VALORIZATION OF CARROT PROCESSING WASTE

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ABSTRACT

Valorization of Carrot Processing Waste

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Commercial carrot processors produce up to 175,000 tons of carrot waste annually. Carrot Mash (CM) is the term referring to the waste by-product of peeled baby carrot processing. Transportation of carrot processing waste is expensive due to its high-water content (approx. 83-95%). High in bioactive compounds (carotenoids) and dietary fibers, it is expected that its conversion into a value-added by-product is of interest to the carrot processing industry. Hemicellulose-rich plant materials have proven to be a source of oligosaccharides, which are known for their beneficial prebiotic activity. The objectives of this research were to: 1) determine the effect of mechanical treatments on the extraction of water and bioactive compounds and evaluate the functional properties of carrot mash; 2) incorporate dried carrot mash into a beef patty and evaluate changes in pH, color, cooking yield, and texture; 3) apply an enzymatic treatment to carrot mash to promote the conversion of polysaccharides to oligosaccharides for prebiotic benefits.

Mechanical separation of liquid and solid fractions by way of expeller pressing was efficient in extracting liquid while simultaneously increasing total solids by nearly 200%, the extraction of carotenoids by 1000%, and polyphenol content by nearly 97%. Mechanical treatments increased the fat binding capacity on average by 183% compared to untreated mash. The addition of unpressed carrot mash or expeller pressed carrot mash increased the cooking yield of a beef patty by 3-13% without significantly changing its textural properties. Enzymatically treating the carrot mash significantly increased the concentration of oligosaccharides up to 2.3%.

These results suggest that carrot processing wastes can be physically and enzymatically modified and have an immense potential to be utilized as a functional ingredient in human food rather than being landfilled, composted or used as animal feed.

Keywords: Carrot mash, Carrot pomace, Mechanical pretreatment, Functional Properties, Enzymatic treatment

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CHAPTER 1 – INTRODUCTION

1.1 Background Information

The production, preparation, and consumption of food results in large quantities of food waste; nearly 1.3 billion metric tons each year, making food waste and loss reduction a significant focus for the food and agriculture sectors (Gustavsson et al., 2011).

The United States Department of Agriculture set a national goal of reducing food loss and waste by 50% of current levels by 2030 (USDA, 2015). The Food and Agriculture Organization of the United Nations (FAO) describes food waste as any discarded food that is safe and edible for human consumption, and food loss as any food that is lost in the supply chain between the producer, processor, and retailer; usually due to inefficiencies in production and processing (Gustavsson et al., 2011). Inefficiencies in fruit and vegetable processing alone can result in 25 to 30% of the edible product going to waste (Sagar et al., 2018). The FAO estimated that waste and losses in fruits and vegetables are the highest compared to all other types of food, almost 60% of the annual loss (Gustavsson et al., 2011).

Majority of food loss and waste happens between the production and retail levels, approximately 180 kg/year per capita in North America (Figure 1-1) (Gustavsson et al., 2011). Production to retail includes everything from in-field harvest to processing and packaging facilities and even to grocery retail stores. North America produces the highest amount of food waste at the consumer level, equating to nearly 110 kg/year/capita.

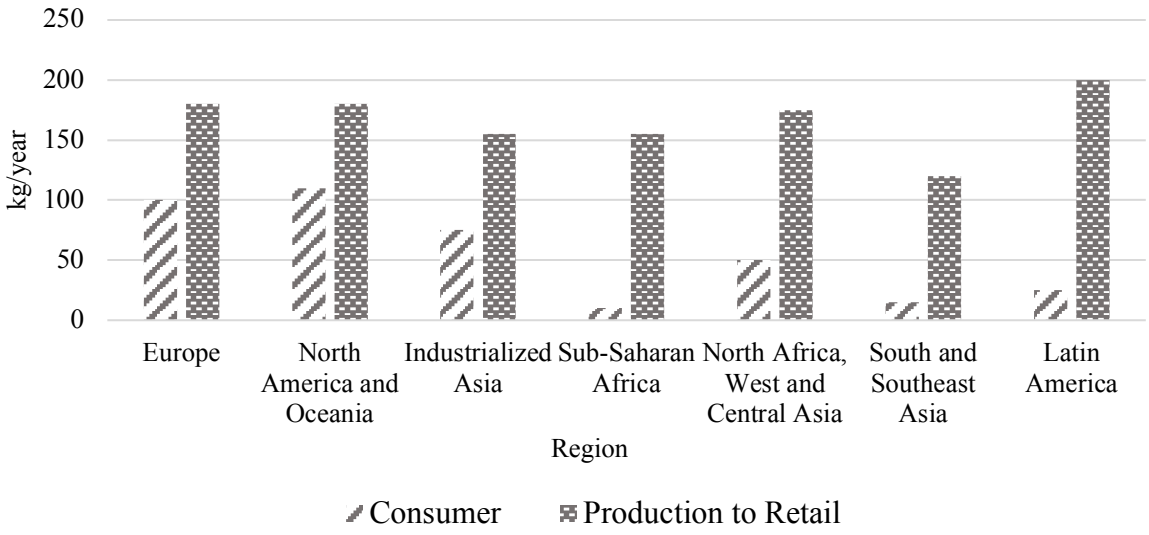

Figure 1-1. Per capita food loss and waste at production/retail and consumption in different regions (adapted from Gustavsson et al. 2011)

Convenience has played an important role in contributing to food waste at the processing and retail levels (Martín-Belloso and Soliva-Fortuny, 2011). Fresh-cut or minimally processed fruit and vegetable products have been available to consumers since the 1930s; however they did not gain popularity until the last two decades when consuming healthy and convenient food became an interest to consumers. In 2016, 49% of US households bought value-added (processed, ready-to-eat, convenient) vegetables (Nielson Perishables Group, 2017). The fresh-cut vegetable category of agriculture is a $1.3 billion industry (excluding pre-packaged salads), and carrots account for the largest share (nearly half) of supermarket sales. Carrots are followed distantly by potatoes and celery (Lin and Lucier, 2007). The same study reported a constant consumption of carrots over the last 30 years. In 2015, the most popular fresh-cut or minimally processed vegetable in the United States were peeled baby carrots (Wells, 2016).

By-products of fruit and vegetable processing include solid residue of peels, skins, seeds, stones, stems, and pulp. Pomace is the by-product of carrot juicing, while

carrot mash refers to the by-product of processing carrots into ready-to-eat products such as chips, sticks, shredded carrots and baby carrots. Carrot processing wastes are known to be a source of beneficial compounds; including phytochemicals such as polyphenols, carotenoids and dietary fibers (Anal, 2018). Carotenoids are precursors to vitamin A in the human body. Deficiency in vitamin A is the leading cause of premature death in children, validating the need to find methods of utilizing a carotene-rich waste product like carrot pomace and mash (National Institutes of Health, 2013). The dietary fibers remaining in carrot pomace are thought to have important functional properties such as water holding and fat binding, which can be beneficial in developing value-added food products. Separating the liquid and solid fractions can lead to the possibility for filtering the liquid for recovery of water to be used for further uses in the processing facility. Currently, carrot processing waste and its vital nutrients are being disposed of in landfills or occasionally used in cattle feed (Sharma et al., 2012). Carotenoids and dietary fiber have the potential to be utilized in new and beneficial ways for human consumption.

1.2 Statement of Research Questions

Can physical extraction methods be applied to separate the liquid from carrot mash? Can carrot mash be dried and used as a functional ingredient to enhance the properties of ground beef patties? Can the carbohydrate profile of carrot mash be modified with an enzymatic treatment to increase oligosaccharide concentration?

1.3 Approaches

The first objective of this project was to determine whether mechanical force by way of expeller press (high shear) or hydraulic press (compressive force) will produce higher yields when separating liquid and solid fractions of the carrot mash and if either

mechanical press affected the extraction of carotenoids or total polyphenols. It was hypothesized that the hydraulic press would produce higher yields when separating liquid and solid fractions and that the expeller press would produce a higher yield in extracting trapped nutrients. To test this hypothesis, percent extractable matter, total solids content, carotenoid content, and polyphenol content were analyzed for the liquid and solid fractions from each press. Dried hydraulic and expeller pressed carrot mashes were analyzed for water holding, swelling, and fat binding capacities with the intent to use as a functional ingredient in foods products. It was hypothesized that the mechanical pressing can enhance the ability for mash to absorb water and bind to fat. To test this hypothesis, water holding, swelling, and fat binding capacity of the pressed mash were each analyzed and compared to the functionality of unpressed mash.

The second objective was to compare the functionality of the dried, ground carrot mash, after mixing into ground beef patties using the best mechanically treated sample from the first objective that was conducted with unpressed carrot mash and a commercial carrot fiber. It was hypothesized that the carrot fiber would enhance cook yield of the beef patty while not changing the textural attributes, color, or pH; producing a patty with similar attributes to one with no carrot fiber. To test this hypothesis, pH, color, cooking yield, and texture analysis were performed on each type of beef patty sample and compared to a control patty where no fiber was added.

The final objective was to determine if the carbohydrate profile of the carrot mash could be modified to increase oligosaccharide concentration in the mash. It was hypothesized that limiting time and concentration of enzymatic treatment could enhance the conversion of polysaccharides into oligosaccharides. An enzyme cocktail containing a

mixture of cellulase, hemicellulase, xylanase, and pectinase, was used to hydrolyze polysaccharides in the carrot mash to test this hypothesis. The enzyme cocktail was used at 2 different concentrations (0.15% and 0.225%) and 2 different times (15 and 30 minutes) to compare to control samples.

This research was conducted in collaboration with Grimmway Farms, a large carrot growing and processing company located in California. Carrot waste used in this study was produced during the manufacturing of peeled baby carrots. Our objectives were to identify long-term benefits to the carrot processing industry and university research.

CHAPTER 2 – LITERATURE REVIEW

2.1 Food Waste

2.1.1 Global Food Waste

The FAO estimated that one-third of all edible foods produced for human consumption is wasted each year, equating to almost 1.3 billion metric tons (Gustavsson et al., 2011). In developing countries, food losses and waste can amount to as much as $310 billion, and roughly $680 billion in industrialized countries annually (Food and Agriculture Organization of the United Nations, 2019). Although food loss and waste have a direct impact on the world's food supply, they also amount to a major dissipation of resources, including water, land, energy, labor, and capital. The FAO (2019) also reported that along with the aforementioned squandering of resources, food loss and waste unnecessarily contributes to the production of greenhouse gas emissions, sequentially contributing to global warming and climate change. Globally, fruits and vegetables, including roots and tubers, have the highest wastage rates compared to any other food category (Figure 2-1) (Food and Agriculture Organization of the United Nations, 2019).

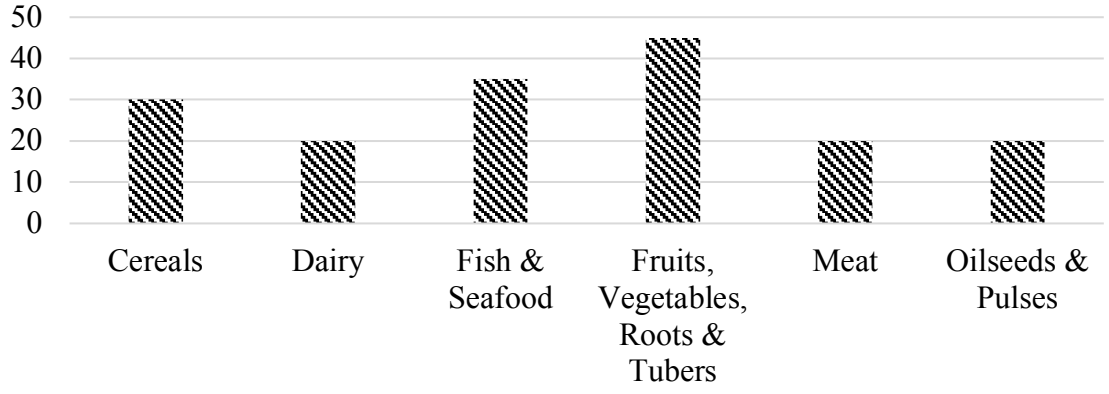

Figure 2-1. Global food losses (%) by category (adapted from Food and Agriculture Organization of the United Nations, 2019)

2.1.2 Food Waste in the United States

The amount of food waste generated in the United States is estimated between 30 and 40 % of the U.S. food supply (USDA, 2015). In 2013, the United States Department of Agriculture (USDA) joined the United States Environmental Protection Agency (EPA) to set a goal to reduce United States food waste by 50% by 2030 (USDA, 2015).

The USDA (2015) reported three important areas impacted by food waste. First, wholesome food is going to waste when it could be used to nourish families in need. Second, land, water, labor, energy, and other resources used to produce, process, transport, store, and dispose of these wastes are being dissipated. Third, food wastes are the largest component going into landfills, in-turn generating methane helping to make landfills the third-largest source of methane emissions in the United States.

In 2016, the EPA reported that 10.5 billion pounds of food waste was diverted from landfills, 60% going to unspecified land applications and 35% to animal feed stocks (Figure 2-2) (Food Waste Reduction Alliance, 2016).

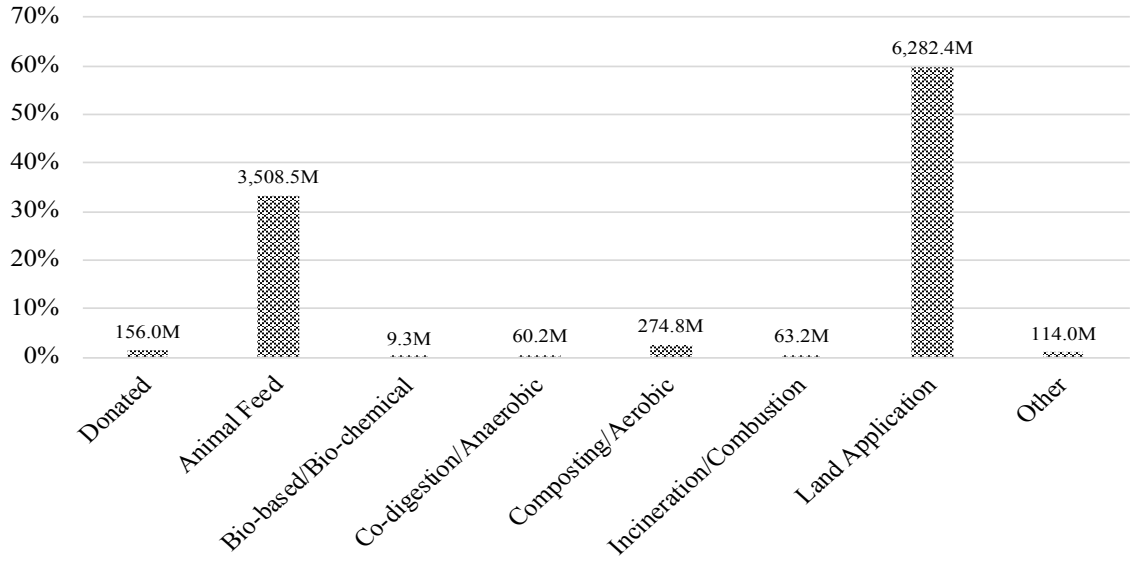

Figure 2-2. Diverted food waste (in pounds) (adapted from Food Waste Recovery Alliance, 2016)

2.1.3 Food Waste in California

CalRecycle (2019) reports that nearly 6 million tons of edible food were wasted or thrown away each year in California, representing about 18% of all the material that goes to landfills. Californians are combatting food waste in many ways at the consumer and retail levels, including programs like the Food Recovery Network and businesses like Imperfect Produce which provides consumers with "ugly" produce at a discounted price.

With all of these efforts to eliminate food waste at the consumer level, the abundance of commodities produced in California, including over a third of the country's vegetables and how they contribute to waste at the production and processing levels, must not be forgotten (CDFA, 2017). California continues to lead the country in fresh-market production, accounting for the production of nearly 53% of fresh market vegetables annually, at nearly $1.4 billion annually. (Wells, 2016). Large scale commercial carrot processors can produce up to 175,000 tons of carrot waste annually (Grimmway Farms, 2018).

2.2 United States Carrot Production and Consumption

The world carrot production equates to nearly 37 million tons, with China responsible for 36% of that production (Singhania et al., 2018). The United States of America is the 3rd largest producer of carrot in the world, over 85% of that coming from California where production is dominated by two large companies based out of the Central and San Joaquin Valleys, Bolthouse Farms and Grimmway Farms (FAO, 2017). Since 2012, the per capita consumption of carrots has remained steady at approximately 10.4 pounds, with 80% coming from fresh carrots and the remaining 20% being canned or frozen (Wells, 2016). The fresh-cut vegetable category of food and agriculture is a

$1.3 billion industry alone (excluding pre-packaged salads), and carrots account for the largest share (nearly half) of supermarket sales. Carrots are followed distantly by squash and celery at one and two percent of supermarket sales, respectively (Lin and Lucier, 2007).

Lin and Lucier (2007) reported that 94% of the U.S. population often purchased carrot juice or a carrot juice blend and 97% purchase fresh-market (whole) and processed carrots (baby, matchstick, coins, etc.) for home consumption.

2.3 Anatomy of a Carrot

The roots of certain vegetables are important nutrient sources. One of the main functions of a root is to absorb nutrients and moisture. Plants have two different avenues for transportation; the xylem (core) transports water from the roots to the leaves, and the phloem (flesh) transports food for the entire plant (World Carrot Museum, 2008).

The carrot root is comprised of six main elements; root cap, epidermis (periderm), root hairs, cortex, endodermis, and central core (Figure 2-3) (World Carrot Museum, 2008). Also known as peel or periderm, the epidermis takes in water to supply to the plant. The cortex is located below the periderm and is comprised of the phloem which serves the root by storing sugars for energy. The endodermis is a thin layer of cells surrounding the xylem and phloem and aids the root by forcing minerals into the vascular tissues (xylem and phloem). Finally, the central core is made up of the xylem, which helps move water from the root to the leaf.

There are over one-hundred different carrot varietals, but four main varietals grouped according to size, shape, and intended use (Lin and Lucier, 2007). The main varietals are Danvers, Nantes, Imperator, and Chantenay (Ernest, 2018). The Imperator

variety are what most commercial growers cultivate for fresh-market consumption and are what is typically found in grocery stores (Ernest, 2018).

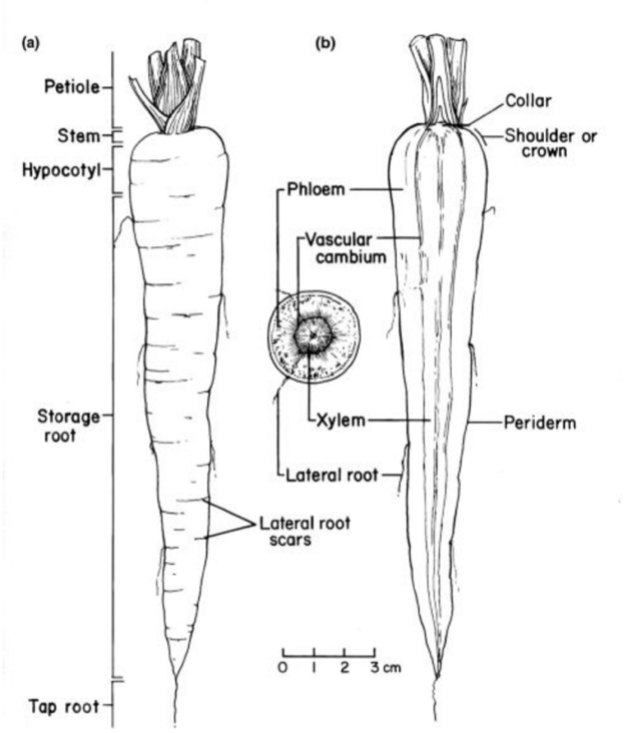

Figure 2-3. Cross section of carrot identifying the periderm, phloem, and xylem (image courtesy of the World Carrot Museum, 2008)

2.2 Carrot Processing

Since the early 1990's, peeled baby carrots have been one of the fastest growing segments of the carrot industry and continue to be one of the most popular produce items in the supermarket. Peeled baby carrots are typically those of the Imperator carrot variety. They are planted close together, forcing them to grow into long, thin, and sweet mature carrots. Post-harvest, these carrots are transferred to a processing plant where they go through a series of washing, sorting, and cutting processes to create the small uniform snacks known as baby carrots (Figure 2-4) (Lin and Lucier, 2007).

Billions of pounds of fresh and processed carrots are produced each year (Figure 2-5). Fresh cut and peeled carrot processing results in large amounts of carrot waste

which contain valuable bioactive compounds that can be utilized for animal and human consumption rather than being discarded (Lin and Lucier, 2007).

Figure 2-4. Processing for Peeled Baby Carrots (adapted from McCarthy, 2014).

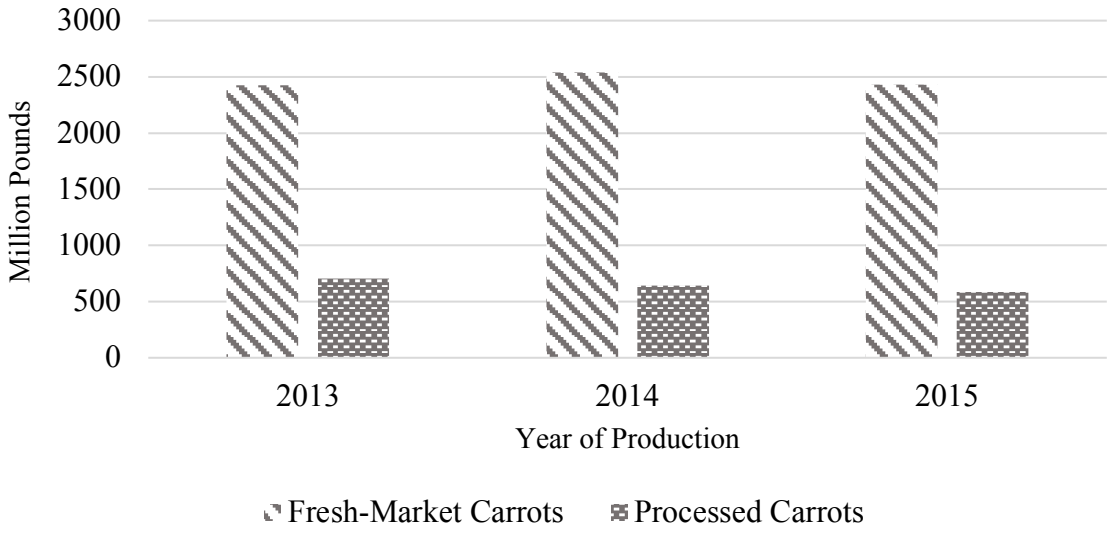

Figure 2-5. Annual U.S. Production of Fresh-Market Carrots Compared to Processed Carrots (adapted from Wells, 2016).

There are two classifications of carrot waste; pomace and mash. Pomace is the waste by-product of carrot juicing and mash is the term referring to the waste byproduct of the peeling and cutting process for ready-to-eat carrot products (i.e. peeled baby carrots, matchsticks, shredded carrots, and carrot chips) (Figure 2-6). Carrot wastes are

unique because they are often edible, nutrient dense wastes, compared to wastes from cauliflower or broccoli, where majority of the waste are leaves and stalks.

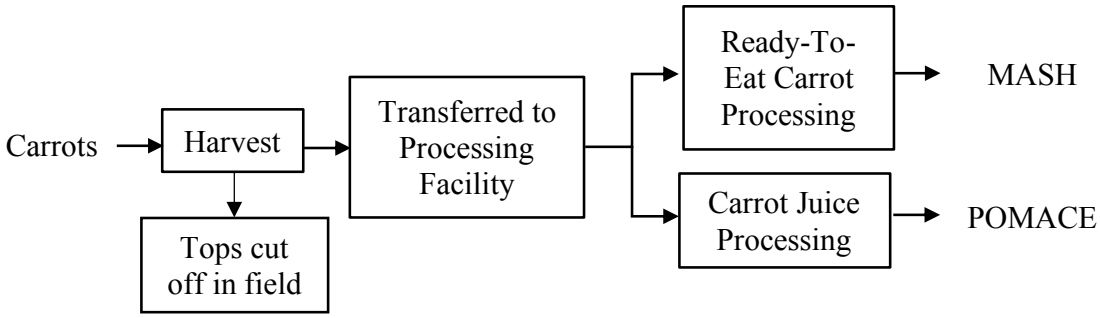

Figure 2-6. Carrot Mash Production.

2.4 Phytonutrients

Carrots are an important root vegetable rich in bioactive compounds like carotenoids (specifically lutein and carotene) and dietary fibers (de la Rosa et al., 2010). The extraction and recovery of these health benefitting compounds could be of interest to carrot processors to utilize their waste for value-added ingredients.

2.4.1 Carotenoids

Carotenoids are organic pigments naturally occurring in the chromoplasts of plants and other photosynthetic organisms (de la Rosa et al., 2010). They are fat-soluble micronutrients; their structure being made up of a repeating, branched five-carbon unit. There are more than 600 derived carotenoids divided into two separate classes; carotenes and xanthophylls. Carotenes are grouped hydrocarbon carotenoids, and xanthophylls are oxygenated carotenoids. In non-green tissues like carrots, carotenoids are found in the chromoplasts (de la Rosa et al., 2010). A chromoplast is a plastid, or a major double membrane organelle that holds chemical compounds (i.e. pigment) in the plant cell. In most fruits and vegetables, carotenoids are concentrated in the peel, and in carrot they

exist as crystals, gradually increasing from the periderm (peel) toward the core (de la Rosa et al., 2010).

Carrots, from which carotenoids derive their name, are rich in four main carotenoids; lutein, lycopene, α-carotene and ß-carotene (Figure 2-7).

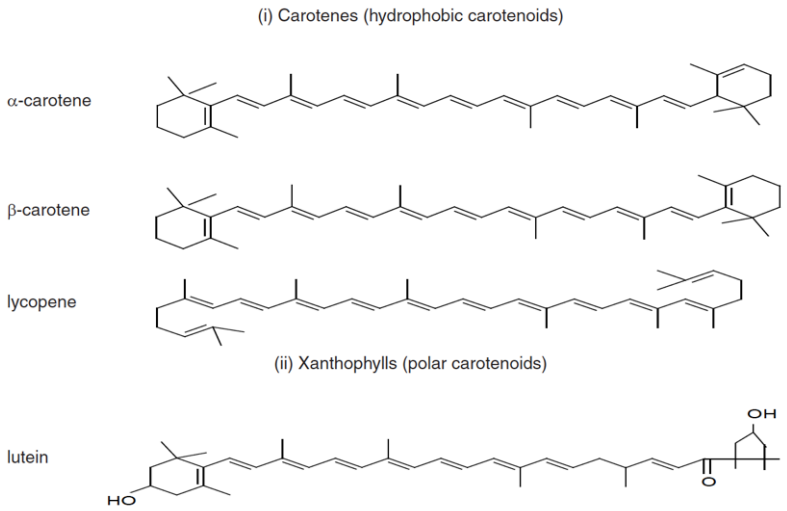

Figure 2-7. Structure of common natural carotenoids (adapted from Kiokias et al., 2016).

In fruits and vegetables, carotenoids impart favorable orange, red, and yellow colors which are perceived by the consumer as being of high quality and freshness (Anal, 2018). Carotenoids and other phenolic compounds have been extracted from fruit and vegetable varietals and this could likely be conducted with carrot varietals as well (Balasundram et al., 2006). Carotenoid content of carrots has been reported as 18.3 mg/100g, higher than spinach, beetroot, and broccoli (5.6, 1.9, and 1.3 mg/100g, respectively) (Rebecca et al., 2014). The carotenoid content of fresh raw carrots was reported to be 14.82 mg/100g and 5.04 mg/100g after dehydration (Al-Dabbas et al, 2015).

Carotene is a precursor to vitamin A in the human body (Figure 2-8). Vitamin A is a fat soluble vitamin important in supporting heart, lung, kidney, and other organs to

work properly and benefitting healthy vision, skin, and bone (de la Rosa et al., 2010). It is also important for the immune system and reproductive health (National Institutes of Health, 2013). ß-carotene is converted into vitamin A faster in the human body compared to other carotenoids. People who eat a significant amount of foods containing ß-carotene might have a lower risk of certain cancers, preventing age-related macular degeneration, and less severe cases of measles in young children compared to individuals who eat less ß-carotene containing foods (National Institutes of Health, 2013). Moreover, deficiency in vitamin A is the leading cause of premature death in children, validating the need to find methods of utilizing a carotene-rich waste product like carrot pomace and mash (National Institutes of Health, 2013).

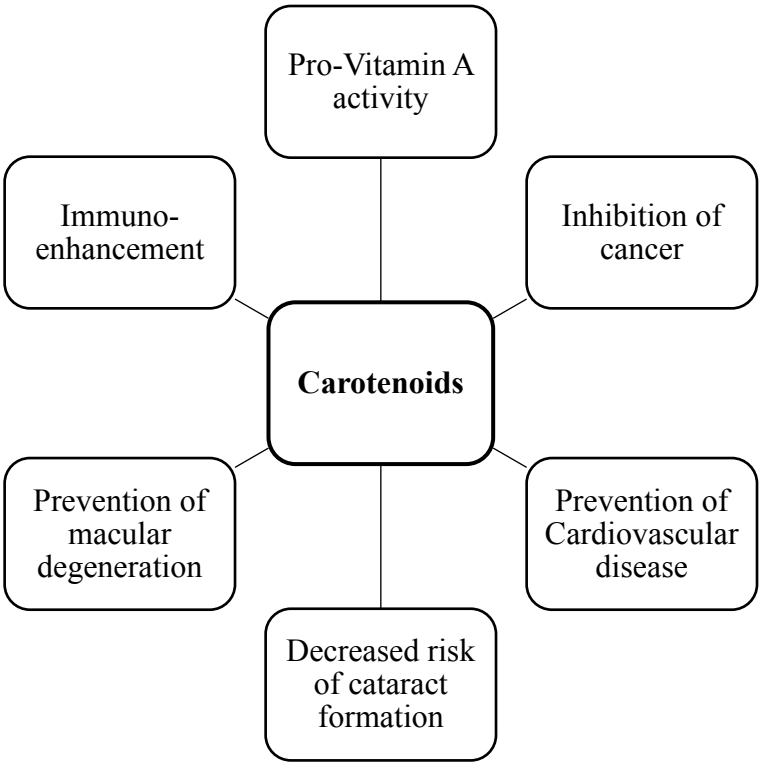

Figure 2-8. Health promoting functions attributed to carotenoids (adapted from Sharma et al., 2012).

Carotenoids are sensitive to heat, light, and oxygen. During processing and storage, isomerization and oxidation can cause color change and loss in biological

activity as well as aid in the formation of volatile compounds that could potentially impart desirable or undesirable flavors. Freezing and freeze-drying can increase the stability of carotenoids by reducing their exposure to heat and reducing the rate of oxidation due to low temperatures (de la Rosa et al., 2010).

The impact of blanching, freezing, and frozen storage on the carotenoid content of two different carrot varietals containing α-carotene, ß-carotene, lutein and lycopene was studied by Behsnilian & Mayer-Miebach (2017). The only loss of carotenoid content in the carrots was reported in α-carotene at approximately by 40% loss after two years storage at the temperatures of -15°C or lower. Lutein and ß-carotene levels remained constant while lycopene levels showed a loss with increasing temperature compared to that of α- and ß-carotene levels. In Kintoki carrots, only 20% of the ß-carotene content was lost after 8 weeks of storage of at 1°C (Spieß and Mayer-Miebach, 2003). Maintaining carotenoid contents through frozen storage of carrot processing wastes are important for researchers and industry personnel when considering storage conditions post-processing to reduce losses in carotenoid bioavailability.

2.4.2 Polyphenols

Polyphenols are known for their inhibitory effects on mutagenesis and carcinogenesis in humans by scavenging free radicals. As a result, they are often highlighted as the most important and largest groups of bioactive compounds produced in vegetables (de la Rosa et al., 2010). They have also shown to reduce the glycemic index, cholesterol, and inflammation (Friedman and Levin, 2009). Different polyphenols include catechins (found in tea leaves), flavonoids, flavanols, and anthocyanins (Scalbert and Williamson, 2000). Polyphenols are known for their antioxidant properties, which protect

cells from free radical damage. Free radicals are responsible for DNA damage, emphasizing that antioxidants are important in cancer prevention. Polyphenols can also react with radicals; such as hydroxyl and lipid peroxyl radicals which are known to cause lipid oxidation, one of the main causes for shorter shelf life in food products (Arvanitoyannis et al., 2006). Aside from their beneficial properties in the human body, polyphenols such as anthocyanins are responsible for the red, purple, and blue colors in fruits and vegetables. They also help with germination and protecting plants from pathogens and predators (Bravo, 1998).

Polyphenols are present in high concentrations in carrots, especially in the periderm, better known as the peel of the carrot. The phenolic contents in different tissues decrease in the following order; peel (periderm) > phloem > xylem. As a result, those compounds are typically in higher concentrations in the peels and stems, which are often discarded after harvest (Jung et al., 2011).

Sharma et al. (2012) reported that the peel of a carrot only accounted for 11.0% of the carrot fresh weight, but it provided 54.1% of the total phenols, while the phloem tissues provided 39.5%, and the xylem tissue provided only 6.4%. The main phenolic compounds found in carrots are chlorogenic acids (Figure 2-9), which contribute to the organoleptic properties of fresh and processed carrots (Rubatzky et al., 1999). Chlorogenic acids present in coffee have been associated with reductions in several chronic diseases (Higdon et al., 2007). Orange, purple, yellow, and white carrots have been associated with 11 different phenolic acids, mainly hydroxycinnamic acid derivatives (Figure 2-10).

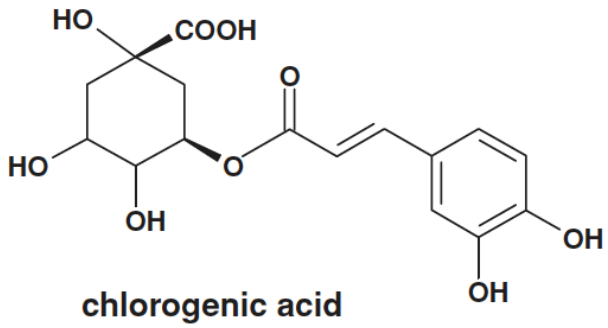

Figure 2-9. The main phenolic compound, chlorogenic acid, found in carrots (adapted from Arscott and Tanumihardjo, 2018)

Alasalvar et al. (2001) identified chlorogenic acid concentrations as 54.1, 8.5, 4.5, and 4.4 mg/100g in purple, orange, white, and yellow carrots, respectively. The same study concluded total phenolic content of 16.2 mg/100 g in orange carrot varietals, which was higher than yellow and white carrots but lower than purple carrot varietals, totaling 7.7, 8.6, and 76.6 mg/100g, respectively. Kaur and Kapoor (2002) reported total phenolics of 55.0 GAE/100g and Chu et al. (2002) similarly reported 56.4 GAE/100g. Total phenolic content in carrots is often expressed in gallic acid equivalents as measured using the Folin-Ciocalteu method (Singleton and Rossi, 1965).

Overall, many of the nutrients present in carrot processing waste would be beneficial to recover for human consumption and health benefits.

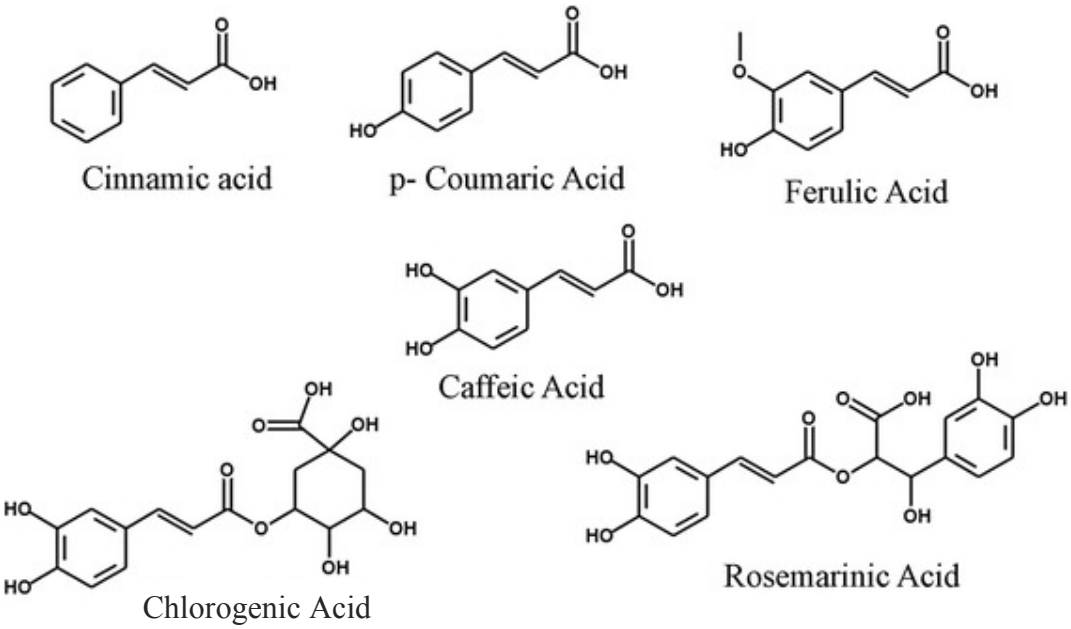

Figure 2-10. Structures of hydroxylcinnamic acid derivatives. Cinnamic acid, p-caumaric acid, ferulic acid, caffeic acid, chlorogenic acid, and rosemarinic acid (adapted from Alam et al;. 2016)

2.5 Dietary Fiber

Fruits and vegetables are known for their high water, low fat contents as well as high vitamin and mineral contents. They also contain significant amounts of dietary fiber and phytochemicals (i.e. polyphenols and carotenoids), all together providing significant beneficial nutrients (de la Rosa et al., 2010).

Plant dietary fibers consist of polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes in the human body (de la Rosa et al., 2010). Hernández-Alcántara et al. (2016) defined dietary fiber as "the remnants of edible plant cells, polysaccharides, lignin, and associated substances resistant to digestion by the alimentary enzymes of humans" also contributing to the "decrease in fecal transit time through the bowel".

Total dietary fiber is broken down into two subcategories depending on

whether or not they are soluble in the human digestive system (Table 2-1). Fiber is not a simple or well-defined chemical compound, but a combination of chemical substances of distinct composition and structure, such as cellulose, hemicelluloses, and lignin. Insoluble dietary fiber consists of cellulose and other polysaccharides along with non-carbohydrate compounds such as lignin, cutin, and other cell-wall constituents. Soluble dietary fiber includes pectins, beta-glucans, arabinoxylans, galactomannans, and other ingestible polysaccharides and oligosaccharides (de la Rosa et al., 2010). The carrot cell wall is composed of pectin, cellulose, lignin, and hemicellulose (Lineback, 1999). The composition of the carrot cell wall is approximately 80.94% cellulose, 9.41% hemicellulose, 7.41% pectin, and 2.48% lignin for whole carrots on a dry weight basis (Nawirska and Kwaśniewska, 2005).

Overall, dietary fiber is associated with a number of health benefits that include prevention of constipation, regulation of blood sugar, protection against heart diseases, and prevention of certain forms of cancers (Sharma et al., 2012). Carrots are one of the few commonly consumed foods with high amounts of dietary fiber. The total dietary fiber content of carrot pomace was 63.6%, with 50.1% insoluble fraction and 13.5% soluble fraction on a dry basis (Chau et al., 2004).

Efforts have been made to utilize carrot pomace in foods such as bread, cake, dressings, pickle wheat bread and high fiber biscuits (Filipini, 2001; Kumari, 2007). Dietary fiber powders from pea and wheat have also been investigated for meat extender in beef burger formulations to improve nutritional characteristics and cooking properties without affecting sensory properties (Besbes et al., 2007).

Table 2-1. Classification of dietary fiber components based on water solubility (adapted from Wichienchot and Ishak, 2018)

Dietary Fibers	Sources	Features
Soluble Fibers		
Gum	Oatmeal, haricot bean, legumes	Generally composed of monomers of hexose and pentose
Pectin	Whole grains, apple, legumes, cabbage, root vegetables	Mainly composed of galacturonic acid, rhamnose, arabinose, high content of galactose, intermediate laminate on the primary wall
Mucilages	Food additives	Compounds which are synthesized in plants, containing glycoprotein
Insoluble Fibers		
Lignin	Is a component of cell walls and mainly consists of aromatic alcohols	Vegetables and flour
Cellulose	Is the main component of cell walls, consisting of glucose monomers	Whole grains, root vegetables, bran, peas, beans family, apples
Hemicellulose	Primary and secondary in cell walls	Bran, whole grains

2.5.1 Insoluble Dietary Fiber

As stated in the previous section, insoluble fibers include lignin, cellulose, and hemicelluloses (Figure 2-11) (Rodríguez et al., 2006). These fibers pass through the digestive system while absorbing water and increase stool bulk. The consumption of insoluble fiber accelerates the movement of stool which can benefit people who struggle with bowel movements (BeMiller and Huber, 2008).

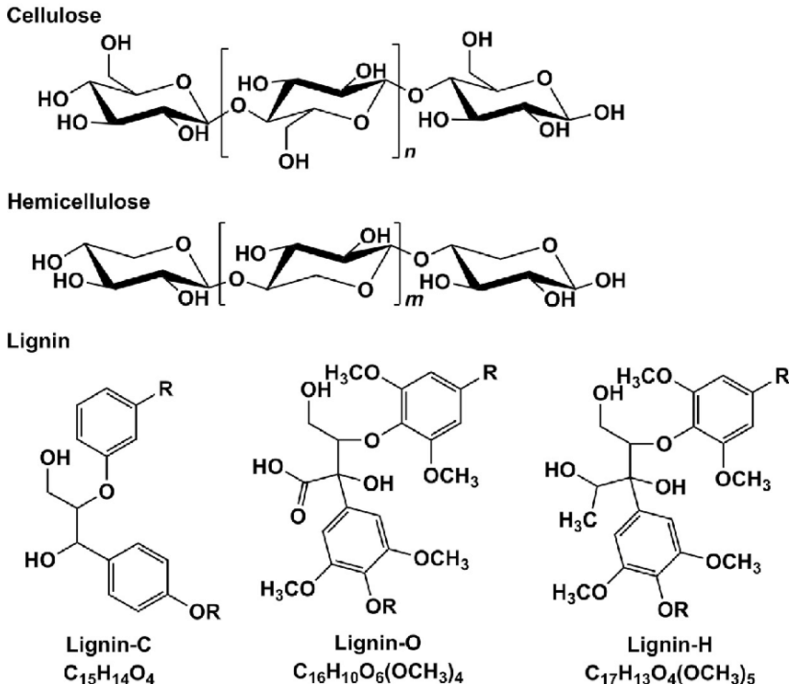

Figure 2-11. Structures of cellulose, hemicellulose, and lignin (adapted from Ranzi et al., 2008).

2.5.2 Soluble Dietary Fiber

Soluble fibers include pectin, gums, and some hemicelluloses (Figure 2-12) (Nawirska and Ukla, 2008). They absorb water to form a gel-like substance inside the digestive system which can block fat and slow down the digestion of cholesterol and sugars that would otherwise be absorbed by the body. This aids in lowering cholesterol and blood glucose levels (Sharma et al., 2012). For example, consuming 10 to 25 grams or soluble fiber a day can lower cholesterol by 18% (Moll 2019).

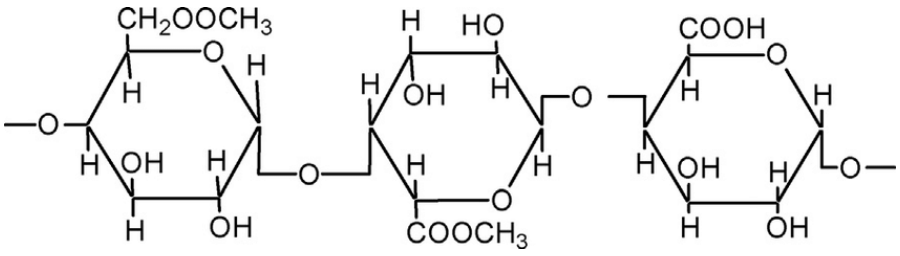

Figure 2-12. Chemical Structure of pectin (adapted from Hassan et al., 2018).

Hernández-Alcántara et al. (2016) reported several health benefits from carrot pomace and other waste products including prebiotic activity, dietary fiber content, total phenolic content and antioxidant capacity as well as bacterial growth and pH parameters of dietary fiber. The carrot pomace was found to have 52% total dietary fiber, 42.1% insoluble dietary fiber and 9.1% soluble dietary fiber content (Table 2-2). Compared to apple and banana waste, carrot pomace has the highest total dietary fiber content.

Table 2-2. Composition of dietary fiber of carrot pomace, apple peel waste, and banana peel waste (adapted from Hernández-Alcántara et al., 2016).

Fiber Content (g/100g)	Carrot Pomace	Apple Peel Waste	Banana Peel Waste
Total Dietary Fiber	52.00 ± 0.081^{a}	35.22 ± 0.49^{c}	46.63 ± 0.27^{b}
Insoluble Dietary Fiber	42.10 ± 0.62^{a}	28.73 ± 0.77^{c}	39.88 ± 0.32^{b}
Soluble Dietary Fiber	9.91 ± 0.43^{a}	6.48 ± 0.28^{c}	6.75 ± 0.05^{b}
IDF[1]/SDF[2]	4.24	4.43	5.90

[a-c] Different letters in the same column indicate a significant difference; $p \leq 0.05$.
[1]Insoluble Dietary Fiber, [2]Soluble Dietary Fiber

2.5.2.1 Oligosaccharides

Oligosaccharides are defined as molecules containing a small number of monosaccharide residues (~ 3 to 10) connected by glycosidic linkages. The partial hydrolysis of various polysaccharides can result in the production of oligosaccharides (Anadon et al., 2016). Enzymatic hydrolysis of insoluble and soluble fibers with endoinulases can be used for the production of prebiotic oligosaccharides (Singh and Singh, 2010). The endoinulases cleave the inulin chain into smaller oligosaccharides (Basso et al., 2010). A variety of studies have looked at the depolymerization of inulin and pectin, specifically derived from a variety of waste products, including lemon peel and sugar beet pulp (Hernández-Alcántara et al. 2016, Gómez et al., 2016). However, no studies have been reported on the partial hydrolysis of polysaccharides to oligosaccharides in carrot wastes.

2.5.3 Prebiotic Fiber

Hemicellulose-rich plant materials are known sources for extraction of oligosaccharides, some of which have the potential of beneficial prebiotic activities (Gullón et al., 2013). The International Scientific Association for Probiotics and Prebiotics defines a prebiotic as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson, 2017). Previously, prebiotic was defined as a "selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora, conferring healthy benefits to the host" (Gibson et al. 2004).

In further defining prebiotics, specific criteria are used to consider a carbohydrate a prebiotic such as; 1) resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption; 2) fermentation by intestinal microflora; and 3) selective stimulation of the growth and/or activity of intestinal bacteria (probiotics) that contribute to health and well-being. Today, majority of determined prebiotics and prebiotic candidates are non-digestible oligosaccharides obtained by the extraction from plants; chicory for example (Hernández-Alcántara et al., 2016).

Prebiotic oligosaccharides resist digestion and reach the colon intact. In the colon, these oligosaccharides are fermented, generating a series of short chain fatty acids which then exert a number of health benefits including constipation relief, blood glucose reductions, mineral absorption improvement, lipid metabolism regularity, decreased likelihood of colonic cancer, and modulation of the immune system (Gullón et al., 2013).

2.6 Functional Properties

The functionality of dietary fibers is directly related to the structure of the polysaccharides. Solubility is increased based on the presence of a substitution group such as COOH or SO_4^{-2}. Solubility and insolubility of dietary fiber involves differences in their technological functionality and physiological effect (Wichienchot and Ishak, 2018). These hydration properties are commonly used by food manufacturers to determine a dietary fiber's optimal usage in a food product in order to maintain desirable textures while keeping cooking yields high (ex. beef patty) (Thebaudin et al., 1997).

2.6.1 Water Holding Capacity

Water holding capacity (WHC) is defined as the amount of water retained by 1 g of dry fiber under specified conditions of temperature, soaking time, duration, and speed of centrifugation or vacuum filtering (Anal, 2018). Water holding capacity of carrot dietary fiber has been reported in the range of 17.9 to 23.3g water/g fiber (Robertson et al. 1980; Fuentes-alventosa et al., 2009). Studies have indicated that high water holding capacity of fibers has the potential to reduce moisture loss, increase cook yields, and tenderness in meat products (Robertson et al., 1980).

2.6.1 Fat Binding Capacity

Dietary fibers bind fats to proteins and carbohydrates, retaining nearly five times its mass in oil (Thebaudin et al., 1997). This is important in determining the functionality of the fiber. Fibers can be utilized to reduce the fat content of food products by reducing the amount of fat lost during cooking. To assess the functionality of dietary fibers in a food product, Slima et al. (2019) added barley-beta glucan and carrot fibers to fresh beef and turkey sausages at 1% concentrations and showed that dietary fibers reduce fat loss in

meat products. Pea fiber added to beef patties showed increases in fat retention and cooking yield, without any changes on juiciness or flavor (Besbes et al., 2007).

2.6.3 Swelling Capacity

Swelling capacity is a measure of the hydration property that allows fibers to absorb high amounts of added liquid (Thebaudin et al., 1997). The act of swelling occurs when the liquid (typically water) moves into the solid structure and expands or spreads the macromolecules (swelling) until they are dispersed, leading to hydration of the molecules. Carrot swelling capacity was reported to be 7.50 mL water/g dry matter (Thebaudin et al. 1997). Carrot dietary fiber and coconut fiber had similar swelling capacity of (18.95 – 23.40 mL water/g dry matter) noticeably higher than reported on carrot and citrus fibers (6.11 mL water/g dry matter) (Raghavendra et al. 2004, Chantaro et al. 2008, Figuerola et al., 2005). The higher swelling values observed in carrot dietary fiber and coconut fiber compared to carrot and citrus may be attributed to the size of the fiber particles or hydrophobic compounds still present in the carrot or citrus.

2.7 Enzymatic Hydrolysis

The fundamental idea behind using enzymes to hydrolyze biomasses consisting of carbohydrate polymers (cellulose, hemicellulose, lignin, pectin) is the elimination of the use of intermediate organisms such as fungi or aerobic microorganism. Enzymes are biological catalysts that can function without nutrients as long as there is the necessary amount of cofactor in the environment and that the conditions (temperature, pH) are not too extreme for the enzymes being utilized (Brummer et al., 2014).

There is no data on the enzymatic hydrolysis of carrot mash polysaccharides into oligosaccharides. However, the enzymatic hydrolysis of insoluble dietary fiber from

carrot pomace with cellulase and xylanase showed an increase in soluble dietary fiber, resulting in water holding capacity by 1.28 times, swelling capacity by 1.06 times, and oil holding capacity by1.09 times (Yu et al., 2018). It was concluded that enzymatically treated pomace had the potential to reduce the glycemic response and plasma cholesterol in the human body, decreasing the risk of cardiovascular disease. Therefore enzymatically treated carrot pomace could be employed as a functional food ingredient in meats, beverages, cereals, and pasta to improve stability, water absorption, and emulsion strength (Yu et al., 2018). The production of a soluble fiber from carrot pomace was also increased using a cellulase-rich crude enzyme isolated from edible snails (Yoon et al., 2005).

There are several examples of enzymatic hydrolysis of other food wastes, including coffee spent waste (Ravindran et al., 2017), oilseeds (Pinelo and Meyer, 2008), and blackberry fruits (Soto et al., 2015) to name a few. The classes of enzymes commonly used in hydrolysis are cellulase, hemicellulase, and pectinase because of the composition of the waste.

2.7.1 Cellulase

Cellulases are a subcategory of glycoside hydrolases. They are composed of a mixture of three classes of enzymes that hydrolyze the β-1,4 linkage in cellulose (Figure 2-13) (Sandhu et al., 2018). First, endo-β-1,4-glucanases hydrolyze internal β-1,4-glucosidic linkages randomly, increasing the number of cellulose chains. Second, exo-1,4-β-D-glucan cellobiohydrolases then advance along the chain hydrolyzing the reducing and non-reducing ends of the cellulose polymer. This activity then releases

glucose (the monomer of cellulose) and cellobiose (the disaccharide of cellulose). Finally, β-glucosidases hydrolyzes the cellobiose into glucose (Binod et al., 2011).

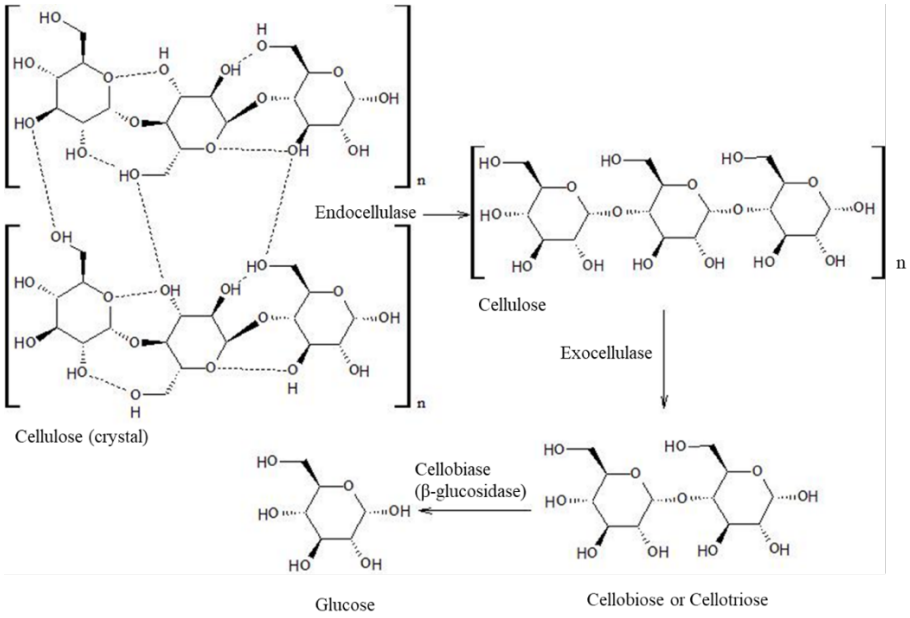

Figure 2-13. General reactions for cellulases (adapted from Binod et al., 2011)

2.7.2 Hemicellulase and Xylanase

Hemicelluloses are a very diverse group of branched and linear polysaccharides. As a result, hemicellulases are a broad group of enzymes that must work together to completely degrade hemicellulose. Similar to cellulases, hemicellulases have carbohydrate-binding domains that focus on and affix to specific carbohydrates. There are 6 major classes of hemicellulase, all of which either hydrolyze glucosidic bonds or ester linkages in acetate or ferulic acid side chains (Shallom and Shoham, 2003).

Xylan is the main carbohydrate present in hemicellulose (Figure 2-14). Xylans are polysaccharides made up of xylose, a pentose sugar. Xylanases are utilized to remove the xylan from lignocellulose, increasing accessibility of cellulose to be hydrolyzed. However, a mixture of xylanases with different specificities and actions are needed to fully degrade the polymer (Binod et al., 2011).

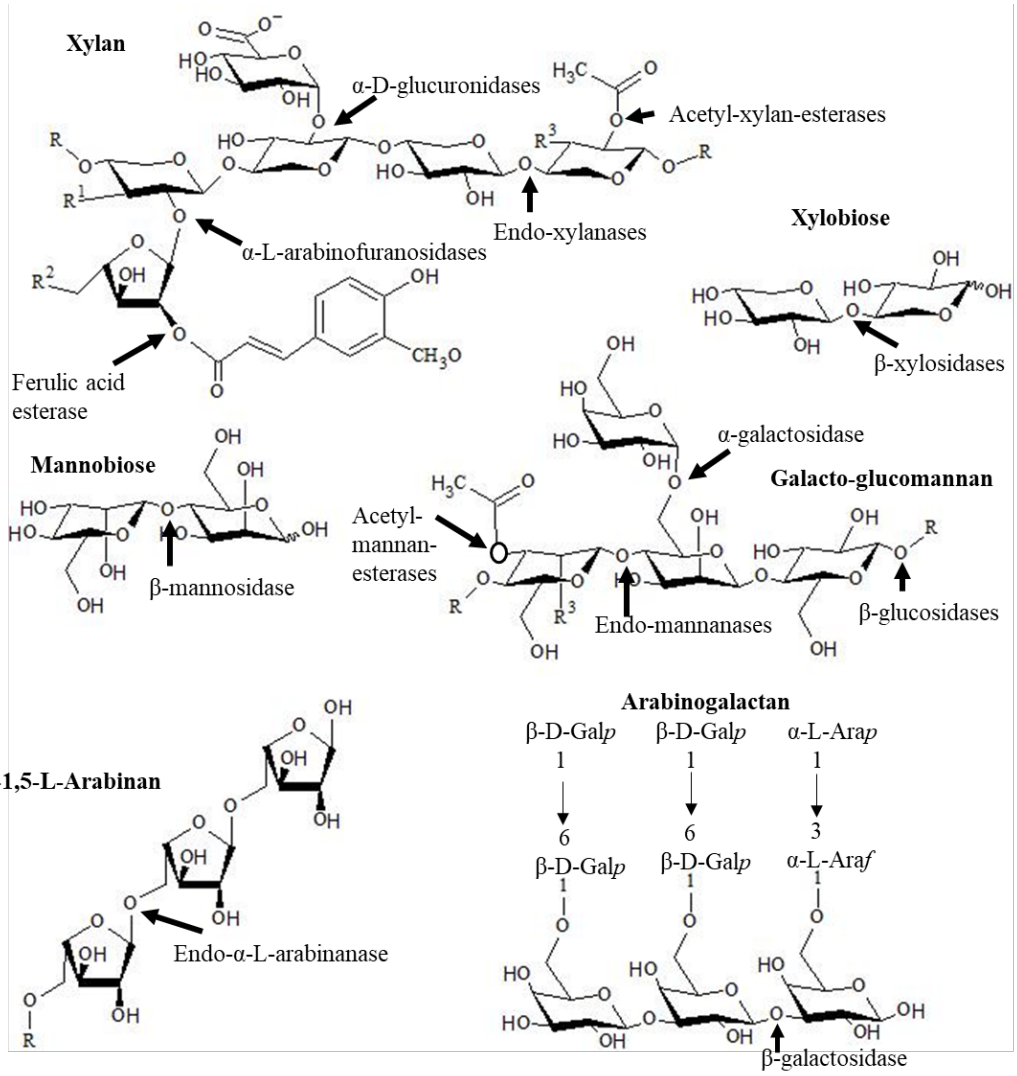

Figure 2-14. Hemicellulases and their targeted linkages, (adapted from Shallom and Shoham, 2003)

2.7.3 Pectinase

Pectins are commonly found in fruits and vegetables which provide structure and firmness to plant cell walls (Massiot et al., 1987). Pectinase is the class of enzymes that hydrolyze pectins and are often used in the maceration, liquefaction, and extraction of vegetable tissues in industry (Gummadi and Panda, 2003). There are four types of pectinase; 1) pectin esterase removes the methoxyl group from pectin (known to decrease gel strength), 2) pectin lyase, 3) polygalacturonase cleave the α-1,4 glycosidic bond

between uronic acid monomers, and 4) polymethygalacturonase removes methoxyl groups and cleaves α-1,4 glycosidic bonds (Figure 2-15) (Gummadi and Panda, 2003).

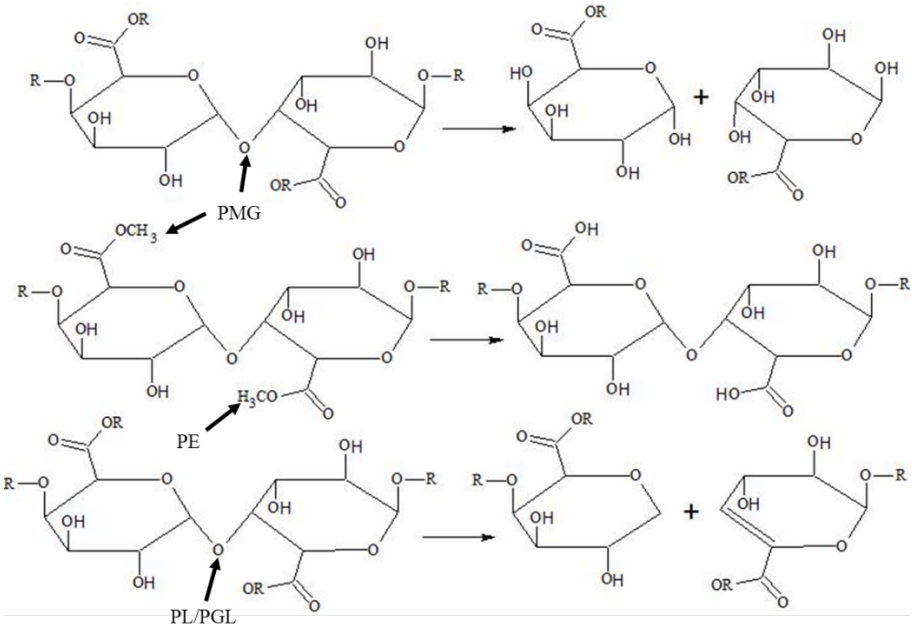

Figure 2-15. General reactions for pectinase
PMG/PGL = polymethylgalacturonase/polygalacturonaselyase; PE = pectin esterase; PL pectin lyase (adapted from Gummadi and Panda, 2003)

2.7.4 Enzymatic Hydrolysis Parameters

Mature carrot cell walls contain high amounts of pectin as well as all the polysaccharides typically found in dicotyledons; cellulose, hemicellulose, xylans, and mannans (Massiot et al., 1987). In many circumstances, a variety of enzymes are used simultaneously to degrade polysaccharides. The most used combinations in the literature to hydrolyze carrot pomace are cellulase and pectinase, however, xylanase has been used as well (Stoll et al., 2003a; Yu et al., 2018). Enzymes are typically used in a 1:1 ratio (substrate: enzyme cocktail), at concentrations ranging from 0.01% to 1% (Table 2-3). Time for enzymatic hydrolysis can last up to 96 h with the goal of fully hydrolyzing the polysaccharides to simple sugars. Table 2-3 was used as a guide to develop specifications

for enzymatic hydrolysis in our research study. Our research study utilized higher enzyme concentrations (0.15% and 0.225%) for shorter enzymatic hydrolysis times because the focus was on the production of oligosaccharides rather than mono and disaccharides.

Table 2-3. Effect of various enzyme treatments on carrot pomace

Substrate	Enzyme(s)	Enzyme Ratio	Conc (%)	pH	Temp (℃)	Time (min)	Measurement	Results	Reference
Commercial Juice Pomace	Pectinex Ultra SP-L -pectinase: Cytolase CL -cellulolytic	1:1	0.10	4.00	35-60	0-180	Change in viscosity over time	Greatest decrease in viscosity 50℃	(Stoll et al. 2003)
	Pectinex Ultra SP-L -pectinase: Cellubrix L-cellulolytic	1:1	0.10	4.50	35-60	0-180	Change in viscosity over time	No significant difference at any temp	(Stoll et al. 2003)
	Pectinex Ultra SP-L -pectinase: Cytolase CL -cellulolytic	4:0; 3:1; 1:1; 1:3; 0:3	0.10	4	50	0-180	Change in viscosity over time	Most pectin broken down in first 30 minutes; overall 1:1 ratio most effective	(Stoll et al. 2003)
	Pectinex Ultra SP-L -pectinase: Cytolase CL -cellulolytic	1:1	0.01; 0.05; 0.1; 0.12; 0.15; 0.25; 0.5	4	50	0-180	Change in viscosity over time	Conc of 0.15% was found to be suitable. Higher conc of enzymes didn't provide significantly better results	(Stoll et al. 2003)
	Pectinex Ultra SP-L -pectinase: Cytolase CL -cellulolytic	1:1	0.15	3.5; 4.0, 4.5	50	0-180	Change in viscosity over time	Optimum pH 4.0	(Stoll et al. 2003)
	Pectinex Ultra SP-L -pectinase: Cytolase CL -cellulolytic	1:01	0.12, 0.15	4	50	0-180	Change in viscosity over time	Viscosity reduced significantly within 60 min	(Stoll et al. 2003)
Carrot Pomace Flour	Cellulase & Xylanase	~ 2:1	Cellulase = 0.009, xylanase = 0.01	4.8	50	150	Compares soluble/insoluble dietary fiber and functional properties	Soluble dietary fiber significantly increased with enzymes; enzymes most effective method; water swelling/retention/oil retention all improved	(G. Yu et al. 2018)
Carrot - cubed	"EC" = Pectinex Ultra SP, Celluclast,"Ec" = Pectinex Ultra SP	1:2:3	"EC" = 16/35/49 g/100g respectively	stable at 4.0 after 1 hr	25	1,3,5,15 h	Yield of alcohol-insoluble residue from filtering through sieves and polysaccharide monomers recovered	Ec alone degraded cellulose slower than EC	(Massiot et al. 1992)
Pretreated Carrot Pomace	Cellulase-rich crude enzyme isolated from Edible Snails	1:1, 1:2, 1:4, 1:6	1% carrot pomace solution	5	50	24, 46, 72, 96 h	Yield of soluble fiber	Yield of soluble fiber increased greatly upon 24 h of enzyme hydrolysis	(K.Y. Yoon et al. 2005)

2.8 Conclusion

Carrot mash and pomace residues are generated during carrot processing. Carrot mash is a by-product of peeled baby carrot processing and carrot pomace is the by-product of carrot juice manufacturing. The increased generation of fresh carrot waste is a concern for carrot processors as they are filling up landfills and reservoirs while various vital nutrients in the carrot waste could be used for human consumption. Carrot processing wastes are known for their high contents of dietary fibers as well as phytonutrient profiles, however little research has been conducted on methods to valorize this waste. Identifying novel methods for the extraction of water and vital nutrients along with applications to use these wastes for consumption, could benefit the carrot industry and decrease landfill disposal, in turn decreasing the environmental impact of these by-products.

CHAPTER 3 – MATERIALS AND METHODS

3.1 Characterization of Carrot Mash

Carrot mash was obtained from Grimmway Farms, a commercial carrot growing and processing company located in Arvin, California. Grimmway carrots are grown in various regions of California, Nevada, and Washington to ensure ideal growing conditions year-round. Once harvested, carrots are transported to the processing facility and sorted into the different processing tracks (Figure 2-6). Two hundred pounds of carrot mash (CM) were obtained from Grimmway Farms (Arvin, CA) and stored in 22 Kg. plastic buckets in the dark at -20 °C until further processed.

3.1.1 Mechanical Separation of Carrot Mash

Mechanical separation of CM into liquid and solid fractions was carried out using either a hydraulic Welles Juice Press (Samson Brands, Danbury, CT) or a Newtry CN-92G Expeller Press (BEAMNOVA, Guangdong, China). Following analyses were performed on the liquid and solid portions of hydraulically pressed and expeller pressed carrot mashes.

3.1.2 Percent Extractable Matter

For each mechanical press, 280.00 g of CM was pressed and the resulting weight of the liquid and solid portions were recorded. The Percent Extractable Matter (PEM) was calculated as

$$PEM\ of\ Liquid = \frac{Liquid\ wt\ (g)}{Initial\ wt\ \ (g)}\ X\ 100 \qquad (3.1.)$$

$$PEM\ of\ Solids = \frac{Solid\ wt\ (g)}{Initial\ wt\ \ (g)}\ X\ 100 \qquad (3.2.)$$

3.1.3 Total Solids Content

Total Solids Content was determined for each fraction recovered from the hydraulic press and expeller press. Approximately 7.00 g of each carrot mash sample were weighed, recorded, and placed in a drying oven (NAPCO Model 620, Thermo Scientific™, Waltham, MA, USA) at 40°C for 24 hours. The weight of the dried samples was recorded, and total solids content was calculated according to the following equation:

$$Total\ Solids\ (\%) = \frac{Dehydrated\ wt\ (g)}{Initial\ wt\ (g)}\ X\ 100 \qquad (3.3.)$$

3.1.4 Carotenoid Content

Carotenoid content was determined on the liquid and solid mash from the hydraulic press and expeller press according to the method described by Lee (2001). Carrot mash samples were homogenized for 30 seconds using a blender (Vitamix Professional Series 500, Cleveland, Ohio, USA) with a mixture of hexane, acetone, and ethanol (50:25:25). They were then centrifuged (Eppendorf 5810 R Centrifuge, Hauppauge, NY, USA) for 5 minutes at 6,500 rpm at 5 °C. After centrifuging, the top solvent layer was transferred to a 25.00 mL volumetric flask and adjusted to 25.00 mL with additional hexane. Absorbance was measured at 450 nm (Genesis-5 Spectronic spectrophotometer, Thermo Scientific™, Waltham, MA, USA). Carotenoid concentration was calculated according to Beer's Law.

$$c = \frac{A}{\varepsilon\ x\ b} \qquad (3.4.)$$

A = Absorbance
ε = 2,505 (mL/mg/cm)
b = pathlength (1 cm)
c= concentration (mg/mL)

3.1.5 Total Polyphenol Content

Phenolic content was determined with the modified Folin-Ciocalteau method described by Waterhouse (2002). A 20% sodium bicarbonate (Na_2CO_3) solution (w/v) was made by weighing 50.00 g anhydrous Na_2CO_3 in a beaker and adding 200.00 mL of deionized water. The mixture was brought to a boil on a hot plate, then removed from hot plate and allowed to cool to room temperature then left undisturbed for 24 hours. The solution was then filtered into a 250.00 mL volumetric flask through Whatman N°1 filter paper. The filtrate was brought to volume with deionized water.

A 0.50 mg/mL gallic acid solution was made with deionized water and used to make a standard curve with concentrations ranging from 50 mg/L to 500 mg/L. Carrot mash samples were extracted using the same procedure as described above in Carotenoid Content (3.1.4).

To 1 mL of sample or standard, 70.00 mL of deionized water and 5.00 mL of Folin-Ciocalteu reagent were added and incubated at room temperature for < 8 minutes followed by the addition of 15.00 mL of Na_2CO_3 solution to each beaker. The final solution was adjusted with deionized water to 100.00 mL and incubated for 2 hours at room temperature; 2.00 mL of the solution was then transferred to a 1 cm, 2.00 mL plastic cuvette and absorbances were read at 760 nm. Results were calculated to express mg GAE (gallic acid equivalent) per gram of sample.

3.1.6 Functional Properties

Functional properties were evaluated on unpressed carrot mash, expeller pressed carrot mash, and hydraulic pressed mash. Mash was dried at 40°C for 24 hours (Harvest Saver R4 drying oven Commercial Dehydrator Systems, Inc., USA), then ground using a

blender (Vitamix Professional Series 500 Cleveland, OH, USA) at speed 4, to pass a 20-mesh sieve (0.85 mm). Dried and ground carrot mash was stored in plastic bags in a dark room at 22 °C, with an aluminum foil cover. .

3.1.6.1 Water Holding Capacity

Water holding capacity (WHC) was determined according to a method described by Raghavendra et al. (2004). Dried carrot mash (0.50 g) was added to 15.00 mL of water in a graduated cylinder. After 24 hours, the supernatant was then filtered through a sintered glass crucible under vacuum. The hydrated residue weight was recorded before being dried at 105 °C for 2 hours to obtain the residue dry weight.

Water holding capacity was calculated as

$$WHC\ \left(\frac{g\ water}{g\ dry\ mash}\right) = \left[\frac{(residue\ hydrated\ weight - residue\ dry\ weight)}{(residue\ dry\ weight)}\right] \qquad (3.5.)$$

3.1.6.2 Fat Binding Capacity

Fat binding capacity (FBC) was determined according to the method of Beuchat (1977) with modification. Canola oil (5.6 g) was added to dried carrot mash (1.00 g) in a 50 mL centrifuge tube. The slurry was vortexed for 30 seconds, allowed to sit for 30 minutes at 22°C, then centrifuged at 1,610 x g for 25 minutes. The weight of decanted supernatant was determined, and g of oil retained per gram of sample was calculated.

$$Fat\ Binding\ Capacity\ \left(\frac{g}{g}\right) = \frac{Weight\ of\ decanted\ supernatant}{Weight\ of\ initial\ sample} \qquad (3.6.)$$

3.1.6.3 Swelling Capacity

Swelling capacity was determined according to a method by Raghavendra et al. (2004). Twenty-five mL of deionized water was added to 1.00 g of dried carrot mash in a 50.00 mL graduated cylinder. Graduated cylinders were covered with parafilm to reduce

evaporation, and the samples were allowed to sit at 22°C for 24 h. After 24 h, the volume of the swollen sample was measured. Swelling capacity is expressed as mL of water per 1.00 gram of carrot mash.

$$Swelling\ Capacity\ \left(\frac{mL}{g}\right) = \frac{Volume\ occupied\ by\ sample}{Original\ sample\ weight} \quad (3.7.)$$

3.1.7 Statistical Analysis

Statistical analysis on all tests was reported as means± SD. Analysis of variance (ANOVA) and Tukey's test was conducted using JMP Pro 12. Statistical significance was determined at $P < 0.05$.

3.2 Development of Beef Patty with added Carrot Mash

3.2.1 Preparation of Beef Patty with Carrot Powder

Unpressed and expeller pressed carrot mash samples were dried for 24 hours at 103°C in Harvest Saver R4 drying oven (Commercial Dehydrator Systems, Inc., USA). Dried samples were then ground using a Vitamix Professional Series 500 blender (Cleveland, Ohio, USA) at speed 4 for 3 minutes before being screened through a 20-mesh sieve (0.85 mm). After sieving, powdered mash was used to prepare beef patties.

Beef chuck with 20% fat from the Cal Poly Meat Processing Center, was ground using a 3/16th inch diameter plate and then separated into 3 groups of 8.2 Kg each. Each 8.2 Kg batch was weighed out to 7 separate 1.2 Kg groups. Each group was hand-mixed with 1% or 3% unpressed powder, expeller pressed powder, or commercial carrot fiber (Hydrobind LP, Bolthouse Farms, Bakersfield CA) and then ground a second time using a 1/8th inch diameter plate to make into 113.4 g. patties (Figure 1). Control was prepared in the same conditions without carrot powder addition. Patties were pre-frozen on trays

overnight at -20 ºC then vacuum sealed and stored at -20 ºC. Samples were thawed for 12 h at 4 ºC prior to pH and color analysis.

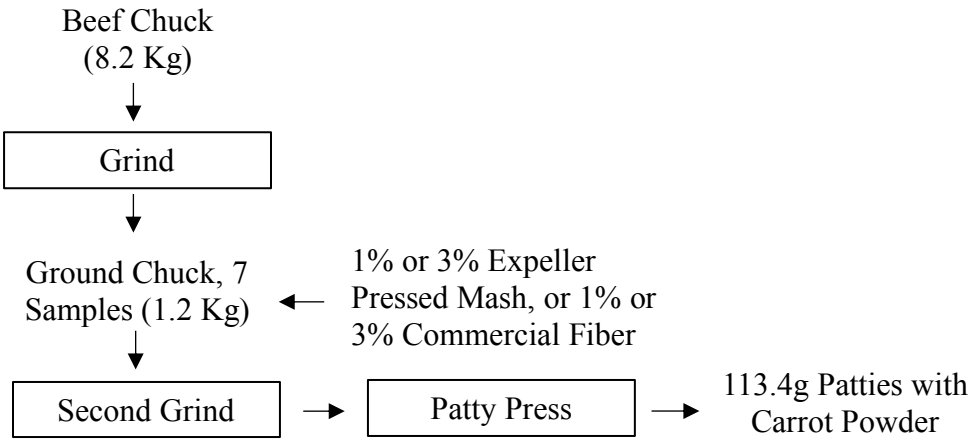

Figure 3-1. Process for development of beef patty with carrot powder

3.2.2 pH

Raw beef patty (control and carrot mash samples) (15.00 g) were blended with 150.00 mL distilled water for 1 minute with a Vitamix Professional Series 500 blender (Cleveland, Ohio, USA). pH was taken in triplicate with an Orion Star™ A211 Benchtop pH meter (Thermo Scientific™, Waltham, Massachusetts, USA).

3.2.3 Color

Raw beef patty color was analyzed using a WR10-8 Series Calorimeter (FRU Industries, Longhua New Area, Shenzhen, China). L* values measure the lightness and ranges from 0 (black) to 100 (white). a* values measure the change from green (-) to red (+) and b* measures the change from blue (-) to yellow (+). Patties were analyzed on 3 different areas (left, middle, and right) on both sides of the patty. Total color difference (ΔE) was calculated using the following equation. Control patty (no mash) was used as a reference.

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3.8.)$$

ΔE = Total color difference

$\Delta L^* = L^*_{sample} - L^*_{ref}$

$\Delta a^* = a^*_{sample} - a^*_{ref}$

$\Delta b^* = b^*_{sample} - b^*_{ref}$

3.2.4 Cooking Yield

Frozen patties were cooked using an Avantco P70S Commercial Grill (Clark Associates Inc., Lancaster, Pennsylvania, USA) preheated to 163°C with no fat addition, to an internal temperature of 71°C. Samples were analyzed in triplicate. Patties were weighed before and after cooking to determine cooking yield as follows:

$$Cooking\ yield\ (\%) = \frac{weight\ of\ cooked\ patty\ (g)}{weight\ of\ uncooked\ patty\ (g)}\ x\ 100 \quad (3.9.)$$

3.2.5 Texture Analysis

Texture of cooked beef patties was analyzed using a Brookfield CT3 Texture Analyzer (AMETEK Brookfield, Middleboro, Massachusetts, USA). Parameters were set to a trigger load of 7 g and speed at 1.70 mm/s, using a TA10 cylinder probe (D: 12.7 mm, L: 35 mm). The following Texture Profile Analysis parameters were determined: hardness-1 (force required for the first compression), hardness-2 (peak force for the second compression), adhesiveness, cohesiveness, springiness (elasticity), gumminess, and chewiness. Hardness is described as the maximum force at the first compression and adhesiveness is the amount of work it takes to pull the probe out of the product. Cohesiveness describes how well the food product retains its form between the first and second compression. Springiness was the distance the sample recovered in height after the first compression. Chewiness was the product of hardness-1, springiness, and

cohesiveness; and gumminess was the product of hardness-1 and cohesiveness (Mittala et al., 1992).

3.2.6 Statistical Analysis

Statistical analysis on all data was reported as means± SD. Analysis of variance (ANOVA) and Tukey's test were conducted using JMP Pro 12. Statistical significance was determined at $P < 0.05$.

3.3 Enzymatic Hydrolysis

3.3.1 Mechanical Pretreatment

Carrot mash was mechanically separated using a Newtry CN-92G Expeller Press (BEAMNOVA, Guangdong, China) at room temperature and the liquid and solid fractions were manually re-mixed in a ratio of 1:16 (wt:wt), liquid to solid (Figure 3-2). Aliquots of 450.00 g were prepared for enzymatic treatments.

3.3.2 Enzymatic Treatment

Expeller pressed carrot mash samples were pretreated with an enzyme cocktail of Cellulase (powder; activity 100,000 CU/g), Hemicellulase (powder; activity 400,000 HCU/g), Xylanase (powder; activity 100,000 XU/g), and Pectinase (powder; activity 8,000 ENDO-PG/g) kindly donated by BIO-CAT (Troy, VA). Total solids content of each sample was calculated to find the enzyme concentration needed at 0.15 % and 0.225% (w/w, dry basis). Concentrations of 0.15 % and the [x1.5] increase to 0.225 % were based on previous studies reported in literature (Table 2-3). Two control samples were used; both unpressed and expeller pressed samples not treated with enzymes.

Samples were initially heated to 50 °C prior to adding enzymes, and either immediately deactivated in a boiling water bath reaching 90 °C for 1 minute (heating rate = 3.05 °C ± 0.24/ min), or shaken in a MaxQ 5000 Floor-Model Shaker (Thermo Scientific™,

Waltham, Massachusetts, USA) at 50 °C for 15 or 30 minutes and then deactivated as before. Resulting samples were cooled in an ice bath before storing at 4 °C and performing the following analyses.

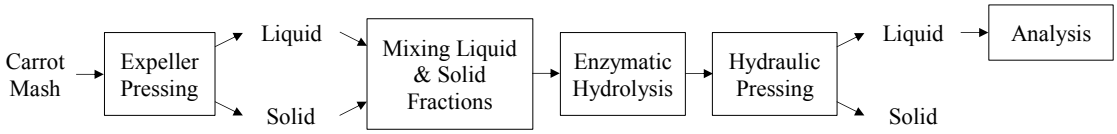

Figure 3-2. Process for pretreatment and enzymatic hydrolysis of carrot mash

3.3.3 Carotenoid Content

Refer to subsection 3.1.4.

3.3.4 Soluble Sugar

Soluble sugar analysis was carried out based on AOAC Method 988.12. A standard curve was prepared using a dextrose standard solution diluted to concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.10 mg/mL. In test tubes, 0.50 mL of 5% phenol solution was added, swirled to mix, and then 5.00 mL of concentrated H_2SO_4 was pipetted into each tube of standard solutions and carrot mash samples (dilution factor of 1000). Blank consisted of phenol solution and H_2SO_4. Absorbance was read at 490 nm using a Genesis-5 Spectronic spectrophotometer. Refer to section 3.3.2 for samples tested.

3.3.5 Total Dietary Fiber Analysis

Samples were sent to Medallion Labs (Minneapolis, MN) for analysis of insoluble, soluble, and total dietary fiber using AOAC 991.43. Refer to section 3.3.2 for samples tested.

3.3.6 Oligosaccharide Profile

Carrot mash samples (Refer to section 3.3.2 for samples tested) treated with/ without enzymes were hydraulically pressed and the liquid portion was vacuum filtered using a Buchner funnel, Whatman Grade 1 filter paper (4.25 cm diameter). The filtered sample (1.80 mL) was diluted with 95% ethanol heated to 60 °C to produce a 78% ethanol solution. After 60 minutes at room temperature, the solution was filtered through a 0.45 μm syringe filter. A 1.00 mL amount of the filtered solution was then transferred to amber HPLC vials and evaporated using a Genevac EZ-2 Evaporator at ambient temperature and 0 mbar. All samples were resuspended with 1 mL nanopore water using a laboratory shaker table at 750 rpm for 25 minutes, before injection (20 μL) into the Ultra Fast Liquid Chromatograph (UFLC) in duplicate.

Quantification of the oligosaccharides was performed using an Aminex HPX-42A column (Bio-Rad, Hercules, California) in a Prominence UFLC (Shimadzu, Kyoto, Japan) equipped with an Agilent 1200 Series Refractive Index (RI) Detector (Santa Clara, California). The mobile phase was nanopore water running at an isocratic flow rate of 0.6 mL/min. D-Glucose at concentrations of 5, 10, and 20 mg/mL were used as standards. D-Glucose provides an LC refractive index (RI) response equivalent to the response factor for non-digestible oligosaccharides. Peaks were integrated with the LabSolutions Analysis Data System (Shimadzu, Kyoto, Japan) and calculated using the below equation to determine Low Molecular Weight Soluble Dietary Fiber (SDFS, water soluble dietary fiber that is soluble in the presence of 78% aqueous ethanol).

$$\%\, SDFS = Rf \times (PA_{SDFS}) \times 1 \times (\frac{100}{\rho \times 1.79}) \quad (3.10.)$$

Rf = (Wt-Glu)/(PA-Glu)
PA_{SDFS} = peak area of oligosaccharide
1 = volume (mL) of final sample
100 = factor to convert to 100g
ρ = density of liquid portion after vacuum filtering used to extract SDFS (modified from AOAC)
1.79 = volume (mL) of liquid portion after vacuum filtering used to extract SDFS (modified from AOAC)

3.3.7 Statistical Analysis

Statistical analysis on all data was reported as means± SD. Analysis of variance (ANOVA) and Tukey's test were conducted using JMP Pro 12. Statistical significance was determined at $P < 0.05$.

CHAPTER 4 – RESULTS AND DISCUSSION

Literature on carrot mash (peeled baby carrot waste) is scarce, however a few studies on carrot pomace (carrot juice waste) have been reported. The largest difference between previous studies and our current study is the manufacturing method of the waste. Previous studies have been reported as laboratory-made pomace, whereas the waste used in this study was from a commercial production in the Central Valley of California. When comparing results to literature, these factors should be taken into consideration.

4.1 Characterization of Carrot Mash

4.1.1 Percent Extractable Matter

Percent extractable matter (PEM) was used to compare the two mechanical presses (expeller and hydraulic) on the separation of liquid and solid fractions (Table 4-1). Expeller and hydraulic pressing both were 93% effective in recovering both liquid and solid fractions from carrot mash. PEM of the liquid fraction of the mash using the expeller press was significantly higher than the PEM using the hydraulic press by approximately 13%. For the solid fractions, PEM from the hydraulic press was significantly higher than expeller press, which would be expected when considering the two different methods of extraction, especially for the liquid fraction from PEM. By observing the equipment at work, expeller pressing looked to be more invasive potentially due to high shear to break the cells in the mash with more liquid release. The auger used for the high shear could contribute to a lower yield of PEM in the solid portion from the expeller pressed samples because carrot mash could easily get caught in the auger and not fully accounted for the final PEM yield. Hydraulic pressing is less

invasive, only using compressive force to release available liquid, leaving some liquid trapped in the solid fraction with a higher weight of the solid fraction.

Prior to extraction, carrot mash moisture content was 95.30%, which was significantly decreased to 86.67% and 83.33% after hydraulic and expeller pressing, respectively (Table 4-2).

While there was no significant difference in moisture content between expeller and hydraulic pressed carrot mash, expeller pressing was more efficient based on PEM. The liquid fraction is not only composed of water but contains carotenoids, soluble carbohydrates, and pectin. Based on PEM, expeller pressing proves to be more efficient at extracting liquid from the carrot mash.

Table 4-1. Impact of Physical Extraction Methods on the Percent Extractable Matter in Commercially Produced Carrot Mash

Extraction Method	PEM Liquid Fraction	PEM Solid Fraction
Expeller Press	76.04 ± 3.44^{a}	16.60 ± 3.09^{a}
Hydraulic Press	69.44 ± 5.16^{b}	23.77 ± 0.36^{b}

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.

Table 4-2. Impact of Physical Extraction Methods on the Moisture Content of Commercially Produced Carrot Mash

Extraction Method	Moisture (%)
Unpressed	95.30 ± 0.51^{a}
Expeller Press	83.33 ± 2.98^{b}
Hydraulic Press	86.67 ± 4.12^{b}

[a-b] Different letters indicate a significant difference; $p \leq 0.05$.

4.1.2 Total Solids Content

Total solids (TS) content of unpressed mash was compared to the liquid and solid fractions of expeller and hydraulic pressed mash (Table 4-3). Total solids in the liquid and solid fractions were statistically different from one another. The liquid fraction had significantly fewer total solids content than the solid fraction. Results also showed that

the solid fraction from the expeller pressing process had a slightly larger total solids content than the hydraulic pressed solid because of the high shearing effect of the expeller pressing process.

Table 4-3. Impact of Physical Extraction Methods on the Total Solids Content in Commercially Produced Carrot Mash

Carrot Mash Sample	Liquid Fraction (%)	Solid Fraction (%)	Total Solids Content (%)
Unpressed	--	--	5.90 ± 0.00^{a}
Expeller	1.03 ± 0.43^{b}	16.62 ± 4.32^{a}	17.65 ± 3.44^{b}
Hydraulic	0.60 ± 0.30^{b}	14.34 ± 5.59^{a}	14.94 ± 4.44^{b}

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.

4.1.3 Carotenoid Content

Carotenoid content (CC) of carrot mash is shown in Table 4-4. Mechanical separation of the carrot mash significantly increased the extraction of carotenoids. Mechanical pressing was successfully used to disrupt the cell walls of *S. pararoseus* and *R. mucilaginosa* yeasts to increase the recovery of carotenoids (Lopes et al., 2017).

The TS results above lead to the expectations that expeller pressing would extract more carotenoids because of the increased solids. Compared to the hydraulic press which uses compressive force, the invasive shearing process of the expeller press extracts higher amounts of carotenoids which could be explained by the breakdown and release of the trapped carotenoid crystals in the cells (de la Rosa et al., 2010). The CC in the liquid fraction of the expeller press was significantly higher (0.69 ± 0.02) than in the liquid portion of the hydraulic press (0.12 ± 0.00). Although carotenoids are fat soluble phytochemicals and insoluble in water, there was more solids in the liquid recovered from expeller press (i.e. high shear). No significant differences in CC were observed when finely ground prior to carotenoid analysis (Stoll et al. 2003) . Higher carotenoid contents compared to our study (40 ppm) was reported for carrot pomace when ground

finely. This could be due to the difference in nature between the two carrot products in the two studies. Stoll's study focused on carrot pomace rather than mash. Pomace is the product of the whole carrot rather than just the outer skins. Considering the gradual increase of carotenoids from the skins to the core, higher CC values in carrot pomace compared to carrot mash could be expected (de la Rosa et al., 2010). Overall, the high shear process of expeller pressing was more effective at extracting total carotenoids compared to both the hydraulic pressed and unpressed mashes due to being more efficient at rupturing cells walls (Knockaert et al., 2012; Jeffery et al., 2012)

Table 4-4. Impact of Physical Extraction Methods on the Carotenoid Content in Commercially Produced Carrot Mash

Carrot Mash Sample	CC[1] (ppm[2]) Liquid Fraction	CC (ppm) Solid Fraction	Total CC (ppm)
Unpressed	N/A	N/A	0.08 ± 0.01^{c}
Expeller Press	0.69 ± 0.02^{a}	0.19 ± 0.00^{a}	0.88 ± 0.01^{a}
Hydraulic Press	0.12 ± 0.00^{b}	0.36 ± 0.04^{b}	0.49 ± 0.03^{b}

[a-c] Different letters in the same column indicate a significant difference; $p \leq 0.05$.
[1] Carotenoid Content
[2] parts per million

4.1.4 Total Polyphenol Content

Polyphenol contents in the liquid fraction of the two pressed samples were similar, but phenolic content significantly increased in the solid fraction of the hydraulic pressed mash (81.76 ± 4.09) compared to the expeller pressed solid fraction (51.75 ± 11.92). The value of total phenolic content in unpressed carrot was 57.54 ± 5.14 mg GAE/100 g which was significantly increased by 196% and 250% after expeller and hydraulic pressing, respectively (Table 4-5).

Alasalvar et al. (2001) reported a total phenolic content in whole orange carrots of 16.21 ± 0.21 mg/100g, which is lower than the result in this study (57.54 mg GAE/100g in unpressed mash).

Table 4-5. Impact of Physical Extraction Methods on the Total Polyphenol Content in Commercially Produced Carrot Mash

Carrot Mash Sample	Polyphenol - Liquid (mg GAE[1]/100 g)	Polyphenol – Solid (mg GAE/100g)	Total Polyphenol (mg GAE/100g)
Unpressed	N/A	N/A	57.54 ± 20.56^{b}
Expeller Press	61.09 ± 8.17^{a}	51.75 ± 11.92^{a}	112.84 ± 13.53^{a}
Hydraulic Press	62.34 ± 5.77^{a}	81.76 ± 4.09^{b}	144.10 ± 1.70^{a}

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.
[1] GAE = Gallic Acid Equivalents

The phenolic content is higher in the peel of the carrot than the core (Sharma et al., 2012). The peel holds 54.1% of the total phenols in the carrot, while the phloem and xylem tissues hold only 39.5% and 6.4%, respectively. Therefore, higher phenolic content reported in carrot mash than whole carrot is expected (Alasalvar et al. 2001).

Our study shows higher amounts of phenolics in the pressed samples, presumably due to the mechanical force that released more bound phenols (Parthasarathi et al., 2005). Similarly, black carrot peels (5170 mg GAE/100 g dw) and black carrot pomace (4151 mg GAE/100 g dw) accounted for a higher percentage of polyphenols compared to whole black carrots (54743 mg GAE/100 g dw) and attributed it to the release of bound compounds with the breakdown of cellular components (i.e., cellulose and cellulose-pectin composites) (Kamiloglu et al., 2016).

4.1.5 Functional Properties

4.1.5.1 Water Holding Capacity

Water holding capacity is defined as the "ability of a matrix of molecules, usually macromolecules at low concentration, to physically entrap certain amounts of water under the application of an external or gravitational force (Reid and Fennema, 2008).

Water holding capacity of the carrot mash significantly decreased with mechanical treatments, with no significant difference observed between the two mechanical treatments (Figure 4-1).

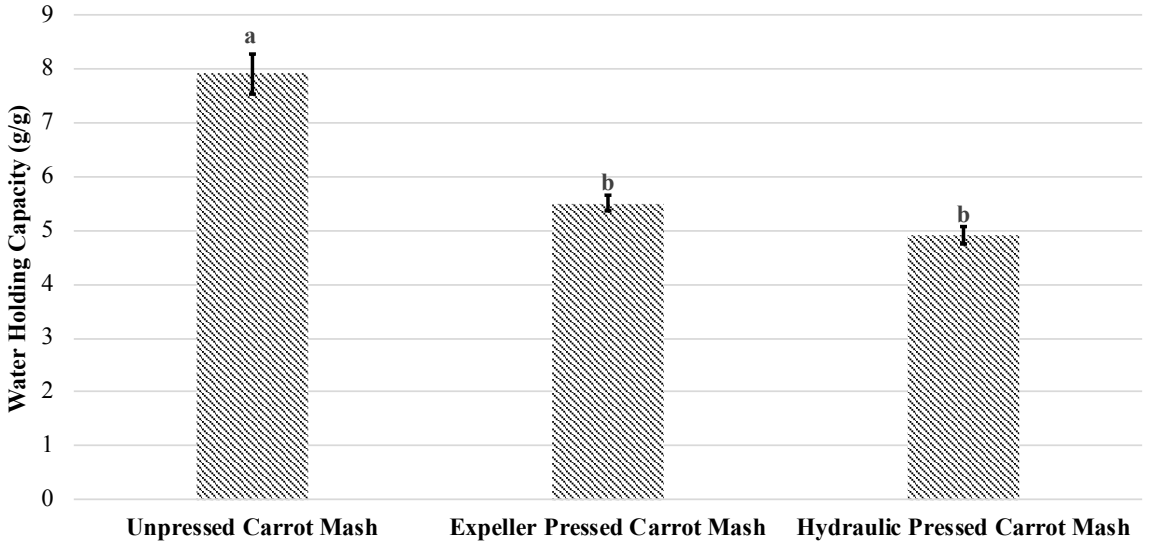

Figure 4-1. Impact of Physical Extraction Methods on the Water Holding Capacity of Commercially Produced Carrot Mash

Thebaudin et al. (1997) reported that as particle size of fibers increase, so does the trapped volume of water. The significantly lower WHC could be explained by smaller particle sizes after the physical press. . (Thebaudin et al., 1997). Additionally, the potential breakage of cell walls after mechanical pretreatment reduced the fibers' ability to hold water (Thebaudin et al., 1997).

Insoluble dietary fiber, alcohol insoluble fiber, and water insoluble fiber of carrot pomace were reported as 13.20 ml/g, 8.73 ml/g, and 18.70 ml/g, respectively (Chau et al., 2004). Water holding capacity of carrot fibers were reported as 17.90 – 23.30 g/g (Robertson et al., 1980). The numbers reported in previous studies are significantly higher than our findings with values of 7.91 g/g for unpressed, 5.51 g/g for expeller

pressed, and 4.91g/g hydraulic pressed mash. As previously stated, these differences could be attributed to the differences in the nature of the substrates.

The porous structure formed by polysaccharide chains in plant materials, have the ability to hold large amounts of water through hydrogen bonds therefore conferring to plant materials beneficial functionality (Sharoba et al., 2013). Two important factors of functionality of these polysaccharide chains are the ratio of insoluble dietary fiber to soluble dietary fiber and the particle size of the product (Jaime et al., 2002). Carrots are known to be high in soluble fibers such as pectin, which have higher WHC than insoluble fibers, and could explain the high WHC in carrot fibers.

The WHC of carrot pomace (19.72 g/g) is higher than those of orange peel waste, potato peels, and green pea peels (16.39, 15.62, and 13.48 g/g, respectively) (Sharoba et al., 2013). Their total dietary fiber content of carrot pomace was reported as 69.85%, carrot pomace which was lower than those of potato and green bean peels (73.25% and 71.30%, respectively). The soluble dietary fiber content of carrot pomace was 3-6% higher than orange peel, potato, and green pea peel wastes. The total dietary fiber content of untreated mash was 75.90 ± 7.24%, which is in the range previously reported for carrot pomace (69.85%).

Dietary fiber content from coconut waste after the extraction of coconut milk (63.25%) was lower than our carrot mash (75.90%). Their water holding capacities however were similar, with the values of 7.1 g/g – 7.9 g/g for coconut residue and unpressed carrot mash, respectively (Raghavendra et al. 2004). The water holding capacity of coconut residue was higher than any other dietary fiber residues including

apple, potato, and wheat bran fibers (Raghavendra et al., 2004). These results implied that the waste from carrot processing could be of equal benefit as the waste of coconut fiber.

4.1.5.2 Fat Binding Capacity

Fat binding capacity (FBC) is the ability of the fibers to absorb and hold fat. There are many factors that impact the FBC of plant polysaccharides, including density, thickness, hydrophobic nature of the particle, particle size, and IDF content to name a few (Sharoba et al., 2013). The FBC values of unpressed and mechanically treated carrot mashes are shown in figure 4-2. Unpressed carrot mash had a fat binding capacity of 1.91 g/g, which increased to 5.26 and 5.56 g/g for expeller pressed and hydraulic pressed carrot mash, respectively.

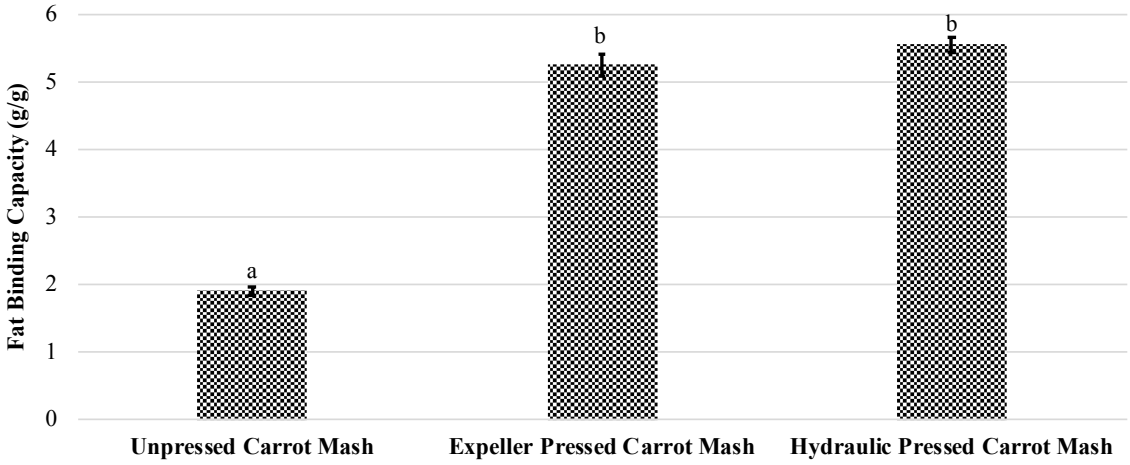

Figure 4-2. Impact of Physical Extraction Methods on the Fat Binding Capacity of Commercially Produced Carrot Mash

Surface properties contribute to the fat binding capacity of dietary fibers (Femenia et al., 1997; Lopez et al., 1996). Mechanical disruption of the fiber particle may change the surface properties by bringing more hydrophobic sections to the surface.

Oil holding capacity of carrot pomace was reported as 3.95 ± 0.17 g/g, which was higher than orange peel waste but lower than potato and green bean peels (Sharoba et al.,

2013). Our results showed higher fat holding capacity of 5.26 ± 0.16 g/g for expeller pressed and 5.56 ± 0.1 g/g for hydraulic pressed mash. It was suggested that fibers from carrot pomace would be able to stabilize food emulsions with a high presence of fat, which supported our results.

4.1.5.3 Swelling Capacity

Swelling capacity (SC) is defined by as the ratio of the volume occupied when the sample is immersed in excess of water after equilibrium to the initial weight (Raghavendra et al., 2004). In clarification of water holding capacity and swelling capacity, water holding capacity includes a step to force water out of the structure (centrifugation or vacuum filtering), whereas swelling capacity does not include the step to force water out of the structure.

The SC of unpressed and mechanically treated carrot mashes are shown in figure 4-3. Swelling capacity of unpressed carrot mash was 29.23 ± 1.81 mL/g and decreased significantly to 14.14 ± 0.45 mL/g and 12.96 ± 0.39 mL/g after expeller and hydraulic pressing, respectively. Swelling capacity was reported to decrease with decreasing particle size which was likely caused by damage to the coconut fiber matrix and collapse of the pores during grinding (Raghavendra et al., 2004).

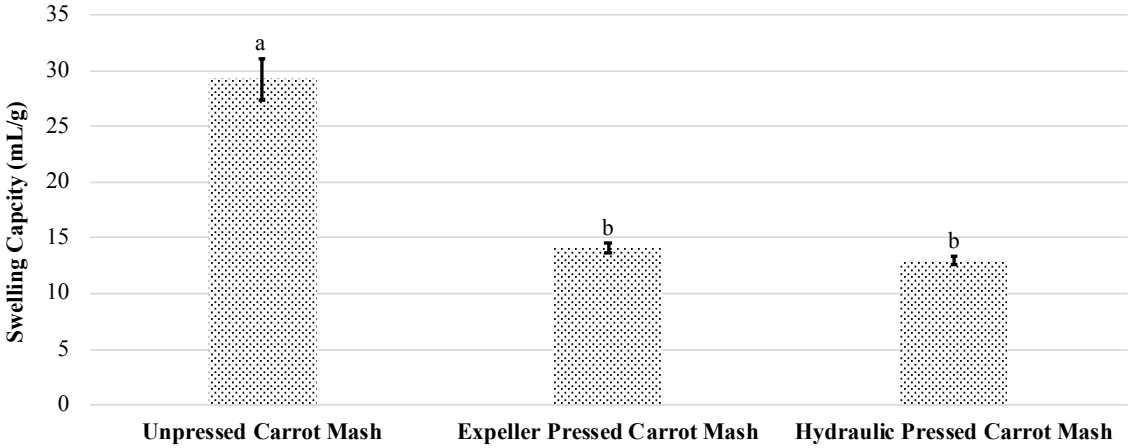

Figure 4-3. Impact of Physical Extraction Methods on the Swelling Capacity of Commercially Produced Carrot Mash

Swelling capacity of carrot insoluble fiber was reported to be 7.50 cm^3 ± 0.50 (Thebaudin et al., 1997). A slightly lower capacity was reported for coconut residue (20 mL/g) than our unpressed carrot mash (29.23 mL/g) (Raghavendra et al., 2004). Coconut fiber content was reported at 63.25%, which was lower than the fiber content of our carrot mash (75.90%). In addition, the soluble dietary fiber content of coconut residue was 4.53%, and soluble dietary fiber content of our carrot mash was 19.70%. Soluble dietary fiber is important to the functionality of dietary fibers because soluble fibers (pectin and gums) possess higher WHC than cellulosic fibers, so this could lead to the assumption that swelling capacity could be affected by this as well (Sharoba et al., 2013). The same study showed SC of carrot pomace to be 23.96 ± 0.58, lower than the unpressed mash in our study but was reported as a "high swelling capacity" matrix (Sharoba et al., 2013). Both expeller pressing and hydraulic pressing of carrot mash do not significantly increase the swelling capacity of carrot mash.

4.2 Development of Beef Patty with Carrot Mash

We established that expeller pressing, overall, was more efficient than hydraulic pressing for increasing the extraction of nutrients; both methods had similar impacts on functional properties. For the following research, expeller pressed and unpressed mash were analyzed.

Our objective was to determine the effect of the addition of carrot mash on the pH, color, cooking yield, and textural properties of beef patties compared to commercially produced carrot fiber and control beef patties with no fiber. Carrot fiber and mash are added at a concentration of 1% and 3%. The lower concentration (1%) was based on the recommended concentration by the company for the commercial carrot fiber, and the high level (3%) was based on the levels used in literature (García et al. 2006; Jiménez-Colmenero and Delgado-Pando 2013).

Table 4-6. Functional Properties of Carrot Mash and Commercial Carrot Fiber[1]

Carrot Additive	TDF[2] (%)	WHC[3] (g/g)	FBC[4] (g/g)	SC[5] (mL/g)
Unpressed Carrot Mash	75.90 ± 7.24	7.91 ± 0.37	1.91 ± 0.06	29.23 ± 1.81
Expeller Pressed Carrot Mash	78.53 ± 3.68	5.51 ± 0.14	5.26 ± 0.16	14.13 ± 0.45
Commercial Carrot Fiber	89.70 ± 1.00	9.15 ± 1.94	5.26 ± 0.22	19.76 ± 1.00

[1] Hydrobind LP, Bolthouse Farms Inc., Bakersfield, CA
[2] Total Dietary Fiber
[3]Water Holding Capacity
[4] Fat Binding Capacity
[5]Swelling Capacity

4.2.1 pH

When comparing the control beef patty to the beef patties with carrot mash and commercial fiber, slight pH differences were found between the control (5.69 ± 0.09) and the expeller pressed mash at 3% (5.50 ± 0.06). The pH of carrot mash prior to addition to

a beef patty was 4.29. There was no significant pH difference between the1% commercial carrot fiber patties and 1% carrot mash patties (Table 4-7).

The pH of the beef patties in our study were similar to the pH of beef patties made with 1%, 3%, and 5% tomato pomace (Savadkoohi et al., 2014). The addition of plant starches (10%) to chicken patties did not have any significant difference in pH of the patties (Das et al., 2015). Ultimately, all pH levels in our study were within the normal pH range of an unstressed animal which is reported at 5.4-5.7 (Miller, 2007).

Table 4-7. pH of Beef Patties with Carrot Powder Compared to a Control Beef Patty

Sample	Concentration (%)	pH
Control	0	$5.69 \pm 0.09^{a,b}$
CM[1]	1	$5.64 \pm 0.04^{a,b,c}$
EPM[2]	1	$5.62 \pm 0.02^{a,b,c}$
CCF[3]	1	5.73 ± 0.07^{a}
CM[1]	3	$5.52 \pm 0.03^{b,c}$
EPM[2]	3	5.50 ± 0.06^{c}
CCF[3]	3	5.71 ± 0.06^{a}

[a-c] Different letters indicate a significant difference; $p \leq 0.05$.
[1]Carrot Mash, [2]Expeller Pressed Mash, [3]Commercial Carrot Fiber

4.2.2 Color

There were no significant differences for L* (lightness) or b* (yellow) between the raw beef patties and carrot – added patties (Table 4-8). The 3% commercial carrot fiber patty and 1% carrot mash patty showed a* values (redness) 38% and 48% higher than the control and expeller pressed mash at 1% and 3%, with intermediate values found for commercial carrot fiber 1% and carrot mash 3%. Adding 1% carrot mash and 3% carrot fiber increased the redness of the patties.

Table 4-8. L*a*b* Color Values of Raw Beef Patties with Carrot Powder

Sample	Concentration (%)	L*	a*	b*
Control	0	16.25 ± 0.43[a]	12.74 ± 1.91[b]	24.12 ± 0.95[a]
CM[1]	1	15.24 ± 1.78[a]	17.65 ± 0.53[a]	28.08 ± 2.42[a]
EPM[2]	1	14.54 ± 1.13[a]	12.66 ± 1.13[b]	25.28 ± 1.03[a]
CCF[3]	1	14.19 ± 2.56[a]	14.98 ± 0.54[a,b]	25.49 ± 0.92[a]
CM[1]	3	13.22 ± 1.78[a]	16.82 ± 1.71[a,b]	27.15 ± 0.44[a]
EPM[2]	3	15.77 ± 0.79[a]	12.85 ± 1.76[b]	26.00 ± 1.68[a]
CCF[3]	3	11.15 ± 1.08[a]	18.91 ± 0.59[a]	26.92 ± 0.66[a]

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.
[1]Carrot Mash, [2]Expeller Pressed Mash, [3]Commercial Carrot Fiber

Table 4-9 summarizes the total color differences of each of the patties when compared to the control patty. The ΔL of the samples are negative, implying that the control patty was darker than carrot patties, regardless of carrot amount. Our data confirmed previous reported observations that beef patties with added dried carrot were lighter than control patties (Saleh and Ahmedb, 1998).

The increase in Δa* showed that the 3% commercial carrot fiber patties were redder in color, followed by 1% carrot mash and 3% carrot mash patties. Similarly, beef patties with dried carrot were shown to be redder (a*) with increases in yellow (b*) values (Saleh and Ahmedb, 1998).

The largest change in color from the control beef patties in our study was with the commercial carrot fiber 3% (ΔE = 8.48) followed by the carrot mash at 1 % (ΔE = 6.39). The patties with the lowest ΔE were expeller pressed mash with 1% (ΔE = 1.95) and 3% (ΔE = 2.07).

Table 4-9. Color Difference of Beef Patties with Carrot Powders Compared to a Control Beef Patty

Sample	Concentration (%)	ΔL*	Δa*	Δb*	ΔE
Control	0	-	-	-	-
CM[1]	1	-1.01	4.91	3.96	6.39
EPM[2]	1	-1.71	-0.08	1.16	2.07
CCM[3]	1	-1.33	2.24	1.38	2.94
CM[1]	3	-3.03	4.08	3.03	5.92
EPM[2]	3	-0.48	0.12	1.88	1.95
CCF[3]	3	-5.10	6.17	2.80	8.48

[1]Carrot Mash, [2]Expeller Pressed Mash, [3]Commercial Carrot Fiber

4.2.3 Cooking Yield

Cooking yield increased with each concentration of carrot mash added (Table 4-10). This result was expected because the fat binding capacity and water holding capacity (Table 4-6) were higher with carrot mash. The addition of fiber likely contributed to the retention of water and fat in the beef patties because of the water holding and fat binding capacities of both carrot mash and commercial fiber. Our results show that all samples except the expeller pressed samples at 1% had significantly higher cooking yield than the control sample. Patties with 3% fibers had the highest cooking yields.

Table 4-10. Cooking Yield of Beef Patties with Carrot Powder Compared to a Control Beef Patty

Sample	Concentration (%)	Average Cook Yield (%)	Increase (%)
Control	0	70.04 ± 3.04^{c}	--
CM[1]	1	76.30 ± 1.51^{b}	8.93
EPM[2]	1	$73.18 \pm 1.48^{b,c}$	4.48
CCM[3]	1	75.76 ± 1.47^{b}	8.17
CM[1]	3	$78.61 \pm 0.29^{a,b}$	12.24
EPM[2]	3	83.97 ± 0.38^{a}	19.97
CCF[3]	3	83.00 ± 1.85^{a}	18.50

[a-c] Different letters indicate a significant difference; $p \leq 0.05$.
[1]Carrot Mash, [2]Expeller Pressed Mash, [3]Commercial Carrot Fiber

Saleh and Ahmedb (1998) compared beef patties with dried carrot and dried sweet potatoes. Cooking yield with the addition of dried carrot was not significantly

different from the control sample. When gari (a product of cassava root processing) was added to beef patties, a significant increase in cooking yield was reported at 10, 15 and 20% levels because of high water holding and fat binding capacity during cooking (Akwetey and Knipe 2012).

With the addition of pea fiber to high and low fat beef patties, an increase in fat retention and cooking yield was observed, without any negative effects on juiciness or flavor (Besbes et al. 2007). Fat binding properties leading to a higher cooking yield were also reported by the addition of apple fiber (Delcour and Poutanen, 2013). Previous studies have indicated that the addition of dietary fiber increased viscosity due to the fat binding capacity creating a weak gel structure, thus giving the product a higher yield (Agar et al., 2016).

4.2.4 Texture Analysis

Little to no difference was seen in textural properties between the control and carrot-added beef patties after cook. Hardness – cycle's 1 and 2 (Figure 4-4), springiness, gumminess, and chewiness (Table 4-11), adhesiveness and cohesiveness (Table 4-12), and are discussed below.

Hardness-1 (g) represents the maximum force measured at the first compression (first bite) and hardness-2 the maximum force at the second compression (second bite). The results showed a decrease in hardness from cycle 1 to cycle 2 for each sample. However, no significant differences between treatments were observed for hardness-1 or hardness-2; all samples were similar to the control patty. Similarly, no significant differences were found between the

hardness of a control patty and any patties with plant starches at 10% w/w (Das et al., 2003).

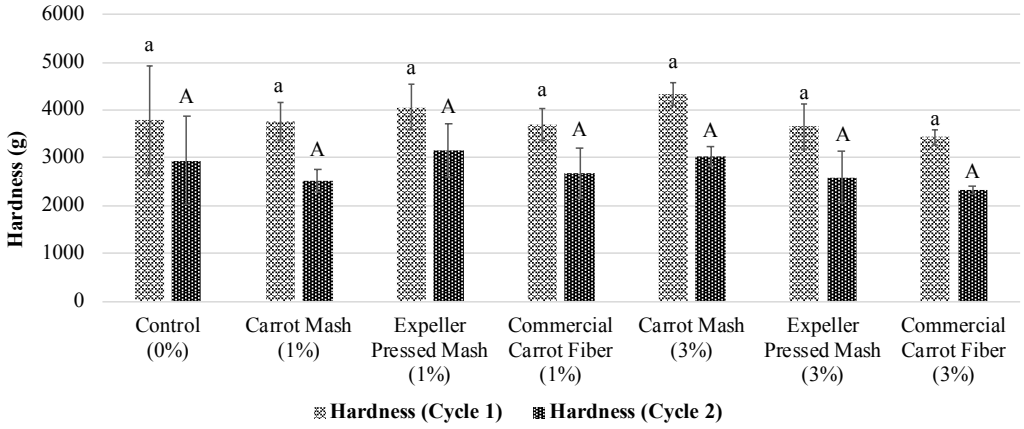

Figure 4-4. Hardness of Beef Patties with Carrot Powder Compared to a Control Beef Patty. Statistical analysis was performed separately for Cycle 1 and Cycle 2.

Springiness, gumminess, chewiness, and adhesiveness were similar regardless of the concentration and carrot fiber type. The only differences observed were for cohesiveness between expeller pressed mash at 1% and commercial carrot fiber at 3%. Addition of fibers to meat products lead to different outcomes. The addition of gari to beef burger patties significantly reduced hardness, springiness, gumminess, chewiness, and cohesiveness by 10 to 20% (Akwetey and Knipe, 2012). When tomato pomace was added to beef frankfurters, a significant increase in hardness was observed with 3 to 7 % pomace addition (Savadkoohi et al., 2014). The texture of beef frankfurters was observed after cooking in a casing, where our study used a ground beef patty without casing, which is more likely to crumble apart than a product with a casing.

Table 4-11. Springiness, Gumminess, and Chewiness of Cooked Beef Patties with Carrot Powder

Sample	Concentration (%)	Springiness (mm)	Gumminess (g)	Chewiness (mJ)
Control	0	6.80 ± 0.54[a]	1651.67 ± 615.07[a]	110.83 ± 38.08[a]
CM[1]	1	7.50 ± 0.39[a]	1355.00 ± 312.64[a]	101.83 ± 29.68[a]
EPM[2]	1	7.11 ± 0.24[a]	1821.67 ± 464.84[a]	128.08 ± 37.28[a]
CCM[3]	1	7.18 ± 0.09[a]	1263.50 ± 226.41[a]	89.17 ± 16.83[a]
CM[1]	3	6.89 ± 0.09[a]	1374.50 ± 127.53[a]	93.33 ± 9.29[a]
EPM[2]	3	7.14 ± 0.51[a]	1234.50 ± 379.51[a]	87.98 ± 31.92[a]
CCF[3]	3	6.73 ± 0.16[a]	923.17 ± 23.27[a]	61.02 ± 3.03[a]

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.

Table 4-12. Adhesiveness and Cohesiveness of Cooked Beef Patties with Carrot Powder

Sample	Concentration (%)	Adhesiveness (mJ)	Cohesiveness
Control	0	0.57 ± 0.25[a]	0.42 ± 0.04[a,b]
CM[1]	1	0.77 ± 0.28[a]	0.36 ± 0.07[a,b]
EPM[2]	1	0.35 ±0.11[a]	0.44 ± 0.06[a]
CCM[3]	1	0.40 ± 0.16[a]	0.34 ± 0.03[a,b]
CM[1]	3	0.43 ± 0.06[a]	0.32 ± 0.02[a,b]
EPM[2]	3	0.42 ± 0.08[a]	0.33 ± 0.07[a,b]
CCF[3]	3	0.87 ± 0.47[a]	0.27 ± 0.01[b]

[a-b] Different letters in the same column indicate a significant difference; $p \leq 0.05$.

No significant differences between the textural properties of the control and carrot powder beef patties support the objective that dried carrot mash can be incorporated into beef patties up to 3% without affecting the texture. The addition of unpressed carrot mash increased fiber to 0.86g at 1% and 2.58g at 3% and expeller pressed increased fiber to 0.89g at 1% and 2.67% at 3%. The similar textural properties and increased cooking yield with the addition of carrot powders make it an attractive ingredient.

4.3 Enzymatic Hydrolysis

The focus of this section was to determine the potential of an enzymatic treatment to modify the carbohydrate profile of carrot mash to increase the concentration of oligosaccharides. By manipulating the carbohydrate profile, a waste substrate can be

converted into a product that is not only edible but with added value. Some oligosaccharides have the potential to be prebiotic, which can be used by the probiotic bacteria in the gut (Martínez et al., 2009).

4.3.1 Carotenoid Content

Neither expeller pressing alone or used in conjunction with an enzyme cocktail at 0.15% and 0.225% significantly increased carotenoid content in carrot mash (Table 4-13).

Carotenoid contents of both fresh and oven dried carrot pomace were reported at ≅ 92 ppm and 65 ppm, respectively, which was higher than our study (≅ 0.82 – 1.36 ppm) (Hernández-ortega et al., 2013). Pomace is a product of the whole carrot, while mash is only made from the peelings of the carrot. As mentioned earlier, carotenoid content in carrots are more prevalent in the periderm of the carrot, decreasing towards the core, so using the whole carrot rather than strictly the peelings, provides more carotenoids overall (de la Rosa et al., 2010). The results of Stoll et al. (2003), although only analyzed for α- and β-carotene rather than total carotenoids, were similar to our study (total carotenoids) as they did not see significant differences in carotenoid content from a fine grinding (40ppm) and enzymatically hydrolyzed (45ppm) treatments with a pectinase and cellulase mixture at 1:1 ratio for 1 hr . Overall, the extraction of total carotenoids was not significantly enhanced or hindered with enzymatic treatments compared to the untreated sample.

Table 4-13. Carotenoid Concentration of Expeller Pressed Carrot Mash treated with a

Carrot Mash Sample	Enzyme Concentration (%)	Treatment Time (min)	Average Carotenoid Concentration (ppm)
Untreated Mash	N/A	0	0.82 ± 0.10^{a}
Expeller Pressed Mash	N/A	0	1.30 ± 0.31^{a}
Control	0.0	15	1.12 ± 0.07^{a}
Treatment 1	0.15	15	1.10 ± 0.01^{a}
Treatment 2	0.225	15	1.33 ± 0.14^{a}
Control	0.0	30	1.12 ± 0.21^{a}
Treatment 1	0.15	30	1.03 ± 0.04^{a}
Treatment 2	0.225	30	1.36 ± 0.14^{a}

cocktail of cellulase, hemicellulose, xylanase and pectinase.
[a-b] Different letters indicate a significant difference; $p \leq 0.05$.

4.3.2 Soluble Sugar

There was no statistical difference between the soluble sugar concentration in the untreated or expeller pressed carrot mash samples, illustrating that expeller pressing did not impact soluble sugar content. Increasing both enzyme concentration and hydrolysis time significantly increased the amount of soluble sugar present in carrot mash (Table 4-14). All samples treated with enzymes were statistically different from the untreated mash, expeller pressed mash, and the control (0.0% enzyme concentration) carrot mash. The largest difference was between the samples treated with 0.225% enzymes at 15 and 30 minutes (4938.75 mg/mL and 4800.44 mg/mL, respectively), compared to untreated mash at (1119.90 mg/mL) an increase of 340% and 328% respectively. Both enzyme concentration and time were statistically significant in altering soluble sugar content ($F = 51.87$ and 5.88, respectively), with enzyme concentration having the largest impact on increasing soluble sugar content.

When alcohol soluble and insoluble dietary fiber portions from carrot pomace were treated with cellulase for 24 hours, total mono and oligosaccharide contents in both

portions of the pomace increased by ≅17% (Yoon et al., 2005). These results are consistent with our study, that enzymatic hydrolysis increases soluble sugars in carrot waste. This was expected, as the enzymatic treatment hydrolyzed polysaccharides into smaller oligosaccharides, disaccharides, and monosaccharides as enzyme concentrations and contact times increase (Martínez et al., 2009)

Table 4-14. Average Soluble Sugar Concentration in Enzymatically Treated Carrot Mash

Carrot Mash Sample	Enzyme Concentration (%)	Treatment Time (min)	Average Soluble Sugar Concentration (mg/mL)
Untreated Mash	N/A	0	1119.90 ± 95.97[b]
Expeller Pressed Mash	N/A	0	1485.69 ± 380.76[b]
Control	0.0	15	1664.80 ± 89.67[b]
Treatment 1	0.15	15	4209.44 ± 419.15[a]
Treatment 2	0.225	15	4938.75 ± 852.10[a]
Control	0.0	30	1883.97 ± 68.13[b]
Treatment 1	0.15	30	4343.96 ± 242.28[a]
Treatment 2	0.225	30	4800.44 ± 787.35[a]

[a-b] Different letters indicate a significant difference; $p \leq 0.05$.

Table 4-15. Significance[1] of Enzyme Concentration and/or Time on Soluble Sugar Concentration in Carrot Mash

Source	*Nparm*	*DF*	*Sum of Squares*	*F Ratio*	*Prob > F*
Enzyme Concentration	2	2	32,931,796	51.87	<.0001[2]
Time	2	2	3,731,054	5.88	0.0109[2]
*Enzyme Concentration*Time*	4	4	3,162,011	2.49	0.0799

[1] Probability Values
[2] Source with significant impact on soluble sugar concentration

4.3.3 Total Dietary Fiber

During the incubation for 30 min, the insoluble dietary fiber content was linearly decreased as the enzyme concentration increased (Table 4-16). The 0.15% enzyme treatment decreased the insoluble fiber by ≅ 17% compared to untreated mash, ≅ 16% compared to expeller pressed mash and ≅ 15% compared to control treated for 30 min.

The 0.225% enzyme treatment decreased the insoluble fiber by ≅ 21% compared to untreated mash, ≅ 20% compared to expeller pressed mash and control treated for 30 min.

The most significant differences in IDF were between the mash treated with 0.225% enzymes at 30 minutes (44.10 ± 4.19) and the untreated mash, expeller pressed mash, and the samples treated with 0% enzymes at 30 minutes (56.20 ± 1.45, 55.70 ± 1.84, and 55.30 ± 0.36, respectively).

The results obtained for untreated carrot mash in our study was consistent with total dietary fiber (TDF) (60.33 ± 0.16), insoluble dietary fiber (IDF) (47.66 ± 2.21), and soluble dietary fiber (SDF) (12.67 ± 0.59) reported for carrot pomace (Hernández-ortega et al., 2013). Total dietary fiber, IDF, and SDF for carrot pomace were also reported as 63.6, 50.1, and 13.5, respectively (Chau et al., 2004). It can be concluded that enzymatic treatments had no significant impact on soluble and total dietary fiber.

Table 4-16. Insoluble, Soluble, and Total Dietary Fiber Content of Enzymatically Treated Carrot Mash

Carrot Mash Sample	Enzyme Concentration (%)	Treatment Time (min)	Insoluble Dietary Fiber (%)	Soluble Dietary Fiber (%)	Total Dietary Fiber (%)
Untreated Mash	N/A	0	56.20 ± 1.45^{a}	19.70 ± 6.06^{a}	75.90 ± 7.24^{a,b}
Expeller Pressed Mash	N/A	0	55.70 ± 1.84^{a}	22.83 ± 1.84^{a}	78.53 ± 3.68^{a}
Control	0.0	15	54.90 ± 1.93^{a,b}	17.60 ± 0.88^{a}	72.50 ± 1.04^{a,b}
Treatment 1	0.15	15	48.83 ± 0.65^{a,b,c}	21.40 ± 1.13^{a}	70.23 ± 1.35^{a,b}
Treatment 2	0.225	15	50.27 ± 3.08^{a,b,c}	20.80 ± 0.99^{a}	71.07 ± 2.17^{a,b}
Control	0.0	30	55.30 ± 0.36^{a}	17.97 ± 1.73^{a}	73.27 ± 1.44^{a,b}
Treatment 1	0.15	30	46.63 ± 3.03^{b,c}	22.73 ± 1.31^{a}	69.37 ± 2.17^{a,b}
Treatment 2	0.225	30	44.10 ± 4.19^{c}	21.80 ± 2.07^{a}	65.90 ± 2.12^{b}

$^{a-c}$ Different letters in the same column indicate a significant difference; $p \leq 0.05$.

4.3.4 Oligosaccharide Profile

Oligosaccharide content in untreated mash (0.19% ± 0.03%) significantly increased to 2.55% ± 0.71% after 30 min of enzymatic treatment with 0.15% enzymatic

cocktail (Table 4-17). When looking at the effect of enzyme concentration, heating time or the combination of enzyme concentration and treatment time on the production of oligosaccharides; enzyme concentration (F = 7.54) had a more significant effect on oligosaccharide content than time of heating (F = 0.00), or a combination of enzymes and time (F = 1.06) (Table 4-18).

Similarly, oligosaccharide content in carrot pomace was increased by 2% with an enzymatic treatment (Edible Snails Crude Enzyme, 96 h hydrolysis) (Yoon et al., 2005). The highest oligosaccharide content was observed in carrot mash treated with 0.15% enzyme for 30 minutes. The carrot mash treated with 0.225% enzyme for 30 minutes, was lower in oligosaccharides, likely due to the carrot mash breaking down further into mono and disaccharides (Figure 4-5).

Table 4-17. Oligosaccharide Content in Enzymatically Treated Carrot Mash

Carrot Mash Sample	Enzyme Concentration (%)	Treatment Time (min)	Oligosaccharide Content (%)
Untreated Mash	N/A	0	0.19 ± 0.03^{b}
Expeller Pressed Mash	N/A	0	0.24 ± 0.04^{b}
Control	0.0	15	$0.71 \pm 0.21^{a,b}$
Treatment 1	0.15	15	$2.21 \pm 1.04^{a,b}$
Treatment 2	0.225	15	$1.89 \pm 1.33^{a,b}$
Control	0.0	30	$0.80 \pm 0.05^{a,b}$
Treatment 1	0.15	30	2.55 ± 0.71^{a}
Treatment 2	0.225	30	$2.11 \pm 1.21^{a,b}$

[a-b] Different letters indicate a significant difference; $p \leq 0.05$.

Table 4-18. Significance[1] of Enzyme Concentration and/or Time on Oligosaccharide Content of Carrot Mash

Source	*Nparm*	*DF*	*Sum of Squares*	*F Ratio*	*Prob > F*
*Enzymes*Time*	10	4	2.16	1.06	0.3980
Enzymes	5	2	7.71	7.54	0.0029[1]
Time	2	0	0.00	-	-

[1] Probability Values

[2] Source with significant impact on Oligosaccharide content

Figure 4-5 shows the impact of expeller pressing and enzymatic treatments on oligosaccharide content determined by HPLC. As expected, compared to the control, expeller pressing alone did not modify the carbohydrate profile. At the enzyme concentration of 0.15%, monosaccharides increased and oligosaccharides with a DP ≥ 10 appeared. An enzyme concentration of 0.15%, the increase in incubation time (15 to 30 minutes), did not significantly modify the carbohydrate profile. Very few studies have aimed to break down plant polysaccharides into oligosaccharides, and none have shown what they look like via an HPLC chromatogram (Martínez et al. 2009).

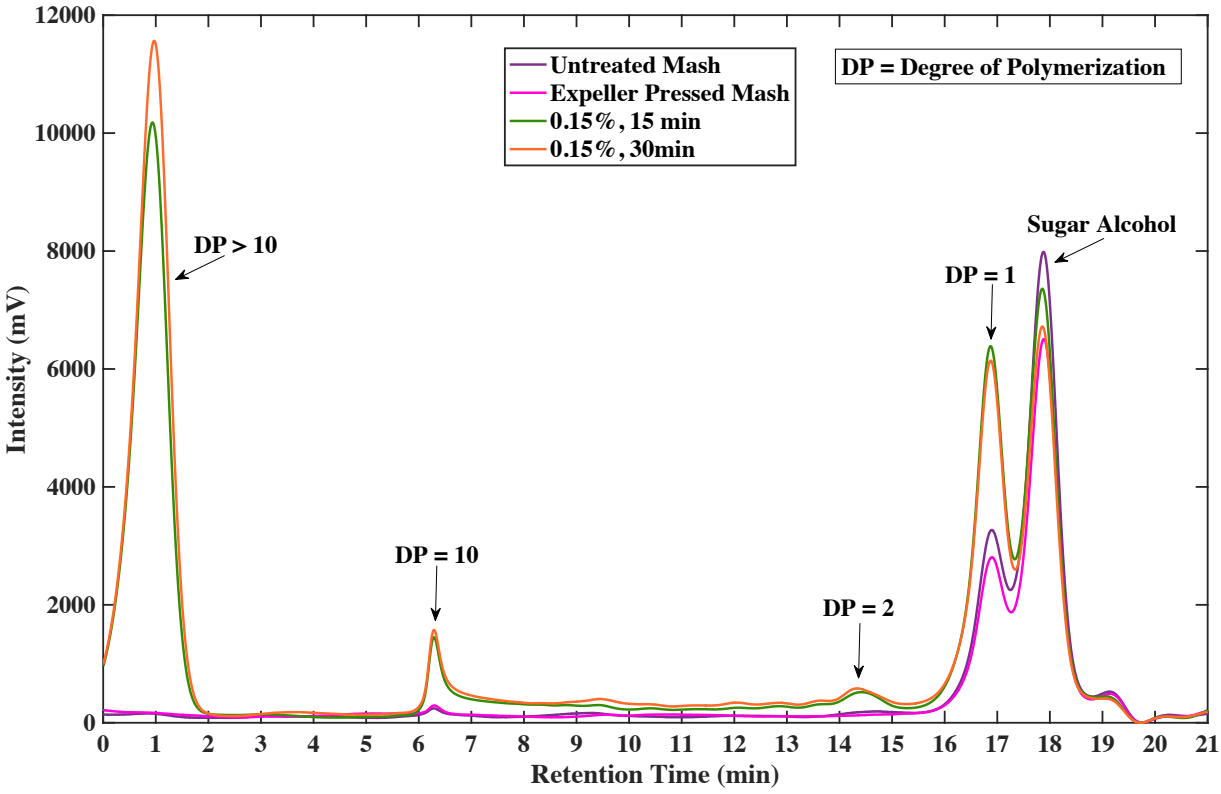

Figure 4-5. Selected HPLC Chromatogram Profile on the Distribution of Expeller Pressing and Enzymatic Treatments on Oligosaccharide Profile

CHAPTER 5 – CONCLUSION

The overall objective of this research was to investigate processing methods to alleviate the excess amount of food waste existing today. Specifically, this project focused on evaluating the opportunity to utilize carrot processing waste as a functional food ingredient. It was hypothesized that mechanical separation would be a viable way to separate liquid and solid fractions of the waste. It was also hypothesized that carrot mash could be used as a functional ingredient in ground beef patties, and that enzymatic treatments can modify the carbohydrate profile to increase oligosaccharide concentration in the mash.

Expeller pressing showed the most promise in extracting liquid based on the results from percent extractable matter and total solids content. Total carotenoid extraction increased by 1000% with the use of expeller pressing. The separation of water from the solids and increased extraction of carotenoids could allow a processing company to have two potential revenue generating streams from their waste. Future research in carrot processing waste should include a viable way to ensure that the recovered water is safe to use for irrigation purposes or to recirculate back into the processing facility.

With a mechanical treatment, increases in fat binding capacity and swelling capacity of dried carrot mash were observed which could beneficiate their use for the development of functional foods. The beef patty with the most desirable qualities was the patty incorporated with 3% expeller pressed carrot mash added. This patty had similar textural properties, pH, and least color change, while having the highest cooking yield. All of these are promising characteristics for carrot mash to be used as a meat filler with

added fiber. Future research on the addition of carrot mash into beef patties is needed for the effects of carrot mash on the oxidation and sensory characteristics of the patties.

The final part of this research demonstrated that an enzymatic treatment of carrot mash can be applied to break down polysaccharides into oligosaccharides. The use of enzymatic treatments in this study did not have any significant effects on carotenoid content or total dietary fiber content, however it was shown to increase soluble sugar content. The confidence interval for predicted oligosaccharide content was found at 0.780 and 4.32 meaning that there is potential to get near 4% oligosaccharide content from the enzymatic treatment with 0.15% for 30 minutes. Future research into the enzymatic hydrolysis of carrot mash is required to determine an optimal condition for extracting oligosaccharides, and their prebiotic property.

Carrot processing wastes hold useful nutrients like carotenoids, polyphenols and dietary fibers, and have an immense potential for utilization in foods and pharmaceuticals. Finding applicable uses for carrot mash will help divert the waste from landfills and to new avenues for carrot producers and processors in California to gain a new revenue stream.

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