Effect of Vacuum Microwave Dehydration on the Off-Flavour Intensity and Functionality of Pea Proteins

by

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Abstract

Pea (*Pisum sativum* L.) has garnered recent attention as a plant-protein source due to its high protein content, nutrient density and low allergenicity. However, pea proteins are difficult to incorporate into food formulations due to undesirable green, grassy and beany aromas and limited functional properties. Direct steam injection is often used in the food industry to decrease off-flavour intensity of pea proteins and improve functional properties. However, induced cooked off-flavours and nutrient losses that are attributed to heating warrant exploration of an alternative process. This research examined the applicability of vacuum microwave dehydration as a pre-processing step for plant protein for use during non-dairy alternative production.

In this thesis, effects of the following process parameters: initial moisture content (5-425% dry basis), vacuum level (40-200 Torr), specific power (10-200 W/g), and process time (1-50 minutes) on volatile compound concentration, functionality and quality parameters such as available lysine and colour, were analyzed. Increasing initial moisture content decreased ($p<0.05$) protein solubility, emulsifying activity index and chemically available lysine content, but increased ($p<0.05$) emulsifying stability index. Having a higher initial moisture content decreased ($p<0.05$) lightness, but increased ($p<0.05$) the a* and b* coordinates and total colour difference compared to untreated pea protein. Generation of specific volatiles related to thermally induced lipid oxidation was observed in some samples, thus, necessitating lower specific energy treatments to be considered.

Results showed that three VMD processes could be developed for use on two sources of pea proteins. VMD-processing pea protein with an initial moisture content of 162% d.b. at 100W/g microwave energy and 200 Torr vacuum-level for 2.5 minutes was found to be the optimal conditions for retaining functional properties and minimizing volatile concentration that contributed to off-flavour intensity.

Descriptive analysis showed that the finalized VMD-process reduced levels of “raw/beany” and “green/grassy” aroma and flavour, but increased overall aroma intensity, “goaty/caproic acid” aroma and “chalky flavour”. Future consumer trials are needed to verify whether these differences in attribute intensities are relevant at the consumer level.

Lay Summary

In recent years, consumers are increasingly opting for plant-based diets. Peas are a nutritious, inexpensive, hypoallergenic, and sustainable source of plant-based protein; however, barriers exist before incorporating them in food formulations. Pea proteins possess undesirable off-flavours and have poor functional properties. Current methods such as direct steam injection may help reduce intrinsic off-flavours, but may add an undesirable cooked off-flavour. Vacuum microwave dehydration was investigated as an alternative to remove the off-flavour causing aroma compounds and its effects on functional properties were assessed. Three vacuum microwave dehydration processes were developed based on aroma analysis and protein functionality data. Flavour profile of pea protein subjected to the optimized VMD process was assessed with descriptive analysis using a trained panel. This study suggested that VMD could be a feasible option for pre-processing plant-based proteins before incorporating them into plant-based dairy analogues.

Preface

Besides the works discussed below, Philip Pui-Li Yen designed and performed all experiments, analyzed and interpreted the results and wrote this Master's thesis, under the supervision of Dr. Anubhav Pratap Singh.

Experiments involving solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME GC-MS) were completed by Lufiani Lina Madilao. Philip Pui-Li Yen prepared the samples and analyzed the data.

Statistical consulting was obtained from Jonathan Agyeman of the UBC Department of Statistics.

Parts of this thesis have been accepted as an original research article entitled "Vacuum microwave dehydration decreases volatile concentration and soluble protein content of pea (*Pisum sativum*, L.) protein" in the Journal of the Science of Food and Agriculture.

Experiments involving human panelists were approved by the University of British Columbia's Behavioural Research Ethics Board [Certificate # H19-02124].

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By God's Grace, I have successfully defended and published my thesis.

Dedication

I dedicate this Master's thesis to my lovely mom Eva Lan Fun Yen who constantly reminds me that every day is a good day.

Chapter 1: Introduction and Literature Review

1.1. Background

In recent years, plant-based foods have vastly grown in popularity due to numerous changes in consumer preferences (Chao and others 2018; Lam and others 2018). Consumers are now more cognisant of their food, nutrition and health, thus plant-based foods are increasingly being preferred (Sadiq Butt and Batool, 2010; Lam and others 2018). Consumers may preferentially select plant-based foods due to social factors, relative inexpensiveness, religious beliefs, and ethical influences, such as sustainability and animal welfare (Stone and others 2015; Lam and others 2018; Lan and others 2018). This surge in interest in plant-based foods has driven the Canadian food industry to develop novel plant-based products that appeal to a multitude of consumers (Pietrysiak and others 2018).

Peas are consumed all around the world and have become recently increasingly popular. The total world production of peas in 2009 was over 10 million tons, with Canada at the forefront (Dahl and others 2012). The incorporation of legumes, such as peas, into food products has been explored in part due to their high protein content (Pietrysiak and others 2018). Peas are also low in fat and contain a plethora of vitamins, minerals and various bioactive compounds, (Lam and others 2018; Nishinari and others 2014). Thus they have strong potential to be incorporated into vegetarian-friendly products, specifically meat analogues (Sandberg 2011). However, pea proteins have inferior functional properties to those of animal-based proteins. Furthermore, raw legumes may have characteristic off-flavours such as grassy or beany notes, hence they are processed to mitigate said off-flavours (Damodaran and Arora, 2013). One prominent method is to use direct steam injection, which involves subjecting food matrices to high-temperature steam for a short period of time (Pietrysiak and others 2018). The steam facilitates the removal of the volatile compounds that are responsible for generating these off-flavours (Lane 1997). Dehydration can also lead to the removal of volatile compounds (McMinn and Magee 1999). However, both direct steam injection and conventional dehydration involve high heat, which adversely affects nutrient retention, sensory properties, as well as functionality. With advances in thermal processing technology, novel methods have been developed; these methods still decrease the concentration of off-flavour causing volatile compounds, with minimal deleterious effects on quality (Michailidis and Krokida 2014). Vacuum microwave dehydration is a relatively novel dehydration

technology and has shown potential to eliminate unwanted volatile flavour compounds without jeopardizing quality (Nöfer and others 2018).

1.2. Pea (*Pisum sativum*, L.) Protein

Peas are trending in consumer popularity due to high protein, dietary fibre, vitamin and mineral contents and low levels of fat (Dahl and others 2012; Lam and others 2018). Pea protein is also popular as of late due to its low allergenicity (Schindler and others 2012). Pea proteins have a high lysine content, but are often limiting in methionine and tryptophan, which are all essential amino acids (Lam and others 2018). To ensure all human amino acid requirements are being met, peas are often consumed with cereals or grains, as their amino acid profiles are complementary (Young and Pellett, 1994). The most prominent proteins in legumes are globulins, which comprise approximately 70-80% of the total pea protein (Nehete and others 2013; Lam and others 2018). Globulins are soluble in salt solutions and include legumin (11S), vicilin (7S) and convicilin (7S) (Barać and others 2010; Lam and others 2018). The former two globulins are the most prevalent in peas and their ratio can influence the overall functionality of the pea protein (Lam and others 2018). Protein extraction methods greatly influence the final composition of the processed pea protein and therefore affect the functional properties (Stone and others 2015).

Pea proteins often have limited water solubility and poor functional properties, prompting the need to process or structurally modify the proteins (Chao and others 2018). Pea proteins are classified as isolates or concentrates depending on the protein content. A protein isolate is generally defined as having a protein content of 90% or higher, whereas a protein concentrate has a lower protein content (Hoffman and Falvo 2004).

Peas have strong, characteristic beany or green off-flavours, making it difficult to produce palatable products when they are incorporated in formulations (Schindler and others 2012; Damodaran and Arora, 2013). According to Jakobsen and others (1998), the extent of off-flavour formation can be reduced greatly depending on the harvesting steps, whether the peas were blanched and affected by storage conditions. Jakobsen and others (1998) and Roland and others (2017) report that many of the identified volatiles in peas were degradation products of fatty acids, leading to the generation of many six-carbon aldehydes, alcohols, ketones and esters. Lipoxygenases are the most prominent enzyme in legumes and are responsible for the generation of these off-notes (Trikusuma and others 2020). A

prominent off-flavour causing volatile compound in raw legumes is n-hexanal, which is derived from the degradation of linoleic acid (Chiba and others 1979; Schindler and others 2012). n-Hexanal is an indicator for lipid oxidation and loss of pea protein quality (Schindler and others 2012). Other common off-flavours reported in peas are earthy, hay-like, mushroom, and fatty (Roland and others 2017; Trikusuma and others 2020). Although only a few compounds in peas may be perceived as beany when standalone, mixtures of specific volatiles such as a 1:100 mixture of 1-octen-3-one (earthy/mushroom-like aroma) and hexanal (green/fatty aroma) characteristically elicit intense beany aromas (Trikusuma and others 2020). Many compounds responsible for off-flavours may be present in very low concentrations but are still easily perceived due to their low odour thresholds (Roland and others 2017). However, in the last few decades, the ability to detect, identify and quantify these off-flavour causing volatile compounds has greatly improved.

1.3. Dehydration

Dehydration is an ancient preservation method (Michailidis and Krokida, 2014; Richter Reis, 2014) From sun-drying, or simply exposing food products under the sun, dehydration methods have vastly evolved, leading to a plethora of complex dehydration technologies in the last few decades (Michailidis and Krokida, 2014; Richter Reis, 2014). Dehydration preserves food by lowering the available water so that the growth of pathogens and spoilage-causing microorganisms and chemical and enzymatic reaction rates are either minimized or inhibited (Chung and Chang, 1982; Michailidis and Krokida, 2014). Dehydrated foods generally have a relatively long shelf-life as microorganisms do not grow below a water activity of 0.6 (Ijabadeniyi and Pillay 2017). Conventional dehydration uses heat to facilitate the migration of moisture from the food product to the dehydration medium (Sagar and Suresh Kumar, 2010). Exposure to prolonged high temperatures generally leads to the degradation of many quality attributes. For instance, colour, flavour and texture may deteriorate substantially, product structure, size and rehydration properties may be adversely altered, and nutrient retention may be lower (Sagar and Suresh Kumar, 2010; Arefin and others 2017; Figiel and Michalska 2017). However, drying proteins may lead to changes in functional properties due to altered chemical structure.

It is paramount to determine the drying kinetics of plant foods so that current drying processes can be optimized to yield more high-quality products in an economic manner (Isik and others 2019). Drying kinetics can be defined as the rates and behaviours of how a foodstuff is dried and can be estimated with simplistic equations and models (McMinn and Magee 1999; Veras and others 2012). The drying

kinetics of a food matrix is highly dependent on many factors, such as the structure of the food and characteristics of the drying medium (Chung and Chang 1982; Krokida and others 2003). There are two main periods during the dehydration process: the constant rate and the falling-rate period, though the latter can be divided into two periods depending on the food matrix (McMinn and Magee 1999). In the constant rate period, the water from the interior migrates to the surface of the food matrix, producing a water film. (Chung and Chang 1982). The surface water then evaporates and is transported as it comes in contact with the drying medium (Chung and Chang 1982). The rate at which water is removed is constant until the moisture content of the food is equal to the critical moisture content, signifying the start of the falling rate period (Chung and Chang, 1982). The dehydration rate starts to decrease because the moisture content in the interior of the food sample is insufficient to maintain the water film on the surface of the food (Chung and Chang, 1982). Dehydration kinetics can be used to establish the drying time of a product, often used to calculate the process time in drying process studies with a targeted final moisture content.

1.4. Dehydration Technologies

1.5. Conventional Solar and Air Drying

Sun-drying is the oldest dehydration technique; however, despite its antiquity, it is still used today (Michailidis and Krokida 2014). Sun-drying involves leaving food outside in a location where sunlight exposure is high and temperatures exceed 30°C (Michailidis and Krokida 2014). There is no economic cost as the sun's radiant energy is harnessed to dehydrate foods (Michailidis and Krokida 2014). However, dehydration times are long, sometimes upwards of a week and are highly dependent on a plethora of factors, such as solar radiation, wind velocity, air humidity and temperature and surface area exposed (Jain and Tiwari, 2003; Bal and others 2010; Michailidis and Krokida 2014). Solar-dried foods are susceptible to invasive pests, insects, and microorganisms, some of which are toxin-producing (Michailidis and Krokida 2014). Furthermore, extensive chemical and enzymatic reactions, such as Maillard browning and ascorbic acid oxidation compromise product quality (Sagar and Suresh Kumar, 2010; Michailidis and Krokida 2014).

Air-drying is another old, commonly used and economical dehydration method used in food industry. It has been considered preferable to solar drying due to its shorter process times, increased control over drying conditions and reduced exposure to pests and microorganisms (Isik and others 2019). A notable

concern associated with air drying is case hardening, where product's surface becomes thick and rigid due to the transition from a rubbery to a glass state on account of faster drying rate of the surface as compared to the interior (Fernando and others 2008; Gulati and Datta 2015). Nevertheless, it has always been recognized to adapt the drying parameters to suit a particular food matrix.

1.5.1. Spray-drying

Industrially, spray-drying is the most preferred and economical dehydration method for producing powdered food products from liquid food matrices (Chen and Patel, 2008; Dobry and others 2009). Spray-drying is also extensively used in the pharmaceutical industry (Dobry and others 2009). The throughput of spray-dried food products is very high, up to several tons per hour; however, one limitation of this method is that the starting food matrices must be in the liquid form (Chen and Patel, 2008). A spray-dryer typically consists of an atomizer, a hot air supply, and a chamber where the hot air and liquid food interacts (Chen and Patel, 2008). Liquid food products are fed through a nozzle into a drying chamber where they come in contact with streams of heated air and get dried (Krishnaiah and others 2014). The drying rate largely depends on the ability of the atomizer to produce consistent droplet sizes, the interaction between the air and the liquid droplets and air temperature and flow (Chen and Patel, 2008). Plant protein isolates are often produced by spray-drying or other drying methods. Sumner and others (1981) found that spray-dried pea protein isolates had an improved foaming capacity and a lighter colour over plant protein isolates subjected to other processing methods. Spray-drying can also be used to encapsulate food matrices, often for masking off-flavours and protecting against lipid-oxidation (Yang and others 2012).

1.5.2. Freeze-drying

Freeze-drying, also known as lyophilisation physically removes water via sublimation and desorption and is considered the golden standard for dried product quality (Roy and Gupta 2004). Freeze-drying often takes a long time because the vapour pressure differential acts as the driving force, as opposed to the driving force being high temperatures (Shofian and others 2011). The efficiency of drying is highly dependent on thermal conductivity (Bhambere and others 2015). Freeze-drying yields very high-quality food products with characteristics resembling those of their fresh counterparts (Shofian and others 2011). Rehydration capacity of freeze-dried foods is often very high compared to those processed with conventional drying methods (Link and others 2017). Freeze-dried products have

higher porosity as the temperatures used are often below the glass-transition temperature, preventing the transition away from the glassy state (Marques and others 2006).

Due to the mild heating and reduced oxygen environment, compounds that are heat-sensitive or sensitive to oxidative deterioration are often dehydrated via freeze-drying to maintain high quality (Shofian and others 2011). However, due to the long drying time and high operational costs, freeze-drying may not be suitable for all food products from an economic perspective (Shofian and others 2011).

1.5.3. Vacuum Dehydration

Vacuum dehydration technology uses low pressures (a vacuum), created by a vacuum pump, to drastically shorten dehydration times by increasing the driving force (water vapour pressure gradient at the product surface) of the mass transfer phenomenon (Richter Reis 2014). Due to a reduction in boiling point of water under vacuum, evaporation of moisture is possible at low to moderate temperatures (Michailidis and Krokida 2014). Thus, this process is very useful for the preservation of heat-sensitive compounds, such as vitamins, pigments and antioxidants (Michailidis and Krokida 2014). In addition, processing in a vacuum environment vastly minimizes undesirable oxidation-dependent reactions, such as enzymatic browning and lipid oxidation as air is driven out of the system (Michailidis and Krokida 2014; Richter Reis, 2014).

1.5.4. Microwave Dehydration

Microwaves are a form of electromagnetic energy with a frequency ranging from 300 – 300,000 MHz, where 2450 MHz is the most common frequency that is used in food industry (Wray and Ramaswamy 2015). The mechanism by which microwaves heat food is volumetric heating, which contrasts greatly from conventional conductive heating. The thermal behaviour of microwaved food products is highly dependent on the dielectric properties of the food (Wray and Ramaswamy 2015). Many factors affect the dielectric properties, which in turn, dictate the efficiency of microwave heating (Heddleson and Doores, 1994). Dielectric constant (ε') is the capacity of a food to store electrical energy, while dielectric loss (ε'') is the capacity for this stored energy to be converted into thermal energy (Chandrasekaran and others 2013). Other influences include food composition, product characteristics, and process parameters such as temperature and microwave frequency (Heddleson and Doores, 1994; Chandrasekaran and others 2013). Microwaves quickly heat the interior of the food to the point

of evaporation, generating a lot of water vapour which readily migrates to the surface and is removed by the dehydrating medium (Wray and Ramaswamy 2015). As this water vapour is removed from the interior of the food, it helps produce a porous structure, preventing product shrinkage, thus improving the drying rate (Andrés and others 2004). Microwaves can also be employed when the drying rate starts to slow down. The high vapour pressure in the interior of the product generated due to the internal heat helps to drive the moisture to the surface (Andrés and others 2004). As a result, microwaves are also efficient in removing bound water without leading to substantial product shrinkage (Wray and Ramaswamy 2015).

Shimelis and Rakshit (2005) reported that the heat generated from microwaves led to an irreversible loss of quaternary structural integrity of globulins. Protein solubility in various beans substantially decreased even after only several minutes of microwave exposure (Shimelis and Rakshit 2005). Similarly, Caprita and Caprita (2010) showed that protein solubility of soybean protein decreased to below 50% after microwave treatment for 3 minutes. They attribute this marked decrease in solubility to the way microwaves heat. Microwaves are absorbed wherever water is present, allowing the localized heating throughout the whole food matrix, as opposed to conventional heating where convectional heat transfer occurs from the surface to the interior of the food matrix, (Caprita and Caprita 2010). This heating enhances protein-protein interactions via disulphide exchange, thus promoting protein aggregation which can result in loss in solubility (Caprita and Caprita 2010). This may have substantial implications for nutritive quality. Caprita and Caprita (2010) concluded that a protein solubility of 74% is the lower acceptable limit for lysine digestibility in humans, as well as animals.

1.5.5. Vacuum Microwave Dehydration (VMD)

Microwaves are typically combined with other dehydration processes, such as air-drying or vacuum-drying, as they can help facilitate more moisture loss (Andrés and others 2004). Vacuum microwave dehydration (VMD) employs microwaves in a vacuum environment (Figiel and Michalska 2017). VMD leads to very high-quality food products because it has all of the advantages of its standalone components (Figiel and Michalska, 2017). For instance, by introducing a vacuum, foods do not reach as high of a temperature as conventional drying nor are they exposed to a high-oxygen environment, nor or they exposed to an oxygen-rich environment (i.e. oxygen partial pressures are low due to vacuum, despite the same oxygen concentration), retaining the quality of both heat and oxygen-sensitive compounds (Michailidis and Krokida 2014; Richter Reis, 2014). Instead of using hot air to

dehydrate food, microwaves can rapidly and effectively facilitate the removal of moisture as it operates via volumetric heating (Andrés and others 2004; Orsat and others 2007; Wray and Ramaswamy 2015). Due to the shorter processing times of vacuum microwave drying, as well as the aforementioned advantages of both vacuum and microwaves, the resultant product quality is very high (Michailidis and Krokida 2014; Richter Reis 2014). Durance and Wang (2002) reported that the drying rate of a VMD process (16 kW, 50 Torr) was 18 times that of air-drying at 70°C at 12% relative humidity. Lin and others (1998) recruited an untrained panel to evaluate the colour, appearance, texture, aroma/flavour and overall hedonic rating of VMD-processed (3 kW for 19 minutes, 1 kW for 4 minutes and 0.5 kW for 10 minutes) and air-dried (70°C) carrot slices and found that VMD-processed carrot slices received significantly higher ratings in all sensory attributes. In addition, they found that the final vitamin C content of VMD-processed carrot slices was slightly more than double that of the air-dried carrot slices (Lin and others 1998).

1.6. Technologies for Volatile Removal

1.6.1. Direct Steam Injection

Direct steam injection injects high temperature steam (up to 150°C) inside the product for several seconds or minutes (Roberts and Dill 1962; Yuan and Chang, 2007). A schematic of a direct steam injection system is shown in Figure 1. Introducing steam can be used to remove volatiles from various food matrices (Yuan and Chang 2007). In the work of Takemitsu and others (2016), the headspace vapour of rice subjected to high-temperature steam contained fewer off-flavour causing volatile compounds than that of normally cooked rice, as determined by GC-MS. Although direct steam injection can decrease concentrations in off-flavour causing volatile compounds, such as hexanal and hexanol, undesirable flavours may also be imparted (Yuan and Chang, 2007). The compounds 2-pentyl furan and trans-4-decadienal, which have green/beany and fried/fatty aroma, respectively, increased after steam treatment (Yuan and Chang, 2007).

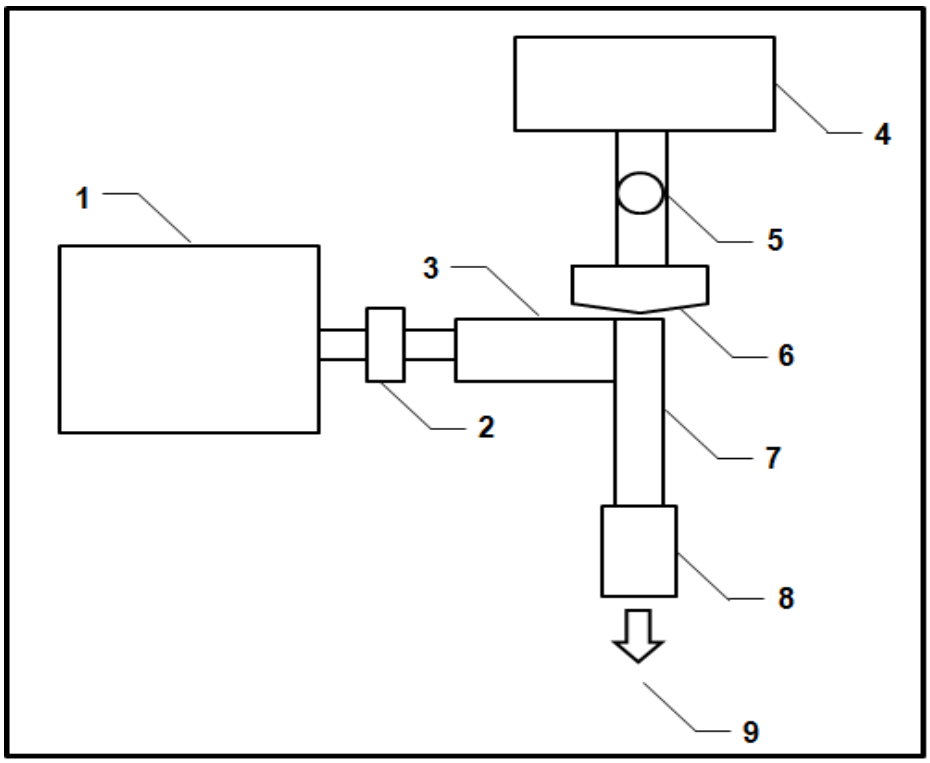

Figure 1. Schematic of a direct steam injection system: (1) feed tank; (2) feed pump; (3) preheating heat exchanger; (4) steam source; (5) steam control valve; (6) nozzle; (7) holding tank; (8) cooling heat exchanger; (9) product discharge

Furthermore, when the steam is introduced to the food product, it condenses due to some losses in sensible and latent heat of vaporization (Lewis and Heppell 2000). This moisture can accumulate and dilute the food product components (Lewis and Heppell, 2000). This increased moisture content needs to be dried after the process, which is not economically favourable. Energy losses also occur due to the need to flash-cool the final product (Robinson 1994). A more suitable process should be considered for removing off-flavour causing volatile compounds in pea proteins due to these limitations.

1.6.2. VMD as a Potential Volatile Removal Technology

VMD has the potential to remove volatile compounds because of the vacuum environment. The differential between the vapour pressure of the volatile compounds and the low pressure of the vacuum chamber acts as the driving force to remove the volatile compounds from the pea proteins (Speight 2017). In addition, since microwaves operate via volumetric heating, water vapour can be generated from within the food matrix (Wray and Ramaswamy 2015). This water vapour creates a large pressure,

which further helps to facilitate the loss of volatile compounds. Mui and others (2002) found that VMD-processed banana chips at 1.5 kW at 50 Torr had significantly less total volatile compounds, total esters, total butanoates and total acetates, than those that were air-dried (70°C for 3 hours), as determined by SPME-GC-MS. The biggest advantage of VMD is that this removal of volatile compounds takes place at temperatures lower than conventional dehydration, preventing the development of an unwanted cooked flavour (Michailidis and Krokida 2014). Since VMD may be able to lower off-flavour intensity without the deterioration of quality, this process should be thoroughly explored. To the best of our knowledge, the utilization of VMD to remove off-flavour causing volatile compounds in plant proteins has not been discussed in the literature; thus more research is needed to assess its feasibility and suitability for pea proteins.

1.7. Solid-Phase Microextraction

Various solvent extraction methods exist, such as liquid-liquid extraction; however, these conventional methods are reagent intensive, labour intensive and have a low selectivity (Rawa-Adkonis and others 2006a). Solid phase extraction (SPE) is an alternative isolation technique that involves passing liquid samples through a cartridge and is based on the principle that analytes with a high affinity for the solid phase will adhere, while those with a low affinity will pass through (Ismail and others 2010). Then, the solute is rinsed with solvents in an order where the solvents are progressively stronger (Ismail and others 2010). SPE is advantageous over conventional liquid-liquid extraction because it reduces the need for solvents, lessening the extent of environmental damage (Arthur and Pawliszyn 1990). This is due to the analyte of interest being collected via adsorption onto a solid phase, as opposed to large amounts of high-purity solvents. Another major advantage of SPE is that the compounds adsorbed onto the solid phase can be transported and analyzed at a later time and location (Rawa-Adkonis and others 2006a). However, even SPE has some disadvantages, such as having low recoveries due to considerable interactions between the sorbent and the food matrix (Ismail and others 2010). Arthur and Pawliszyn (1990) developed the new volatile extraction technique solid-phase microextraction (SPME), which has many advantages over SPE (Kataoka and others 2000). SPME uses a fused-silica fibre that adsorbs volatile compounds onto its coating material, which acts as the stationary phase (Kataoka and others 2000). SPME is advantageous over SPE because of the smaller amount of sample needed and the cost-effectiveness of fibres versus SPE cartridges (Prieto and others 2009). In SPME, the headspace, or the gas above the food matrix, is collected and trapped with a solid-phase coated fibre is

introduced into the headspace and collects the volatile compounds (Jung and Ebeler 2003; Qian and others 2010). SPME is an advantageous extraction technique as it does not require large amounts of toxic organic solvents (Piotrowicz 2016). Furthermore, SPME is automated, highly sensitive and results are often very repeatable (Silva and others 2014). However, one limitation is the high cost and that it may take a long time to standardize all of the important factors, such as equilibration and extraction time-temperature combination, sample volume and concentration and fibre type (Silva and others 2014). Regardless, SPME is still recognized as a powerful method that many researchers are using in lieu of conventional extraction methods (Alam and others 2015).

The fibre coating that is used greatly affects the sensitivity and reproducibility of the SPME performance (Jiang and others 2006). The polymer or combinations of polymers of the fibre must be thoroughly considered as the efficiency of adsorption is highly dependent on the polarity of the analytes of interest (Jiang and others 2006). As the popularity of SPME has been increasing substantially in recent years, there are now a myriad of different fibre coatings being used, all with their own advantages and limitations. For instance, organic coating materials such as polyamines have a high polarity, thermal stability and high wettability, while inorganic coating materials such as activated carbon is used due to its large adsorptive capacity (Jiang and others 2006). SPME fibre coating materials must be thermally stable and have a high adsorption capacity (Spietelun and others 2013). One frequently used SPME fibre coating material is polydimethylsiolxane (PDMS), which is effective in adsorbing non-polar compounds (Piotrowicz 2016). However, flavour compounds can range greatly in polarity, therefore the SPME fibre should consist of multiple materials so that molecules of all polarities can be adsorbed (Piotrowicz 2016). For instance, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) can adsorb compounds of varying polarities so that a representative sample from the headspace can be extracted (Piotrowicz 2016). DVB/CAR/PDMS fibre coating was used to extract polar, semi-polar and non-polar volatile compounds. Thicker fibres (100 μm) are more suitable for volatile compounds, while thinner fibres are more suitable for larger, less volatile compounds (Qian and others 2010).

The food matrix has a prominent role in the efficiency of SPME. For instance, several matrix effects may exist such as fouling effect, the effects of pH and salt, as well as other food constituents binding and competing for the SPME fibre (Jiang and others 2015b). Also, the aroma profile that is generated is highly dependent on sample composition, thus careful control of SPME-related experimental

parameters is vital so that the generated aroma profile is representative of the food matrix (Tholl and others 2006). Since SPME is highly sensitive, unwanted suspended matter can be inadvertently detected (Rawa-Adkonis and others 2006b). Also, unwanted analytes with a high gas-liquid partition coefficient may compete with sorption onto the fibre, which saturates the fibre and displaces analytes of interest that are in trace amounts (Rawa-Adkonis and others 2006b; Qian and others 2010).

Afterwards, the volatile compounds need to be desorbed and injected into either a gas or high performance liquid chromatographic inlet, followed by detection using a mass spectrophotometer (Schindler and others 2012). According to Alam and others (2015), equilibration time, or the time it takes for the volatile compounds of interest to equilibrate to the headspace, is decreased if the hydrophobicity of the analytes is inherently high, at a given concentration of unbound matrix component and radius of sample container. Meanwhile, extraction time is also influenced by a myriad of factors such as temperature, partition coefficient and agitation (Spietelun and others 2013). For instance, increasing temperature can facilitate volatile release into the headspace, decreasing the partition coefficient of the analyte (Spitelun and others 2013). Agitation, either with a stir bar, ultrasonication or other methods, decreases the amount of equilibration and extraction time (Vas and Vékey 2004).

1.8. Protein Functional Properties

Functional properties are the properties of the food or ingredient that affect its use during production, processing and storage (Pour-El 1981). Some important functional properties of plant protein isolates include solubility, emulsification and surface hydrophobicity.

1.8.1. Solubility

Protein solubility is the extent at which proteins dissolve in a solvent (Aryee and others 2018). The amount of soluble protein is important for determining functional properties as well as nutritional properties such as digestibility. When protein-protein interactions are in excess, the proteins cannot readily interact with the solvent and cannot be solubilized (Aryee and others 2018). The water solubility of a protein ranges from completely insoluble to several hundred mg/mL (Kramer and others 2012). Protein solubility is highly dependent on the proportion and composition of exposed side chains, for instance, whether they are hydrophilic or hydrophobic (Aryee and others 2018). To maintain thermodynamic stability, hydrophilic amino acid residues tend to orient themselves so they can interact with water, while hydrophobic amino acid residues tend to bury themselves in the interior of the protein

structure (Lam and others 2018). However, some hydrophobic amino acid residues may still be oriented towards water, leading to a lower solubility (Lam and others 2018). Other factors that influence protein solubility include pH, temperature and presence of salts (Kramer and others 2012). An important solubility-related value to consider is the isoelectric point (pI), which is the pH at which the net charge of the protein is zero (Kramer and others 2012; Lam and others 2018). At the pI, the electrostatic repulsive forces are minimal, enhancing protein-protein interaction and minimizing protein-solvent interaction, leading to aggregation, and eventually to precipitation (Aryee and others 2018; Lam and others 2018). Its pI is approximately 4.5, while its maximum solubility is around pH 8-9 (Barać and others 2015). To improve solubility, the pH of the solution should be as far as the pI of the protein as possible (Lam and others 2018). It is imperative to optimize protein solubility, as it is a necessary pre-requisite for many functional properties (Kudre and others 2018).

Protein solubility is also highly dependent on the ionic strength of solution (Kramer and others 2012). The presence of salts can either improve or worsen protein solubility (Tsumoto and others 2007). Low concentrations of salt can cause “salting-in” and increase the extent of electrostatic repulsive forces, preventing protein-protein interaction and improving protein solubility (Xu and others 2015). The type of salt also contributes to protein solubility. The Hofmeister series approximates the extent of how much a salt can salt-in or salt-out proteins and classifies salts as kosmotropes or chaotropes (Okur and others 2017). Kosmotropes can enhance the hydrophobic effect by binding water and render it unavailable to the protein, leading to the salting-out phenomenon, while chaotropes encourage the “salting-in” phenomenon (Okur and others 2017). Salting-out occurs when ions can compete with proteins for hydration (Kramer and others 2012). Since salts can bind tightly to water, there is less available water for proteins to interact with, which facilitates proteins to associate with themselves and aggregate (Kramer and others 2012). One caveat is that the Hofmeister series is a simplistic model that may not always accurately predict, but only approximates, the ability to salt-in or salt-out.

Organic solvents typically decrease protein solubility by disrupting hydrophobic interactions between nonpolar amino acid residues (Asakura and others 1978). This enhances protein-protein interactions to such an extent that precipitation may occur (Asakura and others 1978). Kramer and others (2012) and Yoshikawa and others (2012) state that the low dielectric constant of organic solvents is associated with reduced protein solubility. However, altering pH, ionic strength and solvent type may not be appropriate in the context of food formulation (Kramer and others 2012). Moreover, changing these

factors may only marginally improve solubility, hence further research is warranted. Pea protein has been reported to have a very low solubility due to its high number of exposed hydrophobic amino acid residues (Lam and others 2018).

1.8.2. Emulsification

Emulsification properties are critical for food quality and texture (Can Karaca and others 2011). An emulsion is a mixture of at least two immiscible phases, where one phase, termed the discontinuous phase, is dispersed into another phase, termed the continuous phase (Smith 2017). In food systems, the most common two emulsion types are oil-in-water or water-in-oil emulsions, such as milk and butter, respectively (Lam and others 2018). Two important emulsification properties are emulsifying activity index (EAI) and emulsifying stability index (ESI). EAI can be defined as the capacity for an emulsion to form and is expressed as the amount of oil that can be emulsified by gram protein (Aryee and others 2018). This is vastly different from ESI, which is essentially the ability for an emulsion to resist changes over time (Can Karaca and others 2011).

As proteins have both hydrophilic and lipophilic moieties, their amphipathic nature allows them to be efficient emulsifiers (Aryee and others 2018). Emulsions are thermodynamically unstable, so over time, they will destabilize, unless a stabilizer is added (Smith 2017). If emulsions are left to destabilize over time, they can undergo creaming or sedimentation, both of which are migration of oil droplets due to differences in density (Goodarzi and Zendehboudi 2019). These oil droplets can form a cluster, without the loss of integrity of each oil droplet, which is also known as flocculation (Goodarzi and Zendehboudi 2019). Eventually, coalescence may occur, which is the phenomenon where smaller oil droplets merge to become larger oil droplets due to the rupture of the interfacial film (Santos and others 2017). Coalescence is an irreversible process and is very detrimental to consumer acceptance (Goodarzi and Zendehboudi 2019). Other mechanisms of emulsion destabilization involve Ostwald ripening where dispersed phase is transferred from small to large-sized droplets, and phase inversion, where the continuous and dispersed phases switch (Santos and others 2017).

Proteins are amphipathic, meaning that they contain both hydrophilic and hydrophobic amino acids, allowing them to aid in the formation of emulsions by adsorbing at the oil-water-interface, lowering interfacial tension (Aryee and others 2018; Lam and others 2018). Proteins are effective emulsifiers when they partially unfold as they expose the buried hydrophobic groups that can act on the oil-water

interface (Nishinari and others 2014). According to Tang (2017), when proteins adsorb at the oil-water interface, a viscoelastic protein film can be formed, contributing to emulsification. Proteins can also stabilize emulsions by increasing the viscosity of the continuous phase, which discourages the dispersed droplets from coming together (Tesch and Schubert, 2002). Addition of salt can help influence emulsion stability as well (Xu and others 2015). When a low concentration is added, electrostatic repulsive forces are enhanced, more effectively dispersing the particles within the solution, improving the stability of an emulsion (Xu and others 2015). When a solution exceeds a certain ionic strength, emulsions tend to destabilize as the charges are insufficient to counterbalance the extent of attractive forces, such as van der Waals forces (Xu and others 2015). However, the presence of salt has a complex effect as salt may also impact protein conformation, thus influencing its solubility (Xu and others 2015). Liang and Tang (2013) found that pea legumin readily formed emulsions, but pea vicilin formed emulsions that were more stable. Pea protein prepared via various extraction methods were found to have a lower emulsifying capacity than whey and egg proteins (Stone and others 2015). The emulsifying capacities of pea and soybean protein isolates have been shown to be comparable (Stone and others 2015). However, differences in processing parameters, such as temperature can greatly influence emulsification properties (Tang 2017).

1.8.3. Sulphur Group Content

Disulphide bonds play a vital role in the way proteins fold and how stable they are, thus influencing a protein's functional properties (Liu and others 2016). More specifically, disulphide interactions are an indicator of the stability of the protein's tertiary structure (Wedemeyer and others 2000). Legumin, a pea globulin has a hexameric structure with each monomer consisting of an acidic and basic subunit linked via a disulphide bond (Lam and others 2018). However, vicilin, another globulin, is deficient in sulphur-containing amino acids and is stabilized mainly by non-covalent bonds (Ye and others 2016; Lam and others 2018). Although pea albumins have cysteine residues, they can also form disulphide bonds, stabilizing their higher order structures, but they only comprise approximately 10-20% of the total protein in peas (Burns and others 2016; Lam and others 2018).

1.8.4. Surface Hydrophobicity

Hydrophobicity is the tendency for a molecule to favour a non-aqueous environment and exclude water (Wilson and others 2000). A surface is considered hydrophobic when its contact angle for water is

greater than 90° and is not readily wettable (Law 2014). Surface hydrophobicity is a structure-related function that has been suggested to be inversely related to other functional properties of protein, such as solubility and emulsifying properties (Alizadeh-Pasdar and Li-Chan, 2000; Jiang and others 2015a; Ma and others 2018). Surface hydrophobicity is also an indicator of the degree of protein aggregation and thus, the extent of the loss of solubility (Wagner and others 2000). Pea protein has a high surface hydrophobicity (20-25 a.u.), so as expected, its solubility in water is limited due to few protein-solvent interactions (Lam and others 2018; Acquah and other 2020). Legumins have a higher surface hydrophobicity than vicilins, explaining why legumins formed emulsions more readily, as they can more easily interact at the oil-water interface (Barać and others 2010). Upon heating, surface hydrophobicity often increases as denaturation exposes more hydrophobic groups (Wang and others 2014).

1.9. Available Lysine Content and Nutritional Quality of Protein

Pea protein is an important source of the essential amino acid lysine (up to 6% of the pea protein, but its nutritional availability depends on a myriad of factors (Gorissen and others 2018; Lam and others 2018). According to the Ball and others (2013), available lysine is defined as “the standardized ileal digestible lysine”. Changes in available lysine are an indicator of the early and end stages of the Maillard reaction (Ferrer and others 2003a). Many biological, chemical, enzymatic and microbial methods have been developed to estimate available lysine, but chemical assays are frequently used due to their speed (El-Sherbiny and others 1980). Fluorometric detection methods are rapid, but may not be suitable for certain food matrices because foods with a high reducing sugar content may form interfering compounds and lead to an overestimation of chemically available lysine (Ferrer and others 2003b). Although available lysine determination assays may shed some light on the nutritional properties of a food product, it is in no way a substitute for measurements of protein quality.

Protein quality is an important, multi-faceted criterion that involves amino acid composition, bio-accessibility, and bio-availability (Jenzer and others 2016). Bio-accessibility is the amount of nutrients that can be extracted from a food matrix and become readily absorbed by the gastrointestinal tract, while bio-availability, which is also known as digestibility, is the amount of nutrients that can be absorbed, distributed and metabolized (Gropper and Smith 2013; Jenzer and others 2016). Some methods to assess protein quality include biological value, protein efficiency ratio and protein digestibility corrected amino acid score (Hoffman and Falvo 2004). The aforementioned protein quality

determination methods involve animal feeding studies, protein digestibility and/or amino acid content determination (Hoffman and Falvo 2004).

1.10. Factors Affecting Flavour-binding

Proteins have a prominent role in the flavour balance of a food formulation (Wang and Arntfield 2015). Proteins bind various flavour compounds through a plethora of chemical interactions such as irreversible covalent linkages as well as non-covalent binding, such as hydrophobic and electrostatic interactions, hydrogen bonding and van der Waals forces (Wang and Arntfield 2015). The extent of a protein's ability to bind flavours greatly depends on the composition and properties of the food matrix (Taylor 1999; Guichard 2002). The pH of the food is a notable factor as it can affect the net charge and secondary structure of proteins, affecting the degree of binding of volatile flavour compounds (Yang and others 2017). If the pH of the environment is at the isoelectric point of the protein, the net charge is zero, facilitating more protein-protein interaction (Lam and others 2018). The proteins may aggregate and expose buried hydrophobic amino acid residues, leading to more potential flavour binding sites (Wang and Arntfield 2017). For example, it was reported that various ketone compounds, such as 2-hexanone, 2-heptanone and 2-octanone, had increased protein-binding when pea proteins were subjected to near-isoelectric pH (Wang and Arntfield 2015).

Proteins have a limited number of binding sites, so when they interact at the oil-water interface of an emulsion, they bind to both hydrophilic and hydrophobic phases, leaving less binding sites for flavour compounds when proteins are participating in emulsification (Guichard 2002).

Another factor that greatly influences the extent of flavour-binding of proteins is ionic strength (Damodaran and Kinsella 1981). It is well established that low concentrations of salt can improve protein solubility as the ions can stabilize the charged protein moieties (Wang and Arntfield 2015). Ionic salts stabilize proteins by neutralizing charged amino acid side groups, surrounding proteins with a double layer of ions, which limit the extent of protein-protein interactions (Wang and Arntfield 2015). Thus, this double layer of ions may prevent proteins from binding with flavour compounds, causing a larger flavour release into the headspace (Wang and Arntfield 2015).

However, as salt concentration increases, ions may compete with proteins for water, decreasing protein solubility (Tsumoto and others 2007). Higher salt concentrations can stabilize proteins, hence facilitating more protein-protein interactions (Tsumoto and others 2007). This may increase the extent

of flavour-binding, leading to lower concentrations of the more hydrophobic flavor compounds in the headspace (Guichard 2002)

Complex carbohydrates may also contribute to flavour retention (Goubet and others 1998). Carbohydrates have varying affinities toward flavour compounds. For instance, low-esterified pectinates can interact with aliphatic ketones via van der Waals interactions between the hydrocarbon tail of the ketone and the hydrophobic areas of the pectinate (Guichard 2002). Starch has a helical structure and some hydrophobic regions, allowing inclusion complexes to formed, binding hydrophobic flavour compounds (McGorrin and Leland 1996). Suratman and others (2004) found that the addition of cyclodextrin reduced the concentrations of beany off-flavour causing volatiles in the headspace of soymilk. This may be attributed to cyclodextrin's hydrophobic interior which can entrap many flavour compounds (Suratman and others 2004). The carbohydrate's physical state also influences the extent of volatile loss, where the crystalline form leads to greater losses in volatiles as they are forced out of the crystalline matrix (Guichard 2002). Riéra and others (2006) state that increasing carbohydrate concentration can lead to increased retention due to increased viscosity of the solution.

Although proteins and complex carbohydrates can bind flavour constituents through various mechanisms, lipids are considerably more effective in retaining these compounds (Guichard 2002). As most flavour compounds are hydrophobic, they are more soluble in lipids (Guichard 2002). Furthermore, increasing fat content can lower the vapour pressure of highly fat-soluble volatiles, leading to a higher odour threshold (McGorrin and Leland 1996; Riéra and others 2006; Guichard 2002). When fat is removed, volatile compounds are released almost immediately, leading to an intense, but rapidly dissipating flavour perception (McGorrin and Leland 1996). However, since pea protein isolates contain only trace amounts of lipid and complex carbohydrates, the major contributor of flavour binding is due to proteins.

1.11. Sensory Evaluation

Sensory evaluation is a scientific discipline that aims to measure and analyze how people respond to food products as perceived through the five senses (Stone and Sidel 2004). Although the field of sensory evaluation was originally rooted in psychophysics in the 19th century, it has quickly evolved into the unique field it is today (Stone and Sidel 2004). Depending on the objective, different types of

sensory evaluations are carried out (Yang and Lee 2019). The three major categories of sensory evaluation are descriptive, discriminative and affective (Poste and others 1991).

Descriptive sensory evaluation is a powerful and sophisticated tool that utilizes a handful of extensively trained panelists to act as analytical instruments to comprehensively describe the attributes of a food product (Andrade de Aguiar and others 2019). However, descriptive sensory evaluation requires an extensive amount of time and resources to train the panelists to accurately and reliably assess attributes (Andrade de Aguiar and others 2019). Descriptive sensory evaluation is very versatile and can be used for quality control purposes, shelf-life determination, assessing effects of product reformulation (Murray and others 2001). Descriptive sensory evaluation results can also be correlated to instrumental analysis results to create models for how volatile data is actually perceived (Murray and others 2001). Descriptive sensory evaluation results can also be correlated to consumer acceptance results to identify which sensory attributes may improve or reduce acceptance (Murray and others 2001).

Discriminative sensory evaluation aims to detect whether any differences exist between food products, whether holistically or in a certain attribute (Lawless and Heymann 2010). The ability to discriminate between products depend on the magnitude of the differences between products, the power of the methodology employed as well as the number of panelists (Ennis and Jesionka 2011). Panelists need to be trained in the various discrimination methodologies, but do not need to have as great sensory acuity as those conducting descriptive sensory evaluation (Rogers 2017).

Lastly, affective sensory evaluation tries to capture consumer preferences and acceptances (Poste and others 1991). As product diversification and market competition has vastly grown in recent years, affective sensory evaluation remains an invaluable tool in determining consumer trends (Stone 2018). Affective sensory evaluation does not require training, but requires a large sample size to generate meaningful and representative results (Stone 2018). The most common methodology is the 9-point hedonic scale, which was developed in 1947 and introduced in 1952 and was rapidly adopted by the food industry, government and academia (Lim 2011).

1.12. Objectives, Hypothesis and Research Design

The overall objective of this Master's thesis was to develop a VMD process that simultaneously decreases the off-flavour intensity while improving the functionality of pea proteins to be used in non-dairy milk alternatives.

1.12.1. Hypothesis

The overall hypothesis of my Master's thesis is that vacuum microwave dehydration can reduce the off-flavour intensity in pea proteins with minimal thermal deterioration. Introducing a vacuum facilitates the vaporization of undesirable volatile compounds without needing to reach as high of a temperature as conventional dehydration. Without extensive thermal damage, protein functionality can be retained or even slightly enhanced.

1.12.2. Specific Objectives

This hypothesis was tested using the following three specific objectives.

Specific Objective 1: Effects of vacuum microwave dehydration on pea protein functionality

Various parameters such as initial moisture content, vacuum level, specific power, specific energy and process time were varied. VMD samples were analyzed for soluble protein content, emulsifying activity and stability indices, available lysine content, surface hydrophobicity, free sulfhydryl group content, moisture content, and colour.

Specific Objective 2: Effects of vacuum microwave dehydration on volatile content of pea proteins

Selected VMD samples were analyzed for key off-flavour causing compounds, volatiles within functional groups commonly attributed to pea off-flavours, and total volatiles.

Specific Objective 3: Descriptive sensory analysis, volatile analysis and functional properties of optimized VMD-processed pea protein

Based on the results from Specific Objectives 1 and 2, an optimized VMD-process was proposed that could minimize the off-flavour by facilitating the loss of undesirable volatile compounds without generating additional volatile compounds, while retaining protein functionality. Appearance, aroma,

flavour, texture and aftertaste, of solutions prepared with the optimized VMD-processed pea proteins were evaluated by a trained panel. Trends between volatile analysis and sensory data were discussed.

Chapter 2: Effects of Vacuum Microwave Dehydration on Protein Functionality

2.1. Background

Peas are a good source of protein as well as dietary fibre, yet do not contain many lipids (Lam and others 2018; Pietrysiak and others 2018). Peas are sustainable, inexpensive and hypoallergenic (Stone and others 2015; Lam and others 2018). Hence, pea protein has gained a lot of attention in recent years and has become a huge focus in research relating to the food sector (Wei and others 2020). However, pea proteins are difficult to incorporate into food products because they are water insoluble and have poor functional properties (Pietrysiak and others 2018). For instance, pea protein has lower emulsifying capacities than whey and egg proteins (Stone and others 2015). Pea protein has a high lysine content, but not all of it may be nutritionally available (Lam and others 2018).

Dehydration extends the shelf-life of foods by reducing the water activity, decreasing the amount of food losses throughout the farm-to-fork continuum (Figiel and Michalska 2017). However, dehydration methods can greatly impact a food product's essential amino acid content and protein functionality, therefore the dehydration method used must be carefully selected (Nöfer and others 2018). Chao and others (2018) state that dehydration methods such as spray drying and air drying generally lead to some heat-induced denaturation of proteins. Vacuum microwave dehydration (VMD) was developed a few decades ago and leads to higher quality products than those processed by conventional dehydration methods as the reduced pressure allows drying at low temperatures (Michailidis and Krokida 2014). Microwave heating during VMD is efficient as there is no come-up time unlike in conventional thermal processing, thus energy costs and thermal damage due to lengthy heating and cooling are mitigated (Drulyte and Orlien 2019). Initial moisture content (M_i) and microwave-related parameters such as microwave power and process time greatly influence how the properties of the food matrix change (Ashraf and others 2012).

Solubility is a key functional property that vastly influences other functional properties, such as emulsification, foaming, gelation, water and oil-holding capacities (Sashikala and others 2015; Aryee and others 2018; Hou and others 2018; Kudre and others 2018; Lan and others 2018). Solubility is also related to protein digestibility and hence protein quality (Sashikala and others 2015). Emulsification properties play a prominent role in food quality, texture and acceptance (Can Karaca and others 2011).

Hydrophobicity and disulphide bonds greatly influence a protein's folding pattern and stability of its tertiary structure (Liu and others 2016).

A review written by Dehnad and others (2016) described how various conventional and novel dehydration technologies impacted protein functionality, but no studies on vacuum microwave dehydration were discussed. However, there is a lot of existing literature on how vacuum or microwave drying affect protein functionality. Protein solubility of microwaved foods can be severely reduced (Caprita and Caprita 2010). Hafez and others (1985) observed that microwaving soy protein at 650W for 9 minutes led to four-fold decrease in solubility. Microwave drying can induce changes in functionality as well as the nutritional and compositional changes in many food matrices (Chandrasekaran and others 2013). Mune Mune and Singh Sogi (2015) observed changes in the water holding and oil absorption capacities of cowpea protein concentrates after vacuum drying at 50 °C and 25 Torr for 24 hours. A review written by Sá and others (2019) concluded that incorporation of microwaves can improve protein quality by inactivating anti-nutritional factors, but may also decrease protein quality by facilitating Maillard browning.

It is necessary to elucidate the impact each VMD processing parameter has on the overall functionality and quality of pea proteins. However, studies pertaining to VMD-processing of proteins and effects on functionality are limited. To the best of our knowledge, no study has investigated the functionality of VMD-processed pea proteins. Thus, this chapter aims to comprehensively understand how initial moisture content, vacuum level, microwave power and process time affect protein functionality.

2.2. Materials and Methods

2.2.1. Materials

Pea protein samples (72.7% crude protein, 7% ash, both reported on a dry basis) were acquired from Daiya Foods Inc. (BC, CA) and stored at room temperature until analysis. Crystallizing dishes (270 mL), bovine serum albumin, ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulphate were purchased from VWR International (PA, USA). Kjeldahl tablets, sulphuric acid (95-98%), sodium hydroxide (32%), sodium hydroxide solution (N/10 certified 0.0995-0.1005N), boiling chips, standardized 0.5N hydrochloric acid, Ellman's reagent and 1-anilino-8-naphthalenesulfonate were obtained from Thermo Fisher Scientific Inc. (MA, USA). A bicinchoninic (BCA) assay kit was purchased from G-Biosciences (MO, USA). Pure corn oil was purchased from MP Biomedicals (CA, USA). O-

Phthalaldehyde (OPA), β-mercaptoethanol (≥ 98%) and casein were purchased from Alfa Aesar (MA, USA).

2.2.2. Vacuum Microwave Dehydration (VMD)

Pea protein (2-10g), placed in 270 mL crystallizing dishes (VWR International, PA, USA) were vacuum microwave dried using a nutraREV vacuum microwave dryer available at the University of British Columbia (BC, CA). Parchment paper was used to prevent sticking of the powder to the glass, as needed. A schematic of this vacuum microwave dehydrator is seen in Figure 2. A turn-table (Enwave Corporation, BC, CA) was incorporated into the VMD chamber so that it was situated at the center of the chamber. The programmable logic controller allowed the microwave power (100-2000W) and process time to be adjusted. The waveguide was directly above the sample. Vacuum level (40-250 Torr) was adjusted manually with a bleed valve. The vacuum pump hose was connected on the opposite side of the chamber door. Crystallizing dishes were placed at the center of the turntable which was rotated at 7 rpm. Samples were cooled to room temperature and were blended with a Waring blender for 30 seconds.

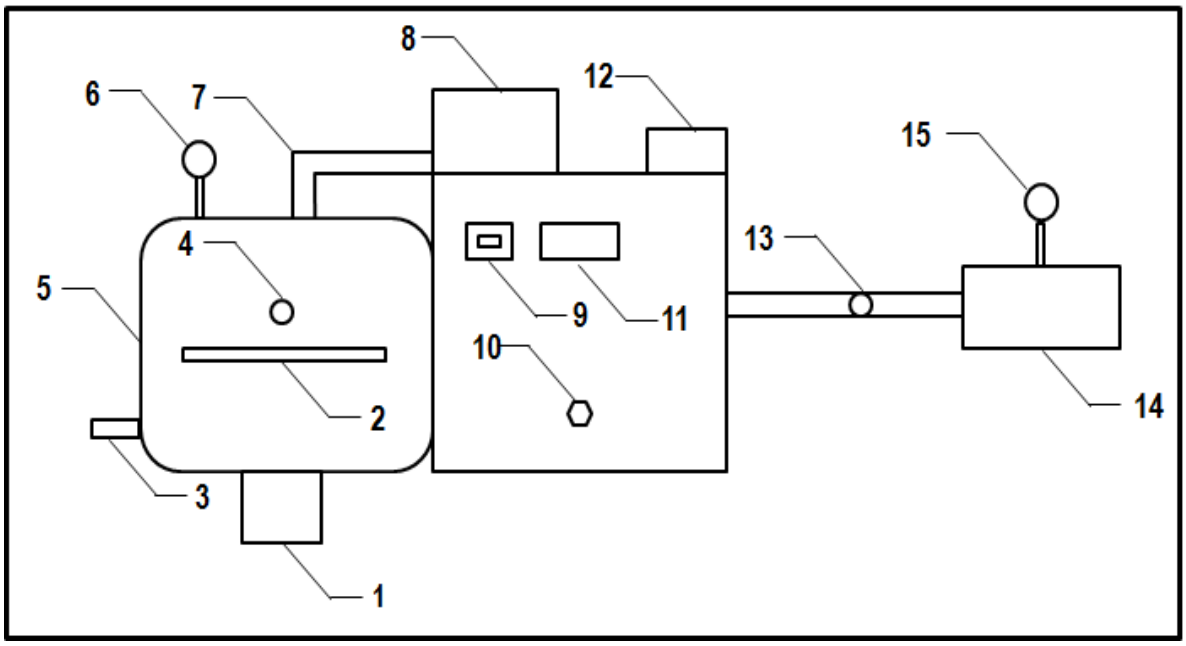

Figure 2. Schematic of the vacuum microwave dehydrator: (1) bottom motor; (2) turntable; (3) bleeder valve; (4) surface temperature probe; (5) chamber; (6) pressure gauge; (7) waveguide; (8) magnetron; (9) on/off switch; (10) emergency stop; (11) programmable logic controller; (12) reflected power reading; (13) valve; (14) vacuum pump; (15) pressure gauge

2.2.3. Research Design

The research in this chapter is organized into five experiments, each focusing on analyzing a particular combination of product and process parameters. A summary of how VMD process parameters were varied can be found in Table 1. Samples were coded in the following way: $M_aP_bV_cT_d$, where pea protein with initial moisture content a% d.b. was processed at a microwave power of b W/g and a vacuum level of c Torr for a process time of d minutes.

In experiments 1-4, each parameter (initial moisture content, microwave power, vacuum level and process time) were individually assessed at constant values of other parameters. In experiment 5, VMD processing was done at combinations of microwave power and process time so that the specific energy was constant at 500W*min/g. Based on previous findings, initial moisture content was fixed at 31% d.b., vacuum level was fixed at 200 Torr, specific power was fixed at 100 W/g and process time was fixed at 5 minutes.

Samples at extreme conditions were found to be burnt in each of the experiments 1-5. Samples processed at very low initial moisture content (5% d.b.), high microwave powers (150 and 200 W/g), high vacuum levels (40 Torr) and extreme combinations of microwave power and process time (100 W/g and 10 minutes) burned and were not reported further. Processed samples were stored at room temperature for further analysis. Untreated pea protein samples were used as control for each of these experiments.

Table 1. Summary of VMD processing parameters (specific power, vacuum level, process time and initial moisture content) varied during this study

Sample ID	Initial Moisture (% Dry Basis)	Vacuum Level (Torr)	Specific Power (W/g)	Process Time (mins)
Experiment 1: Effect of Varying Initial Moisture Content				
$M_5P_{100}V_{200}T_5$–X [2]	5	200	100	5
$M_{31}P_{100}V_{200}T_5$	31	200	100	5
$M_{75}P_{100}V_{200}T_5$	75	200	100	5
$M_{162}P_{100}V_{200}T_5$	162	200	100	5
$M_{425}P_{100}V_{200}T_5$	425	200	100	5

Experiment 2: Effect of Varying Microwave Power				
$M_{31}P_{10}V_{200}T_5$	31	200	10	5
$M_{31}P_{50}V_{200}T_5$	31	200	50	5
$M_{31}P_{100}V_{200}T_5$	31	200	100	5
$M_{31}P_{150}V_{200}T_5$–X [2]	31	200	150	5
$M_{31}P_{200}V_{200}T_5$–X [2]	31	200	200	5
Experiment 3: Effect of Varying Vacuum Level				
$M_{31}P_{100}V_{40}$ T_5–X [2]	31	40	100	5
$M_{31}P_{100}V_{120}$ T_5	31	120	100	5
$M_{31}P_{100}V_{200}$ T_5	31	200	100	5
Experiment 4: Effect of Varying Process Time				
$M_{31}P_{100}V_{200}T_1$	31	200	100	1
$M_{31}P_{100}V_{200}T_2$	31	200	100	2
$M_{31}P_{100}V_{200}T_3$	31	200	100	3
$M_{31}P_{100}V_{200}T_5$	31	200	100	5
$M_{31}P_{100}V_{200}T_{10}$–X [2]	31	200	100	10
Experiment 5: Effect of Varying Microwave Power and Process Time at Constant Specific Energy				
$M_{31}P_{10}$ $V_{200}T_{50}$	31	200	10	50
$M_{31}P_{50}$ $V_{200}T_{10}$	31	200	50	10
$M_{31}P_{100}V_{200}T_5$	31	200	100	5
$M_{31}P_{150}V_{200}T_{3.3}$	31	200	150	3.33
$M_{31}P_{200}V_{200}T_{2.5}$–X [2]	31	200	200	2.5

[1] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[2] "X" denotes that the sample burned and was not analyzed subsequently

2.2.4. Homogenization

Pea protein solutions (1% w/v) were prepared and homogenized at 19000 rpm using a bench top homogenizer (Ultra Turrax® T25, IKA Labortechnik, Staufen, Germany) for 1, 5 and 10 minutes, respectively. These three samples were analyzed only for soluble protein content.

2.2.5. Drying Kinetics

Deionized water was added to pea protein to an initial moisture content of 425% (dry basis). Pea protein slurry (50g) was vacuum microwave dehydrated at microwave powers of 100, 250, 550 and 1000W at vacuum levels of 40 and 200 Torr. The weight of the pea protein slurries was measured at various time intervals during VMD-processing. The moisture ratio (MR) was calculated using Equation 3. All moisture contents were reported on a dry basis.

$$MR = \frac{M - M_e}{M_c - M_e} \quad (1)$$

MR is the moisture ratio; M is the moisture content at a given time, M_e is the equilibrium moisture content and M_c is the critical moisture content.

Drying kinetic parameters A and B were also determined using the following equation and shown in Appendix A (Table 16). Drying kinetics results are shown in Appendix A (Figures 9 and 10).

$$\frac{M - M_e}{M_c - M_e} = Ae^{-Bt} \quad (2)$$

2.2.6. Moisture Content

Moisture content was determined according to Ahn and others (2014), with minor modifications. In brief, pea protein (1g) was added into an aluminum dish and heated in an oven set at 105°C for 6 hours. Moisture content was calculated using the Equation 2.

$$\%\ \text{Moisture content (d. b.)} = \frac{\text{initial weight} - \text{dried weight}}{\text{initial weight}} * 100\% \quad (3)$$

2.2.7. Ash Content

Pea protein (1g) was weighed into a tared crucible. The crucibles were placed into a muffle furnace (Thermolyne™ F62700, Thermo Fisher Scientific Inc., MA, USA), and ignited overnight at 500°C. Once cooled, crucibles were transferred into a desiccator filled with desiccant. Ash-filled crucibles were weighed after ashing. Ash content was determined using Equation 1.

$$\%\ Ash\ (d.b.) = \frac{(w_f - w_{cru})}{(w_i * DMC)} * 100\% \quad (4)$$

where, w_f is the combined weight of the crucible and the sample after ashing, w_{cru} is the weight of the crucible, while w_i is the initial weight of the sample, DMC is the dry matter coefficient (% solids/100).

2.2.8. Crude and Soluble Protein Content

To determine crude protein content, the Kjeldahl method was conducted according to AOAC method 991.20 with minor modifications. A nitrogen-protein conversion factor of 6.25 was used. Digestion at 400°C for 2 hours and distillation (200 mL 32% w/v sodium hydroxide for 30 seconds) was done with a digestion system (Model K-437, Büchi Corporation, DE, USA) and a distillation system (Model K-350, Büchi Corporation, DE, USA).

Soluble protein content was determined according to Jiang and others 2017), with minor modifications. In brief, a 1% (w/v) pea protein solution was prepared with deionized water and centrifuged at 6900 g for 15 minutes. The supernatant was collected, diluted to a working concentration and analyzed for soluble protein content using the BCA assay (G-Biosciences, MO, USA). Bovine serum albumin (BSA) (VWR International, PA, USA) (0-2 mg/mL) was used to construct a standard curve to calculate the soluble protein content, which was reported as mg/g dry matter. Absorbance at 562 nm was measured using a microplate reader (Infinite M200 Pro, Tecan™, Männedorf, Switzerland).

2.2.9. Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI)

EAI and ESI were determined according to Pietrysiak and others (2018), with minor modifications. Protein dispersions (15 mL, 0.5% w/v) were prepared in potassium phosphate buffer (50 mM, pH 7) (VWR International, PA, USA) and added to 5 mL pure corn oil. This mixture was homogenized at 19,000 rpm for 1 minute using a benchtop homogenizer (Ultra Turrax® T25, IKA Labortechnik, Staufen, Germany). Aliquots (50 µL) of emulsion samples from the bottom of the tube were taken after 0 minutes and 10 minutes, and diluted with 10 mL 0.1% (w/v) sodium dodecyl sulphate solution. Absorbances were measured at room temperature at 500 nm in a 1 cm plastic cuvette with a UV spectrophotometer (UV-1800 UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). EAI and ESI were determined with Equations 3 and 4.

$$EAI\ \left(\frac{m^2}{g}\right) = \frac{2*2.303*A_0*DF}{c*\varphi*10{,}000} \quad (5)$$

$$ESI\ (minutes) = \frac{A_0*t}{A_0 - A_{10}} \quad (6)$$

A_0 is the absorbance immediately after homogenization, A_{10} is the absorbance 10 minutes after homogenization, DF is the dilution factor, c is the protein concentration (g/mL) in the aqueous phase prior to emulsification, while φ is the oil volume fraction. EAI was reported as m^2/g dry matter.

2.2.10. Free Sulfhydryl Group Content

Free sulfhydryl content was determined according to the Ellman's reagent user guide (Thermo Fisher, MA, USA) with minor modifications. Pea protein solutions (1% w/v) was prepared in reaction buffer (potassium phosphate buffer (0.1M, pH 8, 1 mM EDTA)) and was centrifuged at 8760 g for 15 minutes. Ellman's reagent was also prepared in reaction buffer. Ellman's reagent solution (50 µL of 4 mg/mL in reaction buffer) was added to 2.75 mL pea protein supernatant. The mixture was incubated for 15 minutes at room temperature in the dark. Absorbance was measured at 412 nm in a 1 cm plastic cuvette with a UV spectrophotometer (UV-1800 UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). Free sulfhydryl content was calculated using the molar extinction coefficient of 14,150/M·cm.

2.2.11. Surface Hydrophobicity

Surface hydrophobicity was determined according to Jiang and others (2017) with minor modifications. 8mM 1-Anilino-8-naphthalenesulfonate (ANS) stock solution was used as a fluorescent probe. ANS (50 mg) was dissolved in 1 mL 1M sodium hydroxide and diluted to a concentration of 8mM with potassium phosphate buffer (0.01M, pH 7). Protein solutions (0-0.2 mg soluble protein/mL) were prepared using potassium phosphate buffer (0.01M, pH 7). ANS stock solution (5 µL) was added to 1 mL protein solution. An aliquot (200 µL) of this mixture was pipetted into a black, 96-well plate. Fluorescence intensity at 340 nm excitation and 440 nm emission was measured with a microplate reader (Infinite M200 Pro, Tecan™, Männedorf, Switzerland). The slope of fluorescence intensity versus protein concentration was used to estimate surface hydrophobicity and expressed as arbitrary units (a.u.).

2.2.12. Chemically Available Lysine Content

Chemically available lysine content was determined according to Ferrer and others (2003b), with minor modifications. Pea protein solutions (1% w/v) were prepared in deionized water and centrifuged at 6900 g for 15 minutes. An aliquot (200 µL) of pea protein supernatant was added to 500 µL 12% (w/v) SDS solution and 300 µL deionized water. Ortho-Phthalaldehyde (OPA) reagent was prepared by adding 40 mg OPA to 1 mL ethanol, 25 mL sodium tetraborate buffer (0.1M, pH 9.7), 2.5 mL 20% (w/v)

SDS and 100 µL ≥ 98% β-mercaptoethanol. An aliquot (5 µL) pea protein solution was added to 200 µL OPA reagent and incubated in a 25°C water bath rotating at 80 rpm for 2 minutes. Fluorescence at 340 nm excitation and 455 nm emission was measured using a microplate reader (Infinite M200 Pro, Tecan™, Männedorf, Switzerland). Chemically available lysine was reported as mg/g dry matter based on a standard curve with 0-2 mg/mL casein. It was assumed that lysine in the insoluble fraction was chemically unavailable.

2.2.13. Colour Analysis

Pea protein was transferred into miniature petri dishes and loosely packed to ensure consistent readings. Colour parameters (L*, a*, b*) were measured using a colourimeter (LabScan XE System, Hunter Associates Laboratory, Inc., VA, USA). Miniature Petri dishes were rotated 90° after each measurement. Three measurements were taken for each of the three biological replicates. Total colour difference (ΔE) was determined using Equation 5.

$$\Delta E = \sqrt{[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]} \quad (7)$$

2.2.14. Statistical Analysis

All experiments were done in triplicate. Levene's test was used to determine whether the assumption of homoscedasticity was met. Data was analyzed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test as a post-hoc test because the assumption of homoscedasticity was met. Statistical significance was defined as $p<0.05$. Pearson's correlation coefficients were determined between available lysine and Hunterlab colour parameters. Regression analysis was conducted to determine whether these correlations were statistically significant. All statistical analyses were conducted using Minitab 19 (Minitab Inc., PA, USA) and Microsoft Excel 2010 with Real Statistics Resource Pack (Redmond, WA, U.S.A.).

2.3. Results and Discussion

2.3.1. Effect of VMD-processing on Colour Parameters and Final Moisture Content After VMD-processing

The final moisture contents and colour characteristics of VMD-processed samples are presented in Table 2. Untreated pea protein had a moisture content of 4.9% d.b. Processing at high power (100W/g) for one minute or low power (10W/g) for five minutes were insufficient in removing enough moisture as

these processes led to significantly higher final moisture contents of 7.7 and 20.7% d.b., respectively. All other VMD processes led to significantly lower final moisture contents, ranging from 0.5-3.0% d.b.

Table 2. Hunterlab colour parameters (lightness (L*), a* coordinate, b* coordinate, total colour difference (ΔE) and final moisture content of pea proteins after VMD-processing

Sample ID	L*	a*	b*	ΔE	Final Moisture (% Dry Basis)
Untreated	81.09 ± 0.46 [a]	2.55 ± 0.59 [c]	23.91 ± 0.93 [c]	[4]	4.91 ± 0.10 [c]
$M_{31}P_{100}V_{200}T_5$	79.65 ± 0.98 [a]	2.59 ± 0.33 [c]	24.31 ± 0.24 [c]	1.63 ± 0.59 [c]	0.49 ± 0.04 [f]
$M_{75}P_{100}V_{200}T_5$	77.92 ± 1.37 [a]	3.14 ± 0.47 [c]	25.43 ± 0.97 [c]	3.57 ± 1.55 [c]	1.02 ± 0.12 [ef]
$M_{162}P_{100}V_{200}T_5$	69.47 ± 2.04 [b]	5.28 ± 0.49 [b]	28.39 ± 0.80 [b]	12.76 ± 1.58 [b]	0.90 ± 0.16 [ef]
$M_{425}P_{100}V_{200}T_5$	57.32 ± 2.59 [c]	8.65 ± 1.06 [a]	34.88 ± 1.64 [a]	26.89 ± 2.45 [a]	1.77 ± 0.08 [ef]
$M_{31}P_{10}V_{200}T_5$	79.94 ± 1.04 [a]	2.36 ± 0.56 [c]	24.23 ± 1.06 [c]	1.28 ± 0.76 [c]	20.73 ± 0.21 [a]
$M_{31}P_{50}V_{200}T_5$	80.06 ± 0.72 [a]	2.49 ± 0.52 [c]	23.98 ± 0.77 [c]	0.68 ± 0.45 [c]	0.97 ± 0.11 [ef]
$M_{31}P_{100}V_{200}T_5$	80.15 ± 0.46 [a]	2.49 ± 0.51 [c]	24.18 ± 0.93 [c]	1.05 ± 0.18 [c]	0.49 ± 0.04 [f]
$M_{31}P_{100}V_{120}T_5$	79.84 ± 0.90 [a]	2.75 ± 0.40 [c]	24.45 ± 0.59 [c]	1.42 ± 0.88 [c]	0.33 ± 0.10 [f]
$M_{31}P_{100}V_{200}T_5$	80.08 ± 0.55 [a]	2.68 ± 0.37 [c]	24.37 ± 0.30 [c]	1.26 ± 0.36 [c]	0.49 ± 0.04 [f]
$M_{31}P_{100}V_{200}T_1$	80.25 ± 0.86 [a]	2.42 ± 0.56 [c]	23.97 ± 0.74 [c]	1.07 ± 0.47 [c]	7.73 ± 1.35 [b]
$M_{31}P_{100}V_{200}T_2$	80.46 ± 0.71 [a]	2.60 ± 0.40 [c]	23.98 ± 0.39 [c]	0.85 ± 0.37 [c]	3.01 ± 0.23 [d]
$M_{31}P_{100}V_{200}T_3$	80.06 ± 0.82 [a]	2.56 ± 0.58 [c]	24.05 ± 0.70 [c]	1.13 ± 0.64 [c]	0.90 ±0.06 [ef]
$M_{31}P_{100}V_{200}T_5$	80.14 ± 1.05 [a]	2.59 ± 0.51 [c]	23.97 ± 0.82 [c]	1.02 ± 0.94 [c]	0.49 ± 0.04 [f]
$M_{31}P_{10}V_{200}T_{50}$	80.26 ± 0.67 [a]	2.60 ± 0.43 [c]	23.98 ± 0.73 [c]	0.89 ± 0.76 [c]	3.45 ± 0.19 [d]
$M_{31}P_{50}V_{200}T_{10}$	80.06 ± 1.18 [a]	2.66 ± 0.51 [c]	24.20 ± 0.80 [c]	1.15 ± 1.20 [c]	0.33 ± 0.18 [f]
$M_{31}P_{100}V_{200}T_5$	80.17 ± 0.41 [a]	2.64 ± 0.52 [c]	24.36 ± 0.82 [c]	1.19 ± 0.20 [c]	0.49 ± 0.04 [f]
$M_{31}P_{150}V_{200}T_{3.3}$	79.97 ± 1.18 [a]	2.75 ± 0.39 [c]	24.56 ± 0.64 [c]	1.39 ± 1.10 [c]	1.09 ± 0.47 [ef]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments, as determined by one-way analysis of variance + Tukey's honestly significant different

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[4] ΔE is the total colour difference relative to the untreated pea protein

Untreated pea protein had a lightness (L*) value of 81, a* coordinate of 3 and b* coordinate of 24. As the initial moisture content increased, lightness significantly decreased ($p<0.05$), while the a* and b* coordinates and overall colour difference significantly increased ($p<0.05$). The L* values for samples with initial moisture contents of 162 and 425% d.b. were 69 and 57, respectively. The increased a* and b* coordinates indicated a redder and yellower product, suggesting extensive Maillard browning had occurred (Wrolstad and Smith 2010). Bornhorst and others (2017) found that a* coordinates were strongly correlated to heating time with an average correlation coefficient of 0.85, thus they focused on a* values instead of other colour parameters. They reported an a* coordinate of unprocessed pea puree was -20 units; however, after a severe thermal process (100°C for 90 minutes), the a* coordinate was approximately 0 (Bornhorst and others 2017). Our untreated pea protein had an a* coordinate of 2.5, which may be explained because our pea protein was already dehydrated and stored, hence an a* coordinate of 2.5 was reasonable. Furthermore, the a* of our samples were darker as it contained primarily proteins, as opposed to puréed peas which have other constituents, therefore Maillard browning occurred more readily in the former. L*, a* and b* of low initial moisture content (≤75% d.b.) samples did not differ significantly ($p\geq0.05$) from the control.

Total colour change (ΔE) of more than 5.0 is considered distinguishable and is considered an indicator of poor product quality (Wrolstad and Smith 2010). Apart from samples that were burnt and not reported, initial moisture content was the only parameter affecting total colour difference of the samples, with high moisture content samples (≥162% d.b.) showing showed a significant ($p<0.05$) colour change. Images of pea protein processed at different initial moisture contents depict the overall impact of VMD on the visual quality of pea protein (Figure 3). High initial moisture content samples and high microwave power samples absorbed more microwaves, and were browner. A low initial moisture content did not provide enough free water for the Maillard browning to occur to a great extent. This explained why those samples looked similar to the untreated protein; however, when the initial moisture content was beyond 162% (d.b.), browning was evident.

Maillard browning is not often reported in conventional microwave systems. The Maillard reaction (MR) occurs at a maximum extent around intermediate water activities, so when using conventional microwaves, where there is excess water, it is expected that MR would decrease (Lund and Ray 2017). However, Maillard browning occurs much more readily in vacuum microwave drying due to the introduction of vacuum, which helps with the mass transfer, thus facilitating the removal of water to

reach intermediate water activities. Many researchers describe the occurrence of the Maillard reaction during microwave treatment (Ibrahim and others 2012; Tu and others 2015; Figiel and Michalska 2017).

Figure 3. Appearance of pea proteins with varying initial moisture contents (5-425% d.b.) after VMD-processing at 100W/g and 200 Torr for 5 minutes

2.3.2. Effect of VMD-processing on Crude Protein Content

Processing pea protein at 2W/g for 68 minutes and 20W/g for 3.5 minutes did not differ from that of the untreated pea protein ($p \geq 0.05$). Naeiny and others (2018) microwaved pea proteins at 1000W for 0-8 minutes and found no significant differences in crude protein content at any of the time intervals. Meanwhile, Purohit and others (2013) microwaved dehulled mungbeans (400-800W for 14-56 seconds) and found no changes in crude protein content. Although no changes in crude protein content were observed, changes in protein solubility, protein functional properties, amino acid composition or digestibility may be altered, warranting further investigation.

2.3.3. Effect of VMD-processing on Soluble Protein Content

According to Chao and others (2018), the solubility of pea protein at near-neutral pH ranges from 25-35%. As the crude protein content of untreated pea protein is roughly 72% (d.b.), a protein solubility of 25-35% equates to around 170-230 mg/g. Our results fell within their range as untreated pea protein had a soluble protein content of 193 mg/g, as shown in Figure 4.

Varying the initial moisture content (M_i) prior to processing for 100W/g for five minutes significantly decreased soluble protein content. Caprita and Caprita (2010) reported that microwaving soy proteins at 800W for 5 minutes led to an 81% percent reduction in solubility. Žilić and others (2006) determined that after 4 minutes of 800W microwave toasting, soybean kernel protein solubility was approximately 20% of that of unprocessed soybean kernel. Pysz and others (2012) observed that increasing microwave energy (500-2000J/g) led to decreases in broad bean seed protein solubility.

As Maillard browning is thought to be a major contributor to the loss of protein solubility, a low initial moisture content (≤75%) (d.b.) did not provide enough free water to facilitate the Maillard reaction to a great extent. Fan and others (2018) reported that Maillard browning significantly decreased milk protein concentrate solubility. Heat generated from microwaves can reorganize the protein structure, leading to altered hydrogen bonding, hydrophobic interactions and disulphide interchange (Teodourowicz and others 2017; Fan and others 2018). Furthermore, the pH of the pea protein slurries was slightly alkaline, which facilitated the initial nucleophilic attack of the amino group on the reducing sugar's carbonyl group. Faciliation of the Maillard reaction explained why the soluble protein contents of samples with initial moisture contents of 31 or 75% (d.b.) were still high (156 and 123 mg/g dry matter) but those with an initial moisture content of 162 and 425% (d.b.) had significantly ($p<0.05$) lower soluble protein contents (55 and 38 mg/g dry matter) than all other samples. The difference between high initial moisture content samples were negligible ($p\geq0.05$) as the extent of Maillard browning did not change past 162% d.b.

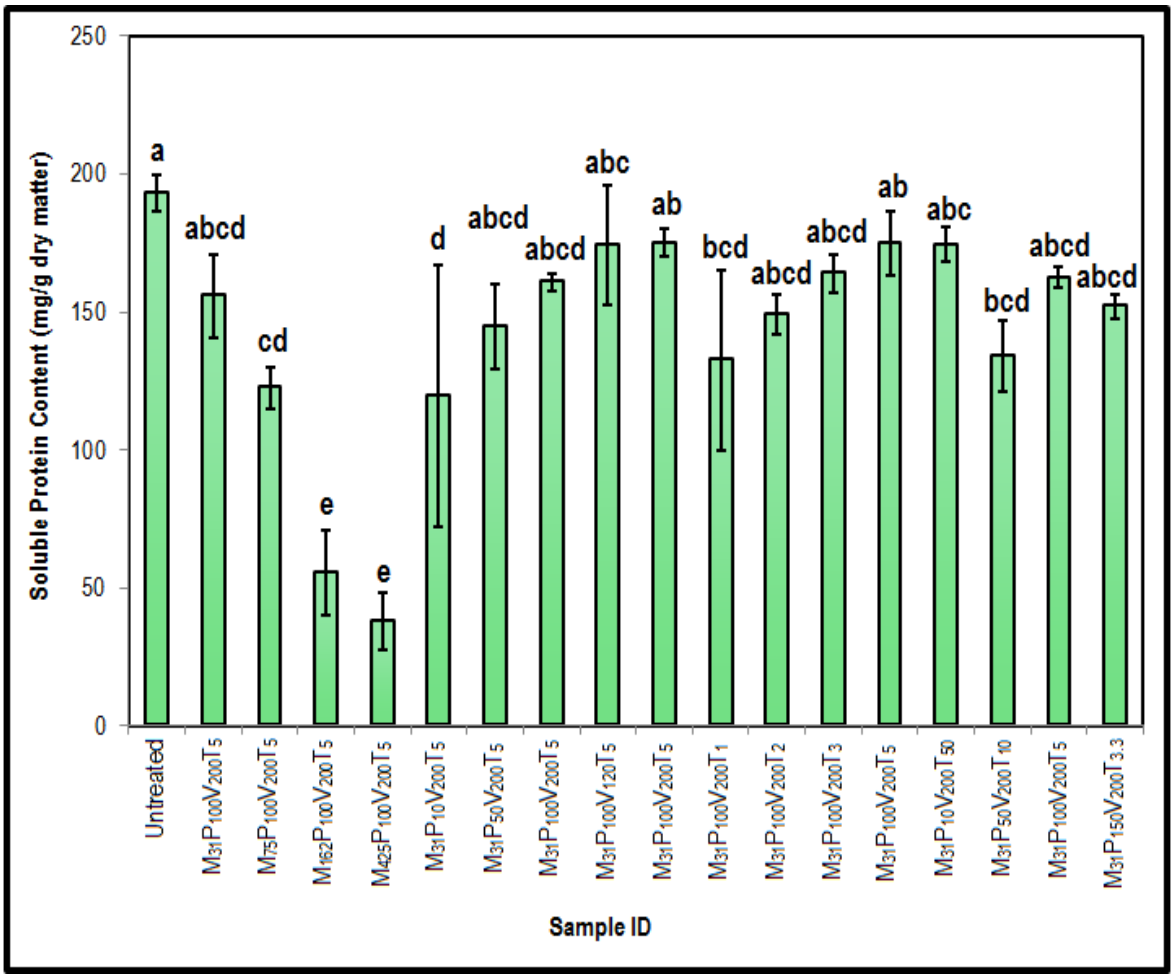

Figure 4. Soluble protein content of pea proteins in deionized water after VMD-processing at various initial moisture contents (5-425% d.b.), vacuum levels (120-200 Torr), specific power levels (10-100W/g) and process times (1-50 minutes)

[1] results are reported as mean ± standard deviation (n = 3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments, as determined by one-way analysis of variance + Tukey's honestly significant different test

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

Specific power, vacuum level and process time did not have an effect on solubility at lower initial moisture contents because the lack of initial moisture did not allow for substantial microwave energy absorption, therefore conformational changes in proteins that reduce solubility may not have occurred to such a great extent. Zayas (1997) stated that the effects of dry heat and moist heat are different.

Samadi and Yu (2011) reported that moist heat can vastly alter the soluble protein content, chemistry and nutritional properties of soybean seeds, more so than dry heat.

The low-energy processes led to slightly but significantly ($p<0.05$) lower soluble protein contents compared to the control, which is contrary to the literature. One explanation for this is because after low energy treatments, the products were clump-like, even after blending. This may take a long time to solubilize which was why our method underestimated the soluble protein content of those samples.

2.3.4. Homogenization After VMD-processing as a Way to Improve Protein Solubility

As reported in the previous section, microwaving can decrease protein solubility. One way to improve protein solubility was with homogenization. Homogenization significantly ($p<0.05$) increased the soluble protein content of pea protein. Even after one minute of homogenization, the soluble protein content increased ($p<0.05$) from 207 to 239 mg/g dry matter, as shown in Table 3. Homogenizing samples for longer periods of time further improved ($p<0.05$) protein solubility. After ten minutes of homogenization, the soluble protein content was 298 mg/g dry matter. Even though homogenization can improve ($p<0.05$) protein solubility, the soluble protein content of extensively VMD-treated samples may still be low, even after homogenization. Other ways to improve protein solubility include altering pH, ionic strength or adding various solvents; however, not all of these methods may be practical for food formulations (Kramer and others 2012). Protein solubility in other buffers used for functionality determination and can be found in Appendix A (Table 17).

Table 3. Soluble protein content of pea protein homogenized at 19000 RPM for 1, 5 and 10 minutes

Treatment	Soluble Protein Content (mg/g dry matter)
Untreated	207.04 ± 5.93 [d]
1 Minute	238.68 ± 1.15 [c]
5 Minutes	276.89 ± 1.74 [b]
10 Minutes	298.34 ± 5.21 [a]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments, as determined by one-way analysis of variance + Tukey's honestly significant different test

2.3.5. Effect of VMD-processing on Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI)

The EAIs and ESIs can be found in Table 4. The EAI of untreated pea protein was 18 m^2/g dry matter. This aligned with the results of Zare and others (2015), as they reported that pea protein had a EAI of 13 m^2/g. Similar to the soluble protein content, initial moisture content was the most influential factor in affecting EAI. Increasing initial moisture content significantly decreased ($p<0.05$) EAI, where samples with an initial moisture content greater than 162% d.b. had an EAI that was approximately half of that of untreated pea protein. Kim and others (2019) report that protein solubility is a major factor affecting EAI. With lower quantities of soluble protein, a protein's effectiveness to form an emulsion is decreased. The diffusion rate to the oil-water interface is reduced when proteins are more insoluble, which aligned with our results (Can Karaca and others 2011). Specific power, vacuum level and process time did not significantly ($p\geq0.05$) affect EAI.

Table 4. Emulsification properties (EAI and ESI), surface hydrophobicity and free sulfhydryl group content of pea proteins after VMD-processing

Sample ID	EAI (m^2/g dry matter)[3]	ESI (mins)[3]	S_0 (a.u.)[3]	Free SH Content (µmol/g soluble protein)
Untreated	18.41 ± 1.43 [a]	25.3 ± 3.5 [b]	21.0 ± 3.3 [ab]	1.38 ± 0.29 [a]
$M_{31}P_{100}V_{200}T_5$	14.80 ± 2.59 [abcd]	34.4 ± 16.7 [b]	21.8 ± 2.9 [ab]	1.24 ± 0.24 [a]
$M_{75}P_{100}V_{200}T_5$	13.33 ± 0.53 [bcd]	34.2 ± 2.9 [b]	17.5 ± 4.6 [ab]	1.18 ± 0.25 [a]
$M_{162}P_{100}V_{200}T_5$	10.58 ± 0.60 [cd]	119.5 ± 30.3 [a]	19.6 ± 4.0 [ab]	1.14 ± 0.15 [a]
$M_{425}P_{100}V_{200}T_5$	10.28 ± 1.07 [d]	137.1 ± 44.0 [a]	13.2 ± 1.3 [b]	0.98 ± 0.08 [a]
$M_{31}P_{10}V_{200}T_5$	14.80 ± 1.62 [abcd]	56.3 ± 24.1 [b]	14.8 ± 2.6 [ab]	1.19 ± 0.10 [a]
$M_{31}P_{50}V_{200}T_5$	15.47 ± 1.94 [ab]	28.1 ± 5.5 [b]	18.5 ± 4.2 [ab]	1.11 ± 0.04 [a]
$M_{31}P_{100}V_{200}T_5$	16.25 ± 0.46 [ab]	25.0 ± 3.1 [b]	22.0 ± 6.9 [ab]	1.35 ± 0.15 [a]
$M_{31}P_{100}V_{120}T_5$	17.21 ± 1.60 [ab]	24.5 ± 3.1 [b]	19.9 ± 2.3 [ab]	1.25 ± 0.24 [a]
$M_{31}P_{100}V_{200}T_5$	16.96 ± 0.35 [ab]	24.4 ± 2.4 [b]	20.7 ± 2.7 [ab]	1.20 ± 0.26 [a]
$M_{31}P_{100}V_{200}T_1$	14.66 ± 1.83 [abcd]	33.5 ± 7.6 [b]	18.2 ± 0.3 [ab]	1.11 ± 0.04 [a]
$M_{31}P_{100}V_{200}T_2$	15.44 ± 1.75 [ab]	35.0 ± 9.6 [b]	23.5 ± 3.0 [a]	1.16 ± 0.11 [a]

$M_{31}P_{100}\ V_{200}T_3$	15.10 ± 2.21 [abc]	32.6 ± 13.1 [b]	21.6 ± 2.9 [ab]	1.26 ± 0.22 [a]
$M_{31}P_{100}\ V_{200}T_5$	16.75 ± 0.12 [ab]	25.5 ± 1.7 [b]	20.4 ± 2.4 [ab]	1.27 ± 0.26 [a]
$M_{31}P_{10}\ V_{200}T_{50}$	16.59 ± 0.20 [ab]	31.1 ± 9.1 [b]	20.9 ± 0.7 [ab]	1.22 ± 0.25 [a]
$M_{31}P_{50}\ V_{200}T_{10}$	14.82 ± 1.04 [abcd]	34.0 ± 13.5 [b]	20.8 ± 1.9 [ab]	1.15 ± 0.14 [a]
$M_{31}P_{100}\ V_{200}T_5$	16.65 ± 0.78 [ab]	26.7 ± 3.3 [b]	17.8 ± 1.7 [ab]	1.24 ± 0.23 [a]
$M_{31}P_{150}\ V_{200}T_{3.3}$	15.37 ± 3.18 [ab]	32.3 ± 19.6 [b]	19.7 ± 1.7 [ab]	1.27 ± 0.24 [a]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments, as determined by one-way analysis of variance + Tukey's honestly significant different test

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes),

[4] abbreviations – EAI (emulsifying activity index); ESI (emulsifying stability index); S_0 (surface hydrophobicity); a.u. (arbitrary units); SH (sulfhydryl)

The ESI of untreated pea protein was 25 minutes. Our range aligns with the results of Zare and others (2015). It was reported that pea protein had an ESI of 33 minutes, whereas the vast majority of our treated pea proteins range from 24-35 minutes. Only samples with an initial moisture content greater than 162% d.b. had significantly higher ($p<0.05$) ESIs than the other pea protein samples. According to Pietrysiak and others (2018), higher soluble protein content has been correlated with increasing emulsifying activity and decreasing emulsifying stability, aligning with our results for samples having a high initial moisture content.

The heat-induced denaturation and aggregation in high initial moisture samples led to increased viscosity (Tavernier and others 2016; Sobhaninia and others 2017). Increased viscosity can immobilize the dispersed oil droplets, preventing them from coalescing together, thus stabilizing the emulsion (Li and Xiang 2019). Protein aggregates may also provide steric hindrance, also preventing flocculation and coalescence (Dapueto and others 2019). Liang and Tang (2014) noticed that a stable Pickering emulsion could be formed with acid-denatured pea protein isolates. Similar to the parameters in our study, a protein concentration of 2% (w/v) with an oil fraction of 0.2, the acid-denatured pea protein isolates prevented creaming for approximately 3 weeks (Liang and Tang 2014). Furthermore, Wu and others (2015) reported that heat-denatured whey proteins could form a stable Pickering emulsion.

These reasons explained why samples with a high initial moisture content had significantly higher ($p<0.05$) ESIs than those with lower initial moisture contents.

2.3.6. Effect of VMD-processing on Surface Hydrophobicity

Surface hydrophobicity is how hydrophobic the surface of a protein is and is highly correlated to other functional properties of protein (Alizadeh-Pasdar and Li-Chan 2000). Surface hydrophobicity of untreated protein was 21 arbitrary units (a.u.). Most of the samples had a surface hydrophobicity in the range of 18-24 a.u., as shown in Table 4. Our results aligned with those collected by Chao and others (2018), where they reported a range surface hydrophobicity range between 14-26, depending on the pea cultivar and the extraction method. Our results also fell within the range of the work conducted by Acquah and others (2020) where they reported yellow pea protein isolate films had a surface hydrophobicity around 20-25 a.u.

No samples significantly differed ($p\geq0.05$) in surface hydrophobicity from the control.

Wang and others (2014) observed a linear increase in surface hydrophobicity of soybean protein isolates after heating. After 30 minutes, they reported a plateau in surface hydrophobicity, which can be explained by the formation of aggregates (Wang and others (2014). Often, the secondary and tertiary structures of thermally processed proteins are disrupted due to the breaking of hydrogen bonds (Acquah and others 2020). This disruption exposes previously buried hydrophobic amino acids, increasing surface hydrophobicity (Acquah and others 2020). Our pea protein samples did not increase ($p\geq0.05$) in surface hydrophobicity despite the thermal energy provided by the microwaves. This energy would have acted to unfold the protein because dehydration during upstream processing may have caused the surface hydrophobicity to increase so that any further heat from the VMD process could not induce any further increases in surface hydrophobicity.

2.3.7. Effect of VMD-processing on Free Sulfhydryl Group Content

Disulphide bonds are covalent bonds that greatly influence the tertiary structure of a protein and hence its functional properties (Wedemeyer and others 2000; Liu and others 2016). Legumin and vicilin are globulins, a class of salt-soluble proteins that compose approximately 70-80% of a pea protein (Lam and others 2018). Disulphide bonds play a large role in the structure of legumin, while vicilin is held together mainly via hydrophobic interactions (Lam and others 2018). Free sulfhydryl group contents

can be found in Table 4. Untreated pea protein has 1.38 μmol free sulfhydryl groups/g soluble protein. There were no significant differences ($p \geq 0.05$) in free sulfhydryl content between any samples, ranging from 0.98-1.35 μmol free sulfhydryl groups/g soluble protein. According to Xu and others (2019), it was found that longer heat treatments led to decreases in sulfhydryl groups in myofibrillar proteins because it could facilitate refolding and aggregate formation, increasing the number of disulphide bonds created. With more energy absorbed due to the higher initial moisture content, more refolding and aggregation occurs. Alonso and others (2000) also measured the free sulfhydyl contents in extruded pea protein and concluded that decreases in free sulfhydryl groups could be due to oxidation or deterioration of cysteine or formation of cysteine cross-linkages.

2.3.8. Effect of VMD-processing on Chemically Available Lysine Content

Chemically available lysine of pea protein before and after VMD-processing are reported in Figure 5. The chemically available lysine content of untreated pea protein was found to be 22.5 mg lysine/g dry matter. This partially aligned with the findings of Supreetha and others (2009) who found that various pulses had 40-48 mg/g available lysine, while microwaved pulses had 20-23 mg/g available lysine. Similarly, Wang and others (1999) reported extruded pea protein to have a chemically available lysine content of approximately 4g/16g N, which equates to 40 mg/g chemically available lysine. Differences in methodology, unknown upstream processing, differences in pea cultivar and potential storage-related deterioration may lead to differences in chemically available lysine content.

Pea protein with initial moisture contents equal or greater than 162% d.b. had significantly lower ($p<0.05$) chemically available lysine contents than the control. Meanwhile, pea protein processed under different vacuum levels, microwave powers and process times did not differ significantly ($p \geq 0.05$) from the control. The decreased chemically available lysine content of high initial moisture content samples aligned with our findings as the soluble protein contents of those samples were significantly lower ($p<0.05$) than all other samples. The lower chemically available lysine content was due to the formation of dark brown, water-insoluble polymerized compounds called melanoidins in the final stages of the Maillard reaction (Lund and Ray 2017). With a higher initial moisture content, the extent of Maillard browning was enhanced.

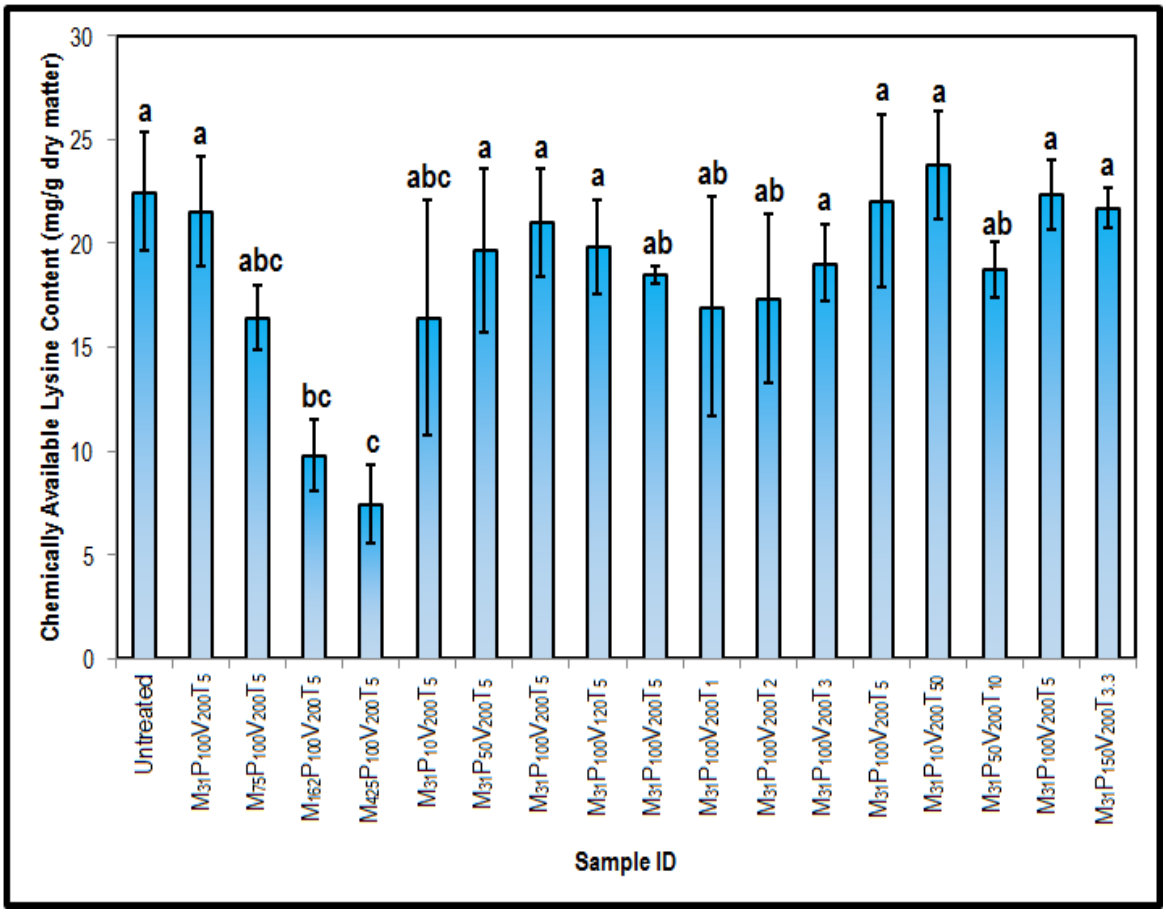

Figure 5. Chemically available lysine content of pea proteins after VMD-processing at various initial moisture contents (5-425% d.b.), vacuum levels (120-200 Torr), specific power levels (10-100W/g) and process times (1-50 minutes)

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments, as determined by one-way analysis of variance + Tukey's honestly significant different

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

As lysine has two amino groups, it was very susceptible to the Maillard reaction. Supreetha and others (2009) found that microwave treatment led to significant reductions in chemically available lysine content of various pulses, approximately a 50% reduction. This seemed contradictory to our results as samples with a low initial moisture content (31% d.b.) did not significantly differ. However, this may be due to the fact that water monolayer does not readily absorb energy as the water molecules are so

tightly bound that their molecular rotations are hindered (Ibrahim and others 2012). Further nutritional studies (animal and human clinical trials can be conducted to verify these results, as chemically available lysine is only an indicator, but not a comprehensive measure of nutritional quality (Ferrer and others 2003a).

It was also found that available lysine content was strongly and significantly correlated to the Hunterlab colour parameters, as shown in Table 5. Available lysine content was positively correlated (0.845) with lightness, but was negatively correlated (-0.843 to -0.822) with a*, b* and ΔE. As mentioned previously, lysine is very susceptible to Maillard browning, which yielded brown pigments, explaining why a* and b* was negatively correlated with available lysine. More browning led to larger ΔEs, also explaining its negative correlation with available lysine.

Table 5. Pearson's correlation coefficients between available lysine and Hunterlab colour parameters of pea proteins after VMD-processing

	Hunterlab Colour Parameters			
	L*	a*	b*	ΔE
Available Lysine	0.845	-0.822	-0.825	-0.843

[1] All correlations are statistically significant ($p<0.05$)

Increasing the initial moisture content also led to a more browned product due to the formation of melanoidins (Lund and Ray 2017). Consistent with our soluble protein content and available lysine results, samples with initial moisture contents of 162 and 425% d.b. were more substantially browned than the other samples, as shown by our Hunterlab colourimeter results (Table 2).

2.4. Conclusions

The effects of initial moisture content and vacuum microwave dehydration parameters (vacuum level, specific power and process time) on protein functionality, available lysine and colour were elucidated. Adjusting initial moisture content led to the most changes in functionality. High initial moisture content samples exhibited enhanced Maillard browning, which was associated with significantly decreased ($p<0.05$) soluble protein and chemically available lysine content and altered the colour of the pea protein. Lightness (L*) was significantly lower ($p<0.05$), while redness (a*), yellowness (b*) and total

colour difference (ΔE) were significantly higher ($p<0.05$) in high initial moisture content (162 and 425% d.b.) samples. Available lysine was found to be strongly correlated to all Hunterlab colour parameters. Increasing initial moisture content also significantly decreased emulsifying activity index ($p<0.05$), but significantly increased ($p<0.05$) emulsifying stability index. Surface hydrophobicity and free sulfhydryl groups content of VMD-processed pea protein samples were found to not be different ($p\geq0.05$) from that of untreated pea protein.

Chapter 3: Volatile Concentration of Selected VMD-Processed Pea Proteins

3.1. Background

The dry-based pea protein segment alone is slated to generate revenue of approximately 150 million Canadian dollars by the end of 2025 (Grand View Research 2019). However, pea proteins are still challenging to incorporate into foods primarily because of their off-flavours, which are typically caused by aldehydes, ketones and alcohols (Schindler and others 2012; Roland and others 2017). The production of some of these off-flavours are partly due to the production of volatile aldehydes (C_6 and C_9) and alcohols generated via enzyme-induced lipid oxidation of unsaturated lipids such as linoleic acid (Ma and others 2016; Roland and others 2017; Zha and others 2019). n-hexanal is highly odour-active and has been identified as a major contributor to undesirable grassy or green off-flavours (Schindler and others 2012; Murat and others 2013; Ma and others 2016; Roland and others 2017; Fahmi and others 2019; Zha and others 2019; Trikusuma and others 2020). Other notable off-flavour causing compounds identified in pea protein include 1-pentanol (Roland and others 2017; Lan and others 2019; Zha and others 2019), 1-hexanol (Schindler and others 2012; Ma and others 2016; Zha and others 2019; Trikusuma and others 2020), 1-octen-3-ol (Schindler and others 2012; Murat and others 2013; Lan and others 2019; Zha and others 2019), and 3,5-octadien-2-ones (Murat and others 2013), Roland and others 2017; Trikusuma and others 2020). n-hexanal and n-hexanol commonly act as a proxies for lipid oxidation (Schindler and others 2012). Pea proteins are also known to have undesirable mushroom/earthy, hay-like off-flavours and impart an unpalatable pasty or gritty mouthfeel (Roland and others 2017; Saint-Eve and others 2019). Generation of off-flavours may also be through thermal deterioration (Ma and others 2016).

Release of volatile compounds from thermally processed proteins is highly dependent on the concentration, volatility, solubility and chemical structure of the chemical compounds (Yousif and others 2000). Volatile compounds can be extracted via solid-phase microextraction. Subsequent quantification and identification can be done with gas chromatography coupled with mass spectrometry (Jung and Ebeler 2003). Solid-phase microextraction is a highly advantageous extraction method that collects volatile compounds present in the headspace, or the gas above a food matrix, onto a solid-phase coated fibre (Jung and Ebeler 2003). It can easily be automated, is highly sensitive, repeatable, and

does not require superfluous amounts of toxic organic solvents (Silva and others 2014; Piotrowicz 2016). Furthermore, collecting the volatiles in the headspace is more representative of aroma, as compared to conventional solvent extraction (Yousif and others 2000).

Their poor organoleptic properties hinder the full potential of the pulse market (Chao and others 2018; Lan and others 2018; Saint-Eve and others 2019). Therefore, processing methods should be used to ameliorate the flavour of pea proteins, while enhancing or at least retaining their functionality (Chao and others 2018). High quality volatile composition studies of vacuum microwave dehydrated legumes were not found; however, some works on other food matrices showed that vacuum microwave dehydration significantly affected volatile composition. Calín-Sánchez and others (2011) analyzed the volatile composition of VMD-processed (0-480W, 0-98 kPa) rosemary via GC-MS and found that VMD processes with a higher vacuum level and microwave power was more effective in decreasing total volatile compounds (d.b.) and key volatiles such as 1,8-cineole and camphene. Nöfer and others (2018) VMD-processed cepe mushrooms at 240 and 480W and observed the hexanal and 1-octen-3-ol contents (d.b.) after processed was 10 and 25% of concentration the fresh cepe, respectively. Chua and others (2019) VMD-processed *Strobilanthes crispus* at 6-12W/g and 30-45 Torr and reported that the total volatile concentrations after VMD-processing decreased from 361.2 mg/g (d.b.) to 3.8-9.8 mg/g (d.b). The primary objective of this chapter was to conduct volatile analysis on various VMD-processed pea proteins to gain an understanding for the resultant aroma profiles.

3.2. Methods and Materials

3.2.1. Materials

Pea protein samples (72.7% crude protein, 7% ash, both reported on a dry basis) were acquired from Daiya Foods Inc. (BC, CA). D-12 hexanal (≥98 atom%, ≥96% (CP)) was purchased from Sigma Aldrich (MO, USA). Graduated micropipettes were purchased from Drummond (AL, USA). HPLC grade methanol was purchased from (VWR International (PA, USA). D-12 hexanal (≥98 atom%, ≥96% (CP), Sigma Aldrich, MO, USA) was used to prepare a 1000 ppm internal standard solution using HPLC grade methanol (VWR International (PA, USA) as a diluent.

3.2.2. Research Design

A full factorial design was used for varying microwave power across 3 levels (2 W/g, 5 W/g and 20 W/g) and vacuum levels across 2 levels (40 Torr and 200 Torr) to assess the effect of VMD for high moisture content samples (425% db). In this set of experiments ($M_{425}P_2V_{40}T_{68}$, $M_{425}P_5V_{40}T_{20}$, $M_{425}P_{20}V_{40}T_{3.5}$, $M_{425}P_2V_{200}T_{88}$, $M_{425}P_5V_{200}T_{21}$ and $M_{425}P_{20}V_{200}T_{3.5}$, where $M_aP_bV_cT_d$ represents experiments conducted on pea protein with initial moisture content a% d.b. at a microwave power of b W/g and a vacuum level of c Torr for a process time of d minutes), process times were based on drying kinetics experiments (Appendix A - Figures A1 and A2) so that a final moisture content of approximately 20% (d.b.) was achieved. A second set of experiments ($M_{56}P_{67}V_{200}T_{2.5}$, $M_{50}P_{20}V_{200}T_5$) was conducted at low moisture content (56% db) to study the effect of process time (0, 2.5 and 5 minutes) at 200 Torr vacuum and 67 W/g microwave power. A third set of six experiments ($M_{31}P_{100}V_{200}T_5$, $M_{75}P_{100}V_{200}T_5$, $M_{162}P_{100}V_{200}T_5$, $M_{31}P_{10}V_{200}T_{50}$,) $M_{31}P_{100}V_{200}T_1$, $M_{31}P_{10}V_{200}T_5$, were conducted to study the effect of initial moisture content (31, 75 and 162 % d.b.), microwave power (10 and 100 W/g) and process time (1, 5 and 50 min) at 200 Torr vacuum. Untreated pea protein served as a control. Samples were VMD-processed as previously discussed in Section 2.2.2.

3.2.3. Volatile Analysis Using Headspace Solid-phase-microextraction Gas Chromatography – Mass Spectrometry (Headspace SPME-GC – MS)

Volatile compounds were collected via solid-phase-microextraction (SPME). Pea protein (1g) was mixed into 7 mL deionized water and vortexed until a suspension was formed. Deionized water (7 mL) was used as a blank. Samples were spiked with 5 µL internal standard (1000 ppm D-12 hexanal) and equilibrated at 40°C for 10 minutes, under frequent agitation. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, PA, USA) was used to extract non-polar, semi-polar and polar volatile compounds at 40°C for 15 minutes from the sample headspace.

Extracted compounds were analyzed with GC (6890N Agilent, CA, USA) coupled with MS (5973MSD Agilent, CA, USA). A J&W DB-WAX 122-7062 capillary column (60m x 0.25 mm ID, 0.25 µL film) (Agilent, CA, USA) was used for separation. Volatiles adsorbed on the SPME fibre were injected at 250°C in pulsed-splitless mode for 5 minutes to be thermally desorbed from the SPME fibre. Helium was used as the carrier gas and was set at a flow rate of 1.4 mL per minute. An additional pressure of

30 psi was applied for half a minute. The temperature was initially programmed to 40°C for 4 minutes, raised to 150°C at a rate of 3°C per minute, raised to 230°C at a rate of 25°C per minute and then lastly held isothermally for 7 minutes. Ion source and transfer line temperatures were set to 230°C and 250°C, respectively. Electron ionization mode at 70eV and a mass range of 40-500 m/z were used for MS.

To identify the volatile compounds, mass spectra were matched to digital libraries (Wiley09/NIST08). Compounds that had no match with the digital spectra were not reported. Volatile compound concentration was quantified using MSD Chemstation E.01.00.237 (Agilent, CA, USA) and reported as area/area internal standard. Only compounds with a signal to noise ratio of equal or above 10:1 were quantified. Compounds with a signal to noise ratio below 10, but greater or equal to 3:1 were reported as below the limit of quantification (LOQ). Compounds with a signal to noise ratio below 3:1 were reported as below the limit of detection (LOD).

3.2.4. Water Activity

Water activity was measured by using a water activity meter (Aqualab Series 3 Water Activity Meter, METER Group Inc., WA, USA). Measurements were taken at 22.0 ± 0.5°C.

3.2.5. Statistical Analysis

Levene's test was used to determine whether the assumption of of homoscedasticity was met. Data was analyzed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test as a post-hoc test. Statistical significance was defined as $p<0.05$. All statistical analyses were conducted using Minitab 19 (Minitab Inc., PA, USA).

3.3. Results and Discussion

Thirty eight volatile compounds were identified in this study. Their retention times and odour descriptors can be found in Appendix B (Table 18). The earliest compound detected was pentane, which had a retention time of 2.95 minutes. Meanwhile, 3,-5 octadien-2-one had the longest retention time – 24.04 minutes. Most of the alkane/alkenes and furans were eluted out within the first six minutes. Over half of the identified compounds were aldehydes, alcohols and ketones, which have been reported in literature to be responsible for pea protein off-flavours (Schindler and others 2012; Roland and others 2017). The identified aldehydes are reported to have a green, grassy or citrus-like odour. There are a plethora of

other compounds with very distinct odour descriptors, such as fruity, mushroom-like, fatty and burnt. Some or combinations of the above odours clearly lead to a lack of palatability, hence, it is paramount to develop a method to reduce pea protein off-flavour intensity.

3.3.1. Effect of Microwave Power on Volatile Compound Concentrations Under High-vacuum (40 Torr) High Initial Moisture Content (425% d.b.) VMD

Only 23 compounds were identified after high vacuum (40 torr) VMD treatment, as shown in Table 6. VMD-processing pea proteins with an initial moisture content of 425% d.b. at 40 Torr led to 50-78% reductions in total volatile compound concentration. Processing pea protein at 2 W/g for 68 minutes led to the greatest total reduction in area, while processing pea protein at 5 W/g for 20 minutes and 20W/g for 3.5 minutes led to lower reductions of approximately 50%. This may be explained because processing at 100W for 68 minutes had the highest specific output energy (6800 W*min). Processing at 250W for 20 minutes (5000 W*min) and 1000W for 3.5 minutes (3500 W*min) were not able to effectively drive off the volatile compounds due to the process times not being adequate. Thus, it was seen that processing at lower power levels for longer times was better for removing volatiles under high-vacuum conditions.

Processing at 2W/g for 68 minutes was very effective in removing alkane/alkenes and furans as compared to the other VMD treatments. Low-power long-time VMD processing (2W/g - 68 minutes) led to approximately 80% reduction in both alkane/alkenes and furans, while the other two VMD processes only achieved approximately a 50% reduction in both compound types. Low-power long-time VMD processing (2W/g – 68 minutes) led to a 70% reduction in aldehydes, whereas higher-power shorter time VMD processing (5W/g – 20 minutes & 20W/g – 3.5minutes) led to 35% and 42%, respectively. Hexanal has a very distinct grassy or beany off-note and was reduced in all treatments, ranging from 34-69% reduction. Jakobsen and others (1998) reported that 55% of the trapped volatile compounds in thawed green peas consisted of hexanal. Ma and others (2016) also stated that the principal aldehyde in pulses was hexanal. Hence, even a 34% reduction may be expected to have a notable beneficial effect on the perceived off-flavour intensity.

Low-power-long-time VMD processing (2W/g - 68 minutes) led to all alcohols being below the limit of quantification and a 96% reduction in ketones. The other VMD treatments also led to substantial

decreases in alcohols and ketones. Evidently, processing pea protein at 2W/g for 68 minutes was the most effective treatment in leading to reductions in each functional group.

Table 6. Effect of increasing microwave power on identified volatile compounds in pea proteins after high-vacuum VMD-processing, as determined by SPME GC-MS

Identity	Retention Time (mins)	Area/Area Internal Standard			
		Untreated	$M_{425}P_2V_{40}T_{68}$	$M_{425}P_5V_{40}T_{20}$	$M_{425}P_{20}V_{40}T_{3.5}$
Total	**N/A**	**7.87**	**1.73**	**3.96**	**3.97**
Aldehydes	N/A	3.19	0.97	2.08	1.84
Alcohols	N/A	0.06	0.00	0.00	0.03
Ketones	N/A	1.11	0.04	0.23	0.29
Alkanes/Alkenes/Alkynes	N/A	0.33	0.06	0.16	0.20
Furans	N/A	3.17	0.67	1.50	1.62
Pentane	2.95	0.06	0.01	0.04	0.03
Heptane	3.13	0.03	0.01	0.03	0.03
Hexane, 2,4 dimethyl	3.42	0.07	0.03	0.05	0.07
Furan, 2-Ethyl	4.16	0.12	0.04	0.07	0.05
Pentanal	4.55	0.10	0.02	0.05	0.05
Furan, 2-ethyl, 5-methyl	5.21	0.03	Below LOQ	Below LOQ	Below LOQ
Toluene	5.37	0.03	Below LOD	Below LOQ	0.01
Hexanal	6.35	2.97	0.93	1.95	1.73
2-Heptanone	9.28	0.70	0.02	0.13	0.18
Furan, 2-Pentyl	10.62	3.02	0.62	1.43	1.56
1-Pentanol	11.55	0.03	Below LOD	Below LOD	Below LOD
2-Octanone	13.11	0.03	Below LOQ	Below LOQ	0.01

Octanal	13.22	0.06	0.02	0.03	0.03
1-Hexanol	15.57	Below LOQ	Below LOD	Below LOQ	Below LOQ
Pentane, 1-Nitro	16.61	0.11	Below LOD	0.03	0.04
2-Nonanone	17.32	0.13	0.02	0.06	0.07
Nonanal	17.47	0.06	Below LOQ	0.04	0.03
1-Octen-3-ol	19.40	0.03	Below LOQ	Below LOQ	0.02
Hexane, 1-nitro	20.83	0.04	Below LOD	Below LOD	0.01
Benzaldehyde	21.48	0.02	Coelution	Coelution	Coelution
2-Decanone	21.48	0.18	Coelution	Coelution	Coelution
3, 5-Octadien-2-one	22.03	0.12	Below LOD	0.01	0.01
3, 5-Octadien-2-one	24.04	0.13	Below LOD	0.03	0.03

[1] Below LOQ denotes that signal to noise ratio was <10:1 but ≥3:1

[2] Below LOD denotes that signal to noise ratio was <3:1

[3] Coelution denotes that two peaks coeluted thus the individual areas could not be determined

[4] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[4] D-12 Hexanal had a retention time of 6.22 minutes

3.3.2. Effect of Microwave Power on Volatile Cmpound Concentrations Under Low-vacuum (200 Torr) High Initial Moisture Content (425% d.b.) VMD

Processing pea protein at low-vacuum levels (200 Torr) led to even more substantial reductions (73-83%) in volatile compound concentration compared to high-vacuum levels. All low-vacuum VMD treatments led to at least a 69, 86, 56 and 69% reduction in aldehydes, ketones, alkane/alkenes and furans, respectively. All low-vacuum level (200 Torr) treatments led to alcohol concentrations being under the limit of quantification. The aroma profile was very similar to that of high-vacuum VMD treatment (40 Torr), as shown in Table 7. Only 25 compounds were identified; however, 2n-butyl furan and propane, 2-methoxy, 2-methyl were identified after low-vacuum treatment but not detected in the samples processed under high-vacuum treatments.

Processing at low-power-long-time (2W/g – 88 minutes) was the most effective treatment for removing volatile compounds in all functional groups. Processing pea protein at 11W/g for 7.5 minutes led to the smallest reduction, although the total reduction was very similar to processing at 5W/g for 21 minutes and 20W/g for 3.5 minutes. Furan reduction after low-vacuum treatment (200 Torr) was comparable to that after high-vacuum treatment (40 Torr). Hexanal was reduced by 80% in pea protein processed at 2W/g (200 Torr), while the other three VMD treatments led to a smaller reduction, by approximately 10% less. Ketone reductions ranged from 86-96%. Processing at 2W/g for 88 minutes led to an 87% reduction in alkane/alkenes.

The improved capacity to remove volatile compounds in low-vacuum processes (200 Torr) as compared to high vacuum processes (40 Torr) may be due to the elevated boiling temperature of water at higher pressure (i.e. lower vacuum levels). As the pea protein was exposed to higher temperatures, proteins can unfold, increasing the likelihood that binding sites from the hydrophobic core were exposed (Wang and Arntfield 2016). Flavour compounds can irreversibly or reversibly interact with these binding sites, explaining why volatile compound concentration decreased (Wang and Arntfield 2017). Denaturation and aggregation of proteins may have also released previously bound flavour molecules (Wang and Arntfield 2016; Xu and others 2019).

Table 7. Effect of increasing microwave power on identified volatile compounds in pea proteins after low-vacuum VMD-processing, as determined by SPME GC-MS

Identity	Retention Time (mins)	Area/Area Internal Standard			
		Untreated	$M_{425}P_2V_{200}T_{88}$	$M_{425}P_5V_{200}T_{21}$	$M_{425}P_{20}V_{200}T_{3.5}$
Total	**N/A**	**10.87**	**1.81**	**2.70**	**2.79**
Aldehydes	N/A	4.06	0.78	1.15	1.21
Alcohols	N/A	0.08	0.00	0.00	0.00
Ketones	N/A	2.27	0.09	0.23	0.18
Alkanes, Alkenes	N/A	0.39	0.05	0.16	0.12
Furans	N/A	4.07	0.89	1.15	1.28
Pentane	2.94	0.08	0.01	0.07	0.04
Propane, 2 methoxy-, 2 methyl-	3.07	0.04	Below LOD	0.01	Below LOD
Heptane	3.11	0.03	0.02	0.02	0.02
Hexane, 2,4 dimethyl	3.40	0.06	0.03	0.04	0.03
Furan, 2-ethyl	4.15	0.12	0.03	0.07	0.04
Pentanal	4.53	0.14	0.01	0.03	0.04
Furan, 2-ethyl, 5 methyl	5.19	0.03	Below LOD	0.01	0.01
Hexanal	6.32	3.62	0.73	1.07	1.12
2n-butyl furan	7.38	0.09	0.04	0.03	0.03
2-Heptanone	9.30	0.94	0.03	0.15	0.10
Furan, 2-pentyl	10.75	3.83	0.83	1.04	1.20
1-Pentanol	11.47	Below LOQ	Below LOD	Below LOD	Below LOD

2-Octanone	13.13	0.06	Below LOD	0.01	Below LOQ
Octanal	13.26	0.10	0.01	0.03	0.02
1-Hexanol	15.53	Below LOQ	Below LOD	Below LOD	Below LOD
Pentane, 1-Nitro	16.61	0.12	Below LOQ	0.02	0.02
2-Nonanone	17.33	0.18	0.03	0.04	0.04
Nonanal	17.48	0.16	0.02	0.03	0.03
3-Octen-2-one	17.85	0.10	Below LOD	Below LOD	Below LOD
1-Octen-3ol	19.38	0.08	Below LOQ	Below LOQ	Below LOQ
Hexane, 1 Nitro	20.84	0.06	Coelution	Coelution	Below LOQ
Benzaldehyde	21.47	0.03	Coelution	Coelution	Coelution
2-Decanone	21.52	0.26	Coelution	Coelution	Coelution
3, 5-Octadien-2-one	22.02	0.31	Below LOD	Below LOD	0.01
3, 5-Octadien-2-one	24.02	0.41	0.03	0.03	0.02

[1] Below LOQ denotes that signal to noise ratio was <10:1 but ≥3:1

[2] Below LOD denotes that signal to noise ratio is <3:1

[3] Coelution denotes that two peaks coeluted thus the individual areas could not be determined

[4] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[5] D-12 Hexanal had a retention time of 6.23 minutes

3.3.3. Effect of Process Time on Volatile Compound Concentrations Under Low Vacuum (200 Torr) Low Initial Moisture Content (56% d.b.) VMD

Pea protein was further processed with a low initial moisture content (56% d.b.). Processing with less initial moisture at 200 Torr did not lead to an appreciable reduction in total volatile compound concentration. VMD-treatments with a low initial moisture content only led to a 2-11% reduction in total area, as shown in Table 8. Processing pea protein with a low initial moisture content led to slight increases in alkane/alkenes. This was not very concerning as they often have weak odours and contribute only a minor role in pulse off-flavours (Ma and others 2016).

Processing low initial moisture content samples for 5 minutes led to a smaller reduction in volatile compound concentration as compared to 2.5 minutes. This may be due to generation of new compounds. Xu and others (2017a) observed that when wheat flour was heated at high temperatures, pyrazine, furan and sulphur-containing compound levels were elevated. This aligned with Ma and others (2016) as they reported that protein isolates tend to generate pyrazines only in low moisture content heat treatment. It was evident that processing samples at high and low initial moisture contents led to major and minor reductions in volatile concentrations, respectively.

Table 8. Effect of increasing process time on identified volatile compounds in pea proteins after high-power, low-vacuum, low initial moisture content VMD-processing, as determined by SPME GC-MS

Identity	Retention Time (mins)	Area/Area Internal Standard		
		Untreated	$M_{56}P_{67}V_{200}T_{2.5}$	$M_{56}P_{67}V_{200}T_{5}$
Total	**N/A**	**14.19**	**12.57**	**13.95**
Aldehydes	N/A	5.67	4.98	5.40
Alcohols	N/A	0.11	0.04	0.05
Ketones	N/A	2.29	1.38	2.26
Alkanes, Alkenes	N/A	0.55	0.65	0.68
Furans	N/A	5.58	5.51	5.56
Pentane	2.95	0.13	0.21	0.22
Heptane	3.12	0.06	0.12	0.10
Hexane, 2,4 dimethyl	3.41	0.03	0.25	0.24
Furan, 2-ethyl	4.13	0.35	0.28	0.04
Pentanal	4.51	0.22	0.15	0.19
Furan, 2-ethyl, 5-methyl	5.16	0.03	0.03	0.04
Toluene	5.31	0.03	Below LOQ	Below LOQ
Hexanal	6.28	5.24	4.69	5.04
2-n-butyl furan	7.31	0.07	0.03	0.02
2-Heptanone	9.23	1.24	0.79	1.55
Furan, 2-pentyl	10.67	5.13	5.18	5.47
1-Pentanol	11.35	0.04	Below LOQ	Below LOQ

2-Octanone	13.05	0.07	0.04	0.05
Octanal	13.19	0.11	0.07	0.08
1-Hexanol	15.40	Below LOQ	Below LOQ	Below LOQ
Pentane, 1-nitro	16.49	0.12	0.08	0.09
2-Nonanone	17.25	0.21	0.16	0.23
Nonanal	17.41	0.10	0.07	0.09
3-Octen-2-one	17.76	0.10	0.04	0.03
1-Octen-3-ol	19.24	0.07	0.04	0.05
Hexane, 1-nitro	20.82	0.05	Below LOQ	0.03
Benzaldehyde	21.51	Coelution	Coelution	Coelution
2-Decanone	21.51	Coelution	Coelution	Coelution
3,5 Octadien-2-one	22.02	0.31	0.15	0.16
3,5 Octadien-2-one	24.00	0.37	0.20	0.24

[1] Below LOQ denotes that signal to noise ratio was <10:1 but ≥3:1

[2] Below LOD denotes that signal to noise ratio is <3:1

[3] Coelution denotes that two peaks coeluted thus the individual areas could not be determined

[4] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[5] D-12 Hexanal had a retention time of 6.18 minutes

3.3.4. Volatile Compound Concentrations of Pea Protein Samples with Varying Initial Moisture Content, Microwave Power and Process Time

The extent of lipid oxidation is elevated at very low water activities, which may have an adverse effect on volatile composition (Simic and Karel 1980). This is due to the lack of free water to quench inherently present free radicals (Simic and Karel 1980). Increased levels of lipid oxidation can generate n-hexanal and other volatile compounds with undesirable odours (Ma and others 2016). Three samples with varying initial moisture contents (31, 75 and 162% d.b.) were processed at 100W/g for 5 minutes and were subsequently analyzed for their volatile composition (Table 9). Thirty four volatile compounds were identified. Increasing initial moisture content led to more significant reductions in volatile compounds. Pea protein with initial moisture contents of 31, 75 and 162% d.b. had a total volatile concentration of 29, 27 and 19, respectively. The total volatile concentration of pea protein with 162% d.b. did not differ significantly ($p \geq 0.05$) from the untreated protein, which had a total area concentration of 16, while pea proteins with initial moisture contents of 31 and 75% had significantly higher ($p < 0.05$) total volatile concentrations than untreated pea protein. Pea protein with an initial moisture content of 162% d.b. did not significantly differ ($p \geq 0.05$) in area of any functional group except ketones, which increased ($p < 0.05$) by about threefold. The other two samples (31 and 75% d.b.) were significantly higher ($p < 0.05$) than the untreated protein in terms of total aldehydes and total ketones compared to the control. Total alcohol content of pea protein (31% d.b.) was higher ($p < 0.05$) than that of untreated pea protein.

The volatile compound most associated with pea protein off-flavour, hexanal, increased ($p < 0.05$) after VMD-processing at high powers. Pea protein with initial moisture contents of 31 and 75% d.b. were both significantly higher ($p < 0.05$) in hexanal, while pea protein with an initial moisture content of 162% d.b. did not differ ($p \geq 0.05$) from that in untreated pea protein. Javidipour and others (2017) microwaved 10 grams soybean and sunflower oils at 600W for 9 minutes, which was very similar to the specific energy output used. This microwave treatment led to 55 and 389-fold increases in hexanal, respectively (Javidipour and others 2017). As linoleic acid is the most predominant fatty acid in peas, soybean oil and sunflower oil, it is reasonable that extensive microwave energy could enhance linoleic acid degradation, forming hexanal and other compounds (Schindler and others 2012; Javidipour and others 2017). The compound 3, 5-octadien-2-one decreased significantly as initial moisture content increased. The concentration of 1-hexanol in the untreated pea protein was below the limit of quantification, but was below the limit of detection when the initial moisture content was 162% d.b.

Trikusuma and others (2020) described 1-hexanol as being a very intense odourant in green peas. Schindler and others (2012) described 1-hexanol as resin-like, flowery and green and also considered 1-hexanol to be a key volatile compound responsible for pea off-flavours. Furthermore, untreated pea protein had a 1-pentanol concentration of 0.06, which did not change ($p \geq 0.05$) after adjusting the initial moisture content to 31 or 75% d.b. When the initial moisture content was 162% d.b., the 1-pentanol concentration was below the limit of detection. The compound 1-octen-3-ol only did not differ ($p \geq 0.05$) after processing at various initial moisture contents. In the untreated pea protein, furans and aldehydes were the most notable categories in terms to sheer area; however, after VMD-processing, ketones emerged as the most notable, while aldehydes and most furans remained high in peak area.

It was hypothesized that the specific energies of these VMD-processed samples were too high which efficiently removed a lot of the moisture, so that it resembled “dry heating” in the latter stages of the VMD-processing. Ma and others (2016) noted that roasting pulses led to significant increases in total volatile concentration. Some compounds were present in VMD-treated pea samples, but not detected in the untreated protein. For instance, 2-butanone, tricholoromethane, 2-octenal, 2-n-butylacrolein and 2-hexenal, 2-ethyl were all below the limit of detection in the untreated pea protein but were present in varying concentrations after VMD-processing. According to Wang and Arntfield (2014), new volatile compounds can either be generated through degradation mechanisms or can be due to various protein-flavour interactions. However, despite generation of new compounds, some compounds were below the limit of detection after VMD-processing. For instance, 1-hexanol, 1-pentanol and 3-octene-2-one were all below the limit of detection when initial moisture content was 162% d.b. One explanation for why higher initial moisture content pea protein samples led to lower volatile concentration was because wet heating induced protein denaturation, which in turn facilitated stronger bonds between the lipid oxidation by-products and the proteins, forming lipoprotein complexes (Ma and others 2016).

Other compounds increased in concentration; however, not all of these may substantially contribute to off-flavours. The most notable increase was 2-heptanone, which initially started with an area of 1.3, but the three VMD-processed samples led to 430-790% increases. The compound 2-heptanone is characterized with a sweet, fruity or soapy aroma (Li and others 2019b). Li and others (2019b) observed formation of 2-heptanone in microwaved yak meat, which was not present in just boiled yak meat. It was deduced that the 2-heptanone was derived from linoleic acid, which is also present in pea proteins (Li and others 2019b). Another compound with a very large peak is 2-pentyl furan, its $A_{2\text{-PENTYL FURAN}}/A_{IS}$ ranged from 4.4-7.3. However, this was already very high the pea protein. Furans are

generated mainly through the thermal deterioration of simple sugars, but also through the degradation of polyunsaturated fatty acids and amino acids (Fromberg and others 2014). The odour threshold of 2-pentyl furan is 6 ppb, which is low; however, it is not regarded as a major contributor to the characteristic off-odours in peas (Buttery and others 1988).

A low specific power, long-time VMD process (processing at 10W/g for fifty minutes) was also analyzed as previous results showed that low specific powers led to larger reductions in volatiles. It was also considered to see whether specific energy was a factor if the specific power was the same. Volatile compounds identified after VMD-processing can be found in Table 13. Twenty-nine volatile compounds were identified. Total area and other functional groups did not differ significantly from that of the untreated protein. Out of the major off-flavour causing compounds, only 3, 5 octadien-2-one (eluted at 21.90 minutes) was reduced slightly but significantly ($p<0.05$). This suggested that despite utilizing a low specific power, the high energy output still facilitated lipid oxidation (Javidipour and others 2017).

Pea proteins subjected to low specific energy processes had significantly lower 1-hexanal and 3, 5 octadien-2-one (eluted at 21.90 and 23.90 minutes) contents. These two samples did not differ in 1-pentanol and 1-octen-3-ol. The sample that underwent a 50W*min/g process only had significantly lower total aldehydes than the control, while the sample that underwent a 100W*min/g process did not differ from the control in any functional group or total volatiles. Contrary to low initial moisture contents discussed in the previous section, a spike in 2-heptanone or 2-pentyl furan was not observed in these samples. This may be due to insufficient energy to induce substantial deterioration of linoleic acid (Javidipour and others 2017; Li and others 2019b). Despite the lower energy outputs, the concentrations of the following compounds butanal, octanal and 1-propen-2-ol, acetate still fell below the limit of detection in some VMD-processed samples. The microwave output was sufficient enough to induce conformational changes in the pea protein, affecting flavour binding (Xu and others 2019). It has been reported that aldehydes tend to irreversibly bind to proteins as the duration of heating increases (Xu and others 2019). Wang and Arntfield (2017) also report that aldehydes can form irreversible covalent bonds or reversible hydrophobic interactions, while ketones tend to only form reversible hydrophobic interactions. Heat generated from the microwaves can unfold and aggregate proteins, leading to the modification of hydrophobic binding sites, freeing volatile compounds (Xu and others 2019). The vacuum environment complemented these heat-induced effects, more effectively driving off the volatile compounds (Speight 2017). Even though low-energy or low-power-long-time treatments decreased volatile concentration ($p<0.05$), it may not have decreased the concentrations by

enough where a difference is perceivable; hence other VMD processes were proposed in Chapter 4. Our findings suggested that VMD-processing at a higher initial moisture content helped to reduce volatile concentrations. VMD-processing at a low specific power for a long period of time was not very effective in decreasing volatile concentrations, but specific energy was a key factor as lower-energy processes led to more volatile reductions, regardless of the specific power employed.

Table 9. Identified volatile compounds in pea proteins after VMD-processing at various initial moisture contents (31-162% d.b.), high specific power, low-vacuum levels for a process time of five minutes, as determined by SPME GC-MS

Identity	Retention Time (mins)	Area/Area IS						
		Untreated	$M_{31}P_{100}V_{200}T_5$	$M_{75}P_{100}V_{200}T_5$	$M_{162}P_{100}V_{200}T_5$	$M_{31}P_{10}V_{200}T_{50}$	$M_{31}P_{100}V_{200}T_1$	$M_{31}P_{10}V_{200}T_5$
Total	**N/A**	**15.62 ± 0.96** [bc]	**29.02 ± 1.09** [a]	**27.20 ± 5.10** [a]	**19.03 ± 2.28** [b]	**14.38 ± 0.57** [bc]	**12.68 ± 1.26** [bc]	**9.98 ± 1.57** [c]
Aldehydes	N/A	5.30 ± 0.22 [bc]	7.99 ± 0.39 [a]	7.33 ± 0.75 [a]	5.88 ± 0.50 [b]	4.87 ± 0.20 [bc]	4.41 ± 0.32 [cd]	3.33 ± 0.48 [d]
Alcohols	N/A	0.16 ± 0.04 [bc]	0.26 ± 0.04 [a]	0.20 ± 0.06 [ab]	0.08 ± 0.02 [c]	0.15 ± 0.01 [bc]	0.12 ± 0.01 [bc]	0.09 ± 0.02 [c]
Ketones	N/A	2.73 ± 0.10 [c]	11.77 ± 0.72 [a]	12.83 ± 2.43 [a]	7.73 ± 1.08 [b]	2.14 ± 0.11 [c]	1.84 ± 0.12 [c]	1.47 ± 0.19 [c]
Alkanes/Alkenes	N/A	0.73 ± 0.08 [ab]	1.05 ± 0.29 [a]	0.72 ± 0.17 [ab]	0.51 ± 0.13 [b]	1.08 ± 0.27 [a]	0.67 ± 0.09 [ab]	0.48 ± 0.10 [b]
Furans	N/A	6.68 ± 0.57 [abc]	7.84 ± 0.37 [a]	6.93 ± 1.63 [ab]	4.81 ± 0.60 [bc]	6.12 ± 0.28 [abc]	5.61 ± 0.74 [abc]	4.61 ± 0.78 [c]
Pentane	2.95	0.16 ± 0.00	0.22 ± 0.09	0.24 ± 0.08	0.16 ± 0.05	0.22 ± 0.04	0.13 ± 0.02	0.08 ± 0.03
Heptane	3.12	0.10 ± 0.03	0.12 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.09 ± 0.01	0.10 ± 0.00	0.07 ± 0.02
1-Propen-2-ol, acetate	3.34	0.03 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	Below LOD	0.02 ± 0.00	Below LOD
Hexane, 2,4 dimethyl	3.40	0.23 ± 0.04	0.20 ± 0.00	0.18 ± 0.04	0.12 ± 0.04	0.25 ± 0.00	0.21 ± 0.07	0.16 ± 0.03
Butanal	3.60	0.03 ± 0.00	0.07 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	Below LOD	0.01 ± 0.00	Below LOD
2-Butanone	3.75	Below LOD	0.05 ± 0.01	0.05 ± 0.02	Below LOD	Below LOD	Below LOD	Below LOD
Furan, 2-ethyl	4.13	0.37 ± 0.10	0.30 ± 0.07	0.30 ± 0.13	0.23 ± 0.08	0.10 ± 0.01	0.32 ± 0.02	0.21 ± 0.04
Pentanal	4.51	0.25 ± 0.01	0.30 ± 0.16	0.26 ± 0.01	0.23 ± 0.01	0.23 ± 0.00	0.20 ± 0.02	0.14 ± 0.02
Trichloromethane	4.75	Below LOD	0.17 ± 0.00[2]	Below LOD	Below LOD	0.15 ± 0.03[2]	Below LOD	Below LOD
Furan, 2-ethyl, 5-methyl	5.16	0.05 ± 0.00	0.08 ± 0.00	0.09 ± 0.03	0.07 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Toluene	5.31	0.04 ± 0.00	0.32 ± 0.21	0.08 ± 0.01	0.03 ± 0.01[2]	CO	0.02 ± 0.01[2]	0.02 ± 0.00[2]
3-Nonen-2-ol	5.91	Below LOD	0.04 ± 0.00	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD
Hexanal [3]	***6.27***	***4.74 ± 0.22*** [b]	***6.79 ± 0.29*** [a]	***6.36 ± 0.76*** [a]	***5.19 ± 0.44*** [b]	***4.46 ± 0.21*** [b]	***4.07 ± 0.29*** [c]	***3.07 ± 0.45*** [c]
2-n butyl furan	7.16	0.11 ± 0.05	0.19 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.13 ± 0.05	0.10 ± 0.05
2-n-Butylacrolein	8.16	Below LOD	0.05 ± 0.03	0.06 ± 0.02	Below LOD	Below LOD	Below LOD	Below LOD

2-Heptanone	9.20	1.31 ± 0.05	10.33 ± 0.71	11.62 ± 2.26	6.95 ± 0.98	1.12 ± 0.07	0.98 ± 0.11	0.79 ± 0.11
Furan, 2-pentyl	10.52	6.17 ± 0.45	7.27 ± 0.29	6.45 ± 1.49	4.38 ± 0.53	5.86 ± 0.26	5.13 ± 0.72	4.27 ± 0.71
1-Pentanol [3]	***11.42***	***0.06 ± 0.00*** [ab]	***0.06 ± 0.00*** [a]	***0.05 ± 0.02*** [ab]	***Below LOD***	***0.06 ±0.00*** [ab]	***0.05 ± 0.00*** [ab]	***0.04 ± 0.01*** [b]
2-Octanone	13.02	0.06 ± 0.00	0.13 ± 0.02	0.11 ± 0.02	0.08 ± 0.01	0.04 ± 0.00	0.10 ± 0.12[2]	0.04 ± 0.00
Octanal	13.13	0.10 ± 0.01	0.34 ± 0.04	0.26 ± 0.03	0.21 ± 0.03	0.07 ± 0.01	Below LOD	0.06 ± 0.00
2-Hexenal, 2-ethyl	14.77	Below LOD	0.20 ± 0.01	0.18 ± 0.06	0.09 ± 0.02	Below LOD	Below LOD	Below LOD
1-Hexanol [3]	***15.41***	***Below LOQ***	***Below LOQ***	***Below LOQ***	***Below LOD***	***Below LOQ***	***Below LOQ***	***Below LOQ***
Pentane, 1-nitro	16.47	0.15 ± 0.01	0.14 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.12 ± 0.00	0.14 ± 0.01	0.11 ± 0.01
2-Nonanone	17.23	0.22 ± 0.01	0.70 ± 0.03	0.66 ± 0.07	0.48 ± 0.07	0.19 ± 0.01	0.16 ± 0.01	0.15 ± 0.02
Nonanal	17.38	0.09 ± 0.01	0.23 ± 0.03	0.22 ± 0.05	0.14 ± 0.01	0.10 ± 0.01	0.08 ± 0.00	0.07 ± 0.00
3-Octen-2-one	17.75	0.11 ± 0.01	0.10 ± 0.00	0.08 ± 0.01	Below LOD	0.08 ± 0.00	0.08 ± 0.00	0.04 ± 0.01
2-Oxooctanoic acid	18.33	0.03 ± 0.00	0.10 ± 0.00	0.09 ± 0.02	0.06 ± 0.02	0.03 ± 0.01	0.03 ± 0.00	Below LOQ
2-Octenal	18.72	Below LOD	0.03 ± 0.01	0.04 ± 0.01	Below LOD	Below LOD	Below LOD	Below LOD
1-Octen-3-ol [3]	***19.24***	***0.07 ± 0.02*** [ab]	***0.08 ± 0.03*** [a]	***0.05 ± 0.01*** [ab]	***0.04 ± 0.00*** [b]	***0.08 ± 0.01*** [a]	***0.05 ± 0.00*** [ab]	***0.05 ± 0.01*** [ab]
Hexane, 1-nitro	20.69	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Benzaldehyde	21.35	0.08 ± 0.01	Coleution	Coleution	Coleution	Coleution	Coleution	Coleution
2-Decanone	21.42	0.24 ± 0.01	Coleution	Coleution	Coleution	Coleution	Coleution	Coleution
3,5-Octadien-2-one [3]	***21.90***	***0.38 ± 0.03*** [a]	***0.21 ± 0.01*** [cd]	***0.13 ± 0.02*** [e]	***0.08 ± 0.00*** [e]	***0.32 ± 0.01*** [b]	***0.26 ± 0.01*** [c]	***0.20 ± 0.02*** [d]
3,5-Octadien-2-one [3]	***23.90***	***0.42 ± 0.01*** [a]	***0.24 ± 0.02*** [bc]	***0.17 ± 0.05*** [cd]	***0.12 ± 0.01*** [d]	***0.40 ± 0.03*** [a]	***0.29 ± 0.03*** [b]	***0.25 ± 0.04*** [bc]

[1] results are reported as mean ± standard deviation (n=3)

[2] denotes that it is displayed as mean ± range (n=2) i.e. one replicate was below LOD or was coeluted

[3] denotes that there is extensive literature suggesting that this is one of the most characteristic off-flavour causing compounds in pea protein (Schindler and others 2012; Murat and others 2013; Ma and others 2016; Roland and others 2017; Fahmi and others 2019; Lan and others 2019; Zha and others 2019; Trikusuma and others 2020)

[4] superscript letters denote significant differences ($p<0.05$) between treatments for each volatile compound, as determined by one-way analysis of variance + Tukey's honestly significant different

[5] Below LOQ denotes that signal to noise ratio was <10:1 but ≥3:1

[6] Below LOD denotes that signal to noise ratio is <3:1

[7] Coelution denotes that two peaks coeluted thus the individual areas could not be determined

[8] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[9] D-12 Hexanal had a retention time of 6.17 minutes

3.4. Conclusions

Pea proteins processed at various initial moisture contents (31, 75, 162% d.b.) at a high microwave power (100W/g) for five minutes led to significantly higher ($p<0.05$) aldehydes, alcohols, ketones and total peak area. VMD-processing at various initial moisture contents also led to an increased hexanal content ($p<0.05$), but decreased ($p<0.05$) 3, 5 octadien-2-one content. Other compounds that were substantially higher after VMD-processing at various initial moisture contents were 2-heptanone and 2-pentyl furan. The higher energy output led to substantial lipid oxidation and other thermally-induced deterioration reactions, generating the aforementioned compounds. Processing pea proteins at higher initial moisture contents significantly decreased ($p<0.05$) volatiles compared to those with lower initial moisture contents. Processing pea protein at low specific power for a long time only decreased ($p<0.05$) 3, 5 octadien-2-one (eluted at 21.90 minutes) content, whereas processing at low specific energies showed slight, but significant ($p<0.05$) reductions in aldehydes, hexanal, and 3, 5 octadien-2-one (eluted at 21.90 and 23.90 minutes) content. Investigation of intermediate specific-energy level processes is warranted.

Chapter 4: Sensorial and functional analysis of Plant-Based Milk Alternatives Formulated with optimized VMD-Processed Pea Protein

4.1. Background

Flavour is one of the most important attributes that determines whether foods are accepted or rejected by consumers (Su and others 2020). Flavour perception is complex and is influenced by many factors such as flavour type, concentration and release (Wang and Arntfield 2017). The food matrix plays a large role in flavour perception as various constituents such as proteins, lipids and carbohydrates can interact with flavour compounds (Wang and Arntfield 2017). Pea proteins are known to have a very notable off-flavour, characterized by beany, vegetative, green or grassy notes (Schindler and others 2012; Damodaran and Arora, 2013). The off-flavours of pea proteins are primarily volatile compounds that can be detected using the nose (orthonasal olfaction) or though the mouth (retronasal olfaction) (Roland and others 2017; Trikusuma and others 2020). These individual volatile compounds can be identified and quantified using gas chromatography coupled with mass spectrometry (GC-MS); however, these methods have limitations. First, not all volatile compounds may be odour-active (Murat and others 2013). Even if a volatile compound is odour-active, it may not be detectable, let alone recognizable, depending on the threshold (Rowe 2004). Volatile compound data may not be representative to how a person perceives the product; for instance, the rate of volatile release, matrix effect and flavour balance are factors that may affect perception (Baek and others 1999). Therefore these findings must be validated with sensory evaluation. Sensory evaluation is a scientific discipline that tries to assess and interpret how people respond to foods as perceived through the five senses (Stone and Sidel 2004).

In sensory evaluation, descriptive analysis (DA) is a powerful tool where trained panelists are treated as analytical instruments to accurately determine the magnitude of perceived sensory attributes in food products (Andrade de Aguiar and others 2019). The most well-known methodology for DA is quantitative descriptive analysis (QDA) (Stone and others 2012). Another widely used methodology is the Spectrum™ method, which is similar to QDA in concept, design and analysis, but is more extensive in training (Stone and others 2012). Several hours of training per day for a period of fourteen weeks may be required (Stone and others 2012).

In Chapters 2 and 3, the effect of vacuum microwave dehydration parameters (initial moisture content, vacuum level, microwave power and process time) on protein functionality and volatile concentrations was assessed. In this chapter, pea protein was subjected to an optimized VMD-process that aimed to minimize the off-flavour and retain protein functionality. Although it was found that vacuum microwave dehydration may decrease the peak area of certain volatiles, it may also generate new chemical compounds. However, volatile analysis may not elucidate whether these volatile compounds bound to the proteins, were released or interacted with other moieties, therefore it is unknown how these changes may actually be perceived (Su and others 2020). Therefore, it is imperative to gain a comprehensive understanding of the flavour profile via descriptive analysis.

4.2. Materials and Methods

4.2.1. Materials

Two pea protein samples (72.7 and 76.2% crude protein, 6.7 and 7.1% ash) were acquired from Daiya Foods Inc. (BC, CA). Reagents for functionality and volatile analyses can be found in sections 2.2.1. and 3.2.1., respectively.

4.2.2. Research Design

Three VMD-processes were developed based on the data from Chapters 2 and 3. These VMD-processes were employed on two pea proteins from different suppliers. The VMD-process parameters for these three processes can be found in Table 10. In this set of experiments, the following samples: $M_{75}P_{100}V_{200}T_{2}$–1, $M_{162}P_{100}V_{200}T_{2.5}$–1, $M_{425}P_{100}V_{200}T_{2}$–1, $M_{75}P_{100}V_{200}T_{2}$–2, $M_{162}P_{100}V_{200}T_{2.5}$–2, $M_{425}P_{100}V_{200}T_{2}$–2, where $M_aP_bV_cT_d$–e represents experiments conducted on pea protein with initial moisture content a% d.b. at a microwave power of b W/g and a vacuum level of c Torr for a process time of d minutes and e represents which pea protein was used as a starting material were analyzed for functionality and volatile concentrations. Only pea protein 2 processed at an initial moisture content of 162% d.b. at 100W/g and 200 Torr for 2.5 minutes ($M_{162}P_{100}V_{200}T_{2.5}$–2) was selected for descriptive analysis, while untreated pea protein 2 acted as the control. Detailed justification on why this sample was selected is discussed in Section 4.3.10.

4.2.3. Functionality

Evaluation of functional properties was done according to sections 2.2.2. to 2.2.14.

4.2.4. Volatile Analysis

SPME GC-MS was done according to section 3.2.2. to 3.2.5.

4.2.5. Descriptive Analysis of Pea Protein Solutions

The flavour profile of pea protein solutions were characterized using a modified Spectrum™ Method. The trained panel was composed of 8 panelists. All were staff members of Applied Consumer and Clinical Evaluations International and have been trained for flavor profiling of pea protein products. The trained panel was trained using the reference standards outlined in Appendix C (Table 19). 120 mL 5% pea protein solutions were served in a transparent 7 oz. lidded plastic cup at room temperature, as shown in Figure 6. Samples were coded with a random three-digit code and served in a randomized order. All samples were swallowed, not expectorated.

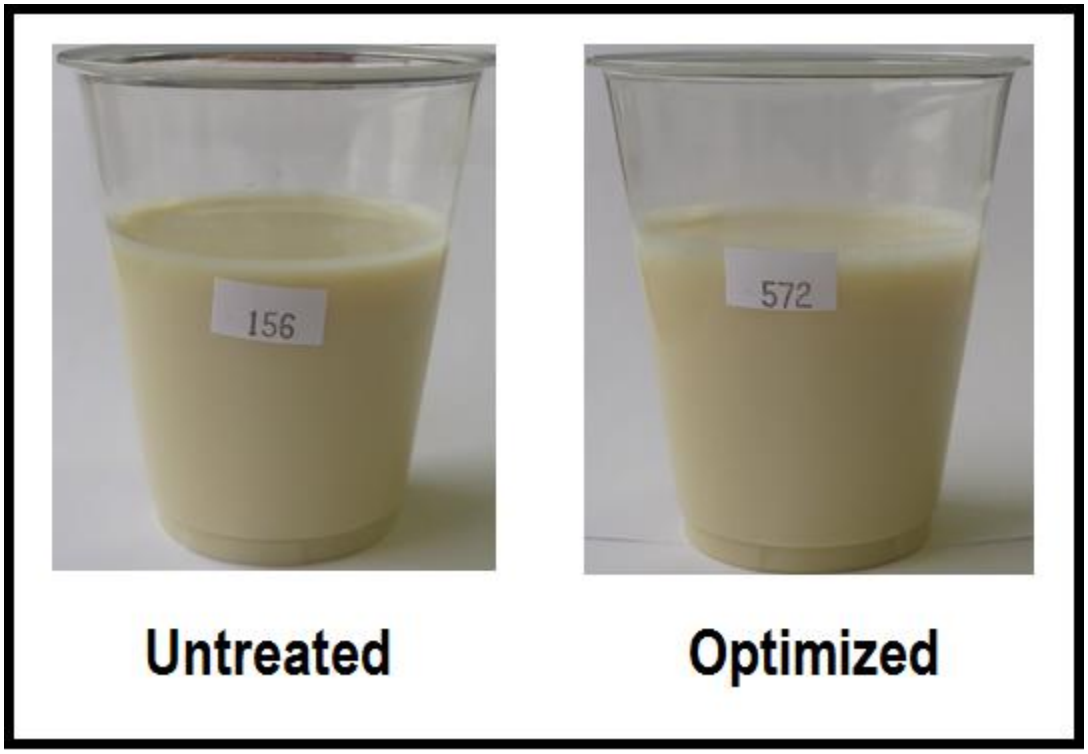

Figure 6. Pea protein solutions (120 mL, 5%) served at room temperature in 7 oz. plastic cups with three-digit randomized codes to prevent bias

All evaluations were performed in duplicate, once in the afternoon and once in the following morning. The intensity of each attribute was assessed using a 15-point continuous scale. Each point on the modified Spectrum™ Method scale was identified with a verbal descriptor Appendix C (Table 20). The

following modalities were assessed: appearance, aroma, flavour, texture and aftertaste. Note that flavour in this case encompasses taste and retronasal olfaction. For evaluation of appearance attributes, both samples were placed side by side and were evaluated in consensus. Panelists were instructed to evaluate aroma attributes by taking three short, shallow sniffs. For flavour and texture attributes, panelists were instructed to take two to three sips prior to evaluation. Lastly, after-taste attributes were evaluated 30 seconds after texture-related attributes were assessed.

4.2.6. Statistical Analysis

All functionality and volatile analyses were done in triplicate. Levene's test was used to determine whether the assumption of of homoscedasticity was met. Functionality and volatile data was analyzed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test as a post-hoc test. Statistical significance was defined at $p<0.05$. For descriptive analysis, ANOVA was conducted using a two-factor model with interaction, evaluating the main (sample, panelist) and interaction (panelist × sample) effects. Statistical analysis was conducted using Minitab 19 (Minitab Inc., PA, USA) and Microsoft Excel 2010 with Real Statistics Resource Pack (Redmond, WA, U.S.A.).

Table 10. Summary of VMD process parameters (initial moisture content, specific power, vacuum level and process time) and obtained water activity for three processes that were implemented on two pea proteins

Pea Protein	Sample ID	Initial Moisture (% d.b.)	Vacuum Level (Torr)	Specific Power (W/g)	Process Time (mins)
1	$M_{75}V_{200}P_{100}T_{2}$–1	75	200	100	2
	M_{162} $V_{200}P_{100}T_{2.5}$–1	162	200	100	2.5
	M_{425} $V_{200}P_{100}T_{2}$–1	425	200	100	2
2	M_{75} $V_{200}P_{100}T_{2}$–2	75	200	100	2
	M_{162} $V_{200}P_{100}T_{2.5}$–2	162	200	100	2.5
	M_{425} $V_{200}P_{100}T_{2}$–2	425	200	100	2

[1] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

4.3. Results and Discussion

4.3.1. Optimized VMD-Processed Pea Protein

Three VMD processes were developed: processing at an initial moisture content of 75, 162 and 425% d.b. at 100W/g and 200 Torr for 2, 2.5 and 2 minutes, respectively. VMD-processes were implemented on two pea proteins to determine if VMD processes were reproducible across different pea proteins. Although employing a specific power of 10W/g seemed to be the most logical, as supported by our previous findings, the long process time, coupled with low sample throughput may make any VMD-processes unfeasible for processing adequate amounts of pea protein from an economic perspective, hence 100W/g was employed. The process time that was proposed was the lowest number of 30-second increments that led to a final product resembling powder rather than a slurry, which was actually 1.5, 2 and 1.5 minutes, respectively. However, the products of these three proposed processes led to water activities above 0.6, some even above 0.9, where most microorganisms can thrive (Ijabadeniyi and Pillay 2017). Although the resultant pea protein could be incorporated into formulations right away, it is more economically feasible to produce shelf-stable pea protein. Hence, a balance between having a reasonable specific energy and an acceptable final water activity was needed. After testing multiple process times, it was determined that 2, 2.5 and 2 minutes achieved this compromise. Upon initial observation, the new pea protein 2 had a cleaner aroma profile than pea protein 1, but its soluble protein content and functional properties were inferior. Detailed comparisons are found later in this chapter.

4.3.2. Water Activity and Moisture Content of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

Water activity of pea protein 1 after VMD-processing did not differ ($p \geq 0.05$) from untreated pea protein, as shown in Table 11. The two pea proteins subjected to the same VMD-processes led to significant differences ($p < 0.05$) in water activity, indicating that each food should have a specific process due to slightly different matrices. The water activities of the untreated pea proteins did not differ ($p \geq 0.05$), as they were 0.26 and 0.30, respectively. However, pea protein 2 after VMD-processing had much higher water activities compared to their pea 1 counterparts. Pea protein 2 (Mi = 425% d.b.) had a water activity of 0.85, which was above the critical water activity value of 0.6, meaning it was not shelf-stable as many pathogens can still grow. The moisture content of VMD-processed pea proteins 1 and 2

ranged from 4-11% and 9-27% d.b., respectively (Table 11). Evidently, the matrix effect is still notable even in very similar food matrices and processes should be designed specifically for each food material.

Table 11. Water activity and final moisture content (% d.b.) of two pea proteins subjected to three different VMD processes (low-vacuum, high-specific power, medium-level specific energy processes)

Sample ID	Water activity	Final Moisture (% d.b.)
Untreated–1	0.26 ± 0.06 [c]	4.91 ± 0.10 [d]
$M_{75}V_{200}P_{100}T_{2}$–1	0.23 ± 0.07 [c]	3.85 ± 0.20 [d]
M_{162} $V_{200}P_{100}T_{2.5}$–1	0.24 ± 0.08 [c]	4.25 ± 0.01 [d]
M_{425} $V_{200}P_{100}T_{2}$–1	0.35 ± 0.04 [bc]	11.09 ± 2.08 [bc]
Untreated–2	0.30 ± 0.06 [bc]	5.19 ± 0.09 [d]
$M_{75}V_{200}P_{100}T_{2}$–2	0.31 ± 0.08 [bc]	9.10 ± 0.28 [c]
M_{162} $V_{200}P_{100}T_{2.5}$–2	0.50 ± 0.10 [b]	13.91 ± 0.09 [b]
M_{425} $V_{200}P_{100}T_{2}$–2	0.85 ± 0.09 [a]	27.09 ± 1.99 [a]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

4.3.3. Volatile Analysis of VMD-Processed Pea Proteins

27 compounds were identified in pea protein 1 samples, while 24 compounds were identified in pea protein 2 samples, as shown in Table 12. The two compounds with the highest area in both untreated and VMD-processed pea proteins were hexanal and 2-pentyl furan. Hexanal has a grassy, green odour, while 2-pentyl furan has a green bean or buttery odour (Flavornet 2004; Roland and others 2017).

The total area of untreated pea protein was 23, while the total areas after VMD-processing pea protein 1 were significantly lower, ranging from 7-9. Interestingly, the total area of untreated pea protein 2 was

7.6, which was significantly lower ($p<0.05$) than that of untreated pea protein 1 – 23.3. The total area after VMD-processing pea protein 2 ranged from 1.5 to 2.6, but no differences were found ($p\geq0.05$), even when compared to the control, possibly due to the large variation among lots. The two pea proteins were from different suppliers so they may have different harvesting, processing, dehydration, handling and storage practices that may account for these differences. According to Jakobsen and others (1999), hexanal is generated more readily in smaller peas than larger peas because smaller peas are more susceptible to mechanical damage. Differences in cultivars could also affect volatile composition (Jakobsen and others 1999). It was also mentioned that most off-flavours were generated via enzymatic reactions within a few hours after harvest, so the time from harvest to blanching may vastly affect volatile composition (Jakobsen and others 1999). Furthermore, differences in composition may also affect volatile composition. If pea protein 1 has more unsaturated lipids, it will be more susceptible to volatile generation via lipoxygenases or other oxidation-related deterioration reactions (Ma and others 2016; Roland and others 2017; Zha and others 2019). Another factor could be storage time. It was unknown when each lot of each pea protein was packaged.

All VMD-processed samples were also significantly lower ($p<0.05$) in hexanal compared to untreated pea protein 1. However, only processing pea protein 2 at an initial moisture content of 162 and 425% d.b. at 100W/g for 2.5 and 2 minutes, respectively, led to hexanal concentrations that were significantly lower ($p<0.05$) than their control. Even though hexanal concentration can be heavily reduced, it could still impart a negative grassy aroma due to its extremely low odour threshold – 4.5 ppb (Schindler and others 2012).

In terms of aldehydes, alcohols, alkane/alkenes, and furans, all VMD-processed samples had significantly lower ($p<0.05$) areas compared to the untreated pea protein 1. For total ketones, the untreated pea proteins did not differ significantly ($p\geq0.05$), but all other VMD-processed samples had significantly lower ($p<0.05$) concentrations compared to untreated pea protein 1. However, for pea protein 2, total alcohols, ketones, alkane/alkenes and furans did not differ ($p\geq0.05$) after VMD-processing.

Alkane/alkenes and alcohols had very low concentrations in most VMD-processed samples. Alkane/alkenes tend to bind to proteins via van der Waals forces (Wang and Arntfield 2017). Even though VMD-processing pea protein 2 had no effect on alkane/alkenes, they possess weak odours and do not contribute much to pulse off-flavours (Ma and others 2016).

The compounds 1-pentanol, 1-hexanol and 1-octen-3-ol had concentrations that were below the limits of quantification or detection after almost every VMD process. However, these alcohols can still impart their unpleasant odours due to their low odour thresholds – 4000, 500 and 1 ppb, respectively (Schindler and others 2012; Trikusuma and others 2020). The compound 1-pentanol has a fruity or floral odour, while 1-hexanol has a very intense green odour (Schindler and others 2012; Trikusuma and others 2020). The compound 1-octen-3-ol has a mushroom-like, earthy or burnt aroma and has an extremely low threshold of 1 µg/L (1 ppb) (Schindler and others 2012; Trikusuma and others 2020). In all VMD-processed pea protein 2 samples, alcohols were almost driven off completely as the alcohols content ranged from 0.01 to 0.02 after VMD-processing. One reason for why many of the identified alcohols were below the limit of quantification was because the enzyme responsible for the generation of many alcohols, alcohol oxidoreductase, is heat labile compared to other deterioration enzymes (Ma and others 2016). Furthermore, alcohols bind to proteins primarily via hydrogen bonds, which are more easily destabilized compared to the interactions of other functional groups (Wang and Arntfield 2017). For instance, aldehydes and ketones primarily interact with proteins via hydrophobic interactions, while the former can also irreversibly covalently bond with proteins, decreasing their volatility (Wang and Arntfield 2017; Xu and others 2019). As ketones have an R-group, while aldehydes only have a hydrogen atom, this steric hindrance may reduce the affinity to bind to proteins, explaining why aldehydes tend to be more retained than ketones (Wang and Arntfield 2017). The greater percent reduction in alcohols and the lower percent reduction in aldehydes may be attributed to conversion into aldehydes via oxidation reactions (Kunjapur and Prather 2015).

The compounds 3, 5 octadien-2-one (eluted at 21.86 and 23.6, respectively) fell below the limits of quantification and detection, especially after processing at higher initial moisture contents. There is currently little to no literature on the odour threshold of 3,5 octadien-2-one in water, but its odour threshold in oil ranges from 200-300 µg/kg (200-300 ppb) (Belitz and others 2009).

Over half of all identified compounds were either below the limits of quantification and detection after VMD-processing pea protein 2. Again, the trend where increasing initial moisture content facilitates effective removal of volatiles was seen. All but six compounds were below the limits of quantification and detection when the initial moisture content was 425%. Although these results were very promising, it is still imperative to validate the extent of the volatile reductions with descriptive analysis.

Table 12. Identified volatile compounds in three VMD-processed pea protein 1 – low-vacuum, high-specific power, medium-level specific energy processes, as determined by SPME GC-MS

Identity	RT (mins)	Area/Area IS							
		Untreated–1	$M_{75}V_{200}P_{100}T_{2}$–1	$M_{162}V_{200}P_{100}T_{2.5}$–1	$M_{425}V_{200}P_{100T2}$–1	Untreated–2	$M_{75}V_{200}P_{100}T_{2}$–2	$M_{162}V_{200}P_{100}T_{2.5}$–2	$M_{425}V_{200}P_{100}T_{2}$–2
Total	**N/A**	**23.31 ± 4.05** [a]	**9.47 ± 0.74** [b]	**7.27 ± 1.04** [bc]	**7.23 ± 1.95** [bc]	**7.62 ± 4.52** [bc]	**2.58 ± 1.50** [bc]	**2.00 ± 1.45** [c]	**1.53 ± 1.05** [c]
Aldehydes	N/A	6.05 ± 0.74 [a]	3.32 ± 0.34 [b]	2.77 ± 0.19 [bc]	2.81 ± 0.65 [bc]	3.30 ± 1.31 [b]	1.47 ± 0.59 [bc]	1.19 ± 0.62 [c]	0.91 ± 0.42 [c]
Alcohols	N/A	0.66 ± 0.42 [a]	0.13 ± 0.08 [b]	0.07 ± 0.06 [b]	0.02 ± 0.04 [b]	0.12 ± 0.10 [b]	0.02 ± 0.02 [b]	0.01 ± 0.02 [b]	0.01 ± 0.01 [b]
Ketones	N/A	4.31 ± 1.96 [a]	1.01 ± 0.29 [b]	0.97 ± 0.29 [b]	1.19 ± 0.69 [b]	1.67 ± 1.51 [ab]	0.30 ± 0.31 [b]	0.19 ± 0.25 [b]	0.06 ± 0.06 [b]
Alkane/Alkenes	N/A	0.89 ± 0.24 [a]	0.36 ± 0.09 [b]	0.19 ± 0.04 [b]	0.16 ± 0.03 [b]	0.27 ± 0.18 [b]	0.07 ± 0.04 [b]	0.04 ± 0.04 [b]	0.05 ± 0.02 [b]
Furans	N/A	11.37 ± 1.62 [a]	4.66 ± 0.72 [b]	3.27 ± 0.56 [bc]	3.03 ± 0.57 [bc]	2.26 ± 1.46 [bcd]	0.70 ± 0.55 [cd]	0.56 ± 0.53 [d]	0.51 ± 0.54 [d]
Pentane	2.95	0.20 ± 0.08	0.08 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.03 ± 0.02	0.02 ± 0.01[2]	0.02 ± 0.02[2]	0.02 ± 0.01
Methanethiol	3.04	0.07 ± 0.03[2]	Below LOD	Below LOD	Below LOD	Below LOD	Below LOQ	Below LOD	Below LOD
Heptane	3.13	0.14 ± 0.05	0.07 ± 0.03	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01[2]	Below LOQ
Hexane, 2,4-dimethyl	3.41	0.29 ± 0.09	0.18 ± 0.07	0.06 ± 0.00	0.06 ± 0.02	0.10 ± 0.04	0.05 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
Furan, 2-ethyl	4.12	0.26 ± 0.03	Below LOD	Below LOQ	Below LOQ	0.13 ± 0.08	Below LOD	Below LOD	Below LOD
Pentanal	4.51	0.20 ± 0.09	0.09 ± 0.03	0.07 ± 0.01	0.06 ± 0.02	0.13 ± 0.09	0.04 ± 0.02	0.03 ± 0.02	Below LOQ
Furan, 2-ethyl, 5-methyl	5.19	0.05 ± 0.01	Below LOQ	Below LOQ	Below LOQ	Below LOD	Below LOD	Below LOD	Below LOD
Toluene	5.30	0.05 ± 0.01[2]	Below LOQ	Below LOD	Below LOQ	0.09 ± 0.07[2]	Below LOQ	Below LOQ	Below LOQ
Hexanal [3]	***6.27***	***5.34 ± 0.50*** [a]	***3.07 ± 0.32*** [b]	***2.59 ± 0.16*** [bcd]	***2.66 ± 0.68*** [bcd]	***2.99 ± 1.13*** [bc]	***1.39 ± 0.52*** [cde]	***1.13 ± 0.54*** [de]	***0.87 ± 0.37*** [e]
2-n-butyl furan	7.14	0.12 ± 0.01	0.10 ± 0.01[2]	Below LOQ	Below LOQ	Below LOD	Below LOD	Below LOD	Below LOD
2-Heptanone	9.17	2.27 ± 0.63	0.55 ± 0.16	0.68 ± 0.12	1.04 ± 0.77	0.53 ± 0.39	0.09 ± 0.07	0.12 ± 0.16[2]	0.04 ± 0.04
Furan, 2-pentyl	10.44	10.94 ± 1.56	4.56 ± 0.63	3.24 ± 0.51	2.99 ± 0.57	2.09 ± 1.32	0.69 ± 0.53	0.55 ± 0.52	0.50 ± 0.51
1-Pentanol [3]	***11.41***	***0.09 ± 0.01*** [a]	***Below LOQ***	***Below LOQ***	***Below LOD***	***Below LOQ***	***Below LOD***	***Below LOD***	***Below LOD***
2-Octanone	12.98	0.09 ± 0.06	Below LOQ	Coelution	0.02 ± 0.00	Below LOQ	Below LOD	Below LOD	Below LOD
Octanal	13.09	0.16 ± 0.02	0.08 ± 0.00	Coelution	0.06 ± 0.02	0.08 ± 0.05	Below LOQ	Below LOQ	Below LOQ
2-Hexenal, 2-ethyl	14.74	Below LOQ	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD

1-Hexanol [3]	***15.39***	***0.48 ± 0.45*** [a]	***0.09 ± 0.08*** [a]	***Below LOQ***	***Below LOQ***	***Below LOQ***	***Below LOD***	***Below LOD***	***Below LOD***
Pentane, 1-nitro	16.41	Below LOQ	Below LOQ	Below LOD	Below LOQ	0.06 ± 0.04[2]	Below LOQ	Below LOD	Below LOD
2-Nonanone	17.18	0.37 ± 0.16	0.14 ± 0.03	0.13 ± 0.03	0.12 ± 0.01	0.11 ± 0.09	Below LOQ	Below LOQ	Below LOQ
Nonanal	17.33	0.17 ± 0.03	0.08 ± 0.1	0.07 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	Below LOQ	Below LOQ	0.03 ± 0.02
3-Octen-2-one	17.71	Below LOD	Below LOD	Below LOD	Below LOD	Below LOQ	Below LOD	Below LOD	Below LOD
2-Oxooctanoic acid	18.33	0.05 ± 0.03	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD	Below LOD
1-Octen-3-ol [3]	***19.19***	***0.16 ± 0.04*** [a]	***0.05 ± 0.01*** [b]	***Below LOQ***	***Below LOQ***	***0.09 ± 0.06*** [ab]	***0.03 ± 0.01*** [b]	***Below LOQ***	***Below LOQ***
Hexane, 1-nitro	20.64	Below LOQ	Below LOD	Below LOD	Below LOD	Below LOQ	Below LOD	Below LOD	Below LOD
Benzaldehyde	21.30	0.16 ± 0.08	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Below LOQ
2-Decanone	21.38	0.38 ± 0.16	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Below LOQ
3,5-Octadien-2-one [3]	***21.86***	***0.40 ± 0.37*** [a]	***0.08 ± 0.07*** [a]	***Below LOQ***	***Below LOQ***	***0.40 ± 0.32*** [a]	***0.04 ± 0.05***[2] [a]	***0.04 ± 0.03***[2] [a]	***Below LOD***
3,5-Octadien-2-one [3]	***23.86***	***0.74 ± 0.58*** [a]	***0.17 ± 0.13*** [a]	***0.11 ± 0.08*** [a]	***Below LOQ***	***0.89 ± 0.89***[2] [a]	***0.19 ± 0.18***[2] [a]	***0.10 ± 0.12***[2] [a]	***Below LOQ***

[1] results are reported as mean ± standard deviation (n=3)

[2] denotes that it is displayed as mean ± range (n=2) i.e. one replicate was below LOD or was coeluted

[3] denotes that there is extensive literature suggesting that this is one of the most characteristic off-flavour causing compounds in pea protein (Schindler and others 2012; Murat and others 2013; Ma and others 2016; Roland and others 2017; Fahmi and others 2019; Lan and others 2019; Zha and others 2019; Trikusuma and others 2020)

[4] superscript letters denote significant differences ($p<0.05$) between treatments for each volatile compound, as determined by one-way analysis of variance + Tukey's honestly significant different

[5] Below LOQ denotes that signal to noise ratio is <10:1

[6] Below LOD denotes that signal to noise ratio is <3:1

[7] Coelution denotes that two peaks coeluted thus the individual areas could not be determined

[8] "RT refers to retention time (minutes). "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

4.3.4. Soluble Protein Content of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

Soluble protein content of pea protein 1 after VMD-processing (Figure 7) was similar to the samples with the same initial moisture content that were outlined in Chapter 2. The only differences between said samples were the process time where those outlined in Chapter 2 were processed for five minutes, while these samples were processed for 2-2.5 minutes. This suggested that the loss in solubility occurs within the first few minutes and the last few minutes of processing do not affect solubility as much. This partially aligns with the results of Caprita and Caprita (2010) as they say that there is a marked decrease in solubility after 3 minutes of processing soybeans; however, the slight difference in process time may have been due to their sample weight used, which was not specified. Alternatively, it could also be due to differences in legumes. Soluble protein content in 0.01M, pH 7 potassium phosphate buffer and potassium phosphate buffer (0.1M, pH 8, 1mM EDTA can be found in Appendix C (Table C3). Pea protein 2 (111 mg/g dry matter) had inherently lower ($p<0.05$) soluble protein content than pea protein 1, approximately 60% of it. This may be due to differences in composition or pre-processing steps. Pea protein 2 may contain more insoluble fibre, decreasing its overall solubility. Also, since pea protein 2 was from a different supplier, their harvesting or dehydration process may have been more intense, leading to a more thermally-damaged product. After VMD-processing, the soluble protein content significantly decreased ($p<0.05$) to 30-46 mg/g dry matter. VMD-processed pea protein 2 samples did not differ significantly ($p\geq0.05$) from each other.

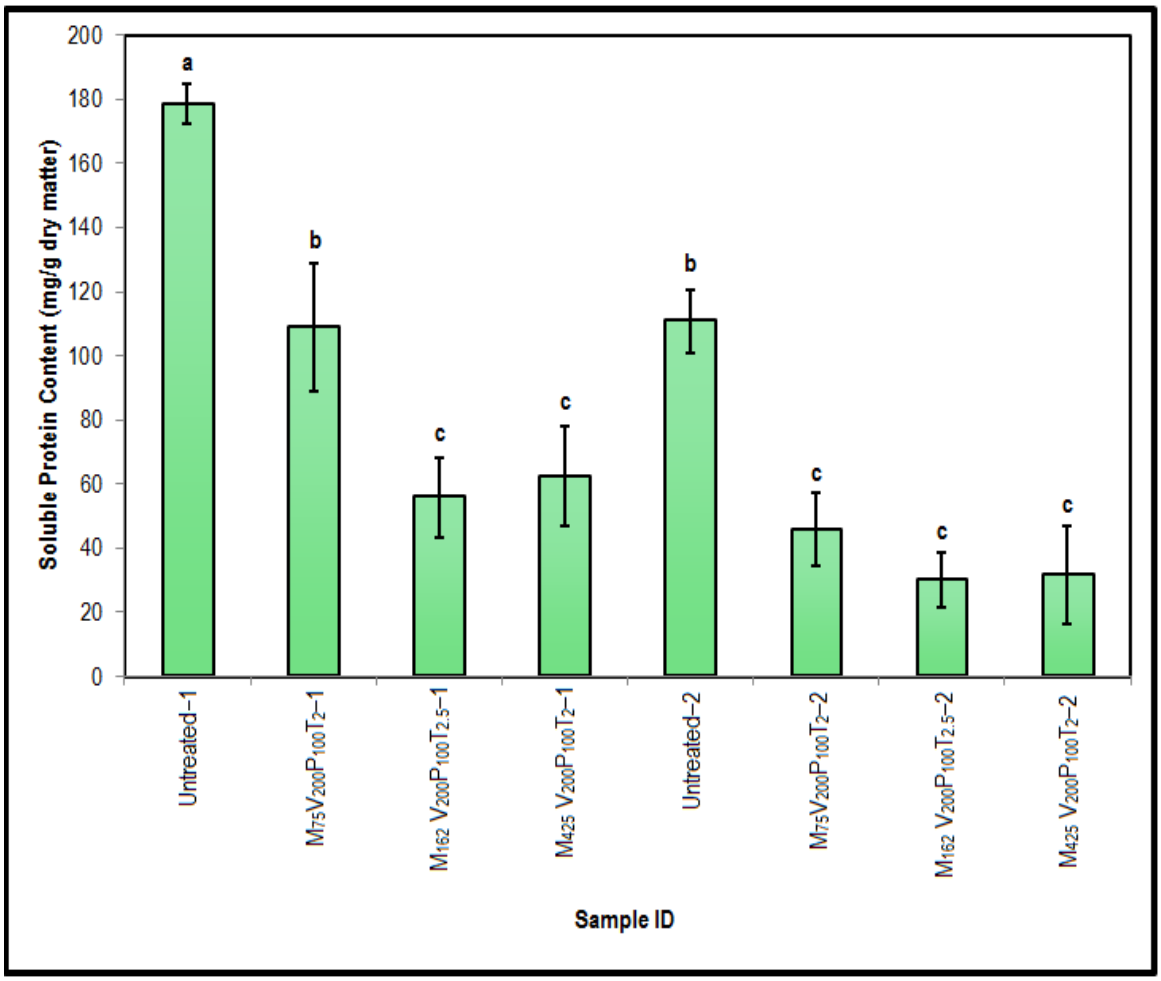

Figure 7. Soluble protein content of of two pea proteins subjected to three difference vacuum microwave dehydration processes (low-vacuum, high-specific power, medium-level specific energy processes)

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test

4.3.5. Emulsification Properties of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

Emulsifying activity index (EAI) and emulsifying stability index (ESI) of pea protein 1 after VMD-processing were very close to the indices of the samples with the same initial moisture content that were outlined in Chapter 2, as shown in Table 13. Untreated pea protein 1 had an EAI and ESI of 16

m^2/g dry matter and 27 minutes, respectively, while untreated pea protein 2 had an EAI and ESI of 11 m^2/g dry matter and 75 minutes, respectively. One reason for why pea protein 2 has a lower EAI and higher ESI than pea protein 1 is the differences in soluble protein content (Kim and others 2019). As expected, pea protein 1 after VMD-processing had significantly lower ($p<0.05$) EAIs than its untreated protein; however, EAI did not differ ($p\geq0.05$) between any pea protein 2 samples. This may be because the untreated pea protein 2 was already inherently poor at emulsifying oils, so not many differences are observed after processing. The samples with lower EAI tended to have higher ESI; however, due to the large variation, there were no significant differences ($p\geq0.05$) in ESI between any pea protein samples, whether treated or untreated.

Table 13. Emulsification properties, surface hydrophobicity and free sulfhydryl group content of two pea proteins subjected to three difference vacuum microwave dehydration processes (low-vacuum, high-specific power, medium-level specific energy processes)

Sample ID	EAI (m^2/g dry matter)	ESI (mins)	S_0 (a.u.)	Free Sulfhydryl Group (μmol/g soluble protein)
Untreated–1	15.93 ± 1.05 [a]	27.1 ± 0.6 [a]	21.3 ± 0.6 [a]	1.42 ± 0.26 [a]
$M_{75}V_{200}P_{100}T_2$–1	12.13 ± 0.94 [b]	67.9 ± 14.3 [a]	19.6 ± 1.4 [a]	1.24 ± 0.25 [a]
M_{162} $V_{200}P_{100}T_{2.5}$–1	10.88 ± 0.41 [b]	209.1 ± 116.3 [a]	17.6 ± 2.6 [a]	1.15 ± 0.19 [a]
M_{425} $V_{200}P_{100}T_2$–1	10.53 ± 0.13 [b]	206.7 ± 162.5 [a]	18.7 ± 0.9 [a]	1.23 ± 0.23 [a]
Untreated–2	11.26 ± 2.15 [b]	74.9 ± 45.1 [a]	16.9 ± 0.9 [a]	1.26 ± 0.03 [a]
$M_{75}V_{200}P_{100}T_2$–2	10.55 ± 0.58 [b]	217.1 ± 135.6 [a]	20.9 ± 3.0 [a]	1.17 ± 0.03 [a]
M_{162} $V_{200}P_{100}T_{2.5}$–2	10.80 ± 0.70 [b]	174.3 ± 35.9 [a]	20.0 ± 3.3 [a]	1.17 ± 0.04 [a]
M_{425} $V_{200}P_{100}T_2$–2	10.97 ± 0.94 [b]	317.2 ± 204.8 [a]	18.8 ± 6.0 [a]	1.18 ± 0.08 [a]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

4.3.6. Surface Hydrophobicity of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

Surface hydrophobicity did not differ ($p \geq 0.05$) between any pea protein samples, whether treated or untreated (Table 13). The surface hydrophobicity values ranged from 17-21, while the samples with the same initial moisture content outlined in Chapter 2 ranged from 13-20, also not showing any differences ($p \geq 0.05$). Again, this may be due to our samples already reaching the "plateau stage" in surface hydrophobicity as it was processed prior to our receiving it (Wang and others 2014). It was not known what dehydration method was used so if a process that led to a lot of thermal deterioration was employed, any further heat treatment would not easily induce any more increases in surface hydrophobicity.

4.3.7. Free Sulfhydryl Group Content of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

As shown in Table 13, the free sulfhydryl group content did not differ ($p \geq 0.05$) between any pea protein samples, whether treated or untreated. Pea protein 1 ranged from 1.15-1.42 µmol/g soluble protein, while pea protein 2 ranged from 1.17-1.26 µmol/g soluble protein. Alonso and others (2000) analyzed the free sulfhydyl contents in extruded pea proteins and reported that there was a decrease in free sulfhydryl groups due to oxidation or degradation of cysteine residues.

4.3.8. Colour Analysis of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

The colour parameters of VMD-processed pea proteins (Table 14) were very similar to samples with the same initial moisture content outlined in Chapter 2. Both pea proteins were similar in all Hunterlab colour attributes. Both pea protein VMD-processed at an initial moisture content of 75% d.b. did not differ significantly ($p \geq 0.05$) from their respective control in terms of lightness (L*) and the b* coordinate value. However, samples with VMD-processed at an initial moisture content of 162 and 425% d.b. were significantly different ($p < 0.05$) from the sample processed with an initial moisture content of 75%, and thus the controls, in all colour parameters. This was expected as it was mentioned previously in Chapter 2 that after a certain initial moisture content, denaturation and Maillard browning both occur extensively, explaining the differences in colour. For each VMD process, the colour parameters between pea proteins did not differ significantly ($p \geq 0.05$) from each other.

Table 14. Hunterlab colour parameters (lightness (L*), a* coordinate, b*coordinate and total colour difference (ΔE)) and chemically available lysine content of two pea proteins subjected to three difference vacuum microwave dehydration processes (low-vacuum, high-specific power, medium-level specific energy processes)

Sample ID	L*	a*	b*	ΔE	Chemically Available Lysine (mg/g dry matter)
Untreated–1	79.60 ± 0.56 [a]	2.44 ± 0.15 [c]	22.87 ± 0.89 [c]	[4]	28.5 ± 4.2 [a]
$M_{75}V_{200}P_{100}T_{2}$–1	74.81 ± 1.91 [a]	3.39 ± 0.47 [b]	25.96 ± 1.04 [bc]	5.79 ± 2.09 [c]	19.7 ± 3.8 [ab]
M_{162} $V_{200}P_{100}T_{2.5}$–1	65.50 ± 3.37 [b]	6.29 ± 0.32 [a]	33.06 ± 0.98 [a]	17.88 ± 2.50 [b]	12.3 ± 2.5 [bc]
M_{425} $V_{200}P_{100}T_{2}$–1	60.71 ± 1.54 [bc]	6.59 ± 0.29 [a]	34.26 ± 1.21 [a]	22.46 ± 0.79 [ab]	14.2 ± 3.5 [bc]
Untreated–2	80.01 ± 0.70 [a]	2.47 ± 0.10 [c]	23.44 ± 2.46 [bc]	[4]	15.9 ± 3.4 [bc]
$M_{75}V_{200}P_{100}T_{2}$–2	74.55 ± 0.26 [a]	3.84 ± 0.21 [b]	27.27 ± 2.48 [b]	8.19 ± 1.56 [c]	9.0 ± 3.1 [c]
M_{162} $V_{200}P_{100}T_{2.5}$–2	66.29 ± 1.24 [b]	6.44 ± 0.22 [a]	33.43 ± 1.30 [a]	18.62 ± 1.70 [b]	7.2 ± 2.7 [c]
M_{425} $V_{200}P_{100}T_{2}$–2	54.68 ± 5.11 [c]	6.25 ± 0.48 [a]	32.46 ± 0.51 [a]	28.06 ± 4.74 [a]	8.3 ± 1.3 [c]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test

[3] "M" refers to initial moisture content (d.b.), "P" refers to specific power (W/g), "V" refers to vacuum level (Torr), while "T" refers to process time (minutes)

[4] ΔE is the total colour difference relative to the untreated pea protein

4.3.9. Chemically Available Lysine Content of Two Pea Proteins Subjected to Three Medium-Level Energy VMD-Processes

As shown in Table 14, pea protein 2 had a chemically available lysine content of 16 mg lysine/g dry matter, which was significantly lower ($p<0.05$) than pea protein 1, which had 28 mg lysine/g dry matter. This may be due to differences in pea cultivar or different stages of seed development, which may lead to differences in legumin to vicilin ratio (Lam and others 2018). Vicilin typically has a higher lysine content than legumin, so pea protein 2 may have a higher legumin to vicilin ratio (Lam and others 2018). The available lysine content of pea protein 1 with initial moisture contents greater than 162% d.b. were significantly lower ($p<0.05$) than their respective controls, whereas pea proteins with an initial moisture content of 75% d.b. were not significantly different ($p\geq0.05$) from their controls. As expected, the lower available lysine contents can be attributed to the enhanced Maillard browning at higher initial moisture contents. It is noteworthy to point out that this browning and marked decrease in chemically available lysine can occur even after 2 or 2.5 minutes at high specific power. In Chapter 2, samples were processed in the same VMD conditions, but for five minutes and had similar chemically available lysine contents, suggesting that most of the lysine degradation occurs within the first few minutes. Although the chemically available lysine contents of pea protein 2 after VMD-processing were lower than that of untreated protein, they did not differ significantly ($p\geq0.05$). However, more comprehensive nutritional measures should be conducted as chemically available lysine is just a crude indicator of nutrition.

4.3.10. Selection of Optimized VMD-Processed Pea Protein

Processing pea protein 2 with an initial moisture content of 162% d.b. at 100W/g at 200 Torr for 2.5 minutes ($M_{162}P_{100}V_{200}T_{2.5}$–2) was selected to be evaluated via descriptive analysis for a myriad of reasons. Its low volatile compound concentration and low-moderate functionality warrant further investigation. Pea protein 2 samples had lower volatile concentrations ($p<0.05$) than pea protein 1 samples after VMD-processing. After VMD-processing ($M_{162}P_{100}V_{200}T_{2.5}$–2), 1-pentanol and 1-hexanol were below the limit of detection, while 1-octen-3-ol was below the limit of quantification. Processing at an initial moisture content of 162% d.b. decreased ($p<0.05$) hexanal and total aldehyde concentrations, while processing at an initial moisture content of 75% d.b. led to hexanal and total aldehyde concentrations that did not differ ($p\geq0.05$) from the control. Also, VMD-processing at 75% d.b. still led

to a 1-octen-3-ol concentration that was still above the limit of quantification. VMD-processed pea protein 2 led to significantly ($p<0.05$) lower soluble protein, but did not differ ($p\geq0.05$) in EAI, ESI, surface hydrophobicity or free sulfhydryl group content.

Although processing pea protein with an initial moisture content of 425% d.b. led to an aroma profile very similar to when initial moisture content was 162% d.b., the former had a significantly higher ($p<0.05$) total colour difference from the control, which may not be perceived so positively by consumers. Furthermore, processing at an initial moisture content of 425% d.b. led to a final water activity is 0.85, making it very susceptible to pathogenic growth, spoilage and deteriorative reactions, while processing at an initial moisture content of 162% d.b. led to a final water activity below the critical limit of 0.6. Furthermore, processing at an initial moisture content 425% d.b. is not as economically feasible because more water needs to be added, just to be dehydrated. Also, the yield is much lower, leading to lower throughput. For these reasons, processing pea protein 2 at an initial moisture content of 162% d.b. at 100W/g and 200 Torr for 2.5 minutes ($M_{162}P_{100}V_{200}T_{2.5}$–2) was selected for descriptive analysis to more comprehensively understand the flavour profile. For the remainder of this chapter, this pea protein sample ($M_{162}P_{100}V_{200}T_{2.5}$–2) was deemed “optimized pea protein”, while the control was deemed “untreated pea protein”.

4.3.11. Descriptive Analysis

Five sensory modalities (appearance, aroma, flavour, texture and aftertaste) were assessed. Several sensory attributes did not meet the ANOVA assumption of homoscedasticity; however, upon closer examination, the observed magnitudes of these differences in variance were within the ranges that are tolerated in sensory evaluation. Therefore, ANOVA can still be used for this sensory experiment due to its robustness against violation of its assumptions (Bathke 2004; Wang and Arkritis 2005; Marin-Galiano and Kunert 2006). The attribute intensities and p-values (sample, panelist, sample x panelist) of untreated and optimized pea protein are shown in Table 15. Prior to interpreting the sample effects, the interaction effects (sample x panelist) were evaluated for each of the sensory attributes. Since all were non-significant ($p\geq0.05$), this confirmed that the attributes were being described consistently by the panelists and therefore interpretation of the main effects can be done. Four attributes differed significantly ($p<0.05$) in aroma, while three attributes differed significantly in flavour ($p<0.05$), all of which can be summarized in Figure 8.

4.3.12. Appearance

As appearance was conducted in consensus, a post-hoc test could not be conducted. These attributes only differed by 0.5 units or less, so they may not be noticed by ordinary panelists. The optimized pea protein had a slightly higher degree of foaming, similar chroma and a slightly lower colour intensity, compared to untreated pea protein.

4.3.13. Aroma

The overall aroma intensity of optimized pea protein was significantly higher ($p<0.05$) than that of untreated pea protein, which was unexpected; however, it cannot be concluded that intensity implies off-flavour intensity, but rather the total intensity. As classified by the modified Spectrum™ Method, both of these samples have a low-medium overall aroma intensity. This may be explained because since the pea proteins were dissolved in nothing but water, flavour retention is low as the product is low in lipids (Guichard 2002). Optimized pea protein had significantly lower ($p<0.05$) ratings of "green/grassy" (2.0 versus 2.4) and "raw/beany" (2.2 versus 2.4). However, there were no significant differences ($p\geq0.05$) in the ratings of "beany/vegetative" (2.9 versus 2.6). According to Roland and others (2017) & The Good Scents Company (20198), furan, 2-pentyl and hexanal impart a beany odour, while furan, 2-ethyl can impart a beany odour. The literature is somewhat conflicting as it also describes furan, 2-ethyl as chemical, bready, malty and coffee-like (The Good Scents Company 2018; Li and others 2019a). The reduction in "raw/beany" aroma aligns with our findings as the hexanal and 2-pentyl furan concentrations were much lower in optimized pea protein. Also, furan, 2-ethyl was below the limit of detection in optimized pea protein. In terms of green/grassy aroma, hexanal and 1-hexanol are the main contributors of this attribute, but pentanal, 2-nonanone and nonanal also contribute to green/grassy aroma (Flavornet 2004; Schindler and others 2012; Roland and others 2017). The hexanal concentration in optimized pea protein was 37% that of the untreated pea protein. Meanwhile, 1-hexanol was already below the limit of quantification in the control sample. However, it was below the limit of detection after VMD processing. Pentanal seemed to slightly decrease after VMD treatment, while both 2-nonanone and nonanal were below the limit of quantification in optimized pea protein. It was unknown whether the perceived beaniness in the aforementioned compounds were more of a "raw" beaniness or a "vegetative" beaniness as there are inconsistencies in descriptions in the literature. Interestingly, panelists detected a very weak goaty or caproic acid note in the optimized pea protein (0.5), where none was detected in the untreated pea protein. Caproic acid, also known as

hexanoic acid, is responsible for a goaty note, but was not identified via GC-MS in any of the pea samples. This may be due to the low odour threshold of caproic acid – 1 ppb (Cometto-Muñiz and Abraham 2010). According to Xu and others (2017b), it also imparts a sour, sweaty, cheese or fatty aroma. Hexanoic acid is produced via hexanal oxidation (Xu and others 2017b). As oxygen is still present in the vacuum chamber, albeit at low concentrations, perhaps the microwave energy catalyzed this oxidation reaction, producing trace amounts of hexanoic acid.

Table 15. Summary of attribute intensities and p-values of a two-way analysis of variance with replication between samples and panelists

Attribute	Intensity [1]		p-value		
	Untreated	Optimized	Panelist (P)	Sample (S)	P x S
Appearance [2]					
Amount of Foam	1.3	1.5	N/A	N/A	N/A
Chroma	2.5	2.5	N/A	N/A	N/A
Colour Intensity	4.8	4.3	N/A	N/A	N/A
Aroma					
Beany/Vegetative	2.6 ± 0.5	2.9 ± 0.3	0.957	0.114	0.816
Cardboard	0.7 ± 0.3	0.8 ± 0.4	0.426	0.059	0.836
Earthy/Mushroomy	0.6 ± 0.3	0.8 ± 0.3	0.059	0.332	0.466
Goaty/Caproic Acid	0.0 ± 0.0	0.5 ± 0.5	0.397	**0.004**	0.843
Green/Grassy	2.4 ± 0.5	2.0 ± 0.2	0.991	**0.018**	0.991
Overall Aroma Intensity	4.5 ± 0.2	4.8 ± 0.3	1.000	**0.013**	1.000
Raw/Beany	2.4 ± 0.3	2.2 ± 0.3	**0.001**	**0.001**	0.176
Flavour					
Beany/Vegetative	2.3 ± 0.7	2.3 ± 0.7	0.797	0.836	0.998
Bitter	1.3 ± 0.6	1.2 ± 0.4	0.188	0.393	0.910
Cardboard	1.1 ± 0.2	1.1 ± 0.5	0.097	0.789	0.792
Chalky	2.7 ± 0.3	2.9 ± 0.3	0.279	**0.033**	0.332
Earthy/Mushroomy	0.8 ± 0.4	0.9 ± 0.4	0.717	0.837	0.643
Green/Grassy	2.0 ±0.2	1.5 ± 0.6	0.884	**0.012**	0.993
Metallic	0.6 ± 0.3	0.6 ± 0.3	**0.001**	1.000	0.851
Overall Flavour Intensity	4.1 ± 0.4	4.1 ± 0.6	0.923	0.841	0.997
Raw/Beany	2.5 ± 0.1	2.1 ± 0.5	0.996	**0.024**	0.999
Sweet	0.8 ± 0.3	0.9 ± 0.3	**0.002**	0.243	0.699
Texture					
Amount of Particles	0.8 ± 0.3	0.8 ± 0.2	0.436	0.435	0.587
Astringency/Mouth Drying	2.5 ± 0.4	2.6 ± 0.5	**<0.001**	0.367	0.971

Fatty/Greasy Mouth Coating	0.7 ± 0.2	0.6 ± 0.3	0.358	0.169	0.694
Thickness/Viscosity	1.7 ± 0.2	1.6 ± 0.3	**0.001**	0.198	0.870
Aftertaste					
Astringency/Mouth Drying	2.3 ± 0.4	2.3 ± 0.4	0.083	0.524	0.940
Beany (Raw & Vegetative)	1.1 ± 0.3	1.1 ± 0.3	**0.003**	0.426	0.697
Bitter	0.4 ± 0.3	0.5 ± 0.3	**0.001**	0.661	0.704
Green/Grassy	1.2 ± 0.3	1.2 ± 0.3	**<0.001**	0.785	0.392
Other	0.3 ± 0.4	0.3 ± 0.5	**<0.001**	0.743	0.394
Overall Flavour Intensity	1.9 ± 0.3	1.9 ± 0.3	**<0.001**	0.501	0.752
Sweet	0.1 ± 0.3	0.1 ± 0.2	0.162	0.710	0.602

[1] results are reported as mean ± standard deviation (n=16)

[2] denotes that it was assessed in consensus, therefore no standard deviation or p-values were determined

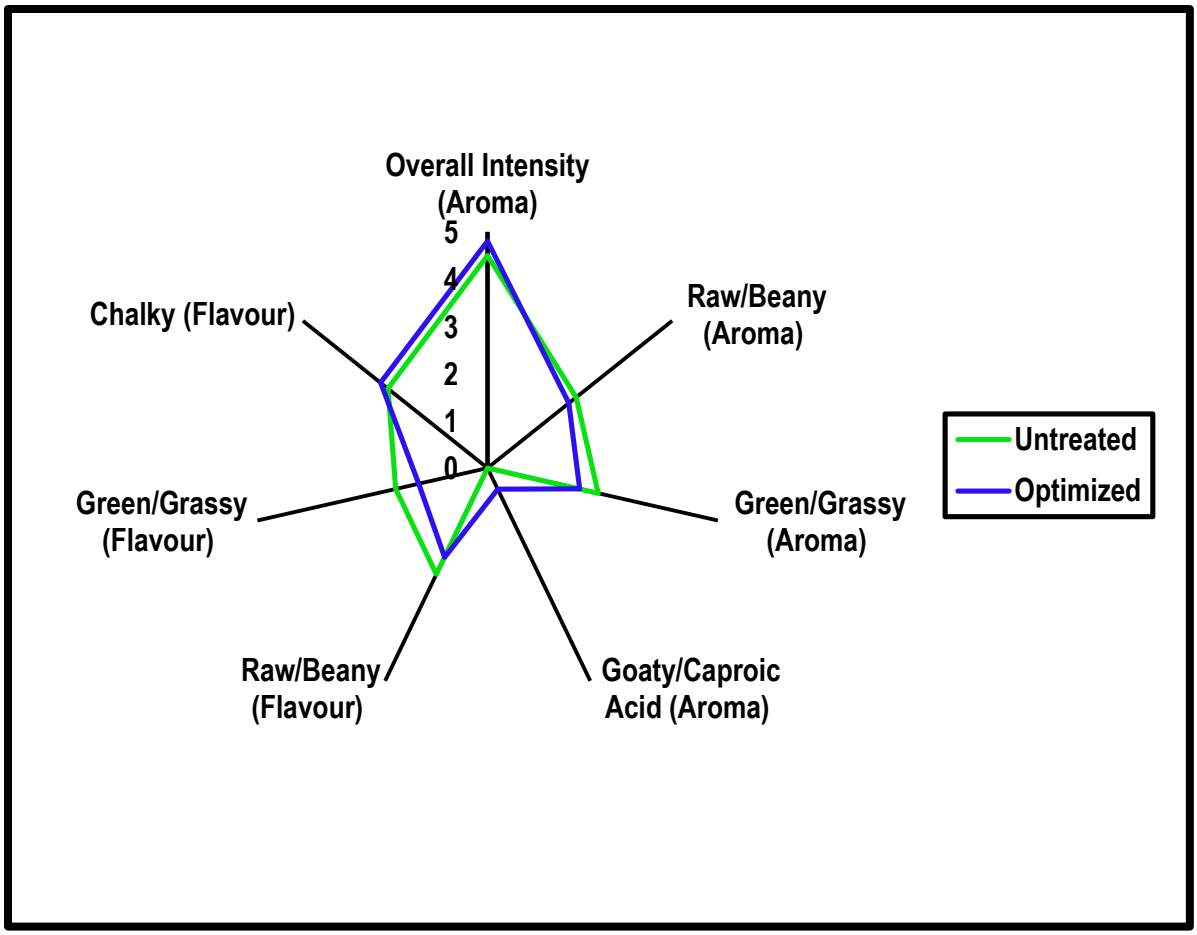

Figure 8. Radar plot of mean sensory scores (n=16) for untreated and VMD-processed 5% pea protein slurries, for statistically significant ($p<0.05$) sensory attributes, as evaluated by 8 trained panelists in duplicate using a modified Spectrum™ method intensity scale (maximum score = 15)

4.3.14. Flavour

There were three significantly different ($p<0.05$) flavour attributes between the two pea samples. Consistent with the findings from the ANOVA for aroma attributes, optimized pea protein was significantly lower ($p<0.05$) in "green/grassy" (1.5 versus 2.0) and "raw/beany" (2.1 versus 2.5) flavour notes; however, VMD-processing seemed to have led to slight but significantly increases ($p<0.05$) in "chalky" flavours (2.9 versus 2.7). It should be noted that flavour in this chapter refers to taste or gustation, combined with retronasal olfaction, which is when volatile compounds are reaching the nasal cavity through the oral cavity (Landis and others 2005). This is different from orthonasal olfaction,

which refers to volatiles directly entering the nasal cavity, for instance during sniffing (Landis and others 2005).

As vacuum microwave dehydration mainly works through the mechanism of removing volatile compounds, it can be deduced that the majority of the differences in flavour attributes can be attributed to retronasal olfactory differences rather than taste differences. None of the compounds identified via GC-MS in this Master's thesis were reported as chalky. However, the trained panelists defined "chalky" as tastes associated with mineral salts. Besides actual minerals, there is little literature on volatile compounds that impart a "chalky" taste. Rodrigues and others (2017) found a strong correlation (r=0.77) between chalky attributes to methanethiol. Although $A_{METHANETHIOL}/A_{IS}$ of both untreated and optimized pea protein were both below the limit of detection, sulphur compounds typically have low thresholds, therefore changes in concentration may not be detectable by GC-MS (Ma and others 2016).

4.3.15. Texture

Despite the many differences in functionality, no significant differences ($p \geq 0.05$) between texture attributes were identified. "Astringency/mouth drying" was the highest rated textural attribute in both pea proteins (2.5-2.6). This may be explained by the presence of saponins in peas (Heng and others 2006). "Fatty/greasy mouth coating" was very low in both pea proteins (0.6-0.7), which was expected as the samples were simply 5% pea protein solutions with no added fats. Despite the vast soluble protein content differences, the amount of particles were both rated as 0.8.

4.3.16. Aftertaste

The two pea proteins did not differ significantly ($p \geq 0.05$) for any of the aftertaste attributes. This may be explained due to the lack of fats in the product. Aftertastes can be due to high levels of flavour compounds residing in the oil phase and then oil droplets subsequently coating the oral cavity (Appelqvist and others 2004). Most flavour compounds are hydrophobic and are retained when fats are present. (Guichard 2002). However, with a lack of lipids in the food matrix, most of the volatile compounds may vaporize immediately, leading to changes in flavour perception (Guichard 2002). Proteins may bind flavours, but once solubilized, proteins are hydrated, some of the volatile compounds that were once bound, such as alcohols, can get released (Wang and Arntfield 2017).

4.3.17. Other Key Off-Flavour Compounds

As mentioned previously, 1-pentanol, 1-octen-3-ol and 3, 5 octadien-2-one are notable volatile compounds responsible for the very characteristic undesirable odours in peas (Schindler and others 2012; Roland and others 2017; Trikusuma and others 2020). The compound 1-pentanol has a fruity, floral aroma (Trikusuma and others 2020). The trained panelists did not detect any fruitiness or floral aroma, which aligned with the fact that the 1–pentanol concentration was below the limit of quantification for untreated protein and below the limit of detection for optimized pea protein. Meanwhile, 1-octen-3-ol has a mushroom/earthy odour (Trikusuma and others 2020). Optimized and untreated pea protein did not differ significantly ($p \geq 0.05$) in mushroom/earthy odour intensity. The 1-octen-3-ol concentration of untreated pea protein 2 was 0.09, while that of optimized pea protein was below the limit of quantification. The compound 3, 5 octadien-2-one is characterized as having a fruity, fat or mushroom odour. The 3, 5 octadien-2-one concentration in optimized pea protein was approximately 10% of that of untreated pea protein. Perhaps another compound that has a really low odour threshold is still contributing to a mushroom/earthy odour or the reductions in 1-octen-3-ol and 3,5 octadien-2-one were insufficient to reduce its detection and recognition.

4.3.18. Panelists

There was no significant ($p \geq 0.05$) interaction between samples and panelist ratings for all attributes. There were several instances when there were significant differences ($p < 0.05$) between panelists. There were two instances in aroma, flavour and texture modalities, while there were five instances in the aftertaste modality. These differences were expected and do not jeopardize our results. Z-score transformations are often done to eliminate the panelist effect; however, they were not necessary in this study (Cheung and others 2015). For any given sensory attribute, the maximum range between panelists was 1.5, where many of the ranges were 1. Given that the scale is a 15-point scale, the narrow range of the scale used was very acceptable. Furthermore, z-score transformations can show which panelists were assessing differently; however, the panel size was small, so eliminating any panelists may lead to inadequate power to determine differences (Lawless and Heymann 2010). Kim and Vickers (2020) clustered panelists based on their orally-related physiological measurements such as saliva flow rate, biting force, etc. The four groups were “low particle-size sensitive”, “high-biting force”, “high-saliva flow rate” and “low-saliva flow & low-chewing efficiency” (Kim and Vickers 2020). This may partially explain why there were differences in panelist ratings in astringency/mouth drying

and thickness/viscosity. Having differences between panelists is not necessarily an undesirable result as it is well known panelists may use scales differently. For instance, descriptive analysis is not particularly useful at quantifying absolute differences as panelists may use different areas of the scale (Lawless and Heymann 2010). However, the relative differences between samples are important (Lawless and Heymann 2010). Overall, the trained panelists from ACCE did a remarkable job as they were still able to elucidate differences between samples despite the small differences between them.

4.4. Conclusions

Three VMD-processes (initial moisture content of 75, 162 and 425% d.b., microwave power of 100W/g, vacuum level of 200 Torr, and process time of 2, 2.5 and 2 minutes, respectively) were implemented on two pea proteins. VMD-processing pea proteins led to significant decreases ($p<0.05$) in soluble protein content, and significant differences ($p<0.05$) in total colour (i.e. lower lightness, higher a* and b* coordinate values). Significant decreases ($p<0.05$) in chemically available lysine and emulsifying activity index only occurred in pea protein 1 after VMD-processing. No changes ($p\geq0.05$) in emulsifying stability index, surface hydrophobicity and free sulfhydryl group content were observed after VMD-processing.

A trained panel from Applied Consumer and Clinical Evaluations International conducted a descriptive analysis on 5% pea protein solutions prepared with untreated and optimized pea protein. The attributes from the following sensory modalities were evaluated: appearance, aroma, flavour, texture and aftertaste. The two solutions were similar in appearance attributes. Optimized pea protein was perceived by panelists to have significantly weaker ($p<0.05$) raw/beany and green/grassy aromas, but had a significantly stronger ($p<0.05$) overall aroma intensity and weak notes of a goaty/caproic acid aroma. The optimized pea protein was also determined to have significantly weaker ($p<0.05$) raw/beany and green/grassy flavours, but a significantly increased ($p<0.05$) chalky flavour. No significant differences ($p\geq0.05$) were found in texture and aftertaste attribute intensities. The 1-pentanol concentrations were low in both untreated and optimized pea protein and fruit-like aromas were not detected by panelists. Although 1-octen-3-ol and 3, 5 octadien-2-one areas were decreased ($p<0.05$) in the optimized pea protein product, there were no perceived differences ($p\geq0.05$) in earthy/mushroom aroma or flavour between the two pea proteins. Although the flavour profiles are comprehensively characterized, it is recommended that a consumer acceptance trial is warranted to determine if

differences in attribute intensity observed in this thesis are detectable by ordinary consumers and whether or not they are relevant to consumer acceptability.

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Chapter 5: Conclusions, Significance, Limitations and Future Research

5.1. Conclusions

The effects that initial moisture content (M_i), vacuum level, specific power, specific energy and process time had on protein functionality, available lysine, and colour were investigated. Increasing initial moisture content led to decreased soluble protein content, emulsifying activity index, available lysine and lightness (L*), but led to increased emulsifying stability index, a* coordinate, b* coordinate and total colour difference. No differences in surface hydrophobicity and free sulfhydryl content were observed. Various samples were analyzed for volatile analysis. Pea proteins subjected to high energy levels led to increased volatile concentrations due to heat-induced lipid oxidation. Pea proteins subjected to lower energy levels led to some reductions in volatiles, but may not have been adequate to lead to substantial changes in perception. Based on this data, three VMD-processes were developed and implemented onto two different pea proteins. Processing pea protein 2 at an initial moisture content of 162% d.b., at 100W/g and 200 Torr for 2.5 minutes was considered optimized due to the low resultant hexanal concentration and low-moderate functionality. Other notable reductions in key off-flavour causing compounds such as 1-pentanol, 1-hexanol and 1-octen-3-ol were observed. This optimized pea protein product was perceived to be less “raw/beany” and “green/grassy” in terms of both aroma and flavour. However, it also had a higher overall aroma intensity, “goaty/caproic acid” aroma and “chalky” flavour. Consumer acceptance trials are warranted to determine whether these perceived differences are relevant to product liking.

5.2. Significance

Long-term effects of this research include reduction of the reliance on animal-based products and the augmentation of agricultural sustainability. By using vacuum microwave dehydration, off-flavour causing volatile compounds in the plant proteins can be removed with fewer deleterious effects on quality. Vacuum microwave dehydration may also improve or retain the functionality of plant proteins that would otherwise be jeopardized in thermal processing, allowing them to be used for a plethora of industrial applications. In addition, more high-protein, plant-based products can be produced, improving the overall health and wellbeing of consumers and offering more dietary options for Canadians with dietary restrictions. By demonstrating the feasibility of vacuum microwave dehydration as a means of removing off-flavours in plant proteins, it is my hopes that our results can be translated onto other

related products. Overall, Canadian farmers and plant-based market will benefit economically. Lastly, utilization of vacuum microwave dehydration vastly reduces energy costs as the process time is short and because steam is not required, thus positively impacting the environment.

5.3. Limitations

The main limitation of this study is that some conditions regarding the pea proteins were unknown. The harvesting method, protein extraction method, and storage conditions were unknown and may have an impact on the aroma composition of the pea protein. Although many volatile analyses were carried out, this may not be an accurate representation of how consumers may perceive the pea proteins. This is because not all compounds are odour-active and the ones that are, may either not be unpleasant in odour or may be below the detection threshold (Murat and others 2013). Moreover, the SPME-GC-MS methodology elucidated the entire aroma profile instead of precisely quantifying a few specific target compounds. Such an approach was beyond the scope and budget of this study. The pea proteins were provided by Daiya Foods Inc. One limitation is that is unknown how the initial drying process from the supplier affected the functionality and volatile composition of the pea proteins. Perhaps if fresh pea protein solutions were VMD-processed, the extent of functionality and volatile concentration changes may be notably different than those reported. Lastly, another limitation of this study was that product temperature during the VMD process could not be monitored.

5.4. Future Research

Although this work provided much insight on many aspects of processing plant proteins, there are still many gaps of knowledge and thus there are many further areas of research to consider. Even though the optimized VMD processes are very efficient in removing volatile compounds, the sample throughput is limited. All VMD processes in the main experiments were conducted with 10 grams starting material. Scaling-up was attempted; however, even when the same parameters were employed on a larger sample size, the resultant products were different visually. Larger samples were drier and had a browner appearance than smaller sample sizes. Evidently, more research is needed to scale up so that this method would be more economically feasible. Implementing a continuous as opposed to a batch process would be desirable.

Future research should only be done on freshly extracted pea proteins as the effects of the initial drying process on functionality and volatile composition are unknown.

Although many factors were tested such as initial moisture content (M_i), vacuum level, specific power, specific energy and process time, many other factors may still exert a minor effect. Exploration of other conditions such as container type and material, mode of rotation, rotation speed, chamber temperature and humidity, and presence or absence of supplementary materials such as parchment paper, is warranted. The interaction effects between various factors should also be explored, perhaps via a response surface design.

This research focused primarily on off-flavour intensity and secondarily on functionality; however, the effects on nutritional parameters were only briefly discussed. Other facets that should be explored include protein quality through in vitro or animal feeding studies. Protein structure should be carefully analyzed and any changes in allergenicity should be assessed.

At the moment, only surface temperature probes are available; however, if product temperature probes are acquired, the ability to pasteurize should be explored. Furthermore, optimization of pasteurization by altering the aforementioned processing parameters could be advantageous in regards to retaining nutritional properties and overall quality of food products.

5.4.1. Other Considerations In Sensory Evaluation

Traditionally, consumers are tasked to provide hedonic ratings, while trained panelists are tasked to comprehensively describe the flavour profile and the intensities of relevant attributes (Ares and Varela 2017). However, as of late, the lines are blurred in regards to the roles of untrained and trained panelists. Ares and Varela (2017) conclude that in the majority of situations, naïve consumers can still replace a trained panel and still provide valuable sensory information. One caveat they mention is that this is highly dependent on the research objective and that in a small number of circumstances such as quality control, a trained panel must always be employed (Ares and Varela 2017). On the other hand, if the research objective is to identify areas that drive consumer preferences, then use of consumers is justified (Bruzzone and others 2015). Ares and Varela (2017) also mention that there is increasing evidence that suggest that the differences in discriminating power and ability to describe products between trained and untrained panelists are negligible. Worch and others (2010) agree that consumers can have a very similar discriminating ability to trained panelists. Lawless (1994) investigated white wine as an example and concluded that the descriptive abilities of consumers and trained panelists were similar. Evidently, the field of sensory evaluation is rapidly evolving and the traditional roles of

naïve consumer and trained panelist are being blurred. A proposed questionnaire to gain insights on how consumers would describe and evaluate the flavour profile and overall acceptability of pea protein products is shown in Appendix D (Figures D1-D5), which was based off the works of Purdy and others (2002), Quan and others (2006), Ares and others (2011), Cadena and others (2014), Cliff and others (2014), Jaeger and others (2015), Oliveira and others (2017), and Cunha and others (2019).

5.4.2. Other Considerations In Flavour Chemistry

Recruitment of so many panelists may not always be feasible. Therefore, one vital area of future research is to develop a robust model relating SPME GC-MS data to sensory data so that no human subjects are required. Although certain odour characteristics can be noted, it is far more important to model the interaction effects between various concentrations of key volatile compounds. To do this, more exact quantification must be done, therefore a new SPME method for each key compound must be developed and validated to ensure accurate and reliable concentrations.

Lastly, vacuum microwave dehydration mostly aims to remove the volatile compounds which are responsible for odour, but does little to the compounds responsible for tastes such as saponins (notable ones in peas include saponins βg and βb), which induce a bitter taste (Heng and others 2006). Steps to ameliorate the undesirable bitterness in peas include selecting different pea cultivars or improving the necessary extraction and purification steps in producing pea protein isolates (Roland and others 2017).

5.4.3. Shelf Life

Shelf-life of the VMD-processed pea proteins should be conducted. According to the suppliers, the expiry date of the untreated pea proteins is around 2 years from a nutritional standpoint; however, depending on the storage conditions, the pea proteins may become unpalatable much quicker than 2 years. Some important factors that may affect the off-flavour intensity of pea proteins would be lipid oxidation, Maillard browning and moisture migration. The volatile concentrations of the untreated pea proteins were variable, which is in part due to biological variation, but could also be due to different stages in its shelf-life. Perhaps the off-flavours in pea protein are palatable in its initial stages. Determining the last day of high quality would be of great use to producers as they could have smaller, but more frequent shipments, so that their final products would be very palatable.

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Appendices

Appendix A: Supplementary Tables and Figures for Chapter 2

Table 16. Drying kinetic parameters (critical moisture content, equilibrium moisture content, A, B, R^2) of pea proteins subjected to four microwave power levels and two vacuum levels

Microwave Power (W)	Vacuum Level (Torr)	M_c (% d.b.)	M_e (% d.b.)	A	B	R^2
100	40	350.95	9.08	0.93	-1.33	0.94
250	40	358.70	8.26	0.80	-1.10	0.96
550	40	271.80	4.00	0.43	-2.66	0.91
1000	40	267.40	6.53	0.25	-1.84	0.98
100	200	78.27	8.22	0.95	-1.54	0.90
250	200	281.64	4.61	0.77	-2.60	0.96
550	200	299.00	1.79	0.51	-2.08	0.99
1000	200	383.34	0.04	0.35	-0.96	0.99

M_c refers to critical moisture content; M_e refers to equilibrium moisture content

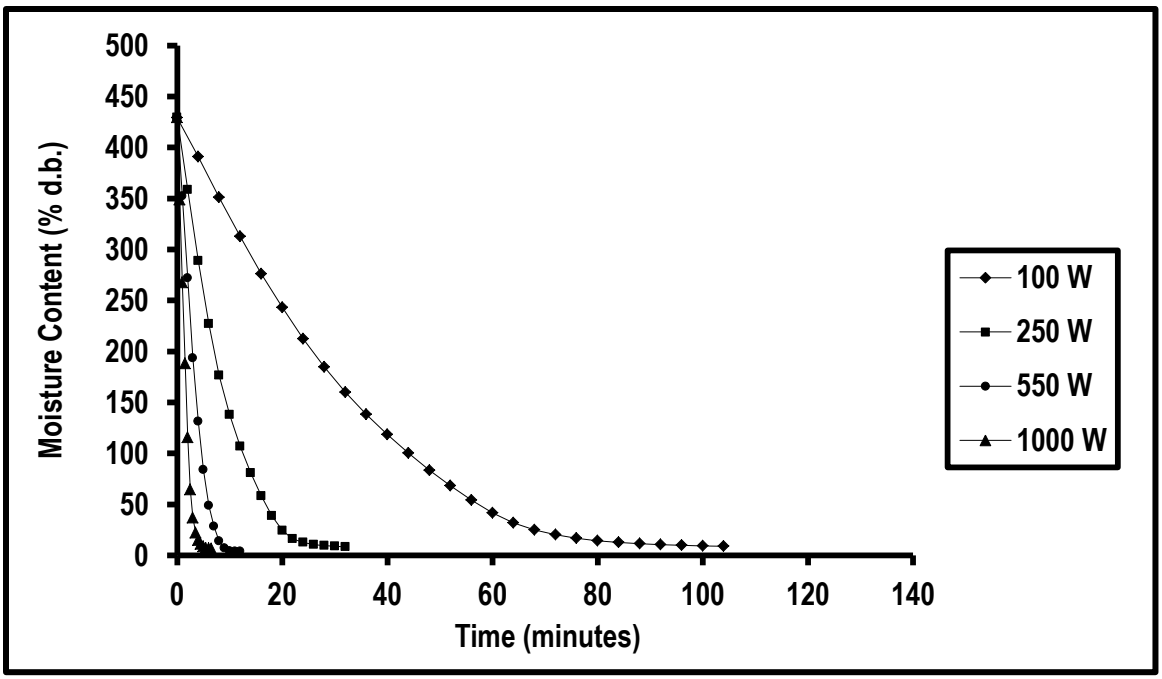


Figure 9. Drying kinetics of high-vacuum (40 Torr) VMD-processed pea proteins

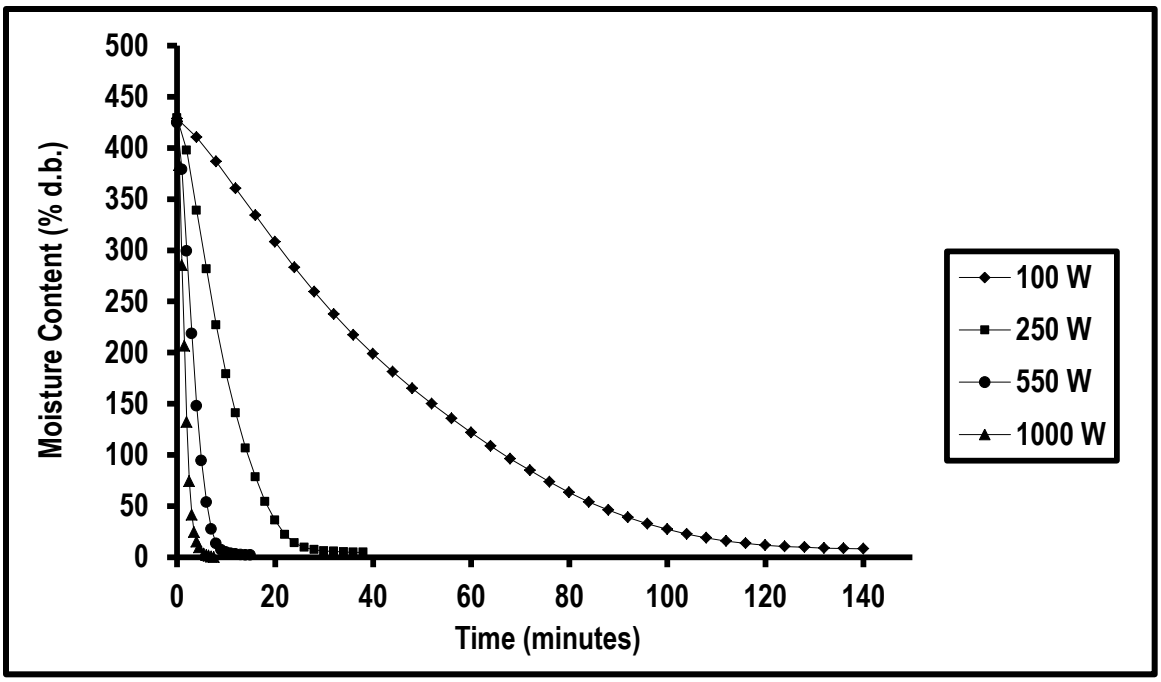


Figure 10. Drying kinetics of low-vacuum (200 Torr) VMD-processed pea proteins

Table 17. Soluble protein content of pea proteins in 0.01M, pH 7 potassium phosphate buffer and potassium phosphate buffer (0.1M, pH 8, 1mM EDTA) after VMD-processing at various initial moisture contents (5-425% d.b.), vacuum levels (40-200 Torr), specific power levels (10-100W/g) and process times (1-50 minutes)

Sample ID	Soluble Protein Content In Potassium Phosphate Buffer (mg/g dry matter)	
	0.01M, pH 7	0.1M, pH 8, 1mM EDTA
Untreated	156.71 ± 10.83 [a]	114.35 ± 18.77 [a]
$M_{31}P_{100}V_{200}T_5$	138.86 ± 20.71 [a]	99.32 ± 7.46 [abc]
$M_{75}P_{100}V_{200}T_5$	97.82 ± 9.66 [abc]	70.33 ± 8.32 [bcd]
$M_{162}P_{100}V_{200}T_5$	59.07 ± 5.50 [bc]	56.47 ± 17.00 [cd]
$M_{425}P_{100}V_{200}T_5$	43.40 ± 9.86 [c]	44.40 ± 16.44 [d]
$M_{31}P_{10}V_{200}T_5$	100.43 ± 34.14 [abc]	79.50 ± 23.87 [abcd]
$M_{31}P_{50}V_{200}T_5$	114.64 ± 33.60 [ab]	85.88 ± 18.43 [abcd]
$M_{31}P_{100}V_{200}T_5$	128.40 ± 24.46 [a]	95.50 ± 8.36 [abc]
$M_{31}P_{100}V_{120}T_5$	139.38 ± 17.72 [a]	102.37 ± 8.18 [ab]
$M_{31}P_{100}V_{200}T_5$	142.38 ± 11.68 [a]	101.66 ± 11.45 [ab]
$M_{31}P_{100}V_{200}T_1$	105.62 ± 22.55 [ab]	71.00 ± 22.03 [bcd]
$M_{31}P_{100}V_{200}T_2$	127.35 ± 6.06 [a]	83.78 ± 9.80 [abcd]
$M_{31}P_{100}V_{200}T_3$	134.46 ± 19.56 [a]	104.64 ± 9.85 [ab]
$M_{31}P_{100}V_{200}T_5$	125.13 ± 4.76 [a]	96.74 ± 1.06 [abc]
$M_{31}P_{10}V_{200}T_{50}$	144.96 ± 11.95 [a]	101.46 ± 6.72 [ab]
$M_{31}P_{50}V_{200}T_{10}$	128.24 ± 20.03 [a]	84.48 ± 7.59 [abcd]
$M_{31}P_{100}V_{200}T_5$	130.43 ± 9.48 [a]	94.11 ± 18.09 [abc]
$M_{31}P_{150}V_{200}T_{3.3}$	133.25 ± 32.00 [a]	101.57 ± 15.16 [ab]

[1] results are reported as mean ± standard deviation (n=3)

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test

Appendix B: Supplementary Tables and Figures for Chapter 3

Table 18. Retention times and odour descriptors of volatile compounds identified in pea proteins, before and after VMD-processing

Retention Time (mins)	Compound	Functional Group	Odour Characteristics
2.95	Pentane	Alkane	alkane
3.04	Methanethiol	Thiol	sulphur, gasoline, garlic
3.07	Propane, 2-methoxy, 2-methyl	Alkane	minty
3.13	Heptane	Alkane	alkane
3.34	1-Propen-2ol, acetate	Alcohol	fruity
3.40	Hexane, 2,4-dimethyl	Alkane	[2]
3.60	Butanal	Aldehyde	green, pungent
3.75	2-Butanone	Ketone	ether
4.16	Furan, 2-ethyl	Furan	chemical, beany, bready, malty, tum, coffee, chocolate
4.55	Pentanal	Aldehyde	almond, malt, pungent, green, milky
4.80	Trichloromethane	Alkane	woody, cedar-like
5.21	Furan, 2-ethyl, 5-methyl	Furan	fresh, gassy, burnt
5.37	Toluene	Alkene	paint
5.91	3-Nonen-2-ol	Alcohol	[2]
6.34	Hexanal	Aldehyde	grass, tallow, fat, green, strong
7.38	2n-butyl furan	Furan	fruity, winey, sweet, spicy
8.16	2n-Butylacrolein	Aldehyde	[2]
9.20	Heptanal	Aldehyde	fat, citrus, rancid, floral

9.28	2-Heptanone	Ketone	soap
10.62	Furan, 2-pentyl	Furan	green bean, butter
11.55	1-Pentanol	Alcohol	fruit
13.11	2-Octanone	Ketone	soapy, fruity
13.22	Octanal	Aldehyde	fat, soap, lemon, green, orange, sweet
14.86	2-Hexenal, 2-ethyl	Aldehyde	lemon, apple, fruity
15.57	1-Hexanol	Alcohol	resin, flower, green
16.61	Pentane, 1-nitro	Alkane	pleasant, fruity
17.32	2-Nonanone	Ketone	hot milk, soap, green
17.47	Nonanal	Aldehyde	fat, citrus, green
17.84	3-Octene-2-one	Ketone	mushroom, mouldy, burnt
18.33	2-Oxooctanoic acid	Carboxylic acid	[2]
18.72	2-Octenal	Aldehyde	dusty, mouldy, musty
19.40	1-Octen-3-ol	Alcohol	mushroom, earthy, burnt
20.83	Hexane, 1-nitro	Alkane	[2]
21.06	1-Hexanol, 2-ethyl	Alcohol	rose, green
21.47	Benzaldehyde	Aldehyde	almond, burnt sugar, marchpane
21.52	2-Decanone	Ketone	orange, floral, fatty, peach
22.03	3, 5-Octadien-2-one[1]	Ketone	fruit, fat, mushroom
24.04	3, 5-Octadien-2-one[1]	Ketone	fruit, fat, mushroom

(Chang and others 1995; Jakobsen and others 1998; Flavornet 2004; Schindler and others 2012; Asikin and others 2018; The Good Scents Company 2018; Alfrebo 2019; Li and others 2019a)

[1] Structural isomers may exist

[2] Odour descriptor data is very limited

Appendix C: Supplementary Tables and Figures for Chapter 4

Table 19. Sensory attributes, definitions, evaluation instructions and references for profiling 5% pea protein solutions with a modified Spectrum™ attribute intensity scale in terms of appearance, aroma, flavour, texture and aftertaste

Attributes and Definitions	Aroma References and Anchors
Appearance *(Remove the lids before evaluation of both samples side by side)*	
Amount of Foam: The extent of foam or bubbles visible throughout the surface of the sample	**N/A** (None to a lot; 0-15)
Chroma: The degree to the colour is bright/pure (absence of gray) throughout the sample	**Observation/Paint chips** (Dull to bright/pure; 0-15)
Colour Intensity: The intensity or strength of the colour, ranging from light to dark	**Observation** (Light yellow to dark yellow; 0-15)
Aroma *(Smell immediately after the lid is removed; Take 3 short, shallow sniffs; Replace lid and evaluate*	
Beany/Vegetative: The extent to which the sample smells like cooked beans or green vegetables	**Canned green beans** (None to strong; 0-15)
Cardboard: Aroma associated with wet cardboard packaging	**Cardboard** (soaked in water overnight) (Not to very; 0-15)
Earthy/Mushroomy: Aroma associated with damp soil or mushrooms	**Sliced mushrooms** (None to strong; 0-15)
Goaty/Caproic Acid: Aroma associated with caproic acid, goat and game	**Unripened goat's milk cheese** (None to strong; 0-15)
Green/Grassy: Green, slightly sweet aroma associated with cut grass	**Alfalfa sprouts** (None to strong; 0-15)

Overall Aroma Intensity: The totality of aroma that is perceived in sample	**Modified Sensory Spectrum™ Universal Intensity Scale (**None to strong; 0-15) [Aromatics in vegetable oil – 2.0] [Aromatics in apple sauce – 5.0] [Aromatics in orange juice – 7.5]
Raw/Beany: Aroma associated with unprocessed and/or uncooked legumes	**Raw green beans in water** (None to strong; 0-15)
Flavour (Taste + Retronasal Olfaction) *(Take 2 to 3 sips and evaluate)*	
Beany/Vegetative: The extent to which the sample tastes like cooked beans or green vegetables	**Raw green beans in water** (None to strong; 0-15)
Bitter: Taste on the tongue associated with caffeine and other bitter substances such as quinine and hop bitters	**Caffeine** (Not to very; 0-15) [0.05% caffeine solution – 2.0] [0.08% caffeine solution – 5.0] [0.15% caffeine solution – 10.0]
Cardboard: Flavour associated with wet cardboard packaging	**Cardboard** (Not to very; 0-15)
Chalky: Flavour associated with mineral salts such as chalk	**5 mL Chalk dust** (gypsum powder) in 100 mL water (None to strong; 0-15)
Earthy/Mushroomy: Flavour associated with damp soil or mushrooms	**Sliced mushrooms** (None to strong; 0-15)
Green/Grassy: Green, slightly sweet flavour associated with cut grass	**Alfalfa sprouts** (None to strong; 0-15)
Metallic: Flavour associated with metals, tin cans, or iron	**1 iron capule in 250 mL water** (None to strong; 0-15)
Overall Flavour Intensity: The totality of flavour that is perceived in the sample, including basic tastes	**Modified Sensory Spectrum™ Universal Intensity Scale** (None to strong; 0-15) [Aromatics in vegetable oil – 2.0] [Aromatics in apple sauce – 5.0] [Aromatics in orange juice – 7.5]
Raw/Beany: Flavour associated with unprocessed and or/uncooked legumes	**Raw green beans in water** (None to strong; 0-15)
Sweet: The taste on the tongue stimulated by sucrose and high potency sweeteners	**Sucrose** (None to strong;0-15) [2% sucrose solution – 2.0]

Texture/Mouthfeel *(Take 2-3 sips and evaluate)*	
Amount of Particles: the quantity of particples felt throughout the sample	**Tomato Juice** (None to much; 0-15) [Heinz Tomato Juice – 2.0]
Astringency/Mouth Drying: The shrinking or puckering of the surface of the tongue caused by substances such as tannins or alum i.e. green banana peel, strong black tea	**Alum** (None to strong; 0-15) [1 gram alum in 1000 mL water – 2.0] [2.5 g alum in 1000 mL water – 5.0]
Fatty/Greasy Mouth Coating: The amount of fatty/greasy residue felt by the tongue when moved over the surfaces of the mouth	
Thickness/Viscosity: The extent to which the sample feels thick or dense in the mouth as opposed to thin and watery	**Various solutions** (Thin to thick; 0-15) [5% sucrose solution – 2.0] [Heinz Tomato Juice – 4.0]
Aftertaste *(Wait 30 seconds after Texture/Mouthfeel evaluations and then evaluate)*	
Astringency/Mouth Drying: Sensations of shrinking or puckering of the surface of the tongue caused by substances such as tannins or alum i.e. green banana peel, strong black tea, detected 30 seconds after tasting	**Alum** (None to strong; 0-15) [1 gram alum in 1000 mL water – 2.0] [2.5 g alum in 1000 mL water – 5.0]
Beany (Raw & Vegetative): The overall intensity of raw/beany and beany/vegetative flavours detected 30 seconds after tasting	
Bitter: Taste on the tongue associated with caffeine and other bitter substances such as quinine and hop bitters detected 30 seconds after tasting	**Caffeine** (Not to very; 0-15) [0.05% caffeine solution – 2.0] [0.08% caffeine solution – 5.0] [0.15% caffeine solution – 10.0]
Green/Grassy: Green, slightly sweet flavour associated with cut grass that is detected 30 seconds after tasting	**Alfalfa sprouts** (None to strong; 0-15)

Overall Flavour Intensity: The totality of flavour that is perceived in the sample, including basic tastes detected 30 seconds after tasting	**Modified Sensory Spectrum™ Universal Intensity Scale** (None to strong; 0-15) [Aromatics in vegetable oil – 2.0] [Aromatics in apple sauce – 5.0] [Aromatics in orange juice – 7.5]
Sweet: The taste on the tongue stimulated by sucrose and high potency sweeteners detected 30 seconds after tasting	**Sucrose** (None to strong;0-15) [2% sucrose solution – 2.0]

(Applied Consumer and Clinical Evaluations International 2020)

Table 20. Verbal descriptors of the Modified Spectrum™ Method intensity scale indicating the intensity of a specific sensory attribute

Descriptor	Attribute Intensity Rating
None	0
Low	1-3
Low-medium	4-6
Medium	7-8
Medium-high	9-11
High	12-14
Very high	15

(Applied Consumer and Clinical Evaluations International 2020)

Table 21. Soluble protein content of pea proteins subjected to three different vacuum microwave dehydration processes (low-vacuum, high-specific power, medium-level specific energy processes) in 0.01M, pH 7 potassium phosphate buffer and potassium phosphate buffer (0.1M, pH 8, 1mM EDTA)

Sample ID	Soluble Protein Content (mg/g dry matter)	
	0.01M, pH 7 Potassium Phosphate Buffer	Potassium Phosphate Buffer (0.1M, pH 8, 1mM EDTA)
Untreated–1	129.62 ± 9.33 [a]	107.63 ± 10.53 [a]
$M_{75}V_{200}P_{100}T_{2}$–1	62.93 ± 11.02 [b]	67.22 ± 8.92 [b]
M_{162} $V_{200}P_{100}T_{2.5}$–1	38.07 ± 3.58 [bc]	38.16 ± 4.27 [cd]
M_{425} $V_{200}P_{100}T_{2}$–1	49.72 ± 9.77 [bc]	41.83 ± 3.82 [bcd]
Untreated–2	61.75 ± 13.71 [b]	56.85 ± 12.36 [bc]
$M_{75}V_{200}P_{100}T_{2}$–2	30.22 ± 10.53 [c]	33.67 ± 8.50 [cd]
M_{162} $V_{200}P_{100}T_{2.5}$–2	22.65 ± 8.40 [c]	23.97 ± 6.00 [d]
M_{425} $V_{200}P_{100}T_{2}$–2	25.15 ± 13.67 [c]	26.46 ± 12.10 [d]

[1] results are reported as mean ± standard deviation

[2] superscript letters denote significant differences ($p<0.05$) between treatments in each experiment set, as determined by one-way analysis of variance + Tukey's honestly significant different test.

Appendix D: Supplementary Tables and Figures for Chapter 5

Figure 11 was adapted from the works of Cadena and others (2014), Jaeger and others (2015) and Cunha and others (2019).

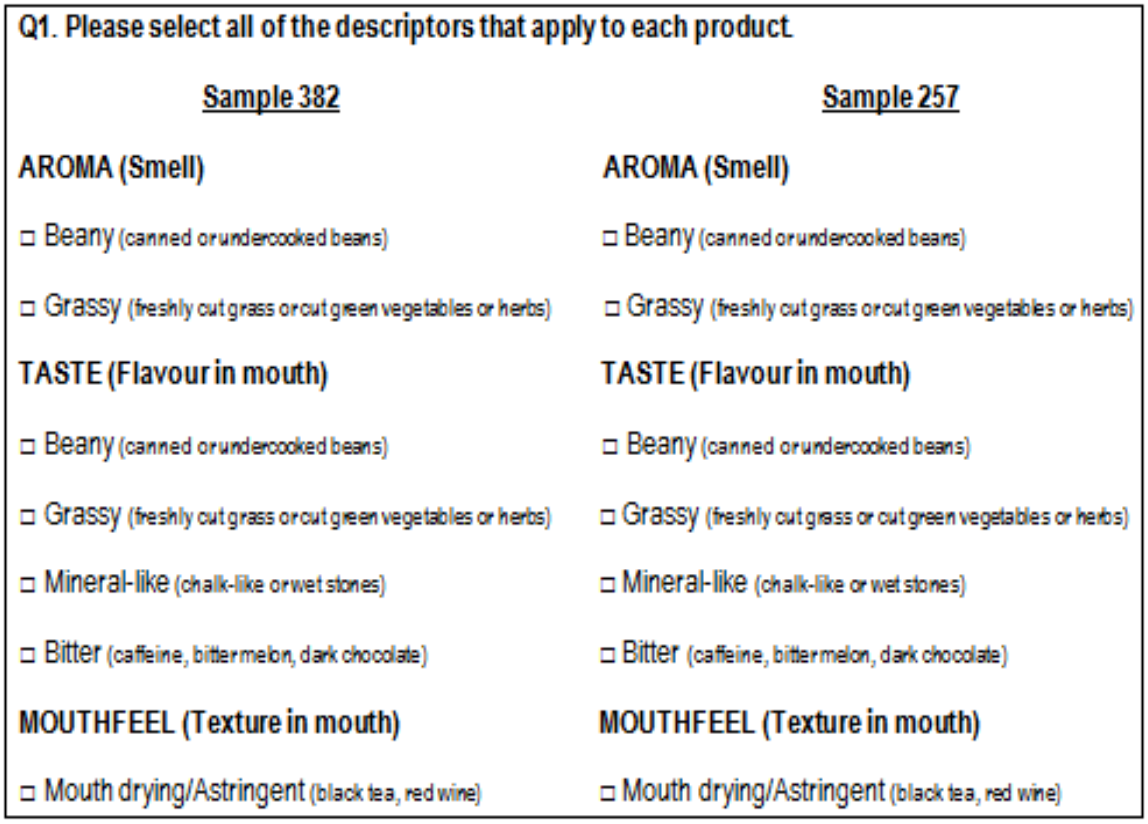
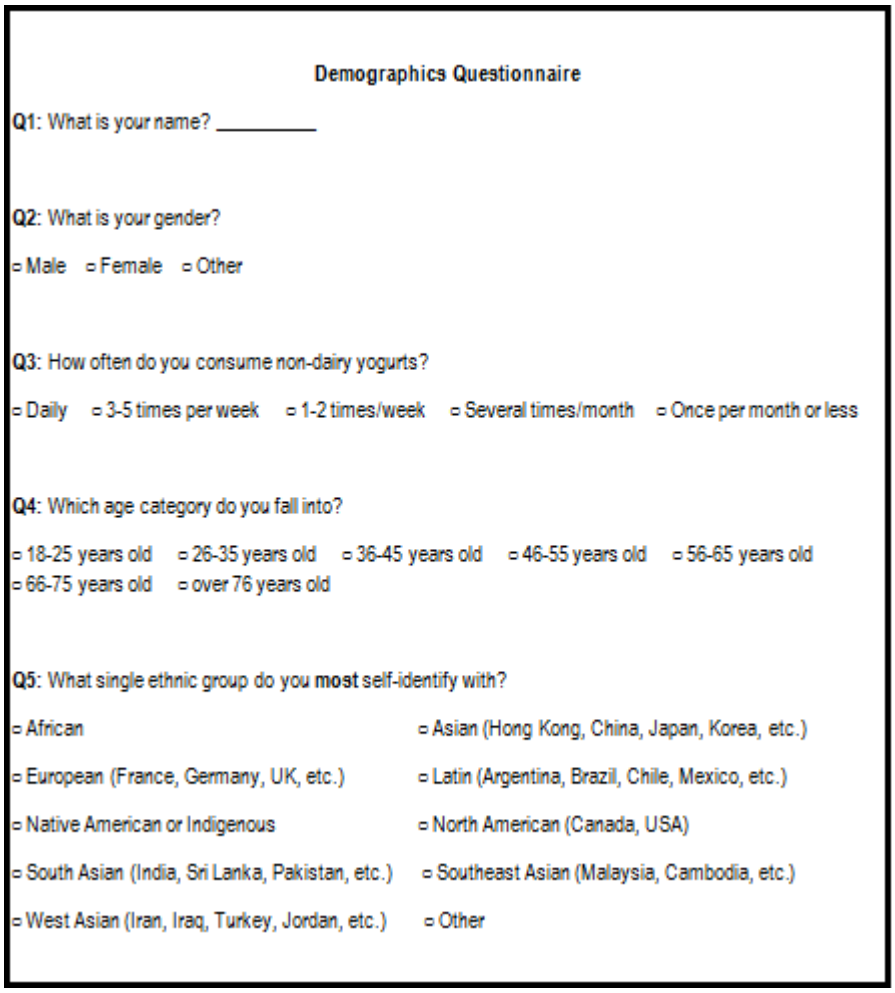


Figure 11. Sensory questionnaire, including a check-all-that-apply question (CATA), designed for consumers to provide flavour attribute information

Figure 12 was adapted from the works of Quan and others (2006) and Cliff and others (2014)



Demographics Questionnaire

Q1: What is your name? _____

Q2: What is your gender?

▫ Male ▫ Female ▫ Other

Q3: How often do you consume non-dairy yogurts?

▫ Daily ▫ 3-5 times per week ▫ 1-2 times/week ▫ Several times/month ▫ Once per month or less

Q4: Which age category do you fall into?

▫ 18-25 years old ▫ 26-35 years old ▫ 36-45 years old ▫ 46-55 years old ▫ 56-65 years old
▫ 66-75 years old ▫ over 76 years old

Q5: What single ethnic group do you **most** self-identify with?

▫ African ▫ Asian (Hong Kong, China, Japan, Korea, etc.)

▫ European (France, Germany, UK, etc.) ▫ Latin (Argentina, Brazil, Chile, Mexico, etc.)

▫ Native American or Indigenous ▫ North American (Canada, USA)

▫ South Asian (India, Sri Lanka, Pakistan, etc.) ▫ Southeast Asian (Malaysia, Cambodia, etc.)

▫ West Asian (Iran, Iraq, Turkey, Jordan, etc.) ▫ Other

Figure 12. Questionnaire collecting demographic data including name, gender, consumption frequency, age category and self-identified ethnicity

Figures 13-15 were adapted from the works of Purdy and others (2002), Ares and others (2011) and Oliveira and others (2017).

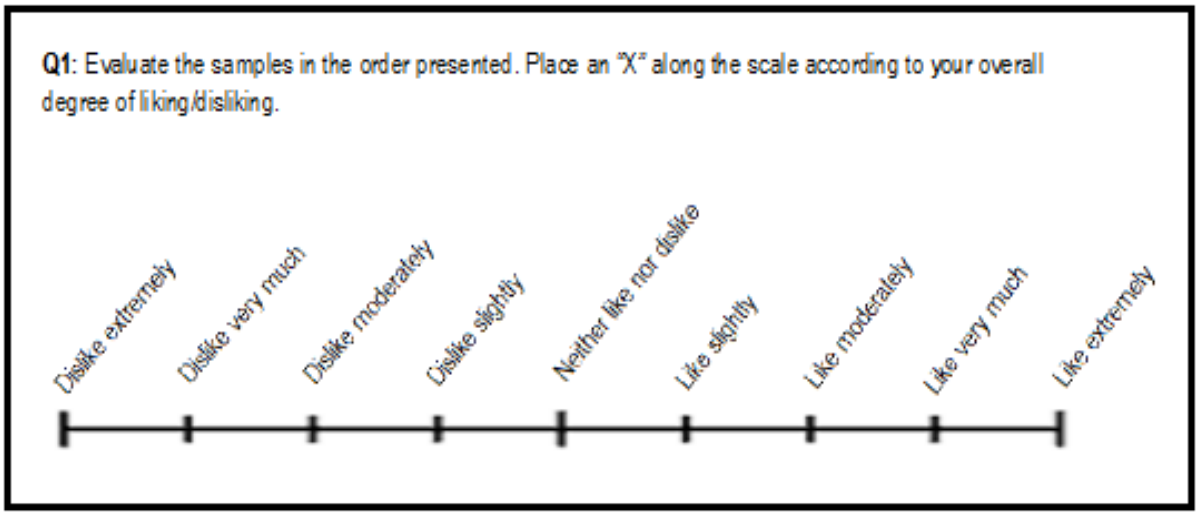


Figure 13. 9-point continuous hedonic scale, collecting data for overall degree of liking/disliking

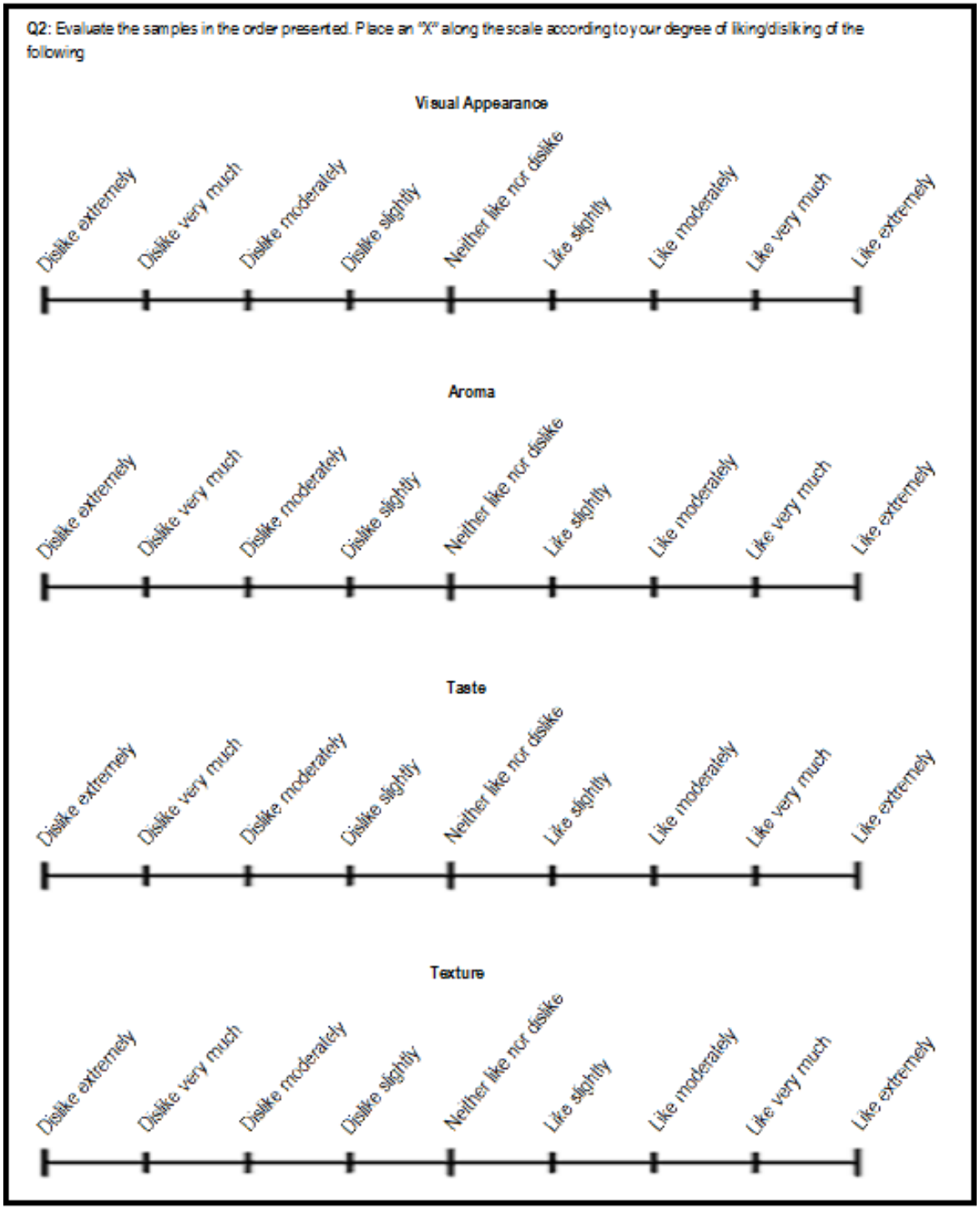


Figure 14. 9-point continuous hedonic scales collecting data for degree of liking/disliking of sensory modalities – visual appearance, aroma, taste and texture

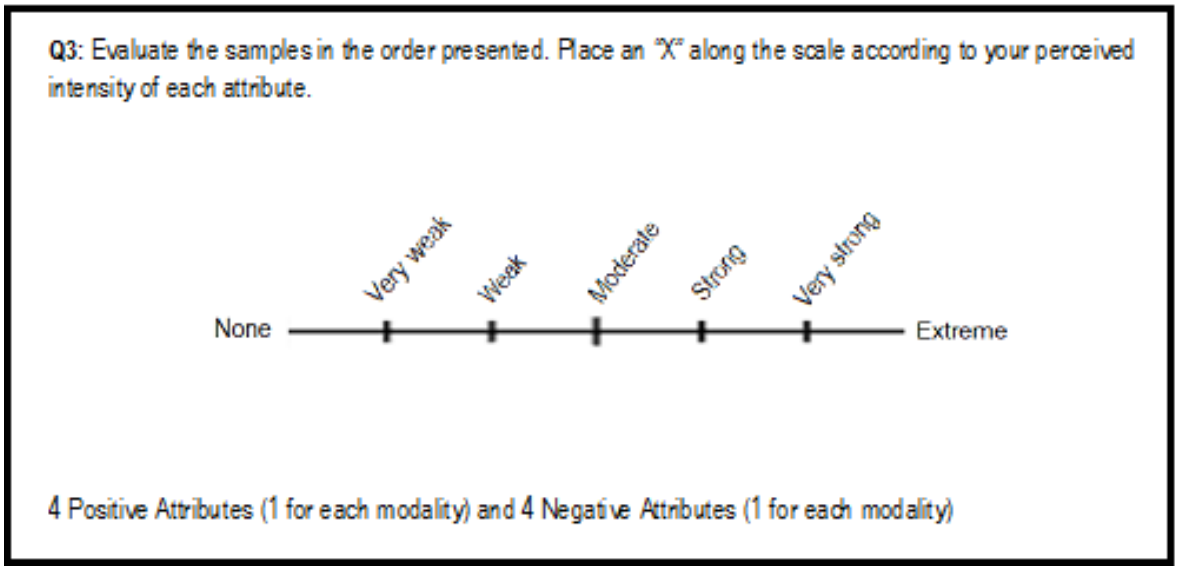


Figure 15. 5-point continuous intensity scale collecting data for perceived intensity of important attributes