</tbody>
</table>

In comparison with the collective traditional solvent extraction of purslane, SFE significantly increased the yield of total ARs to 577.9mg/100g of DM from a range of 34.2-61.1mg/100g of DM yielded from solvent extractions. SFE of triticale bran however was significant in increasing the yield to 1220.1mg/100g of DM in contrast to all other methods with the exception of the ethyl acetate extraction (1423.2mg/100g of DM).
2.4.3 Relative % homologue composition

The relative % homologue composition of ARs in purslane sample was determined using the data collected from GC-FID analysis. In reference to the supercritical extraction of purslane with co-solvent, the highest homologue relative to others was C21, which constituted 40.94% of the total AR content. The relative % homologue composition for this extract was C21 > C23 > C19 > C17 > C15. The method of extraction had an effect on which AR homologue was extracted in relative amounts to others in purslane sample, however with triticale bran each extraction method resulted in C21 being the compound in highest relative % composition for the sample.

2.4.4 Relative extract % yield

The percent yield of extract from the original sample mass was determined and outlined in Table 2.6. Traditional solvent-based extractions of purslane yielded 0.08-0.74% of the original sample mass. Among these, ethyl acetate yielded the highest amount of extract. Overall the supercritical CO₂ extraction of PA with 10% ethanol co-solvent resulted in the greatest yield amongst all methods of extraction at 4.24% w/w, which is greater than the average yield (3.55%) extracted with traditional solvents from triticale bran.
Table 2.6 Quantification of % extract yield from purslane and triticale bran samples. Results expressed as mean values.

<table>
<thead>
<tr>
<th>Extraction Solvent</th>
<th>Purslane</th>
<th>Triticale Bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>0.27</td>
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<td>Ethyl Acetate</td>
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<tr>
<td>Ethanol</td>
<td>0.08</td>
<td>4.91</td>
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<tr>
<td>Supercritical CO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without co-solvent</td>
<td>3.12</td>
<td>6.71</td>
</tr>
<tr>
<td>with co-solvent</td>
<td>4.24</td>
<td>7.44</td>
</tr>
</tbody>
</table>

2.5 Discussion

The extraction methods and their respective quantification of ARs yielded from purslane samples were compared to those in triticale bran, however it is noted that these findings are significant and stand alone in relevance as there has been no previous reporting of even the identification of alkylresorcinol in purslane seeds. The comparison to triticale bran was made simply to observe a comparison of purslane seed AR content to another natural source of ARs, triticale bran, which is the 'gold standard' for ARs in plants, but also to observe if the extraction methods would have an impact on yield and AR content on another plant material known to contain ARs. Although HPLC is a classic method of compound identification, GC-FID analysis was undertaken as an additional and more precise measure for the confirmation of ARs present in purslane sample and quantification of these homologues.
The solvent used for extraction naturally effects which compounds are able to be isolated from the matrix. Ethanol is usually deemed as the solvent that is able to extract almost all ARs from a plant sample although in this present study this solvent was not the most effective at extracting the greatest quantity of ARs amongst the traditional solvents used. However, when 10% ethanol was coupled with supercritical CO\(_2\) the yield of ARs from purslane seeds did increase drastically in comparison to SFE alone, indicating its efficacy as a solvent.

Overall, supercritical fluid was the most efficient at extracting alkylresorcinol from purslane seeds and also increasing the % extract yield. This is likely due to its ability to diffuse and dissolve compounds within the sample, which would otherwise not be accomplished with a traditional liquid solvent. It is postulated that the increased temperature of 40-50°C of the SFE method may have also aided in the extraction of these compounds. The extraction process of phytochemicals is often affected by temperature due to its ability to effect solubility and diffusion coefficient (Spigno, Tramelli, & De Faveri, 2007). In addition to the many benefits of SFE such as efficiency, efficacy and environmentally friendly, it is noted during experimentation that the extraction of purslane seeds was performed without any milling process to the seeds. With traditional solvents, plant material is usually broken down in order to optimize the surface area to volume ratio to produce maximum extraction. However, with supercritical fluids’ characteristic of diffusion and dissolution of sample this milling process is not necessary. Not only does this remove an extra process associated with extraction but it prevents the loss of volatile compounds that result from breaking down material (García-Rodríguez, Carro-Díaz, & Lorenzo-Ferreira, 2008).

The most logical next extension of this work would be to study AR derivatives that differ in ways of saturation of the alkyl chain, and in functional groups such as keto groups. It is likely
that they may be present within this portion of PA plant now that ARs have been identified, and can also serve other, valuable biological activities (Ross, Åman, Andersson, & Kamal-Eldin, 2004).

The introduction of alkylresorcinol in purslane seeds expands horizons to its many uses and as a nutritional source of ARs. In particular, *Portulaca oleracea* seeds can now be identified as a valuable source of alkylresorcinol for individuals with gluten intolerance or sensitivities, who otherwise could not reap the benefits of this phytochemical.

### 2.6 Conclusion

The results of this study are the first report of identifying alkylresorcinol in *Portulaca oleracea* seed. Ethyl acetate extraction of total ARs was the most successful amongst traditional solvent methods, however supercritical fluid extraction using CO$_2$ and 10% ethanol co-solvent performed better in retrieval of these phenolic lipids. These finding not only provide insight into a novel source of alkylresorcinols but suggest a more efficient, economical and environmentally friendly method of extraction of these valuable phytochemicals. This information can be used to further the use of purslane seeds and promote its intake as part of a healthy diet.
CHAPTER 3: ANTIOXIDANT CAPACITY OF PURSLANE SEED AND APPLICATION IN FOOD SYSTEM

3.1 Abstract

The antioxidant capacity of purslane seed extract was evaluated by measure of oxygen radical absorbance capacity, total phenolic content and DPPH inhibition. With an ORAC value of 136.1 μM TE/g, TPC value of 7.67 GAE/g, and 14.6% DPPH inhibition, purslane demonstrated antioxidant ability and comparable results to triticale bran. The potential of ARs (C15, C17, C19, C21, C23 and C25) and purslane extract to prevent rancidification of a food product via their antioxidant characteristic was evaluated via Rancimat. Alkylresorcinol homologue C19 was able to delay spoilage of enhanced-margarine most effectively to an induction time 11.52h, although all AR homologues drastically increased induction time from a baseline value of 1.18h. Purslane seed extract also demonstrated an increase of induction time to 2h, in comparison triticale bran-enhanced margarine with an induction time of 5.52h. This study was conclusive in confirming antioxidant ability of purslane seed extract both by evaluation of classic assays and ability to prolong the stability of a food product.

3.2 Introduction

Antioxidants have been associated with a wide range of beneficial health effects including anti-cancer, anti-inflammation, antibacterial and antimutagenic, amongst more (Cai, Luo, Sun, & Corke, 2004; Mitscher, Telikepalli, McGhee, & Shankel, 1996; Sala et al., 2002). Many of the ways in which antioxidants aid in promoting health is by combatting the free radicals generated by environmental and biological processes that cause damage to cells and
organisms in our bodies. Thus, the consumption of antioxidants as a major part of our dietary background may aid in improving health but also preventing disease.

Antioxidants may also serve the purpose of preventing food spoilage and increasing the shelf life of manufactured foods. However, the most dominant antioxidants used in the industry for this purpose, such as BHT, BHA and TBHQ, are associated with deleterious effects that put humans at risk of disease that can otherwise be avoided. Thus, the use of alternative and safe sources of antioxidants is of interest and study within food chemistry. Antioxidants of natural source may serve as this substitute option for acting as a preservative in manufactured food but also as a nutraceutical. Alkylresorcinols have the potential to be one of these antioxidants that enhance foods without any associated adverse effects. With the identification of alkylresorcinol in purslane seeds, we hypothesize that PA seeds possess antioxidant capacity which can also be applied to the preservation of foods.

In this study, the goal was to examine antioxidant activity and application of purslane seed extract and specifically alkylresorcinol. The objective for this collection of research was to A) evaluate the antioxidant capacity of purslane extract and compare with triticale bran extract, B) determine which AR homologue is most efficient at preventing rancidification of margarine and C) evaluate the capability of purslane to provide oxidative stability of fats in margarine.

### 3.3 Materials and Methods

#### 3.3.1 Purslane seed sample

The purslane seed sample was provided by Agriculture Research Institute in Karaj, Alborz, IR. No alterations were made to these samples for our research purposes.
3.3.2 Triticale bran sample

The triticale bran sample was provided by Agriculture Research Institute in Lethbridge, Alberta, CA. No alterations were made to these samples for our research purposes.

3.3.3 Oxygen radical absorbance capacity assay

The following method for assessing antioxidant capacity via ORAC was adopted from Gunenc and colleagues (Gunenc, HadiNezhad, Farah, Hashem, & Hosseinian, 2015). AAPH (Acros Organics, New Jersey, USA) was used to induce peroxyl radicals. The fluorescence probe used was fluorescein (J. T. Baker, Phillipsburg, New Jersey, USA). The control standard was the vitamin E analog 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma-Aldrich, St. Louise, Missouri, USA) prepared in concentrations of 100, 50, 25, 12.5 and 6.25 μM. The positive control used at 10 and 20 μM was Rutin (Sigma, St. Louise, Missouri, USA). All reagents and test samples were suspended in 75 mM potassium phosphate buffer (Mallinckrodt, Paris, Kentucky, USA) at a pH of 7.4. A 96-well microplate was loaded with 20 μL of buffer, standard, black (potassium phosphate buffer) or control solution as well as the sample of interest in respective wells. One hundred and twenty μL of 0.086 μM fluorescein solution was added to each well, except the blank. Gen5™ software was used to control the FLx800™ Multi-Detection Microplate Reader (Biotek Instruments, Ottawa, Canada) to incubate the microplate at 37°C for 20 minutes. The excitation and emission wavelengths of the system were set to 485 nm and 525 nm, respectively. After the incubation period was complete, 152 mM AAPH was added to each well with exception to the blank. The absorbance of each well was read at 1 minute intervals for a total of 60 minutes. A fluorescein decay curve was generated and
the area under the curve (AUC) was used to determine the ORAC value of each sample. The AUC is calculated using the following equation, where $f_0$ represents the initial fluorescence reading and $f_i$ is the fluorescence reading at time i:

$$AUC = 1 + \frac{f_1}{f_0} + \frac{f_2}{f_0} + \frac{f_3}{f_0} + \ldots + \frac{f_{89}}{f_0} + \frac{f_{90}}{f_0}$$

The Trolox standard curve generated from the results were used to create a regression equation that determined the samples in terms of Trolox equivalent (TE). The final results are reported in $\mu$mol TE/g of ARs.

### 3.3.4 Total phenolic content assay

Phenolic extractions of TB and PA samples were performed in a 1:20 w/v of acidified (1% acetic acid) methanol solution (80%) under constant stirring at room temperature for three hours. The mixture was then centrifuged at 4000 RPM for 15 minutes at 24°C in a Thermo Sorval centrifuge (Legend XT Series, Fisher Scientific, Nepean, Ontario, CA). The supernatant was passed through a syringe filter (Nylon, 0.25 mm, 0.45 $\mu$m). Different concentrations of the extracts were prepared in ddH$_2$O with serial dilution at two-fold, four-fold and eight-fold. Gallic acid standards were prepared in ddH$_2$O at concentrations of 0.75, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL. 50 $\mu$L of all standards and samples were stored in amber 1.5 mL Eppendorf tubes.

Four hundred and seventy five $\mu$L of 10 times diluted Folin-Ciocalteu reagent (FC) was added to all standards and samples, except the blank that was filled with 700 $\mu$L of FC. The tubes were vortexed and left to stand for five minutes. Subsequently, 475 $\mu$L of 60 g/L sodium carbonate solution was added to all tubes, with exception of the blank where 700 $\mu$L was added.
All samples, standards and blank were incubated in the dark for 2 hours and then vortexed and plated at a volume of 200 μL in the microplate.

The absorbance was measured at 725 nm wavelength using a Cary 50Bio UV-Visible Spectrophotometer (Varian Inc., Australia). All samples were performed and analyzed in triplicate.

3.3.5 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH assay for assessing antioxidant activity utilized gallic acid standards in 80% methanol solution at concentrations of 1, 10, 20, 30, 40, 50, and 60 μg/mL. Dried samples of interest were also dissolved in 80% methanol solution. Two hundred μL of the methanol solution was used as a blank and 200 μL of 50 μM DPPH was used as a positive control in the microplate. The remainder of the standards and samples were plated in the microplate at a volume of 20 μL. The same DPPH solution was added to each well at 180 μL, with the exception of the blank and positive control, and then gently mixed. The 96-well plate was then covered and stored in the dark for 60 minutes. After incubation, the absorbance of each well was read at 519 nm using the FLx800 with Gen5 software (BioTek Instruments, Vermont, USA). Each sample was analyzed in triplicate. Antioxidant activity was determined based on the percent discoloration of the sample, calculated by the following equation:

\[
\text{DPPH Scavenging Activity} (\%) = \left[ 1 - \frac{A_{\text{sample}}}{A_{\text{control t=0}}} \right] \times 100\%
\]
The percent DPPH scavenging activity was graphed as a function of the different concentrations of gallic acid standards. The calculated sample values were compared to the standards, and their antioxidant capacity expressed in mg GAE/g of sample.

3.3.6 Rancimat

Samples of margarine at 3g were mixed with 1mg AR standard analogues C15, C17, C19, C21, C23 and C25. Two margarine samples were enhanced with 100 μL of triticale bran or purslane seed extract. One sample of margarine without treatment of AR or extract was evaluated, as well. An accelerated aging test was performed on margarine samples using a 743 Rancimat (Metrohm AG, Switzerland). Measuring vessels were filled with 60mL of ddH2O and the heating block was raised to a temperature of 121.6°C and a gas flow rate of 20L/h was applied. The induction time was evaluated to determine the oxidation rates.

3.3.7 Statistical Analysis

Three trials for each sample was performed. Results are summarized as mean values ± SEM. T-test was applied to the results in order to determine statistical significance of the values. P values less than 0.05 are conclusive of significant results.
3.4 Results

3.4.1 Antioxidant activity

The ability of *Portulaca oleracea* to inhibit or decrease the formation of free radicals was measured by three different antioxidant activity assays. Oxygen radical absorbance capacity, total phenolics and DPPH assays were used to assess antioxidant activity from a multimodal perspective. Figure 3.1 displays the ORAC value of purslane, 136.1μM TE/g, in comparison to triticale bran, 194.8μM TE/g. The TPC results for PA and TB are outlined in Figure 3.2 at 7.67 and 6.83mg GAE/g of sample, respectively. Lastly, the % inhibition of the radical DPPH was measured at 14.6% and 16.4% correspondingly for purslane and triticale bran.

**Figure 3.1** ORAC antioxidant activity of phenolic extracts of triticale bran and purslane in μmol TE/gram. Results are expressed as mean values ± SEM. Values marked by asterisk are statistically significant (*P < 0.05*).
Figure 3.2 Total phenolic content of phenolic extracts of triticale bran and purslane in mg GAE/g. Results are expressed as mean values ± SEM. No statistical difference between samples were found ($P > 0.05$).

Figure 3.3 Antioxidant activity of phenolic extracts of triticale bran and purslane expressed by percent inhibition of DPPH radical (%). Results are expressed as mean values ± SEM. No statistical difference between samples were found ($P > 0.05$).
Table 3.1 Summary of antioxidant activities of triticale bran and purslane as determined by ORAC, TPC and DPPH scavenging activity. All values are expressed as mean ± SEM. Asterisks identifies statistical significance.

<table>
<thead>
<tr>
<th>Antioxidant assay</th>
<th>Sample</th>
<th>Triticale bran</th>
<th>Purslane</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC (μmol TE/g)</td>
<td>194.8 ± 1.84*</td>
<td></td>
<td>136.1 ± 8.72*</td>
</tr>
<tr>
<td>TPC (mg GAE/g)</td>
<td>6.83 ± 0.04</td>
<td></td>
<td>7.67 ± 0.64</td>
</tr>
<tr>
<td>DPPH (% inhibition)</td>
<td>16.4 ± 2.65</td>
<td></td>
<td>14.6 ± 1.02</td>
</tr>
</tbody>
</table>

Table 3.1 summarizes the results of the all three antioxidant assays performed on purslane and triticale bran along with their SEM values. The values measured by ORAC were the only one of three assays that were deemed as statistically significant, although all antioxidant values between PA and TB were closely comparable in measure. Purslane performed better than triticale bran in antioxidant capacity measured by TPC assay. Overall, the results were conclusive for demonstrating the presence of antioxidant ability of purslane.

3.4.2 Oxidative stability

The oxidative stability of margarine enhanced with six different AR homologues were evaluated to assess the phenolic lipid’s capacity to delay the onset of rancidification in a common food product, in this case margarine. The induction period was determined as a function of the conductivity of the ddH2O in the vessels of each sample. As samples decay, the reaction products gathered in deionized water increases electrical conductivity.
In comparison to a baseline induction period value of 1.18h, which was measured for store-bought margarine with no additives, all samples enhanced with the six AR homologues were able to significantly increase the induction period of margarine samples to 10.98 – 12.80h (Figure 3.4). Homologue C15 was the most effective inhibitor of rancidification among the AR homologues tested.

In another Rancimat study outlined in Figure 3.5 margarine samples mixed with purslane and triticale bran extract were evaluated for their capability to hinder decay of sample. Purslane was able to increase the induction time to 2.00h, while triticale bran did the best at preventing rancidity by prolonging the induction time to 5.52h.
Figure 3.4 Rancimat curves displaying induction periods of AR homologues C15, C17, C19, C21, C23 and C25 at gas flow rate of 20L/h.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Induction Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margarine</td>
<td>1.18</td>
</tr>
<tr>
<td>Margarine + purslane</td>
<td>2.00</td>
</tr>
<tr>
<td>Margarine + bran</td>
<td>5.52</td>
</tr>
</tbody>
</table>

**Figure 3.5** Rancimat curves displaying induction periods of samples margarine, margarine mixed with purslane extract, and margarine mixed with bran extract at gas flow rate of 20L/h.
3.5 Discussion

As postulated, *Portulaca oleracea* demonstrated antioxidant potential by three different methods of analysis. The antioxidant potential of purslane demonstrated from ORAC, TPC and DPPH inhibition were closely comparable to that of triticale bran, which with similar values has been recognized as a positive antioxidant source for overall health and nutraceutical application (Agil & Hosseinian, 2012). Although the antioxidant activities of these two plants were similar, the findings from our preceding chapter indicate that total AR content of purslane seeds are lower than that of triticale bran. This suggests that there may be non-alkylresorcinol compounds present within the extracted material that contributes to the scavenging capacity of purslane.

Although we still believe that alkylresorcinol is the main antioxidant phytochemical in this plant as its combination of resonance quinone structure, functional groups such as –OH and benzene ring are the most likely cause of its powerful potential. In addition, generally saturated fats result in greater resistors to oxidative rancidity, which is another indication as to why ARs are such a strong antioxidant.

The study of AR homologues mixed with margarine demonstrated its ability to hinder oxidative decay of this common store-bought food product. Although all homologues demonstrated potential within a close range, C15 best prevented rancidity of the sample. These findings become important in order to identify which AR homologues are the most efficient in acting as a food preservative. This information, along with that of the previous chapter, can aid in identifying the extraction method that would yield the AR homologue that is most efficient at increasing shelf life of a product, if chosen for this purpose.

The ability of TB extract to prolong the period of time before decay of margarine was superior to that of PA extract. If we attribute alkylresorcinol to the ability of extract to prevent
rancidity in margarine, then these findings are valid given purslane’s smaller concentration of total ARs. Although, again, antioxidant potential may be correlated to other phytochemicals present in purslane seeds as other plant extract have also been shown to exhibit antioxidant capacity (Azizkhani & Zandi, 2010).

Overall, these findings are insight to a new source of antioxidants that have now been demonstrated to be a valuable functional food ingredient to prolong the preservation of food and promote the health of individuals given its radical scavenging potential.

### 3.6 Conclusion

With the number of disfavourable effects associated with the use of synthetic antioxidants in the preservation of manufactured food, the adoption of a natural antioxidant within a food system poses as a safe alternative. Purslane seeds were demonstrated in this study to possess antioxidant activities with commensurable values to that of triticale bran. Although the antioxidant capacity of PA can be attributed to the ARs present, it is likely that other phytochemicals contribute to such radical scavenging activity. There was no correlation observed between the length of alkyl chain in ARs and the ability to prevent rancidity in manufactured margarine. The Rancimat test of margarine enhanced with purslane seed extract was conclusive that PA extract does have real potential in food manufacturing to delay the onset of oxidation and thus spoilage of food.
CHAPTER 4: PHYSICAL APPLICATION OF ARs AND PURSLANE SEED EXTRACT

4.1 Abstract

The ability of alkylresorcinol to incorporate and permeate through a membrane, representative of a biological membrane, was demonstrated by HPLC analysis of ARs passed through the membrane and photomicrographs of the same membranes visualized by SEM. Homologue C19 demonstrated the greatest permeability of 95.8% and C17 the greatest incorporation of 23.2%. AR and purslane seed extract were able to develop crystal structures within margarine and chocolate, demonstrating their potential to increase quality of food products.

4.2 Introduction

The ability of added compounds in foods to develop crystal structures can result in a range of benefits including increase melting point of the product, enhancing taste and texture. Fats and lipids have the ability to form these crystalline structures and the study of their form are significant to food chemists for determining ways to enhance foods based on what is advantageous from a consumer and manufacturer perspective.

The interaction of alkylresorcinol with membranes is a study of interest given its unique structure that characterizes this compound as amphipathic: the hydrophobic alkyl chain and hydrophilic benzene ring. These compounds have been demonstrated to possess the ability to exhibit their antioxidant properties within a membrane, indicating their incorporation (A. Kozubek & Nienartowicz, 1995). Further study to evaluate the direct incorporation of ARs within a membrane is needed to confirm this ability of resorcinolic lipids. It is hypothesized that
due to their amphipathic nature ARs have the potential to interact with membranes and incorporate well within food products.

The objectives of this study were to A) evaluate ARs permeability through and integration within a membrane and B) determine if purslane seed extract has the ability to result in crystal formation within chocolate and margarine based on their phytochemical constituents. These objectives collectively signify the functionality of alkylresorcinol in food and membranes

4.3 Materials and Methods

4.3.1 Purslane seed samples

The purslane seed sample was provided by Agriculture Research Institute in Karaj, Alborz, IR. No alterations were made to these samples for our research purposes.

4.3.2 Membrane incorporation

The LiposoFast microextrusion device was used to pass 500 μL of 10 μL AR/mL of methanol through a 200 nm porous polycarbonate membrane (Avestin, Inc., Ottawa, Ontario, CA). This was done back and forth between the membrane 21 times, with the last transfer ending at the opposite end of the membrane from which it started. HPLC analysis of the solution prior to and after being passed through the membrane was performed in order to determine the concentration of AR before and after. The identical method outlined in section ‘2.3.5 High performance liquid chromatography’ was used for HPLC analysis. The polycarbonate membrane was then coated with a 15nm layer of gold at a density of 8.96 g/m³ using a Quorum G150T ES.
The membranes were then visualized using a scanning electron microscope (SEM) under high vacuum conditions (10⁻³ Pa) at 10 kv voltage (Tescan, Brno, Kohoutovice, Czech Republic).

4.3.3 Incorporation of ARs and purslane extract in margarine and chocolate

Purslane phenolic extract was incorporated in store-bought margarine and chocolate at 1% after being melted down in a hot water bath of 40°C and left in 4°C refrigerator for 24 hours on a microscope slide. The same procedure was undertaken with chocolate and margarine samples without the addition of purslane extract and as well with the addition of AR homologue C19, for comparison. After 24 hours the samples were then coated in 15nm layer of copper at a density of 8.96 g/m³ and photomicrographs of the sample were taken using SEM technology in 10⁻³ Pa vacuum and 10 kv voltage.

4.4 Results

4.4.1 Membrane incorporation

Alkylresorcinols suspended in solution were passed through a polycarbonate membrane and visualized with SEM. Figure 4.1 displays the polycarbonate membrane prior to treatment with ARs using the Liposofast device. It is noted that each pore is unblocked by any substance. Figure 4.2 displays the six individual membranes after treatment with 10 μg/mL of AR homologues C15, C17, C19, C21, C23 and C25. It is noted that some pores of this membrane post-treatment are obstructed, as indicated by the lighter colour within these pores in comparison to the completely black colour of unobstructed pores. These microscopy photos are conclusive of alkylresorcinol integration within these polycarbonate membranes.
HPLC analysis of ARs suspended in solution prior- and post-treatment were performed in order to determine the percentage of ARs that were passed through the membrane and the percentage that was incorporated within the membrane (Table 4.1). Homologue C17 demonstrated the greatest incorporation within the polycarbonate membrane with 23.2% integration while homologue C19 exhibited the greatest potential to permeate through the membrane at 95.8%. Overall, there was no general trend that could be concluded between the length of the fatty acid chain of ARs and the degree of incorporation or permeability of the membrane.

![Cryo-SEM imaging of 200 nm porous polycarbonate membrane prior to treatment with resorcinolic lipids.](image)

**Figure 4.1** Cryo-SEM imaging of 200 nm porous polycarbonate membrane prior to treatment with resorcinolic lipids.
Figure 4.2 Cryo-SEM imaging of 200 nm porous polycarbonate membrane post-treatment of 10μL/mL of AR standards with LiposoFast Device. Incorporation of the following AR analogues within the membrane were visualized: (A) C15, (B) C17, (C) C19, (D) C21, (E) C23 and (F) C25. Photomicrographs taken at a magnification of 20.00kx.
Table 4.1 Summary of percentage of ARs incorporated and passed through polycarbonate membrane, as determined by HPLC analysis of solution prior- and post-treatment.

<table>
<thead>
<tr>
<th>AR analogue</th>
<th>Membrane incorporation (%)</th>
<th>Permeability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C15</td>
<td>9.20</td>
<td>90.8</td>
</tr>
<tr>
<td>C17</td>
<td>23.2</td>
<td>76.8</td>
</tr>
<tr>
<td>C19</td>
<td>4.25</td>
<td>95.8</td>
</tr>
<tr>
<td>C21</td>
<td>15.8</td>
<td>84.2</td>
</tr>
<tr>
<td>C23</td>
<td>9.97</td>
<td>90.0</td>
</tr>
<tr>
<td>C25</td>
<td>12.6</td>
<td>87.4</td>
</tr>
</tbody>
</table>

4.4.2 Crystal structure of AR-enhanced chocolate

Margarine and chocolate enhanced with alkylresorcinol homologue C19 and purslane extract were visualized with SEM and compared to margarine and chocolate without such additions. Figure 4.3 displays the photomicrographs of margarine samples. Figure 4.3 (A) sample of unaltered margarine contains no crystals. Figure 4.3 (B) of margarine with the addition of alkylresorcinol C19 contains many crystal structures between 10-30 μm. Consecutively, Figure 4.3 (C) displays margarine enhanced with purslane seed extract resulting in the same size crystal but in less quantity and less complete development. Both margarine samples enhanced with either alkylresorcinol standard or purslane seed extract exhibit single grown crystals as oppose to clusters. Figure 4.4 (A) exhibits unaltered chocolate while Figure 4.4 (B) presents chocolate enhanced with AR C19. These crystals have a more uniform organized arrangement and appear
to have grown on and from each other. Figure 4.4 (C) shows chocolate incorporated with purslane extract with few crystals present. Both chocolate samples that were enhanced with either extract or AR standard exhibit a wide range of crystal size from 30-200 μm.

**Figure 4.3** Cryo-SEM imaging of A) plain margarine, B) 1% AR-enhance margarine and C) 1% purslane extract-enhance margarine. Photomicrographs taken at a magnification of 1.00kx.

**Figure 4.4** Cryo-SEM imaging of A) plain chocolate, B) 1% AR-enhance chocolate and C) 1% purslane extract-enhance chocolate. Photomicrographs taken at a magnification of 1.00kx.
4.5 Discussion

The qualitative and quantitative data in this study was successful in revealing the potential of alkylresorcinol to incorporate within a membrane and permeate through it, as well. The size of the polycarbonate membrane used was 200nm in order to model the blood-brain barrier (Ben-Zvi et al., 2014). Thus, these findings suggest that alkylresorcinol has the potentiality to permeate through the blood brain barrier which in turn gives it the ability to prevent or inhibit the oxidative stress which leads to the degeneration of dopaminergic neurons associated with Parkinson’s disease.

Further study would be needed to evaluate the direct effect of alkylresorcinol within the blood brain barrier. This antioxidant’s unique structure may be what gives rise to its ability permeate through membranes that other antioxidants would otherwise not be capable of given their sterics and functional groups.

The capacity of alkylresorcinol to integrate within a membrane itself means that it can serve an entirely different purpose as well. The tissues of animals are mainly composed of unsaturated fatty acids in the phospholipid membrane of cells. Specifically, these lipids are vulnerable to oxidation due to their electron-deficient double bonds (Brewer, 2011). Thus, the ability for an antioxidant, such as alkylresorcinol, to incorporate into and interact with a membrane can signify potential to prevent this oxidation. The capacity of ARs to assimilate within membranes is likely due to their amphiphilic character (Gubernator, Stasiuk, & Kozubek, 1999). In addition, this membrane incorporation means that ARs can contribute other characteristics to membranes such as fluidity as a result of their long alkyl chain.

Finally, visualization of margarine and chocolate enhanced with alkylresorcinol and purslane extract revealed crystal structure that is valuable in food manufacturing. The value in
the resulting crystal structure as a function of alkylresorcinol and a natural plant extract means that these substances can be used as a method for improving the physical characteristics of food but also as a nutraceutical. In terms of physical characteristics, these crystals can increase the melting point of chocolate and margarine and enhance the texture of the product, as well. The small size of crystals within the margarine sample signifies rapid cooling of the substance while the larger crystals visualized within chocolate represents a slower cooling process. This may not be indicative of alkylresorcinol as a crystal but simply due to the fact that margarine of less density cooled more quickly than a higher-density material like chocolate.

4.6 Conclusion

The incorporation and permeation of antioxidants within and through a biological membrane can serve important modes of action amongst these two regions. The findings in this study demonstrated the ability of alkylresorcinol to perform both these activities. As a powerful biologically active compound, ARs ability to permeate through membranes means it has the potential to exert its antioxidative abilities in difficult to access regions, such as the blood brain barrier. In addition, the experiments performed in this chapter are conclusive of a unique characteristic of crystal formation by alkylresorcinol and PA extract. This functionality of ARs and PA extract can result in yielding desirable traits in manufactured food such as high melting point and smooth texture, amongst others.
CHAPTER 5: CONCLUSION AND FUTURE DIRECTION

With detailed study and analysis using the discussed experiments and theories in this present project, it is concluded that seeds of the *Portulaca oleracea* plant and alkylresorcinols of homologues C15, C17, C19, C21, C23 and C25 are of valuable use based on both their biological and non-biological capacities.

The identification of alkylresorcinols within purslane seeds, for the first time ever studied, reveals the potential to utilize this source of valuable antioxidants for the benefit of human health. The unique structure of ARs allows this compound to be a powerful, yet stable antioxidant. Thus, the consumption of these phytochemicals, from sources such as purslane seeds, can prevent or impede oxidation reactions that are associated with many diseases. The ability of this compound to interact with phospholipid membranes, as well as penetrate through, allows it to exert its capabilities in parts of biological systems that are otherwise not easily accessible by other compounds. Yet, alkylresorcinols within purslane seeds are not only limited to this mode of action.

In non-biological systems, alkylresorcinols and PA seeds can be used for their antioxidative capacity to aid in the preservation of manufactured food. This poses as a unique alternative to synthetic antioxidants, which although widely used, are associated with a number of deleterious effects in regard to human health and consumption. The incorporation of PA extract and ARs in common foods, such as margarine and chocolate, demonstrated their ability to form crystalline structures. Not only is this beneficial from a manufacturing perspective, but also enhances the quality of the product from a consumer point-of-view.

What further makes these characteristics and applications so special is that they are encompassed within a single compound and plant material. So, when integrating PA seeds in diet
or manufactured foods, the benefit is never just one aspect. This unique plant has many multi-modal uses without any perceived negative effects. This research has been insightful in revealing novel uses for this plant, however this is just the starting point for further investigation to come.

Now that alkylresorcinols have been identified in purslane seeds, it is very likely that there remains unsaturated ARs and other analogues that have yet to be characterized. The research into this area may reveal these compounds and their associated characteristics as well. A valuable investigation would be to undergo x-ray diffraction of purslane- and AR-enhanced chocolate to determine the method of incorporation that would yield crystalline morphology in beta form. This application would yield information in the food industry that is highly favourable for manufacturing foods with these compounds. A further study in order to assess the integration of this plant extract in food material from a different perspective would be to evaluate the sensory perceptions from a consumer point-of-view. This research would include evaluating flavour, taste, odor, texture and colour, which are all valuable considerations when introducing food product on the market.

With consideration to all the above-mentioned work and conclusions within this study, Portulaca oleracea has become a plant of growing intrigue and poses as a promising nutraceutical and food additive. This research not only displays novel findings but demonstrates real world application for this newly discovered information.
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