Comparison of New Zealand and commercial wakame (*Undaria pinnatifida)* in terms of physicochemical characteristics, sensory properties and volatile composition

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Jessica Marie G. Balbas

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Chapter 1: Abstract

The brown algae, *Undaria pinnatifida*, known as wakame is native to Japan, Korea, and China, was accidentally introduced to the New Zealand waters in 1987 and the species was categorised as an unwanted organism under the provisions of the Biosecurity Act 1993. Attempts were made to eradicate and/or control its growth in New Zealand with little success. Meanwhile, it has been recognized as having economic importance in wakame producing countries owing to the amount produced, consumed, and exported. The main aim of the project was to compare New Zealand wakame produced from *U. pinnatifida* in terms of physicochemical, sensory and volatiles analysis with commercial Japanese and Korean samples. This research investigated the free carbohydrates in processed *Undaria* (wakame). Furthermore, an investigation was made into the sensory properties of New Zealand wakame, its texture and colour profiles were compared to commercially available varieties from Japan and Korea. A volatile profile analysis was also carried out to help identify key flavour and odour compounds present in the seaweed. Results showed that the only free carbohydrate in *Undaria* is mannitol, the main photosynthetic by-product in phaeophytes. Mannitol concentration is also higher in freeze-dried samples compared with commercially prepared ones that have undergone blanching, salting, and oven-drying. New Zealand wakame processed in August was perceived to be different from commercially available wakame and was described as being fishy, thin, soft, and watery. Further analysis on the texture profile of the different samples confirmed this finding and an improvement in the processing method showed an increase in hardness for the processed New Zealand samples in October. The volatile profile analysis also identified 105 compounds present in the different wakame products which provided an insight as to what volatiles characterise one sample from the rest.

Chapter 2: Introduction

Marine algae, collectively known as seaweed, are plants of the sea (Venugopal, 2008). They have been cultivated and utilised by man for years as food, components of food and pharmaceutical, fertilisers, and feed among others. Ancient Chinese records dating as far back as 500 B.C. showed that people collected algae for food, a practice that spread to Europe a thousand years later through the migration of people from China, Japan, Korea, Indonesia and Malaysia who had always consumed algae as food. In addition to being consumed as food, algae are also cultivated as sources of food modifiers, such as agars and carrageenans (Barsanti & Gualtieri, 2006).

Undaria pinnatifida is a brown seaweed that is of economic importance (Barsanti & Gualtieri, 2006). The Food and Agriculture Organization of the United Nations (2010) reported that the world production of wakame in 2008 was 1.8 million tonnes. Wakame (processed *Undaria*) is often consumed as an ingredient in soups and other products (Barsanti & Gualtieri, 2006), including salads. *U. pinnatifida*, which was introduced to New Zealand in 1987 (Hay & Luckens, 1987; Stuart, 2004) was earlier placed in the list of unwanted organisms in the Biosecurity Act 1993. This saw several attempts at eradicating the species from New Zealand waters (Stuart, 2004). However, a review of the policies on *Undaria* in 2010 was undertaken that allowed a limited commercial harvest of *Undaria* in New Zealand. This paved the way for AUT University researchers to examine the species that grew in New Zealand as a potential food

Chapter 3: Literature Review

3.1 Undaria pinnatifida

One of the most cultivated algae worldwide is the brown *Undaria* sp. algae. Together with *Laminaria* sp, they make up the most important seaweed based on its economic contribution (Barsanti & Gualtieri, 2006). *Undaria* is native to Japan, Korea, and China (Hay, 1990; Hay & Luckens, 1987; Stuart, 2004) and has been used as a food item in Japan as far back as 700 A.D, where its market value is currently estimated at US $400 million. Coincidentally, Japan was also the first to cultivate *Undaria* at the beginning of this century when customer demands could not be met by wild stock harvest alone; China soon followed then Korea in the 1970's. As of 2006, Korea was considered the largest *Undaria* producing nation with yields of 800, 000 tonnes of wet seaweed per year, with half of it produced through cultivation (Barsanti & Gualtieri, 2006). Meanwhile, *Undaria* can also be found in France, New Zealand, and Australia (Hay, 1990; Stuart, 2004; Yamanaka & Akiyama, 1993). In New Zealand, it is believed to have been introduced either via ballast ship waters (Barsanti & Gualtieri, 2006; Hay, 1990) or via ships' hulls (Hay, 1990).

The main species of *Undaria* that is cultivated is *U. pinnatifida*, which grows on rocky shores and bays in the sublittorial zone (near or just below the tidal level) up to 7m below sea level (Hay & Luckens, 1987). Figure 1 shows a frond of *U. pinnatifida* which can grow up to 3 metres with a midrib that can range from 1-3 centimetres wide. The naked basal part of the midrib is also referred to as the stipe. Meanwhile, the reproductive part of *Undaria* is the sporophyll. It is often viewed as a single entity when it is actually two discrete pieces divided by the stipe (Hay, 1990). Figure 2 shows the life cycle of *Undaria* which is an annual plant with a life cycle that is divided into a macroscopic sporophyte stage which lasts for about 6 months and

a microscopic gametophyte stage which is perennial (potentially viable) for at least 24 months (Stuart, 2004). Suitable growth temperatures range from 5-15 °C, while temperatures above 25 °C stops the growth of the seaweed (Barsanti & Gualtieri, 2006). On the other hand, Hay and Luckens (1987) reported that the best growth temperatures ranged from 17 to 20 °C and that *Undaria* died within days when exposed to 30 °C.

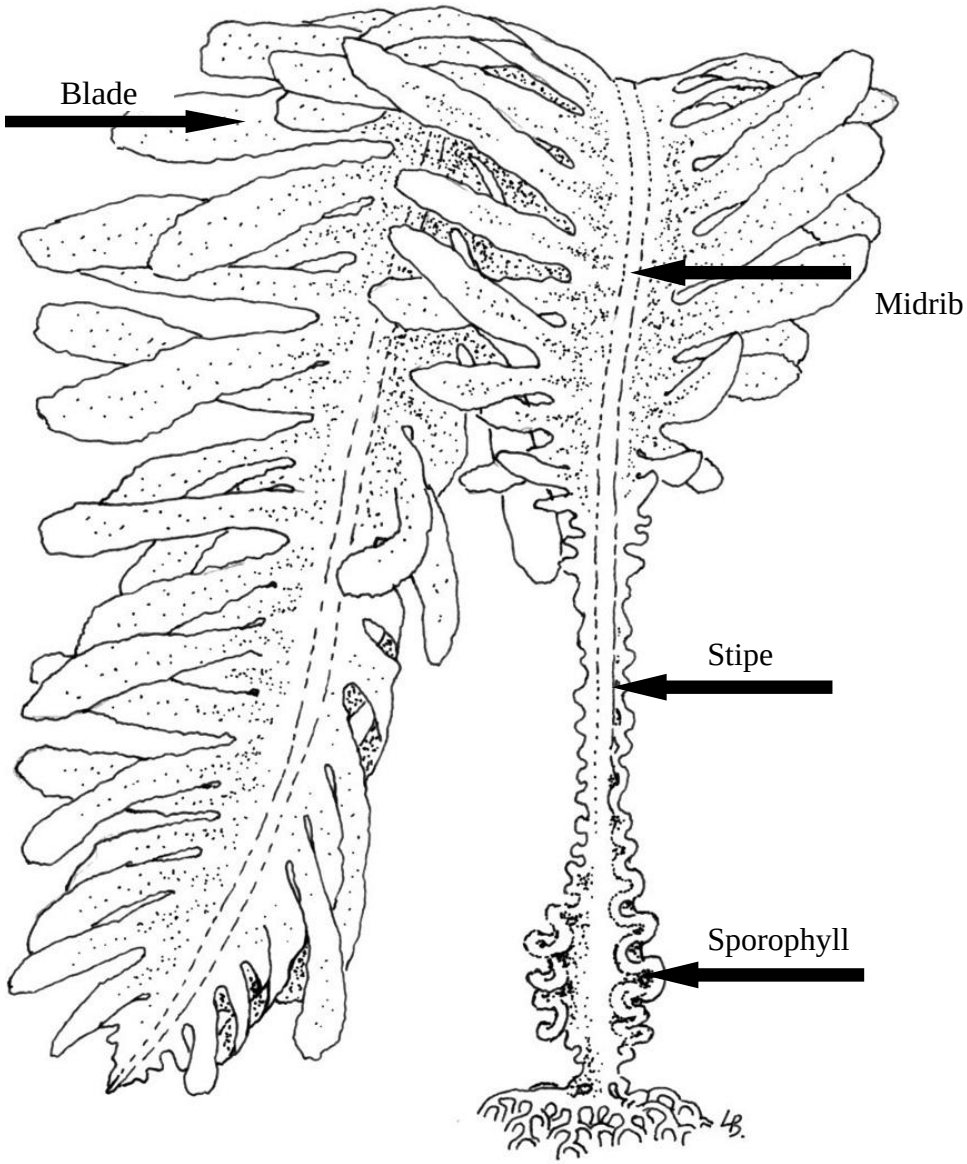

Figure 1. Mature *Undaria pinnatifida* frond (Barsanti & Gualtieri, 2006).

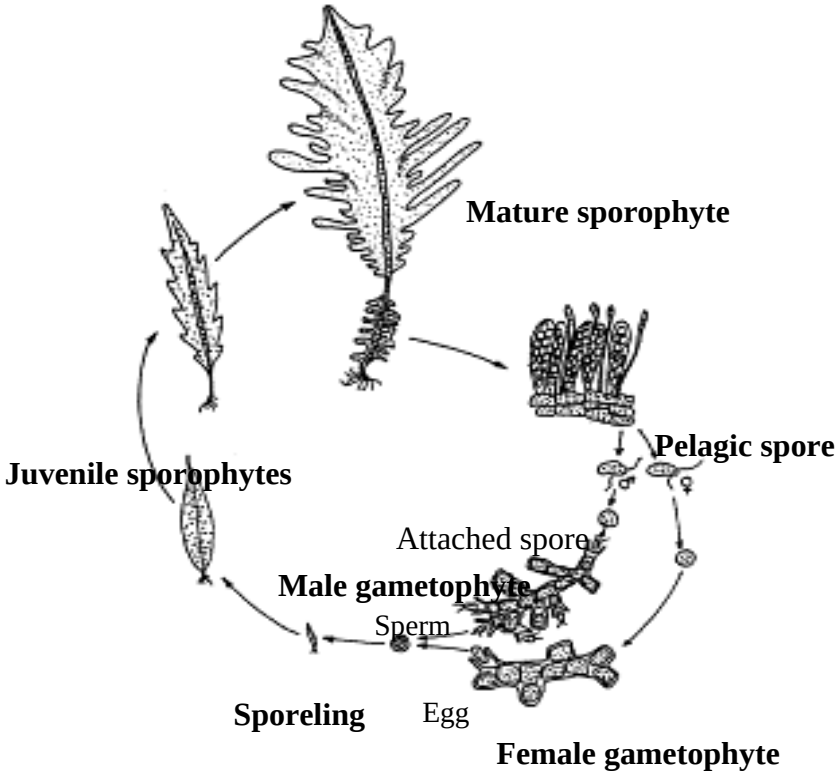

Figure 2. The life cycle of *Undaria pinnatifida* (Stuart, 2004).

3.2 Status of Undaria in New Zealand

Undaria in the New Zealand waters was first reported by Hay & Luckens (1987) who collected seaweed that was growing unusually near the Oriental Bay in Lambton Harbour in Wellington (Hay & Luckens, 1987; Stuart, 2004). It was later determined that the presence of that seaweed was confined to that area. They also found the seaweed growing on wood, bottles, ropes, tyres, cobbles, and boulders in depths that ranged from low water up to 7m in sheltered habitats as well as shores exposed to prevailing northerly gales. As was previously mentioned, *Undaria* grew best at temperatures of between 17 and 20 °C in Asia. In New Zealand, however, *Undaria* persisted longer since the temperature of New Zealand waters was the ideal temperature

range for growth (Hay & Luckens, 1987). *Undaria* was also reported as being able to release zoospores at 9°C (Campbell & Burridge, 1998).

Concerns were raised in New Zealand soon after the discovery of *Undaria* as it grew in thick canopies, which competed for space and light with large, native brown seaweeds like *Carpophyllum* and *Cystophora* (Hay & Luckens, 1987; Stuart, 2004). Colonisation of *Undaria* was seen to result in the dispersion of species that would otherwise normally inhabit the areas or recruitment of species that were likely to thrive in thick canopies. Either way, it displaces the biodiversity of areas that it colonises. As a result, *U. pinnatifida* was classified as an unwanted organism under the Biosecurity Act 1993, and was included in the list of pest species in several regional pest management systems with the aim of slowing down the rate of its spread around New Zealand. In subsequent years, there were several attempts to control *Undaria*, one of which was the manual removal of *Undaria* by divers in Big Glory Bay to remove it at the sporophyte stage. This helped reduce the total number of sporophytes but did not completely eradicate *Undaria.* In the same area, there was also a failed attempt at eradicating all *Undaria* by sterilising floating structures with sodium hypochlorite granules, while the structures were enclosed in polythene sheets at low tide. Another technique of eradication involved the use of brominated microbiocides, which was less corrosive, less likely to evaporate at high temperatures, and broke down more rapidly than chlorine-based biocides, while being more active at seawater pH. This technique proved to be ineffective, however, and attention was directed to other eradication techniques. These other techniques include the use of heat treatment that resulted in a 100% mortality rate of *Undaria* gametophytes in vitro when exposed to hot water at 60 °C for 5 seconds; and a vessel monitoring programme, which identified hulls of ships that were contaminated with *Undaria* spores (Stuart, 2004). In 2004, a review of the

policy on *Undaria* was undertaken and as a result, limited commercial harvest was allowed. This was again reviewed in 2010 and the results are summarised in Table 1 below. The only activity not allowed under the new policy is the harvest of *Undaria* that is not a part of a control programme or a by-catch of another activity since such activities could disturb or remove the existing native canopy species which would lead to the proliferation of *Undaria* (Ministry of Agriculture and Forestry, 2010).

Table 1. A comparison of the scope of the 2004 and 2010 policies on the commercial harvest of *Undaria* (Ministry of Agriculture and Forestry, 2010).

Activity	**2004**	**2010**
Harvesting as part of an *Undaria* control programme	✓	✓
Harvesting as a by-catch of another activity i.e. mussel farming	✓	✓
Harvesting from natural surfaces, if not part of a control programme or by-catch of another activity	×	×
Harvesting from artificial surfaces, if not part of a control programme or by-catch of another activity	×	✓
Harvesting as beach cast *Undaria,* if not part of a control programme or by-catch of another activity	×	✓
Farming in selected marine farming areas heavily infested with *Undaria*	×	✓

3.2.1 Utilisation of Undaria

Seaweeds are nutritionally important because they contain fibre (phycocolloids, hydrocolloids, and gums), protein, lipids, vitamins and minerals. Additionally, seaweeds are also good sources of bioactive compounds including carotenoids, sterols, tocopherol, vitamins and phycocyanins, which are believed to have beneficial effects on human and animal health due to their potential to act as antioxidants, antibacterial or antiviral agents. Furthermore, they can also potentially help control hyperlipidaemia, thrombosis, tumours, and obesity (Venugopal, 2008). Wakame products have grown in popularity over the years because of their high fibre and low energy content. At present, the demand for wakame products in Japan exceeds supply, therefore raising the need to import products from Korea and China. *Undaria* is processed in many ways, which determine the type of wakame end-product. Ultimately, the quality of the product is determined by its thickness and hardness (determined by cultivation and processing conditions), colour (fresh green wakame is preferred by consumers), stability during storage and the absence of foreign materials (Yamanaka & Akiyama, 1993).

The green colour of wakame that is preferred by consumers is achieved by briefly blanching the blade in seawater at no less than 65°C, which causes a change in chlorophyll-related enzymes (Yamanaka & Akiyama, 1993). Yamanaka & Akiyama (1993) also investigated the effect of different blanching times and temperatures on the colour of wakame being produced and its effect on storage. It was determined that temperatures between 80 and 90 °C for short periods of between 30 and 60 seconds yielded the ideal coloured processed wakame. If the product were blanched for too long at a high temperature, the chlorophyll degraded to phaeophytin, which results in a brown colour (Yamanaka & Akiyama, 1993). Table 2 is a list of the different processing methods applied to *Undaria* to produce a variety of wakame products.

Table 2. Different wakame products in Japan (Yamanaka & Akiyama, 1993).

Wakame Variety	Process	Quality
Suboshi	Raw → sun-dried	Brownish green High in foreign materials Poor storage quality
Haiboshi	Raw → mixed with ash →sun-dried → washed → sun-dried	Fresh green High in foreign materials Poor storage quality
Salted	Raw → salted → dehydrated → midrib removed → visual selection → packaging	Brownish green
Boiled and salted	Raw → boiled → cooled → salted → dehydrated → midrib removed → visual selection → packaging	Fresh green
Dried cut	Boiled and salted → sifted → washed → dehydrated → cut → washed → dehydrated → salt removed → dried via rolling dryer → mechanical selection → visual check → metal detection → packaging	Fresh green Low in foreign materials Good storage quality

3.3 Mannitol in Seaweed

Mannitol is a low digestibility carbohydrate that is classified either as a sugar alcohol or a polyol. Its planar configuration is represented in Figure 3. Commercially, it can be used as a humectant, sugar-free bulking agents, crystallisation modifier in different foods, in oral and personal care, pharmaceutical, and industrial products. Other applications include its use as a texturising agent, anticaking agent, as a sweetener for sugar-free gums, and for dusting chewing gum sticks. Mannitol is slowly absorbed by the body and excessive intakes of it (20-30 g) have a laxative effect on humans. (Seppo & Anja, 2001).

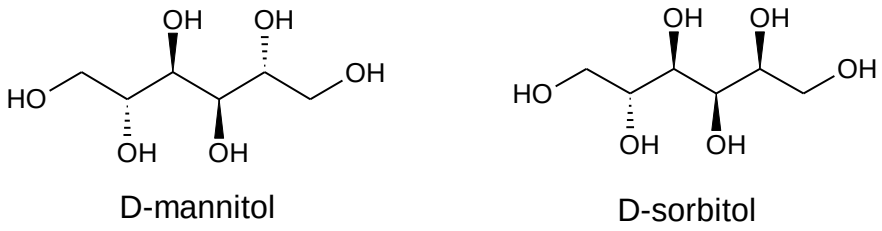

Figure 3. Configurations of D-mannitol and its epimer D-sorbitol.

Sources of mannitol in nature included algae (gold, brown and some red types) (Jamieson, 2011; Seppo & Anja, 2001; White, Coveny, Robertson, & Clements, 2010), bacillariophytes (diatoms), higher plants (White et al., 2010) and some fungi i.e. fresh mushrooms (Jamieson, 2011; White et al., 2010), and marine fungi (Alga & Julian, 2005). It has a water solubility of 22 g/100g in water (Jamieson, 2011), an energy content of 6.70 kJ/g (sucrose = 16.74 kJ/g), and about 50% the sweetness of sucrose (Seppo & Anja, 2001; Y.-J. Wang, 2003). It is commercially produced either by hydrogenation or fermentation of sugars such as fructose or sucrose, or by extraction from specific seaweed species, with hydrogenation of sugars being the most common method (Jamieson, 2011; Y.-J. Wang, 2003). Hydrogenation

of the sugars produces mannitol as well as sorbitol (a stereoisomer of mannitol) and purification is achieved on the basis that the two have different solubilities in water (Jamieson, 2011).

In phaeophytes (brown algae), mannitol can be found as the main by-product of photosynthesis. It also plays a role in osmoregulation of phaeophytes that are exposed to significant changes in seawater salinity (White et al., 2010). Ikeda (2002) reported that as much as 200g of mannitol can be obtained from high-quality seaweed, which makes it ideal as the main source of mannitol in countries where seaweeds are abundant.

3.4 Sensory Projective Mapping

The primary reason for conducting sensory analysis is to perform tests that are valid and reliable to produce data on which sound decisions can be made. Sensory tests have existed for as long as humans have existed as they evaluate whether food, water, shelter, weapons, etc. are good for use or consumption (Meilgaard, Civille, & Carr, 1999). The increase in trading later on called for more formal and structured sensory techniques as prices were based on the results gained from such tests. Grading schemes for wine, tea, coffee, butter, fish, and meat were among the first to be developed, some of which are still used today. Grading also led to the rise of professional tasters and consultants in the early 1900s in the food, beverage, and cosmetics industries. At present, sensory evaluation techniques are devised to serve economic interests, since they can establish the worth and acceptability of a certain product. The technique also contributed to the use of alternative processing methods to make the product better. In the food industry, sensory evaluation techniques are employed in quality control, research, and product development (Meilgaard et al., 1999) to evaluate a food product's texture, appearance, and flavour (Marsili, 2006).

The use of sensory projective mapping emerged after observation that sensory profiling, a class of methods that describe and quantify sensory attributes as perceived by humans, and (dis)similarity scaling, another class of methods that describe overall differences between products, yielded similar maps. In sensory profiling, assessors investigated the similarities and differences of the products based on specific sensory attributes, e.g. texture and colour, rather than considering the whole product. Thus, this method produces a breakdown of the different components or sensory attributes that are quantified by trained assessors' perceived scores for the given sensory attributes. The scores for all attributes are later on combined to assess the similarities and differences between products as a whole. On the other hand, in (dis)similarity scaling, the assessors are not given any sensory attributes that serve as guides and are required to quantify the differences between product pairs as a whole. Although the two methods have different philosophies behind them, it was observed that they yielded similar maps (Risvik, McEwan, Colwill, Rogers, & Lyon, 1994). However, there is a disadvantage with (dis)similarity scaling when the differences between products are subtle, since only those who are trained to perform sensory profiling methods such as descriptive analysis would be able to identify and describe the subtle differences. Furthermore, (dis)similarity scaling is harder to moderate since assessors, owing to lack of training, may use different references and degrees of similarities between repetitions. Overcoming this problem through training, however, may lead to discussions regarding sensory attributes, thereby creating a potential for bias and making it a sensory profiling rather than (dis)similarity scaling since (dis)similarity scaling operates on products being assessed as a whole and not broken down into attributes (Risvik et al., 1994). As a solution to the continuing concerns and limitations of sensory profiling and (dis)similarity scaling, projective mapping was adopted. At that time, it was a method only used in qualitative

market research. However, it was a cost-effective and rapid method used to assess product similarities and differences through a map (Lawless & Heymann, 2010). This method employed naive (untrained) consumers and allowed them to arrange products on a two-dimensional plane (Risvik, McEwan, & Rødbotten, 1997) without being given structures, points of view, or attributes prior to testing, thus allowing the researcher to figure out what criterion was important to the panellists when grouping the products. For example, one panellist may consider the taste of the product as being more important compared with appearance while another may give more importance to appearance over taste (Lawless & Heymann, 2010). The positions and distances of the products on the maps reflect their similarities and differences, therefore giving vague and unstructured ideas about them (Risvik et al., 1994). The coordinates of each product in the map constitute the data set (Lawless & Heymann, 2010).

When sensory projective mapping was reintroduced as "napping" (French for tablecloth), Multiple Factorial Analysis (MFA) was a statistical tool for the analysis of sensory data. This analytical tool helped uncover more than two dimensions in the data based on how panellists considered the different attributes. For example, if half of the panellists grouped the products according to taste and texture, and the other half based the groupings on taste and appearance, then the MFA would come up with a group configuration with three dimensions, with 50% of the variance coming from taste, 25% comes from texture, and 25% from appearance (Lawless & Heymann, 2010). MFA also produced RV coefficients in its output, which are used in multivariate techniques to measure similarities between squared symmetric matrices (Abdi, 2007). RV coefficients reflect the degree of similarity between multivariate configurations (maps) with values ranging from 0 to 1. Numbers close to 1 indicated high similarity (Nestrud & Lawless, 2009). Data acquired was also analysed using Principal Component Analysis (PCA).

In a PCA, variables which are often correlated with one another, thus containing the same information, are consolidated for easy interpretation (F. Wang, 2009). In this study, MFA and PCA were applied in the profiling of New Zealand processed seaweed against similarly processed commercial seaweed samples. This was because no sensory analysis has ever been carried out on the processed seaweed (wakame). Sensory analysis has only been carried out on pasta with seaweed added as a functional ingredient (Prabhasankar et al., 2009), and to a rice-based Korean dish called Bacsulgi (Jun, Kim, & Han, 2008). Table 3 summarizes studies that utilised projective mapping as a sensory tool, with the majority of studies comparing projective mapping with other sensory analysis methods such as descriptive analysis (Kennedy & Heymann, 2009), profiling and (dis)similarity scaling (Risvik et al., 1994), and sorting (Nestrud & Lawless, 2009).

Table 3. Studies carried out using sensory projective mapping.

Product	Experimental Aim	Findings	Reference
Chocolate	To compare maps obtained from projective mapping, profiling and (dis)similarity scaling.	Higher consistency was achieved over repeated trials from projective mapping compared with the other two methods.	Risvik et al., 1994
Blueberry soups	To compare maps obtained from projective mapping using naive consumers and descriptive analysis that utilised a trained panel.	Results showed that mapping replicates showed visually similar maps although RV coefficients indicated that panellists perceived products differently which highlighted the dimensionality of consumer perception compared with trained panellists	Risvik et al., 1997
Chocolate milk desserts	To compare results obtained from projective mapping and a check-all-that-apply (CATA) questionnaire.	Results from the two methods yielded very similar maps, indicating that both methods differentiated the samples. The differences perceived were also highly correlated with the differences in formulation.	Ares, Deliza, Barreiro, Giménez, & Gámbaro, 2009
Milk and dark chocolates	To compare results from projective mapping and descriptive analysis. An untrained panel was used for projective mapping. Once completed, the same panel was trained for descriptive analysis and results were compared.	Maps obtained were visually similar and RV coefficients for all maps were > 0.8, indicating that the untrained panel for projective mapping came up with maps similar to the ones obtained from descriptive analysis. Results also showed that panellists	Kennedy & Heymann, 2009

		perceived the products similarly in terms of separating products with sweet and dairy notes with those with bitterness and astringency.	
Apples and cheeses	To compare results obtained from projective mapping and sorting techniques.	Maps obtained from the two methods were similar, although panellists had more difficulty with apples compared with the cheeses.	Nestrud & Lawless, 2009
Granola bars	To obtain maps and descriptions (terms) of berry flavoured granola bars using projective mapping and evaluate the consistency of results obtained from three different sessions.	The repeat maps for each consumer did not show a high degree of similarity in all consumers. However, maps showed that the products were perceived similarly in terms of how the products were grouped.	Kennedy, 2010
Fish nuggets	To utilise descriptive analysis, flash profiling, and projective mapping on the evaluation of hot served foods with the use of panellists with varying training levels.	Maps obtained from the three methods were correlated well and showed that flash profiling and projective mapping may be used as quick substitutes for descriptive analysis, especially for food that has to be consumed above room temperature.	Albert, Varela, Salvador, Hough, & Fiszman, 2011

3.5 Volatiles in Seaweed

Very little has been reported on the volatile composition of edible seaweed. One of the earliest studies on seaweed odour conducted by Boland and Müller (1987) reported the presence of seven hydrocarbons, 3-undecanone, dictyoprolene, 4-((1E)-1-hexenyl)-cyclopenene, and 6-((1E)-1-butenyl)-cyclohepta-2, 5-diene in the essential oils of a Mediterranean seaweed, *Dictyopteris membranacea.* Kajiwara, Hatanaka, Kawai, Ishihara, & Tsuneya (1988) reported on the flavour compounds found in the oil extracts of different kinds of edible seaweed. The study only detected β-ionone and a sesquiterpene alcohol called cubenol. Cubenol comprised 88% of the volatiles detected in the *Undaria* oil extracts. Sniff tests by experienced flavourists determined that cubenol had odour intensity between 100 and 250 ppm and was described as being "kelp", "hay", "mint", and "ocean". Other compounds found in the other seaweed in the study such as *Laminaria angustata, L. japonica, Kjellmaniella crassifolia, Costaria costatam, Ecklonia cava, and Alaria crassifolia* included (*E,Z)*-2,6-nonadienal, *(E)*-2-nonenal, *(Z,Z)*-3,6-nonadienal, *(E,Z)*-2,6-nonadienol, *(E)*-2-nonenol, myristic acid, and ω-hexadecanoic acid (Kajiwara et al., 1988).

A study by Le Pape, Grua-Priol, Prost, & Demaimay (2004) on the red algae *Palmaria palmata* found seven of each of halogenated compounds and aldehydes, two ketones, three alcohols, and four miscellaneous compounds that included cyclic and noncyclic hydrocarbons. Of these, the halogenated compounds iodoethane, trichloromethane, 2-fluoroprop-1-ene, iodopentane, chlorobenzene, and tribromomethane were reported to be characteristic of the red alga (Le Pape et al., 2004). Kajiwara, Matsui, Akakabe, Murakawa, & Arai (2007) investigated the antimicrobial browning-inhibitory effects of volatile compounds in the essential oils of several seaweed species such as *Laminaria japonica, Kjellmaniella carrifolia, Gracilaria*

verrucosa, and *Ulva pertusa* and reported the presence of flavour compounds such as (3Z)-hexenal, (2*E*)-hexenal, and (2*E*)-nonenal, which have strong antimicrobial effects. In 2005, the *n*-alkanes octadecane (C_{18}), icosane (C_{20}), docosane (C_{22}), tetracosane (C_{24}), and octacosane (C_{28}) in three diverse *U, pinnatifida* samples collected from the different areas Galician coast on different occasions (Punín Crespo & Lage Yusty, 2006).

Nor Qhairul Izzreen & Vijaya Ratnam (2011) recently investigated the extraction and analysis of volatile compounds from three species of seaweed, namely *Kappaphycus alvarezii* (red), *Caulerpa lentillifera* (green), and *Sargassum polycystem* (brown) using headspace solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS). Apart from extraction and characterisation of volatiles, optimisation of conditions at which extractions were carried out was also reported. It was found that 1) incubation temperatures that exceeded 80 °C caused the formation of artefacts; 2) volatile compounds showed an increased response when heated between 40 and 45 °C, above which the volatiles started to migrate out of the fibre; 3) changing heating temperatures from 50 °C to 60 °C caused fluctuations in the types of the compounds in the chromatograph obtained, with higher concentrations of aldehydes, hydrocarbons and higher molecular compounds recorded; and 4) increasing extraction time and temperature overloaded the fibre with high boiling point compounds, thus replacing the low boiling point compounds in the fibre. A total of 233 volatile compounds were identified in this study for three species of seaweeds (82 for the red, 91 for the green, and 50 for the brown) and results were reported as percentage peak areas with retention times (Nor Qhairul Izzreen & Vijaya Ratnam). The reporting of volatiles against retention times rather than retention indices made it difficult for other researchers to use their results since "raw" retention times are highly dependent on various analysis conditions such as the type of stationary phase used, conditions of

the column used such as the content in packed columns and film thickness, column length, carrier gas flow, and oven temperature among others. Therefore, a standardization of the experimental conditions was necessary and can be achieved by calculating retention indices (Zenkevich, 2005).

Extraction of volatile compounds was done using solid phase microextraction (SPME), a method developed to satisfy the need for a quick, solvent-free and field-compatible method of sample preparation. An SPME device is illustrated on Figure 4 below. Applications include environmental, clinical, forensic, food, drug, and industrial hygiene analysis (Pawliszyn, Pawliszyn, & Pawliszyn, 1997).

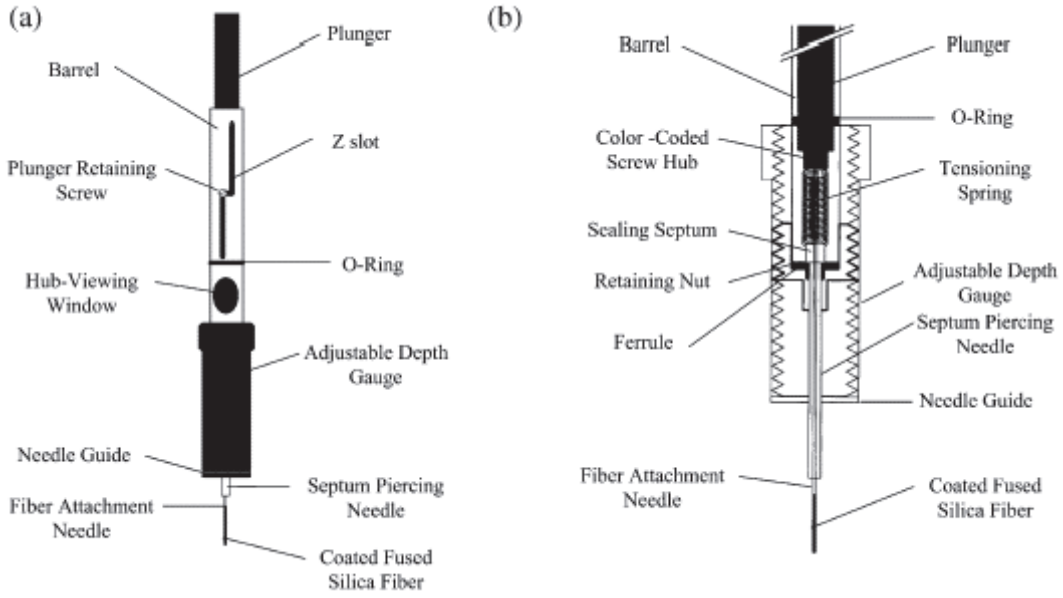

Figure 4. A commercial SPME device. (a) shows the SPME fibre holder and (b) shows the SPME holder and the fibre assembly (Mester, Sturgeon, & Pawliszyn, 2001).

Modes of extraction using an SPME can either be direct or headspace extraction. The direct extraction involves inserting the fibre into the sample medium, where the analytes are adsorbed onto the fibre. On the other hand, headspace extraction requires the analytes to be

volatilised and transported through air prior to being absorbed into the SPME fibre. This mode protects the fibre from being contaminated by saturation with high molecular weight and non-volatile compounds in the sample media. The two methods, however, yield similar results as long as the volumes of the liquid phase and gaseous headspace volumes are the same, and equilibrium in the sample vial is achieved prior to introduction of SPME (Mester, Sturgeon, & Pawliszyn, 2001). Yang (1994) also reported that the addition of salt to the sample matrix helped enhance the adsorption onto the SPME fibre, while a larger sample:headspace ratio increased the sensitivity of analysis. Headspace SPME also had an advantage over static headspace sampling, where an aliquot of the headspace is injected into a gas chromatograph because it can absorb and concentrate analytes onto the fibre as opposed to the low recovery rate of flavour volatiles in static headspace sampling (Miller & Stuart, 1998).

SPME has been used in the characterisation of volatiles drinking water (Cho, Kong, & Oh, 2003; Watson, Brownlee, Satchwill, & Hargesheimer, 2000), wine (Rocha, Ramalheira, Barros, Delgadillo, & Coimbra, 2001), juices (Miller & Stuart, 1998), oils (Yang & Peppard, 1994), truffles (Pelusio et al., 1995), and bananas (Liu & Yang, 2002), and pesticide residues in food (Aulakh, Malik, Kaur, & Schmitt-Kopplin, 2005), to name a few.

Chapter 4: Methods

4.1 Harvesting and Sampling

Undaria pinnatifida seaweeds were harvested from the Wakatu Inc. mussel farms, when available, in the Marlborough Sounds between the months of June and October 2011. A total of six farms were harvested from the Pelorus Sounds and three from Port Underwood. Pelorus Sound was considered a sheltered site and Port Underwood was considered an exposed site Pelorus Sounds is found in the inner peninsula and Port Underwood is located more toward the open seas. Figure 5 maps the harvest sites.

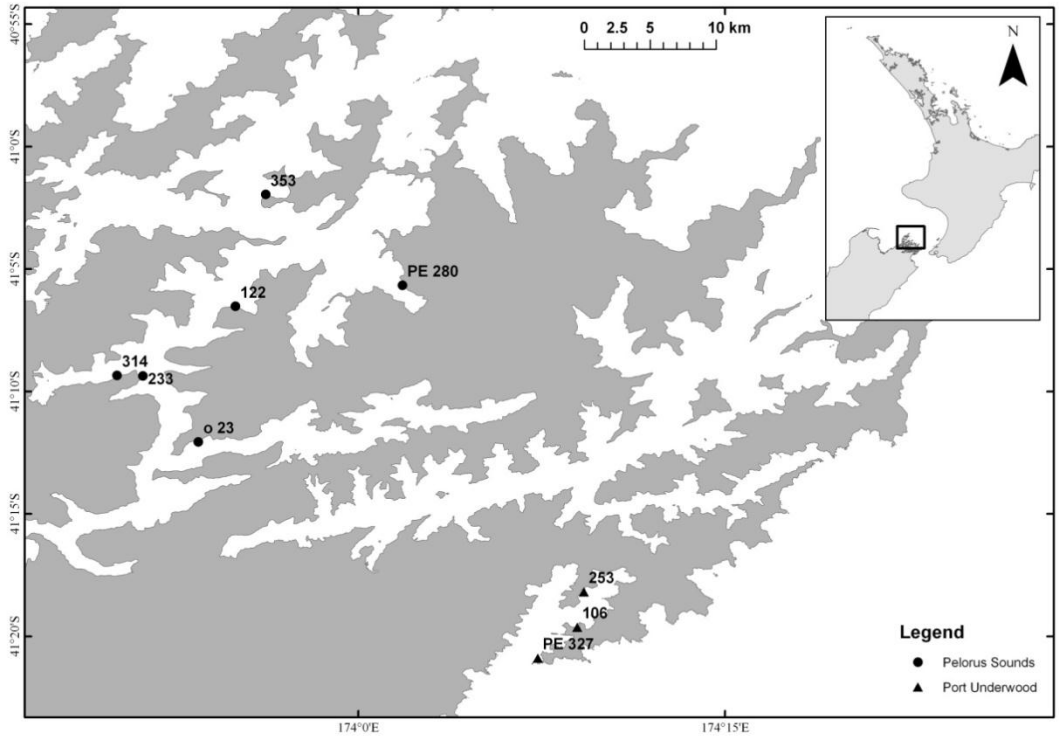

Figure 5. Location of mussel farms in the Marlborough Sounds from which *Undaria pinnatifida* seaweed was harvested.

The blades of the seaweed were separated from the sporophyll on the boat, and each sample was placed in numbered and labelled bags. These samples were then frozen overnight

prior to being air freighted to Vitaco Limited, a freeze-drying plant in Auckland, to be lyophilised in bulk within 48 hours of frozen storage. Dry weights of the freeze dried samples were recorded prior to the samples being milled using a coffee grinder (Breville CG2B Coffee 'n' Spice Grinder) and sieved using a 600 micron sieve. The powdered samples were then stored in 200 mL plolystyrene urine sample containers and kept in a dark cupboard at room temperature prior to analysis.

4.2 Mannitol Analysis

Eight individual plants from each of the farms 327, 106, 353 and 122 collected in the months of June, July, August, September and October, 2011 were analysed. Extraction of mannitol was carried out according to White et al (2010). Freeze-dried powder (0.1g) mixed with 10 mL of water in a 50 mL polypropylene Nalgene centrifuge tube was vortexed and placed in an 80°C water bath for one hour for free sugar extraction. The tubes were then centrifuged at 17000 g at 4°C for 15 minutes. The supernatant (4 mL) was collected from each tube and passed through a 100mg/3mL Phenomenex Strata C18-E SPE column and transferred into a 4 mL capped glass sample vial. The supernatant was then diluted with Millipore water and HPLC-grade acetonitrile (Fisher Scientific) to make up a mixture of 70% acetonitrile:water (70:30 v/v). The solutions were then filtered using a 0.45 µm Phenex RC filter. The remaining supernatants were frozen and kept as stock samples. Extracts were analysed for sugar content isocratically at a flow rate of 1.5ml/min with a 97:3 v/v acetronitrile:water mobile phase using a Shimadzu HPLC-10AD equipped with a Luna 5µ HILIC column (100 x 4.6mm, 5 micron), and an Agilent Technologies 385-ELSD (evaporative light scattering detector).

Standards that ranged from 0.00000025 mg/mL to 0.0003 mg/mL in water were made using analytical grade D-mannitol (BDH Laboratory Supplies, Palmerston North, NZ). A new standard curve was determined each day of analysis. Statistical analysis was performed using One-way ANOVA (α=0.05) with Tukey's significance tests after testing for Equal Variances using Minitab 16.1.0. Some data sets had to be transformed to an exponential function prior to testing with One-way ANOVA to achieve homogeneity of variance. These data sets are marked by * and ** in the mannitol results section.

4.3 Sensory Analysis

4.3.1 New Zealand seaweed processing to produce wakame

Wakame processing of New Zealand seaweed was carried out according to Kantono (2011). Fresh New Zealand *U. pinnatifida* harvested from Farm 327 from Port Underwood in the Marlborough Sounds were rinsed and placed into plastic bags on the boat. The samples were then transported to the AUT laboratory via airfreight overnight to be further processed into wakame. The samples were washed in freshwater for 5 minutes to remove epiphytes and any other foreign matter prior to being blanched at 80 °C for 1 min. The seaweed was then quickly cooled using cold water. Following this, the seaweed was salted with normal table salt in a 3:10 salt to seaweed wet weight ratio and left to cure for 48 hours. After curing, the samples were rinsed with freshwater to remove excess salt and the midrib was removed. This was followed by oven-drying at 60 °C for 24 h. After drying, the dried wakame preparations were packed in resealable plastic bags and stored in a cupboard at room temperature.

4.3.2 Commercial and NZ wakame preparation for projective mapping

Five (5) commercial samples (two Korean samples from One-mart in Auckland: Chung Jung Won and Ottogi; three Japanese samples from Japan Mart in Auckland: Wakou Shokai, Fue Fue and Riken) and one New Zealand sample (from the month of August) processed as described above were rehydrated using cold water for 2 min and then drained. Approximately 2-3 g of each sample was added into individual glass soup bowls containing a mild flavoured chicken soup (9.92g of Knorr powdered chicken stock per litre of boiling water). The chicken soup was kept at 70 °C in a slow cooker set on high prior to being served to panellists in the tasting booths. Green tea was used as a palette cleanser and testing was conducted under red light to mask colour differences between the New Zealand and the commercial samples, since optimum wakame colour was not achieved at the time of testing (Kantono, 2011).

4.3.3 Projective Mapping

Panellists were recruited on the basis that they were regular consumers of wakame (processed *Undaria)*. Those eligible – those who consumed wakame at least once a week – were invited to attend projective mapping sessions at the AUT Sensory Lab for three consecutive Mondays between 10 am and 3 pm. Panelists were given verbal instructions before being led into the sensory booths. The same set of instructions was also displayed on the computer terminals using a FIZZ programmed sensory projective mapping test (FIZZ Network v2.46C, Biosystemes). Panellists then tasted the randomised and coded samples in the sensory booths, in the order presented from left to right. Panellists grouped the samples according to their similarities and differences, with those grouped close together being more similar to each other. Additionally, they were asked to write descriptors and/or attributes that corresponded to their

groupings. Individual panellist maps were keyed in by panellists and recorded using the FIZZ Network v2.46C system. Analysis of results was performed using Multifactorial Analysis (MFA) to get overall product maps, General Proscrustes Analysis to obtain overall product coordinates, and Principal Component Analysis to obtain product and attribute biplots using Addinsoft XLSTAT-MX version 2011.5.01. Sensory attributes that occurred a minimum of five times across panellists per product were included in the PCA biplots.

4.4 Texture Analysis

Texture analysis was performed on five commercial and two New Zealand wakame (August and October samples from Farm 327) samples. Samples were immersed in a bowl of water for about three minutes until fully hydrated and then drained and cut into 3 cm x 3 cm sheets. Texture analyses were performed using a Stable Micro Systems TA.XTplus Texture Analyser equipped with a Film Support Rig (HDP/FSR) on a Heavy Duty Platform (HDP/90) with a 5 mm stainless steel probe (P/55) and a 5 kg load cell. The texture analyser was set to measure force in the compression mode with a pre-test speed of 2.0 mm/s, a test speed of 1.0 mm/s, and a post-test speed of 10.0 mm/s. Target mode was set to distance set at 5 mm. Data acquisition rate was set at 500 pps. Significant differences between texture readings of the different samples were tested using One-way ANOVA on Minitab v 16.1.0 with Tukey's post hoc comparison tests where $p \leq 0.05$ ($\alpha=0.05$) indicates significant differences between means.

4.5 Colour Analysis

Colour analysis was performed on each of the five commercial wakame samples and two New Zealand processed wakame (August and October samples from Farm 327) according to

(Whale, Singh, Behboudian, Janes, & Dhaliwal, 2008) using a HunterLab ColorFlex machine, and *L*, *a*, and *b* values were recorded. Figure 6 shows the L a b colour space where *L* corresponds to the lightness and darkness of the sample with values between 0 and 100, with 100 being the lightest. *a* differentiates between green and red with negative values indicating a higher abundance of green pigments, while a positive value indicates a higher abundance of red pigments. Lastly, *b* corresponds to either yellowness or blueness of a product, where negative values indicate more blue pigments and positive values indicates more yellow pigments (HunterLab, 2008). Values for *a* and *b* readings range from -120 to 120 (León, Mery, Pedreschi, & León, 2006). Each product was sampled three times and their means were compared using One-way ANOVA in Minitab 16.1.0 with Tukey's post hoc significance tests where $p \leq 0.05$ ($\alpha=0.05$) indicates a significant difference between means.

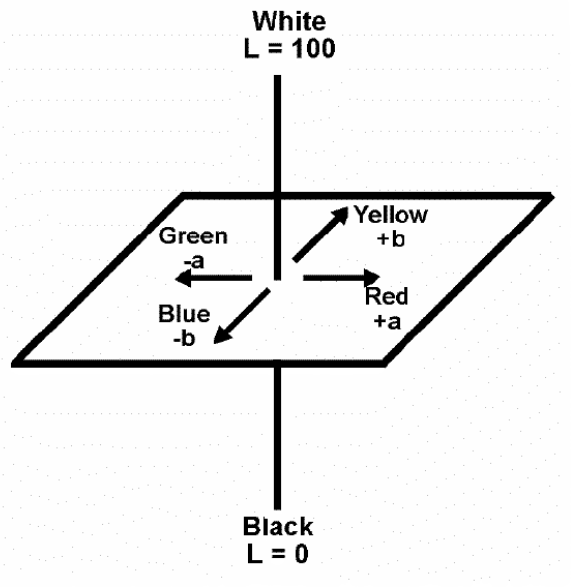

Figure 6. The Hunter L, a, b colour scale (HunterLab, 2008).

4.6 Volatile Profile Analysis

The same six commercial and New Zealand samples used for sensory projective mapping plus an October New Zealand sample were ground and stored in the same manner as for mannitol analysis. For headspace SPME extraction, 0.1 g of the powdered sample was measured into a 20 mL Agilent flat bottom headspace vial and mixed with 10 mL deionised water and 5g of salt. A stir bar was placed in the vial before capping and crimping it with a 20mm tan silicone septum with a PTFE face. One microlitre of one part per thousand (ppt) 1,2-dichlorobenzene was also injected through the septa as an internal standard. The samples were then incubated for 30 min in a Supelco heater block for 28mm diameter vials (Sigma-Aldrich) set at 70 °C on a magnetic hot plate. After 30 min, the vials were quickly cooled to room temperature using cold tap water and then re-incubated at 40 °C for 15 minutes to reach equilibrium temperature before extraction of headspace volatiles using a Supelco DVB/CAR/PDMS SPME fibre for 15 min. The SPME was then injected into the Trace Ultra GC (Thermo Scientific, USA) equipped with 30m×0.25mm×0.25□m VF-5 ms low bleed/MS fused-silica capillary column (Phenomenex, Inc, Torrance, USA) at a helium flow rate of 1.5 mL min^{-1}. The injector was operated in splitless mode for 30 s then at a 10:1 split ratio. The detector was a DSQ Series single quadrupole mass spectrometer (Thermo Scientific, , USA). The temperature program was: Hold for 2 min at 40 °C, increase at 5 °C/min, and finally hold for 3 min at 250 °C. The mass spectrometer operated in the electron impact mode with a source temperature of 200 °C, an ionising voltage of 70eV, and the transfer line temperature was 250 °C. The mass scan was from m/z 48 to m/z 400 at 3.41 scan s^{-1}

Peak identification was carried out by comparison of their mass spectra with spectra from authentic compounds previously analysed, NIST/EPA/NIH Mass Spectral Database (Version

2.0a, 2002), or NIST web book (http://webbook.nist.gov/chemistry/) and comparison of retention indices from previous literature. The approximate quantities of the volatiles were estimated by comparison of their peak areas with that of the 1,2-dichlorobenzene internal standard using a response factor of 1. Analysis for significant differences in peak areas at specific retention indices was performed using One-way ANOVA with in Minitab 16.1.0 with Tukey's post hoc comparison tests where $p \leq 0.05$ indicates significant differences between means. These were then plotted against the different wakame products using Principal Components Analysis in the Addinsoft XLSTAT MX package version 2011.5.01.

Chapter 5: Results

5.1 Mannitol Analysis

Table 4. Mannitol content (mg/g) of *Undaria* by month and harvest area (farm).

MONTH	327, Exposed	106, Exposed**	122, Sheltered	353, Sheltered
June	5.43 ± 3.07^{A}	7.99 ± 3.56^{A}	N/A	N/A
July	5.94 ± 2.17^{A}	7.06 ± 2.01^{A}	N/A	N/A
August	8.68 ± 6.23^{Ab}	20.06 ± 6.15^{Aa}	8.68 ± 2.79^{Bb}	18.41 ± 8.50^{a}
September*	5.63 ± 3.72^{Aa}	15.19 ± 10.52^{Aa}	5.97 ± 1.81^{Ba}	N/A
October	N/A	N/A	18.64 ± 6.19^{A}	N/A

Samples were collected from June to October 2011 from Port Underwood and Pelorus Sound.
All the values are mean ± SE of eight samples (period when seaweeds were not collected are indicated with NA, not available). Significant differences ($p < 0.05$) for farms 327 and 106, and 122 in a location are indicated by different superscript capital letters. Significant differences ($p < 0.05$) between farms for August and September are indicated by different superscript lower case letters.
P values are obtained from one-way Analysis of Variance.

*****Mannitol concentrations were transposed to an exponential function to achieve homogeneity of variance so as to compare the different farms in September.
****** Mannitol concentrations were transposed to an exponential function to achieve homogeneity of variance so as to compare mannitol content for Farm 106 for different months.

5.1.1 *Effect of location on mannitol content of Undaria pinnatifida*

Analysis of the difference in mannitol content of samples drawn from four different farms in August showed that mannitol content varied from farm to farm. Farms 106 and 353 showed a higher concentration of mannitol compared with Farms 327 and 122. Farms 106 and 327 were both from Port Underwood which was an exposed site where seaweeds were more exposed to tidal flows while farms 122 and 353 were located in Pelorus Sounds and were considered sheltered sites. The location, whether exposed or sheltered did not affect mannitol content of *Undaria*. This may be due to the fact that mannitol is a by-product of photosynthesis which is mainly determined by the availability of sunlight and nutrients (Dean & Hurd, 2007). Figure 7 below shows the mannitol content of the different farms for August.

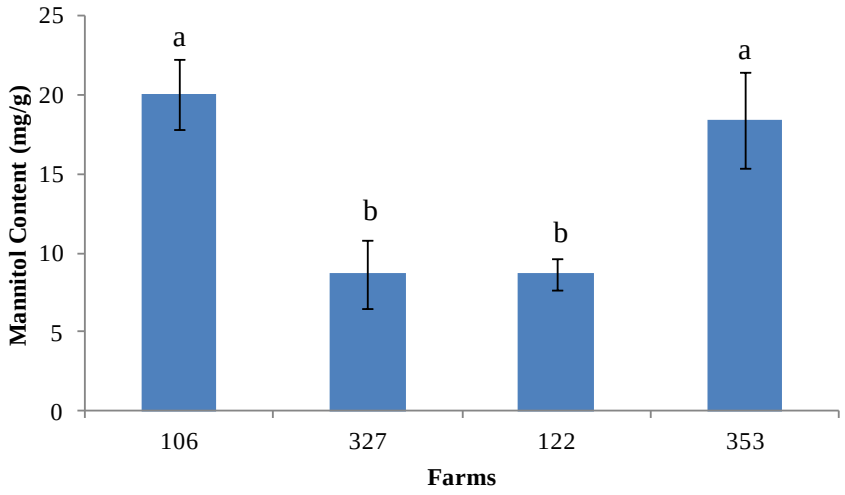

Figure 7. Mannitol concentrations for the month of August for farms 327, 106, 353, and 122.

Results from the analysis of variance for September samples shown in Figure 8 below indicated that there were no significant differences in mannitol concentration between farms 106, 327, and 122.

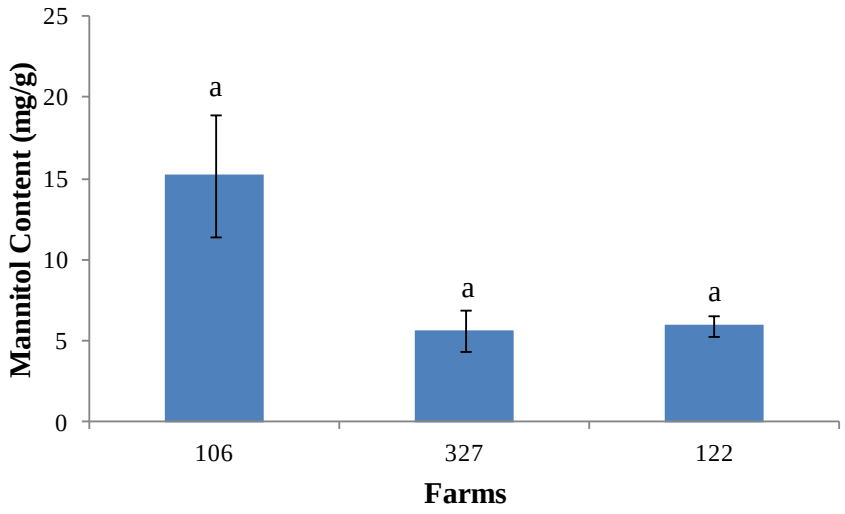

Figure 8. Mannitol concentrations for the month of September for farms 327, 106, and 122.*
***Mannitol concentrations were transposed to an exponential function to achieve homogeneity of variance so as to compare the different farms for September

5.1.2 Monthly changes in the mannitol content of Undaria pinnatifida

Choi et al. (2009) suggested that photosynthesis occurred most when plant growth was most active which coincides with when seawater temperature is decreasing and before the coldest period of the year. Furthermore, Dean & Hurd (2007) determined that *Undaria* growth is not light limited which means that *Undaria* in New Zealand can still thrive in winter when there is less sunlight as well as in sub-canopies and depths of up to 8 meters. Dean & Hurd (2007) also noted large *Undaria* individuals in the Otago Harbour during a survey in 2005.

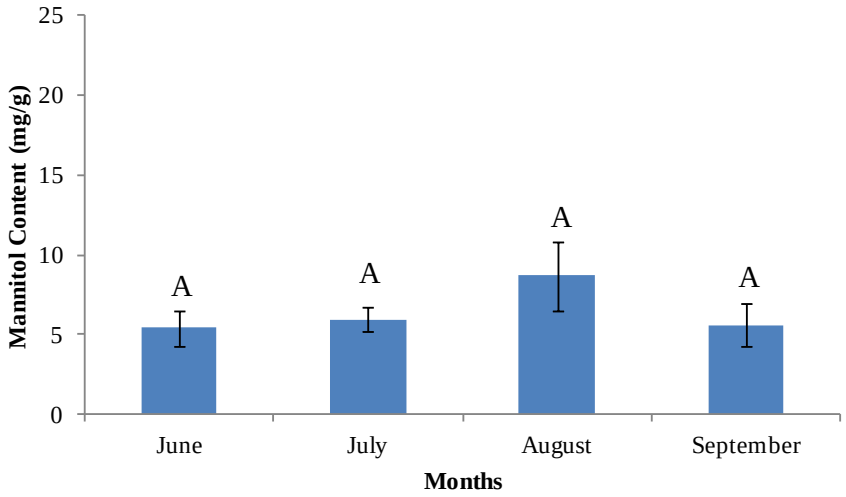

Figure 9. Comparison of mannitol concentrations between the months of June, July, August, and September for Farm 327.

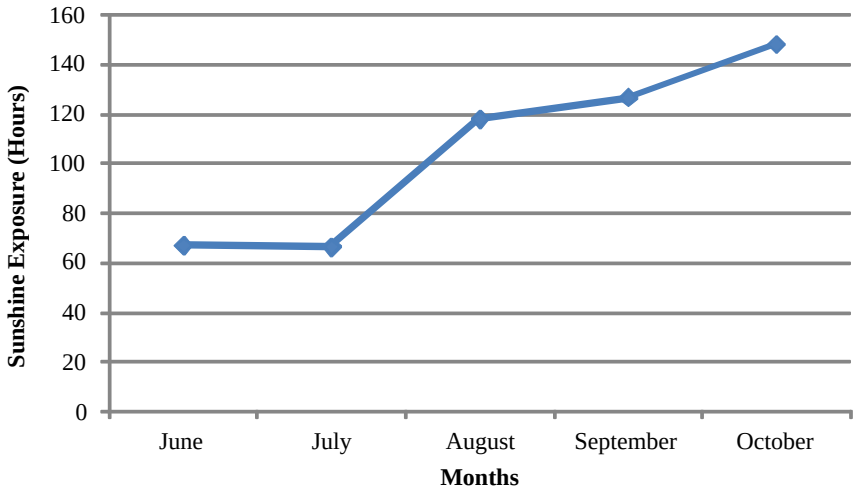

Figure 10. Total sunshine data for a NIWA station in Blenheim obtained from http://cliflo.niwa.co.nz/.

Figure 10 shows the mean total sunshine in hours in a station close to where samples from the exposed sites, farms 327 and 106, were collected. There was an increase in the amount of sunshine from July to August which coincided with the New Zealand spring season. For both farms 327 and 106, while the statistical analysis showed no significant differences across four months in the mannitol content of seaweed, Figures 9 and 11 of the mannitol content for each

farm showed an increase in mannitol from July to August. This may be explained by the increase in the amount of sunlight the plants were exposed to, which could have led to an increase in the photosynthetic activity of the plants. The same was found for Farm 122, shown in Figure 12, which showed a significant increase in mannitol content for October while concentrations between August and September showed no significant differences.

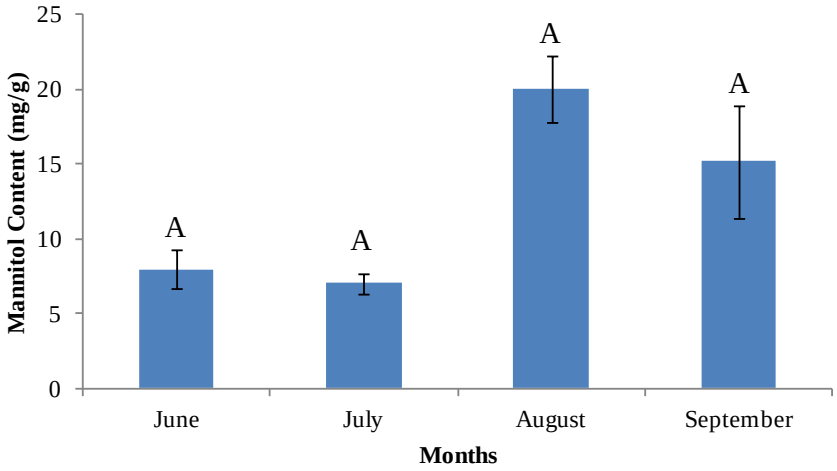

Figure 11. Comparison of mannitol concentrations between the months of June, July, August, and September for Farm 106. ***

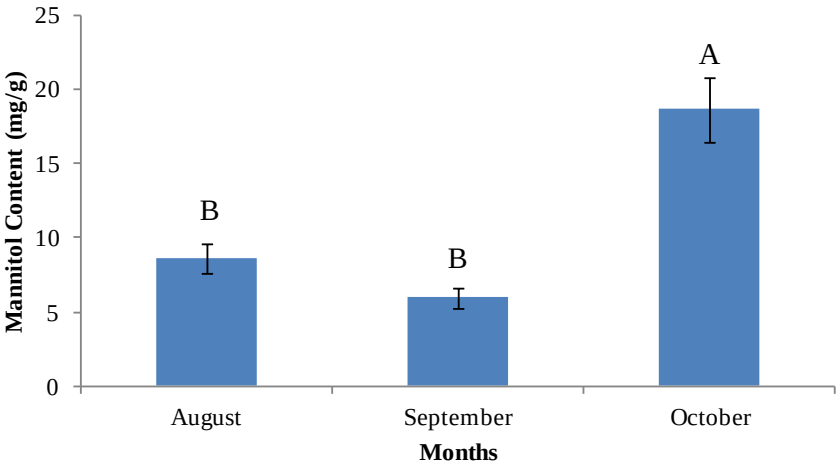

Figure 12. Comparison of mannitol concentrations between the months of August, September, and October 2011 for Farm 122.

5.1.3 Effect of processing on the mannitol content of Undaria pinnatifida

Table 5. Mannitol content (mg/g) of processed *Undaria* (wakame) samples.

Sample	Mannitol content (mg/g)
New Zealand (August)	6.34 ± 0.35^{A}
Ottogi	1.71 ± 1.08^{B}
Fue Fue	2.49 ± 0.33^{B}
Chung Jung Won	0^{C}
Wakou Shokai	0^{C}
Riken	0^{C}

Results for mannitol analysis of the processed wakame samples (5 commercial and 1 NZ), summarised in Table 5 showed that there are significant differences between the New Zealand wakame and the commercial ones ($p=0.000$). The New Zealand sample had the most mannitol, followed by Ottogi and Fue Fue. No mannitol was detected in the other three products. Mannitol taste threshold has been determined to be 20 mmol/L which converts to 3.643 g/L (Rotzoll, Dunkel, & Hofmann, 2006). The mannitol present in New Zealand *Undaria* was expressed as 6.3354 ± 0.3478 mg/g dry weight. However *Undaria* harvested in New Zealand has a 90-95% water content. Assuming 100% rehydration, then the mannitol concentration will be 20 times less or about 0.32 mg/g prior to consumption.

5.2 Sensory Analysis

A total of 17 panellists took part and 16 completed the sensory projective mapping of the six wakame products over three weeks. The RV coefficients were used to determine how well a panellist's map fitted with the consensus maps. Out of the 16 panellists, one – panellist, N6, scored badly in terms of fit with the rest (RV < 0.500) for 2 out of the 3 weeks (wk1 = 0.453, wk2 = 0.746, wk3 = 0.414). Therefore, a decision was made to remove the data gathered from this particular panellist from further statistical analysis. Panellist 16 was also not included due to failure to complete the three weeks of testing. RV coefficients less than 0.500 are in red in Tables 6-8.

Table 6. RV coefficients between projective maps and multifactor analysis (MFA) for week one where 80% of panellists scored >0.500.

Panelist #	1	2	3	4	5	7	8	9	10	11	12	13	14	15	17	MFA
1	1.000	0.781	0.210	0.193	0.180	0.081	0.464	0.317	0.022	0.168	0.139	0.230	0.279	0.313	0.166	0.483
2	0.781	1.000	0.283	0.160	0.106	0.257	0.420	0.473	0.184	0.136	0.061	0.131	0.245	0.291	0.362	0.532
3	0.210	0.283	1.000	0.618	0.763	0.218	0.109	0.365	0.287	0.088	0.498	0.565	0.516	0.596	0.546	0.743
4	0.193	0.160	0.618	1.000	0.574	0.490	0.067	0.107	0.248	0.047	0.317	0.474	0.460	0.869	0.525	0.700
5	0.180	0.106	0.763	0.574	1.000	0.049	0.097	0.092	0.025	0.065	0.137	0.176	0.633	0.658	0.488	0.568
7	0.081	0.257	0.218	0.490	0.049	1.000	0.251	0.338	0.698	0.403	0.417	0.430	0.513	0.473	0.463	0.689
8	0.464	0.420	0.109	0.067	0.097	0.251	1.000	0.375	0.173	0.460	0.051	0.103	0.389	0.082	0.341	0.471
9	0.317	0.473	0.365	0.107	0.092	0.338	0.375	1.000	0.148	0.024	0.488	0.323	0.387	0.069	0.613	0.578
10	0.022	0.184	0.287	0.248	0.025	0.698	0.173	0.148	1.000	0.577	0.670	0.654	0.069	0.205	0.036	0.531
11	0.168	0.136	0.088	0.047	0.065	0.403	0.460	0.024	0.577	1.000	0.144	0.223	0.219	0.046	0.053	0.380
12	0.139	0.061	0.498	0.317	0.137	0.417	0.051	0.488	0.670	0.144	1.000	0.906	0.101	0.215	0.129	0.569
13	0.230	0.131	0.565	0.474	0.176	0.430	0.103	0.323	0.654	0.223	0.906	1.000	0.117	0.409	0.133	0.633
14	0.279	0.245	0.516	0.460	0.633	0.513	0.389	0.387	0.069	0.219	0.101	0.117	1.000	0.581	0.691	0.714
15	0.313	0.291	0.596	0.869	0.658	0.473	0.082	0.069	0.205	0.046	0.215	0.409	0.581	1.000	0.434	0.708
	0.166	0.362	0.546	0.525	0.488	0.463	0.341	0.613	0.036	0.053	0.129	0.133	0.691	0.434	1.000	0.693
MFA	0.483	0.532	0.743	0.700	0.568	0.689	0.471	0.578	0.531	0.380	0.569	0.633	0.714	0.708	0.693	1.000

Table 7. RV coefficients between projective maps and multifactor analysis (MFA) for week two where 93% of panellists scored >0.500.

Panelist #	1	2	3	4	5	7	8	9	10	11	12	13	14	15	17	MFA
1	1.000	0.541	0.690	0.031	0.661	0.297	0.005	0.134	0.779	0.175	0.478	0.573	0.339	0.407	0.491	0.691
2	0.541	1.000	0.612	0.225	0.793	0.458	0.280	0.441	0.678	0.473	0.178	0.620	0.388	0.130	0.325	0.744
3	0.690	0.612	1.000	0.131	0.718	0.463	0.067	0.423	0.486	0.140	0.448	0.445	0.581	0.307	0.325	0.717
4	0.031	0.225	0.131	1.000	0.017	0.311	0.536	0.448	0.340	0.154	0.255	0.281	0.350	0.467	0.322	0.526
5	0.661	0.793	0.718	0.017	1.000	0.223	0.114	0.133	0.587	0.190	0.168	0.516	0.484	0.082	0.314	0.619
7	0.297	0.458	0.463	0.311	0.223	1.000	0.220	0.414	0.313	0.296	0.191	0.585	0.032	0.097	0.281	0.559
8	0.005	0.280	0.067	0.536	0.114	0.220	1.000	0.216	0.181	0.083	0.054	0.316	0.131	0.046	0.106	0.350
9	0.134	0.441	0.423	0.448	0.133	0.414	0.216	1.000	0.144	0.247	0.105	0.221	0.482	0.418	0.181	0.529
10	0.779	0.678	0.486	0.340	0.587	0.313	0.181	0.144	1.000	0.496	0.548	0.592	0.381	0.430	0.661	0.804
11	0.175	0.473	0.140	0.154	0.190	0.296	0.083	0.247	0.496	1.000	0.523	0.210	0.382	0.376	0.649	0.576
12	0.478	0.178	0.448	0.255	0.168	0.191	0.054	0.105	0.548	0.523	1.000	0.259	0.393	0.711	0.772	0.654
13	0.573	0.620	0.445	0.281	0.516	0.585	0.316	0.221	0.592	0.210	0.259	1.000	0.270	0.087	0.558	0.688
14	0.339	0.388	0.581	0.350	0.484	0.032	0.131	0.482	0.381	0.382	0.393	0.270	1.000	0.355	0.521	0.637
15	0.407	0.130	0.307	0.467	0.082	0.097	0.046	0.418	0.430	0.376	0.711	0.087	0.355	1.000	0.595	0.593
17	0.491	0.325	0.325	0.322	0.314	0.281	0.106	0.181	0.661	0.649	0.772	0.558	0.521	0.595	1.000	0.759
MFA	0.691	0.744	0.717	0.526	0.619	0.559	0.350	0.529	0.804	0.576	0.654	0.688	0.637	0.593	0.759	1.000

Table 8. RV coefficients between projective maps and multifactor analysis (MFA) for week three where 93% of panellists scored >0.500.

Panelist #	1	2	3	4	5	7	8	9	10	11	12	13	14	15	17	MFA
1	1.000	0.487	0.541	0.195	0.433	0.435	0.210	0.278	0.492	0.298	0.099	0.343	0.143	0.145	0.759	0.595
2	0.487	1.000	0.342	0.346	0.681	0.361	0.512	0.167	0.211	0.333	0.257	0.521	0.277	0.552	0.472	0.662
3	0.541	0.342	1.000	0.306	0.216	0.198	0.198	0.162	0.415	0.419	0.470	0.318	0.416	0.120	0.218	0.535
4	0.195	0.346	0.306	1.000	0.335	0.554	0.676	0.879	0.328	0.787	0.345	0.464	0.314	0.753	0.213	0.799
5	0.433	0.681	0.216	0.335	1.000	0.325	0.556	0.074	0.068	0.359	0.216	0.553	0.045	0.488	0.375	0.577
7	0.435	0.361	0.198	0.554	0.325	1.000	0.563	0.614	0.626	0.575	0.244	0.127	0.210	0.417	0.664	0.730
8	0.210	0.512	0.198	0.676	0.556	0.563	1.000	0.468	0.259	0.731	0.386	0.541	0.246	0.560	0.168	0.732
9	0.278	0.167	0.162	0.879	0.074	0.614	0.468	1.000	0.513	0.669	0.221	0.241	0.329	0.653	0.349	0.717
10	0.492	0.211	0.415	0.328	0.068	0.626	0.259	0.513	1.000	0.235	0.347	0.176	0.154	0.201	0.477	0.574
11	0.298	0.333	0.419	0.787	0.359	0.575	0.731	0.669	0.235	1.000	0.456	0.324	0.499	0.514	0.296	0.783
12	0.099	0.257	0.470	0.345	0.216	0.244	0.386	0.221	0.347	0.456	1.000	0.511	0.596	0.313	0.157	0.566
13	0.343	0.521	0.318	0.464	0.553	0.127	0.541	0.241	0.176	0.324	0.511	1.000	0.044	0.512	0.092	0.587
14	0.143	0.277	0.416	0.314	0.045	0.210	0.246	0.329	0.154	0.499	0.596	0.044	1.000	0.283	0.106	0.475
15	0.145	0.552	0.120	0.753	0.488	0.417	0.560	0.653	0.201	0.514	0.313	0.512	0.283	1.000	0.252	0.718
17	0.759	0.472	0.218	0.213	0.375	0.664	0.168	0.349	0.477	0.296	0.157	0.092	0.106	0.252	1.000	0.580
MFA	0.595	0.662	0.535	0.799	0.577	0.730	0.732	0.717	0.574	0.783	0.566	0.587	0.475	0.718	0.580	1.000

RV coefficients obtained during the three weeks of testing indicated an improvement in the consensus maps of the panellists. In week 1, 80% of the 15 panellists scored >0.500 while weeks two and three showed a 93% agreement between the panellists.

5.2.1 *Product and attribute maps*

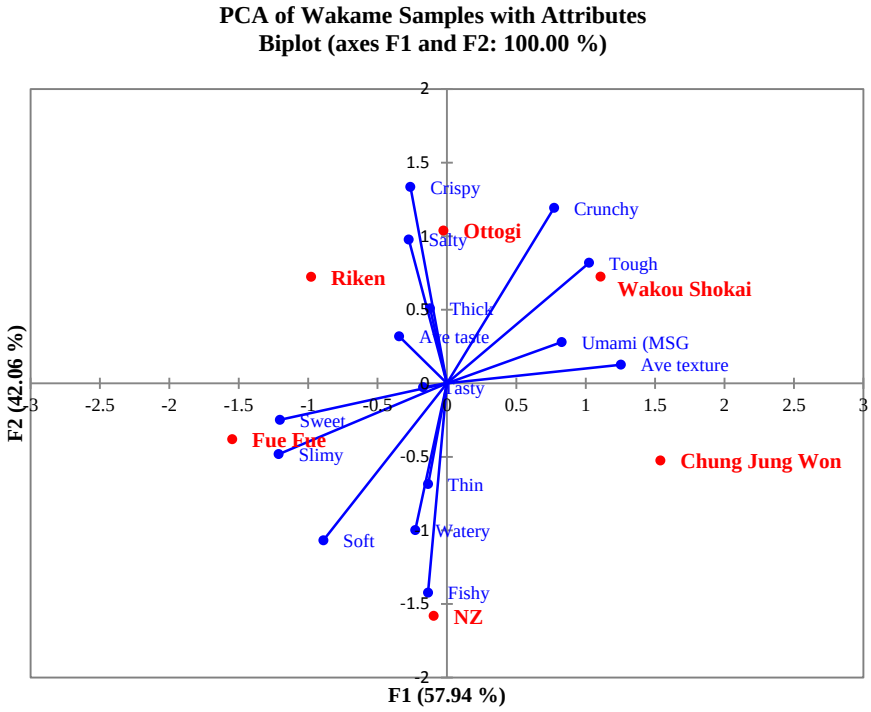

Figure 13. Principal component analysis of 15 panellists over three weeks of testing.

Factor 1 (F1) on Figure 13 above explained 57.94% of the variation between Fue Fue and Chung Jung Won samples which differentiated sweet from umami, slimy from average texture, and soft from tough, while factor 2 (F2) which explained 42.06% of the variation between samples differentiated between thick Ottogi and thin New Zealand samples. The New Zealand sample was also characterised as being watery and fishy on top of being thin, while Wakou Shokai is characterised as being crunchy, tough, and having umami flavour. Riken and Ottogi are considered thick and crispy while Fue Fue is described as being sweet and slimy. Lastly, Chung Jung Won is characterised as having average texture. The quality of wakame is based on its thickness, hardness, and colour (Yamanaka & Akiyama, 1993). Looking at the biplot above, it was evident that there are textural differences among the products.

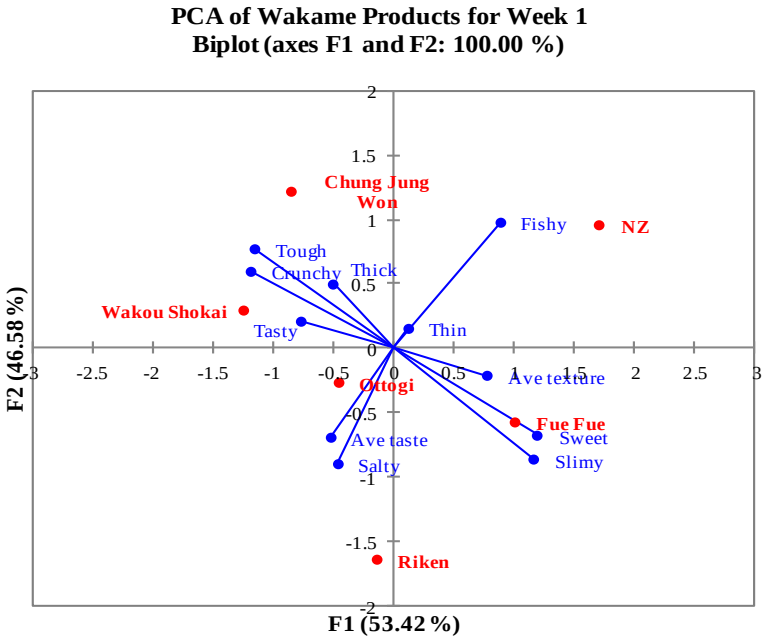

Figure 14. PCA biplot of products and attributes for week 1.

The product biplot in Figure 14 differentiated NZ and Fue Fue from Chung Jung Won, Wakou Shokai, and Ottogi along Factor 1 which explained 53.42% of the products' variation on the basis of the former being fishy, sweet and slimy, while those on the left are tough, crunchy and thick. Factor 2, on the other hand, which explains 46.58% of the variation between samples, separated Riken, Ottogi and Fue Fue from NZ, Chung Jung Won, and Wakou Shokai.

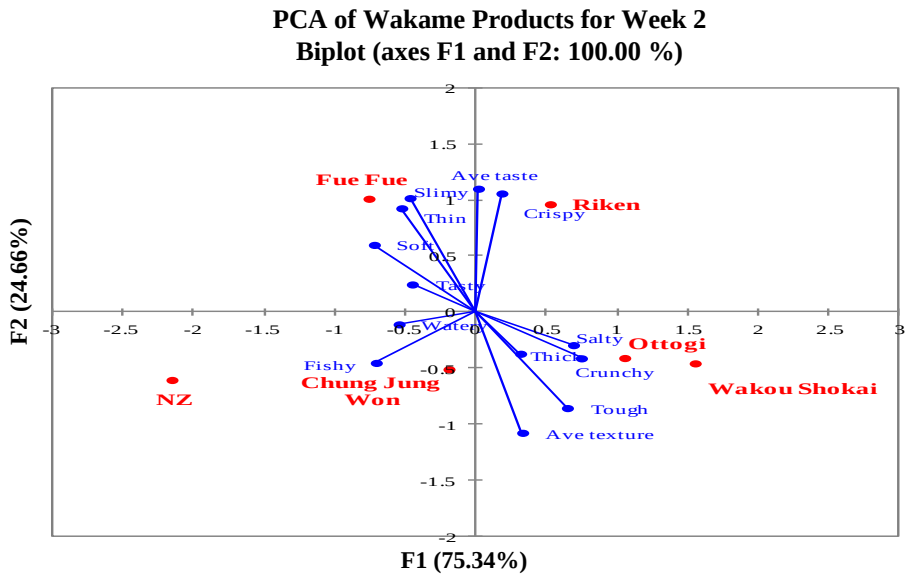

Figure 15. Principal component analysis of products and attributes for week 2.

The biplot for week 2 (Figure 15) showed slightly different groupings compared with the map obtained from week 1. Chung Jung Won was now grouped with NZ and Fue Fue and was separated from the three other samples namely Riken, Ottogi, and Wakou Shokai along Factor 1 which explained 75.34% of the variation. Chung Jung Won, NZ, Wakou Shokai, and Ottogi were separated from Fue Fue and Riken along Factor 2, which explained 24.66% of the variation.

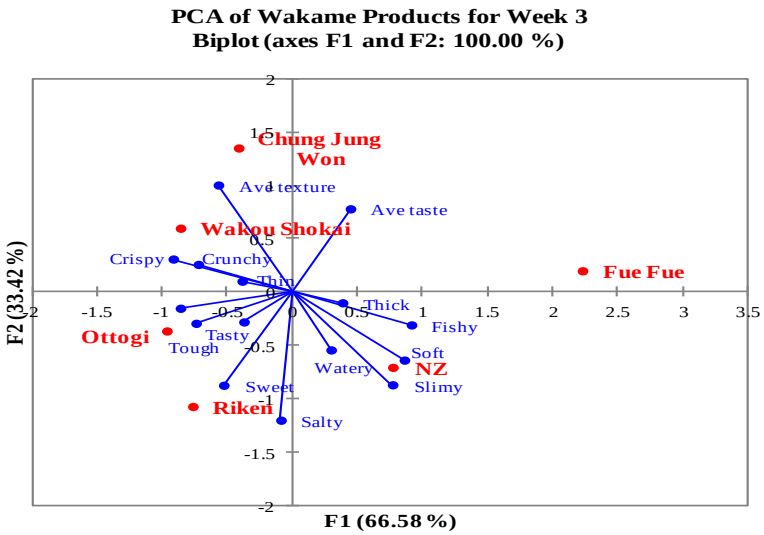

Figure 16. Principal component analysis of products and attributes for week 3.

Figure 16 above indicated a product grouping similar to that obtained from week 1. Factor 1 explained 66.58% of the variation, and separated the Fue Fue and NZ samples from the four other products namely Chung Jung Won, Wakou Shokai, Ottogi, and Riken. This factor mainly differentiated the crispy, crunchy, and tough attributes that described Riken, Wakou Shokai and Ottogi samples from the slimy, soft, and fishy that described NZ and Fue Fue samples. Factor 2, on the other hand, separated Chung Jung Won and Wakou Shokai that had average taste and texture from Ottogi, Riken, and NZ which were salty, soft, slimy, and sweet.

5.3 Texture Analysis

Texture profile analyses on the processed wakame samples were conducted to further investigate the textural differences obtained through projective mapping. Values for hardness, resilience, cohesiveness, springiness, gumminess, chewiness, and adhesiveness were obtained using a texture analyser. Of these values, only hardness showed significant differences, as summarised in Table 9.

Table 9. Hardness of wakame samples expressed as means ± SD with Tukey's Comparisons.

Sample	Hardness (g)
Riken	76.55 ± 31.74^{b}
Chung Jung Won	161.27 ± 18.37^{a}
Fue Fue	7.43 ± 3.33^{c}
Ottogi	88.25 ± 5.58^{b}
Wakou Shokai	88.08 ± 29.19^{b}
NZ August	25.55 ± 4.76^{c}
NZ October	83.53 ± 5.12^{b}

Hardness is defined as the maximum peak force during the first compression cycle which corresponds to the first bite (Isabel, Marta, & Mercedes, 2009). In other words, it is a measure of the force required to penetrate the sample with the molar teeth (Ak & Gunasekaran, 2002), which can also be referred to as firmness, with units either in Newtons, grams, or kilograms.

The texture profile analysis results indicated that there are significant differences in the textural qualitites in terms of hardness of the different wakame samples. Fue Fue and New Zealand samples required the least amount of force to penetrate. This related well with the results obtained from projective mapping discussed earlier and shown on Figure 13. The actual values for hardness indicated that Chung Jung Won was the sample that required the most force to penetrate, followed by Ottogi, Wakou Shokai, NZ October, and Riken. A consumer sensory testing study of the commercial wakame products and NZ August conducted by Kantono (2011) showed significant difference in the liking scores of 96 respondents in terms of texture and colour. In terms of texture, panellists liked the texture of Ottogi and Chung Jung Won more than

the rest of the samples. From the texture analysis results, the texture of Chung Jung Won and Ottogi, both of Korean origin, were preferred by consumers possibly because their hardness.

5.4 Colour Analysis

Testing for colour was not incorporated during sensory testing since obvious colour differences were observed prior to sensory testing. Hence sensory evaluation was carried out under red light so as not to influence the judgement of other attributes like flavour and odour. Further investigation and changes in the processing method of wakame for October samples yielded a dried product that held its green colour as well as its structural integrity better after rehydration. This was achieved by blanching the seaweed on the boat soon after harvesting rather than blanching them after airfreighting and arrival to AUT. This method is probably closer to how wakame is produced industrially since processing plants are often located close to the shore, to allow shorter delay in the processing of wakame. Results from colour analysis are shown in Table 10.

Table 10. Mean colour space values ± standard deviation of the different wakame samples.

Sample	*L*	*a*	*b*
Riken	11.430 ± 1.172^{c}	-7.0567 ± 0.3287^{e}	16.233 ± 1.292^{c}
Chung Jung Won	13.140 ± 4.261^{bc}	-6.1700 ± 0.1664^{de}	15.780 ± 4.163^{c}
Fue Fue	15.277 ± 0.885^{abc}	-4.9400 ± 0.3122^{cd}	18.167 ± 1.142^{abc}
Ottogi	14.983 ± 2.086^{abc}	-3.2100 ± 1.1173^{bc}	17.663 ± 0.895^{bc}
Wakou Shokai	15.693 ± 1.640^{abc}	-6.9233 ± 0.5006^{de}	18.307 ± 2.181^{abc}
NZ August	21.597 ± 3.039^{a}	2.1400 ± 1.1995^{a}	23.120 ± 2.323^{ab}
NZ October	19.497 ± 2.784^{ab}	-1.2967 ± 0.8358^{b}	24.337 ± 2.130^{a}

Table 10 indicated that there were significant differences in colour between the samples since p=0.002. *L* values for the New Zealand sample processed in August indicated that it was the lightest in colour (values ranged from 1 to 100, 100 being lightest). The October sample from New Zealand was not significantly different from the August sample and most of the commercial samples, except for Riken, which came out darkest in colour.

The *a* values indicated the presence of either green or red pigments. All samples, apart from the New Zealand August sample, were negative values, therefore indicating that green pigments were more dominant than red. The NZ sample for August which had a positive *a* reading indicated the presence of red pigments in small amounts that rendered the seaweed a slightly brown colour. Overall, Riken had the most green pigments while NZ August had least while having some red pigments.

The *b* readings indicated that all samples had more yellow pigments than blue and that the NZ October samples had the most, followed by NZ August, Fue Fue, Wakou Shokai, and Ottogi. These five samples were considered not significantly different from each other as indicated by the Tukey's subscripts. Only Riken and Chung Jung Won were significantly different from the two New Zealand samples.

The colour swatches in Figure 17 represent an average colour representation of the different samples as seen by the naked eye, with each sample tested three times. These swatches were obtained from a website by inputting the *L*, *a*, and *b* values obtained from the ColorFlex machine. Good quality wakame preferred by consumers is partly determined by its green colour (Yamanaka & Akiyama, 1993). Riken, Wakou Shokai, and Chung Jung Won had the most green pigments while the August New Zealand sample had the least. A change in the processing method of wakame for October when samples were blanched on the boat straight after harvest

and prior to being transported to AUT yielded a sample that was less brown in colour. This, however, still came out significantly different from the three samples that had the most green pigments namely Riken, Wakou Shokai, and Chung Jung Won.

Figure 17. Simulation of the L* ab colour space values for all samples. (From www.rgb.com).

5.5 Volatile profile analysis

5.5.1 Volatile compounds found in the headspace of cooked wakame samples

A total of 105 compounds were found in the headspace of the wakame samples using the SPME-GC-MS method. These compounds are listed in Table 11, where 1, 2-dichlorobenzene served as the internal standard. Of these, 21 were aldehydes, 16 alcohols, 15 ketones, 11 alkanes, 8 alkenes, and 10 esters. A small number of halogens, carboxylic acid, furans, ethers, amines, amides, and a sulfate were also present. Some compounds in smaller quantities were also detected but could not be tentatively identified by comparison of the MS spectra with the NIST library database.

Table 11. Volatile compounds identified in the commercial and New Zealand seaweed samples using SPME coupled to GC-MS.

Peak #	RI*	Compound**	Functional group	Characteristic Flavours/Aromas
2		2-Butanone	Ketone	Sweet, apricot-like odour [o]
4		Formyl acetate	Ketone	
5		1-Penten-3-ol	Alcohol	Bitter, mild green odour [o]
6	704	2-Ethylfuran	Furan	Powerful, sweet, burnt odour [o]
8	741	3-Penten-2-one	Ketone	Fruity odour that turns pungent on storage [o]
9	755	trans-2-Pentenal	Aldehyde	Fruity aroma, astringent taste [a]
10	756	3-Ethyl-2-methyl-1-pentene	Alkene	No descriptor but found in tea [o]
12	770	cis-2-Penten-1-ol	Alcohol	Green, fruity[b]
13	789	Hex-5-enal	Aldehyde	Oily, fatty, insect-like green and herbal odours[d]
14	803	Hexanal	Aldehyde	Fatty, green, grassy, powerful fruity odour and taste [o]
16	830	2-Butenal, 2-ethyl-	Aldehyde	
17	831	2-Methyl-2-pentenal	Aldehyde	Grassy-green, slightly fruity odour [o]
18	853	trans-2-Hexenal	Aldehyde	Bitter almond[b]
19	861	Ethylbenzene	Alkane	Musty, plastic, resin, oily, chemical, styrene, stale[e]
21	871	Cyclopropane,propyl-	Alkane	
22	878	2-(methylthio)Benzimidazole		
24	900	Triethyl orthoformate	Ester	
25	901	cis-4-Heptenal	Aldehyde	Fried, buttery flavour [o]
26	903	Heptaldehyde	Aldehyde	Fatty, harsh, pungent odour, unpleasant fatty taste [o]
27	905	2-Butoxyethanol	Alcohol	Spicy, woody [f]
28	914	Oxime-,methoxy-phenyl-_	Imine	
29	958	2-Heptenal	Aldehyde	Pungent green somewhat fatty odour. Pleasant in extreme dilutions [o]
30	963	Benzaldehyde	Aldehyde	Bitter almond [o]
31	974	3,5,5-Trimethyl-2-hexene	Alkene	Characterises freshness[n]

Peak #	RI*	Compound**	Functional group	Characteristic Flavours/Aromas
32	978	1-Hepten-3-one	Ketone	Geranium-like[g]
33	981	1-Octen-3-ol	Alcohol	Herbaceous note similar to lavender, rose and hay. Sweet, herbaceous taste [o]
34	986	2,2,7,7-Tetramethyloctane	Alkane	
35	988	6-Methyl-5-hepten-2-one	Ketone	Fatty, green, citrus-like odour, bittersweet taste [o]
36	990	2-Pentylfuran	Furan	Green bean, metallic, vegetable odour [o]
37	1004	Octanal	Aldehyde	Fatty, citrus, honey odour when diluted [o]
38	1014	trans,trans-2,4-Heptadienal	Aldehyde	Fatty, green odour [o]
39	1034	1,2-Dichlorobenzene	Halogen	
40	1059	(E)-2-Octenal	Aldehyde	Green walnut, sawdust[i]
41	1060	1H-Pyrazole		
42	1069	(E)-2-Octen-1-ol	Alcohol	Unpleasant, musty-oil odour [o]
43	1071	(E,E)-3,5-Octadien-2-one	Ketone	Pungent, herbaceous odour [o]
45	1094	3,5-Octadien-2-one	Ketone	Fatty, fruity odour notes[h]
46	1105	n-Nonanal	Aldehyde	Fatty odour and flavour. Citrus-like flavour [o]
47	1110	1-Methylcycloheptanol	Alcohol	
49	1146	2,6,6-Trimethylcyclohex-2-ene-1,4-dione	Ketone	Sweet honey aroma[j]
50	1154	(E,E)-2,6-Nonadienal	Aldehyde	Green, cucumber-like[c]
51	1154	2,3-Dihydrofuran	Furan	
52	1161	trans-2-Nonenal	Aldehyde	Green walnut, sawdust[i]
53	1165	Benzaldehyde, 3-ethyl-	Aldehyde	
54	1177	2,5-Dimethylbenzaldehyde	Aldehyde	
55	1179	5-Methyl-2-(1-methylethyl)cyclohexanol	Alcohol	
56	1189	Cyclohexanol,2-methylene-5-(1-methylethenyl)-	Alcohol	Menthol[m]

Peak #	RI*	Compound**	Functional group	Characteristic Flavours/Aromas
57	1190	1,6-Dimethylheptan-1,3,5-triene	Alkene	
58	1196	3-Cyclohexene-1-methanol, alpha,alpha,4-trimethyl-	Alkene	Pine terpene lilac citrus woody floral odour; citrus woody with a lemon lime nuance flavour[m]
59	1197	Dodecane	Alkane	
60	1206	n-Decanal	Aldehyde	Sweet, waxy, floral, citrus, pronounced fatty odor. Fatty, citrus-like taste [o]
62	1220	1-Cyclohexene-1-carboxaldehyde,2,6,6-trimethyl-	Alkene	Tropical, saffron, herbal, clean, rose oxide, sweet tobacco, fruity odour[m]
63	1238	Clozapine		
65	1243	1H-Indene,2,3-dihydro-1,1,3-trimethyl-	Alkene	
67	1257	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	Aldehyde	Woody [o]
68	1263	2-decenal,(Z)-2-decenal	Alkane	Bug[i]
70	1318	2-Oxo-1-methyl-3-isopropylpyrazine		*Found in apricots*[k]
71	1347	Propanoic acid,2-methyl-, 1-(2-hydroxy-1-methylethyl)-2,2-dimethylpropyl ester	Ester	
72	1357	17alpha-Hydroxy-yohimban-16alpha-carboxylic acid methyl ester	Ester	
73	1372	Propanoic acid,2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	Ester	
75	1394	Trimethylamine	Amine	Pungent, fishy, ammoniacal odour [o]
76	1397	Tetradecane	Alkane	
78	1448	6,10-Dimethyl-5,9-undecadien-2-one	Ketone	Green, rosy floral odour [o]
80	1475	1-Undecanol	Alcohol	Floral, citrus-like odour; fatty flavour [o]
81	1480	3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	Ketone	Violet-like odour; Fruity, woody flavour [o]

Peak #	RI*	Compound**	Functional group	Characteristic Flavours/Aromas
82	1483	4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one	Ketone	Sweet berry, fruity, woody, violet orris, powdery odour[m]
83	1497	N-pentadecane	Alkane	Waxy[m]
85	1533	5,6,7,7-*alpha*-Tetrahydro-4,4,7-*alpha*-trimethyl-2(4*H*)-benzofuranone	Ketone	Tea-like odour [o]
86	1536	(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid lactone	Ketone	Musk coumarine[m]
87	1576	1-Tridecanol	Alcohol	Musty[m]
88	1585	Propanoicacid,2-methyl-,	Carbocylic acid	Cheese-like[l]
90	1623	Dodecanoic acid,1-methylethyl ester	Ester	
91	1650	Methyl 3-oxo-2-pentylcyclopentaneacetate	Ester	Sweet-floral, jasmine-like, somewhat fruity odour [o]
92	1681	Hexanedioic acid,1,6-bis(2-methylpropyl) ester	Ester	
93	1703	Potassium sulfate	Sulfate	
94	1722	4-tert-Amylphenol	Alcohol	
95	1752	Phenol,4-(1,1,3,3-tetramethylbutyl)-	Alcohol	
96	1804	Cyclohexane carboxylic acid, (1H-tetrazol-5-yl) amide	Carboxylic acid	
97	1821	Isopropyl myristate	Ester	Virtually odourless, slightly fatty but not rancid [o]
98	1845	Ethanone,1-(3-ethyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-	Ketone	Sweet intense musk ambrette macrocyclic[m]
99	1859	1,2-Benzenedicarboxylicacid, 1,2-bis(2-methylpropyl) ester	Ester	
100	1881	1-Hexadecanol	Alcohol	Odourless [o]

Peak #	RI*	Compound**	Functional group	Characteristic Flavours/Aromas
101	1888	Homomenthyl salicylate		Mild menthol[m]
102	1955	Dibutyl phthalate		Faint odour[m]
103	2033	5,8,11,14-Eicosatetraenoicacid, ethyl ester, (5Z,8Z,11Z,14Z)-	Ester	
104	2039	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene,trans-2,9-anti-9,10-trans-1.10-	Alkene	
105	2060	1-Naphthalenepropanol, a-ethenyldecahydro-a,5,5,8a-tetramethyl-2-methylene-,(aS,1S,4aS,8aS)-	Alcohol	

* RI on a VF-5MS column, was calculated in relation to the retention time of *n*-alkane (C–C_{30}) series

**MS, tentative identification by comparison of mass spectrum with the NIST library spectrum

[a](Moshonas & Shaw, 1973),[b](Angerosa, Mostallino, Basti, & Vito, 2000) ,[c](Ullrich & Grosch, 1988), [d](Hatanaka, 1993),[e](Young, Horth, Crane, Ogden, & Arnott, 1996), ,[f](Vejaphan, Hsieh, & Williams, 1988), [g](Buettner & Schieberle, 1999), [h](Morales, Rios, & Aparicio, 1997), [i](Chatonnet & Dubourdieu, 1998), [j](Rogerson et al., 2000), [k] (Gomez, Ledbetter, & Hartsell, 1993), [l](Münch, Hofmann, & Schieberle, 1997), [m](The Good Scents Company), [n](Duflos et al., 2010), Characteristic flavours/aromas obtained from 'Fenaroli's handbook of flavour ingredients' (Burdock, 2004)[o]

5.5.2 Multivariate study of the volatiles found in the headspace of cooked wakame

In order to illustrate differences between the different wakame samples, PCA was carried out to assess the variation in the volatile compounds. The PCA in Figure 18 described 39.57% and 17.56% of the variation in factor 1 (F1) and factor 2 (F2), respectively. Fue Fue, Wakou Shokai and Ottogi had positive values and were separated from Riken and the two New Zealand samples along factor 1. On the other hand, Riken and Fue Fue had high positive loadings along factor 2 and were separated from New Zealand October, Chung Jung Won, Ottogi, and Wakou Shokai. While the grouping of samples in the PCA do not show the expected groupings of the commercial products based on origin, a group of volatiles indicated by the red circle were considered characteristic of the two New Zealand samples. This group, circled in red, comprised of formyl acetate (4), 3-ethyl-2-methyl-1-pentene (10), cyclopropane,propyl- (21), 2-(methylthio)benzimidazole (22), Triethyl orthoformate (24), 2-butoxyethanol (27), 2,2,7,7-tetramethyloctane (34), octanal (37), 1H-pyrazole (41), dodecane (59), clozapine (63), 2-decenal,(Z)-2-decenal (68), tetradecane (76), and n-pentadecane (83). Of these compounds, five were considered significantly different from each other ($p \leq 0.05$) based on one-way ANOVAs performed for individual peaks. These were cyclopropane,propyl-, dodecane, clozapine, tetradecane, and *n*-pentadecane. Post-hoc (Tukey's) analyses on these compounds are shown in Table 12. *n*-pentadecane concentration which has been described as waxy (The Good Scents Company), was significantly higher in the NZ October sample than the NZ August and commercial samples. As for tetradecane, which was highest in the NZ October, followed by Ottogi and Riken were significantly higher than the other samples. The post-hoc comparison for the other three compounds cyclopropane,propyl-, dodecane, and clozapine, found grouped with

the New Zealand samples in the PCA did not reflect the significance found in the ANOVA which may be due to large residual standard deviations (RSD) of the means.

The presence of n-alkanes namely dodecane (C_{12}), tetradecane (C_{14}), and pentadecane (C_{15}) in the headspace of the wakame samples was consistent with the findings of Sartin et al (2002) who reported their presence in the seaweed *Fucus spiralis*. However, the bigger n-alkanes octadecane (C_{18}), icosane (C_{20}), docosane (C_{22}), tetracosane (C_{24}), and octacosane (C_{28}) found in lyophilised fresh *Undaria* (dried after harvesting without further processing such as salting or blanching) harvested from the Galacian coast (Punín Crespo & Lage Yusty, 2006) were not found to be present in the wakame samples tested. Compounds found such as trans-2-hexenal and trans-2-nonenal (Kajiwara et al., 1988), and 2,6-nonadienal (Kajiwara et al., 2007) have also been reported to be present in *U. pinnatifida*.

A number of silicon-containing compounds such as dimethylsilanediol, hexamethylcyclotrisiloxane, decamethylcyclopentasiloxane, octamethylcyclotetrasiloxane, and styrene were also found to be present in all samples. However, since these compounds were found to be abundant in all samples, it was concluded that they may have been contaminants from the various storage vessels used during harvest (resealable plastic bags) and storage (PET bottles).

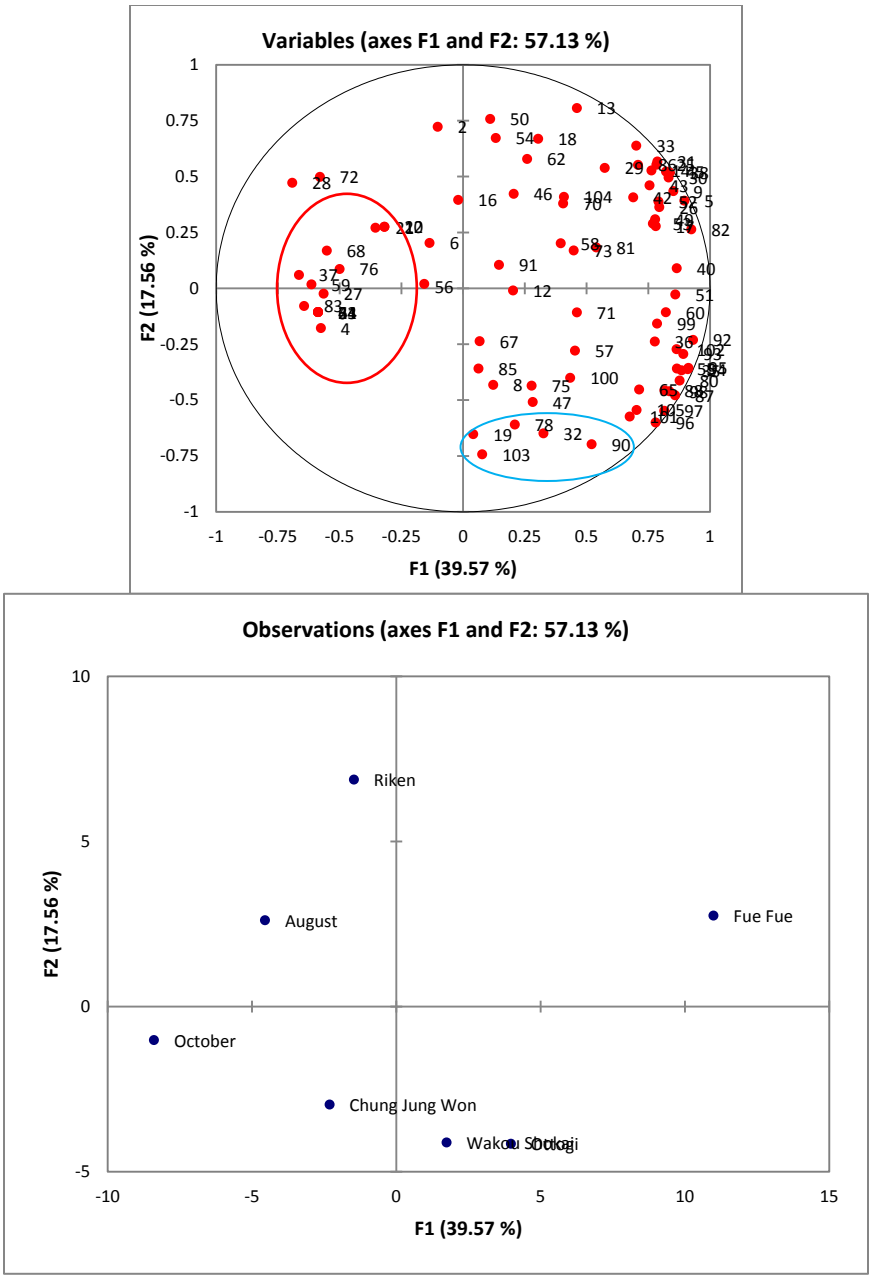

Figure 18. Principal component analysis of the volatile compounds found in the different wakame products.

Volatile compounds that showed significant differences between samples ($p \leq 0.05$) are summarized in Table 12. Some of them, however, should be interpreted with caution since they showed no significant differences when Tukey's post hoc comparisons tests were carried out due

to large RSDs of the means as well the fact that some of the compounds found in Table 12 were not present in all the samples.

A number of the significant peaks in Table 12 characterised the Fue Fue. 1-penten-3-ol, which has been found is seaweed, is a compound with a bitter, mild green odour (Burdock, 2004) is a degradation by-product of ω3 polyunsaturated fatty acids (PUFA) (Le Pape et al., 2004). This compound was found to be significantly most abundant in Fue Fue followed by Ottogi and Riken compared to other samples. Hexanal and 2-methyl-2-pentenal described as being grassy-green and fruity respectively (Burdock, 2004), were also significantly ($p<0.05$) higher in Fue Fue. Aldehydes in plants materials are usually formed through enzymatic oxidation of lipids when plant tissues are disrupted. Lipoxygenase which is widely distributed among plants catalyses the hydroperoxidation of PUFAs (Josephson, Lindsay, & Stuiber, 1983). In the case of hexanal, it could have come from lipid peroxidation of linoleic acid or other ω3 PUFAs such as eicosapentanoic acid (C20:5ω3) (Le Pape et al., 2004). Two other aldehydes, namely trans-2-pentenal and heptaldehyde were also significantly higher in Fue Fue ($p<0.05$). Trans-2-pentenal has been characterised as having a fruity aroma and an astringent aftertaste (Burdock, 2004), while heptaldehyde exhibited a harsh and pungent odour (Burdock, 2004). Both the alkanes tetradecane and n-pentadecane were found to be characteristic of the NZ October sample. However, some of the volatile compounds found to have a significant p-value ($p<0.05$) in the analysis of variance such as 3-ethyl-benzaldehyde, dodecane, clozapine, propylcyclopropane, and cis-4-heptenal did not show significance in the post-hoc analysis based on groupings as indicated by the superscripts in Table 12 which may be due to large RSDs as well as the fact that some of these compounds were not detected in all samples.

The compounds circled in blue in the PCA shown in Figure 18 comprised of ethylbenzene (musty, oily, stale (Young et al., 1996)), 1-hepten-3-one described as geranium-like (Buettner & Schieberle, 1999)), 6,10-dimethyl-5,9-undecadien-2-one described as green, rosy and floral odour (Burdock, 2004)), as well as 1-methylethyl ester dodecanoic acid, and 5,8,11,14-eicosatetraenoic acid ethyl ester (5*Z*,8*Z*,11*Z*,14*Z*) characterised two Korean samples (Chung Jung Won and Ottogi) and one Japanese sample (Wakou Shokai). On the other hand, Riken is characterised in the PCA by 2-butanone, possibly produced from the oxidation of 2-butanol (Traiger & Bruckner, 1976) which has a sweet, apricot-like odour (Burdock, 2004). 6,10-dimethyl-5,9-undecadien-2-one may arise from oxidative cleavage of carotenoids (Simkin, Schwartz, Auldridge, Taylor, & Klee, 2004) that have also been found to be present in *U. pinnatifida* (Riccioni, D'Orazio, Franceschelli, & Speranza, 2011). None of these compounds, however, were found to be significantly different ($p>0.05$) for the seven different wakame samples.

Table 12. Names, descriptions and relative concentrations of compounds found to be significantly different between wakame samples.

Compound	Description	NZ August (µg/g)	NZ October (µg/g)	Riken (µg/g)	Ottogi (µg/g)	Wakou Shokai (µg/g)	Chung Jung Won (µg/g)	Fue Fue (µg/g)
1-Penten-3-ol	Bitter, mild green odour[o]	0.84±1.45^{B}	0^{B}	4.29±2.40^{AB}	9.48±6.30^{AB}	2.49±2.49^{B}	1.37±2.38^{B}	16.24±3.63^{A}
trans-2-Pentenal	Fruity aroma, astringent taste[o]	0^{B}	0^{B}	1.91±1.69^{AB}	3.29±3.06^{AB}	0.71±1.22^{AB}	0.90±1.56^{B}	7.25±2.50^{A}
Hexanal	Fatty, green, grassy, powerful fruity odour and taste[o]	19.12±8.04^{AB}	3.15±0.41^{B}	14.36±6.88^{AB}	32.22±17.70^{AB}	6.57±7.20^{B}	17.34±21.07^{AB}	59.77±21.74^{A}
2-Methyl-2-pentenal	Grassy-green, slightly fruity odour[o]	0^{B}	0^{B}	0^{B}	0.09±0.15^{B}	0^{B}	0^{B}	2.00±0.33^{A}
trans-2-Hexenal	Bitter almond[b]	11.95±7.08^{A}	0.41±0.24^{B}	5.21±3.51^{AB}	8.87±6.74^{AB}	1.97±2.23^{AB}	2.95±2.61^{AB}	13.92±4.83^{AB}
Cyclopropane,propyl-		1.67±1.58^{A}	0.08±0.13^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
cis-4-Heptenal	Fried, buttery flavour[o]	0.50±0.87^{A}	0^{A}	2.15±1.23^{A}	3.46±1.83^{A}	0.85±1.48^{A}	0.66±1.15^{A}	5.09±1.86^{A}
Heptaldehyde	Fatty, harsh, pungent odour, unpleasant fatty taste[o]	1.23±0.64^{B}	0^{B}	0.58±1.00^{B}	2.98±1.39^{B}	0^{B}	0.61±1.06^{B}	6.22±2.23^{A}
Benzaldehyde,3-ethyl-		0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0.81±0.81^{A}
Dodecane		0^{A}	1.54±1.49^{A}	0.25±0.43^{A}	0^{A}	0^{A}	0^{A}	0^{A}
Clozapine		0^{A}	0.32±0.31^{A}	0^{A}	0^{A}	0^{A}	0^{A}	0^{A}
Tetradecane		0^{B}	0.74±0.51^{A}	0.38±0.26^{AB}	0.94±0.67^{AB}	0^{B}	0^{B}	0^{B}

N-pentadecane	Waxy[m]	2.71±1.71 [B]	13.68±9.32 [A]	1.00±0.30 [B]	2.62±0.87 [B]	1.14±0.82 [B]	0.27±0.46 [B]	0.45±0.50 [B]

Note: Concentrations were calculated based on the peak area of 1ppt 1,2-dichlorobenzene for that particular sample. The amounts stated therefore were used as a guide and were not considered as absolute values. Two samples that do not share a letter are significantly different from each other.

[b](Angerosa et al., 2000), [m](The Good Scents Company), [o](Burdock, 2004)

Chapter 6: Conclusion

The main aim of the project was to compare New Zealand wakame produced from *U. pinnatifida* in terms of physicochemical, sensory and volatiles analysis to commercial Japanese and Korean samples. Additionally, the free sugar profile of the seaweed was to have been investigated. However, only mannitol was detected from the HPLC method and differentiation of mannitol content of *U. pinnatifida* in different farms and months was carried out.

Mannitol content of the fresh freeze-dried *Undaria* samples from New Zealand showed significant differences between the different farms in August where Farms 106 and 353 had significantly higher mannitol content compared with Farms 327 and 122. Mannitol content from Farm 122 in the Pelorus Sound was significantly higher in October compared with August and September. The increase in mannitol concentration observed in October for Farm 122 may have been a result of an increase in the amount of sunshine which in turn increased the photosynthetic activity of the seaweed, resulting in mannitol production – its main photosynthetic by-product. As for the differences within the months, it was initially thought that differences in the sites' exposure to tidal flows may cause changes in the plants' photosynthetic activity and growth. However no evidence of this was found since the differences were not dictated by farm location. As the water temperature, sunshine exposure per site, amount of rainfall, and other environmental factors in each site were not monitored, it was not possible to fully understand the differences in mannitol content. On the other hand, the amount of mannitol in processed *Undaria,* wakame was below the threshold at which it could be detected by humans. Further work on the characterisation of the polysaccharides that could be present in *U. pinnatifida* may also be carried out for future study.

Sensory projective mapping differentiated the New Zealand August wakame sample from the commercial ones in terms of fishy, watery, thin, and soft attributes. Texture analysis confirmed that the August sample was significantly softer than the other samples. Furthermore, colour analysis indicated that the NZ wakame was significantly lighter and browner in colour compared to the commercial samples. Previous work by Kantono (2011) showed that the Korean product, Chung Jung Won was most preferred by consumers as determined by consumer sensory testing. This may be due to the fact that Korean wakame products are more dominant in the market with more wakame products from Korea and China coming into even the Japanese market. With an annual production of 300 000 tonnes of fresh *Undaria* per annum, Korea is now considered the major producer of wakame (Yamanaka & Akiyama, 1993) with more consumers exposed to Korean wakame. A preliminary trial on New Zealand wakame carried on October samples that were blanched and salted in the boat resulted in a product with improved colour and texture compared to the August samples that were only blanched and salted after being airfreighted to Auckland. This suggests that the delay in processing may have resulted in deterioration of the unprocessed seaweed whilst the October sample that was processed on the boat had improved textural and colour properties.

Lastly, the volatile profile analysis showed that there were volatile compounds that diffrentiated the New Zealand samples from the commercial ones. The two New Zealand samples were characterised by 6 alkanes, an ester, an alcohol, an aldehyde, a ketone and an alkane. These were formyl acetate, 3-ethyl-2-methyl-1-pentene, cyclopropane,propyl, 2-(methylthio)benzimidazole, triethyl orthoformate, 2-butoxyethanol, 2,2,7,7-tetramethyloctane, octanal, 1H-pyrazole, dodecane, clozapine, 2-decenal,(Z)-2-decenal, tetradecane., and n-pentadecane while the Japanese wakame Fue Fue was characterised by one alcohol and four

aldehydes namely 1-penten-3-ol, hexanal, 2-methyl-2-pentenal, trans-2-pentenal and heptaldehyde.. Two ketones, an ester and a carboxylic acid namely ethylbenzene, 1-hepten-3-one, 6,10-dimethyl-5,9-undecadien-2-one, 1-methylethyl ester dodecanoic acid, and 5,8,11,14-eicosatetraenoic acid ethyl ester (5*Z*,8*Z*,11*Z*,14*Z*) characterised Chung Jung Won, Ottogi, and Wakou Shokai while Riken is characterised by the ketone 2-butanone.

The presence of alkanes was interesting. However it would be important to ascertain their source, whether they were from the seaweed, environment, or storage conditions. Individual standards for the GC-MS work should be further employed for a more robust compound identification and quantification work. Similarly, the possible contamination with silicon-containing compounds must be eliminated for future studies to preserve the integrity of the samples since large amounts of silicon-containing contaminants were found in the wakame samples used in the study. Furthermore, characterisation of *U. pinnatifida* must be carried out for a longer period in parallel with the gathering environmental data such as water temperature, nitrogen content and other factors that may impact the growth of seaweed. Allowing for a longer period of harvest rather than the five months in this study would mean that a full life cycle of *U. pinnatifida* could be documented. As for the production of wakame, if the new Zealand samples were to compete with the commercially available products, sensory attributes that include colour and texture must be improved. This may be achieved by processing the seaweed as soon as it is harvested. The blanching on the boat applied to the October sample showed an improvement in sensory properties.

References

Abdi, H. (2007). *RV Coefficient and Congruence Coefficient*. Retrieved 6 December 2011, 2011, from http://wwwpub.utdallas.edu/~herve/Abdi-RV2007-pretty.pdf

Ak, M. M., & Gunasekaran, S. (2002). Cheese Texture. In *Cheese Rheology and Texture*: CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420031942.ch7. doi:10.1201/9781420031942.ch7

Albert, A., Varela, P., Salvador, A., Hough, G., & Fiszman, S. (2011). Overcoming the issues in the sensory description of hot served food with a complex texture. Application of QDA®, flash profiling and projective mapping using panels with different degrees of training. *Food Quality and Preference, 22*(5), 463-473. doi:10.1016/j.foodqual.2011.02.010

Alga, Z., & Julian, M. (2005). Fungal Communities of Seaweeds. In J. Dighton, J. F. White, & P. Oudemans (Eds.), *The Fungal Community* (pp. 533-579): CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420027891.ch27. doi:10.1201/9781420027891.ch27

Angerosa, F., Mostallino, R., Basti, C., & Vito, R. (2000). Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry, 68*(3), 283-287. doi:10.1016/s0308-8146(99)00189-2

Ares, G., Deliza, R., Barreiro, C., Giménez, A., & Gámbaro, A. (2009). Comparison of two sensory profiling techniques based on consumer perception. *Food Quality and Preference, 21*(4), 417-426.

Aulakh, J. S., Malik, A. K., Kaur, V., & Schmitt-Kopplin, P. (2005). A Review on Solid Phase Micro Extraction—High Performance Liquid Chromatography (SPME-HPLC) Analysis of Pesticides. *Critical Reviews in Analytical Chemistry, 35*(1), 71-85. doi:10.1080/10408340590947952

Barsanti, L., & Gualtieri, P. (2006). Algae and Men. In *Algae Anatomy, Biochemistry, and Biotechnology* (pp. 251-291): CRC Press. Retrieved from http://dx.doi.org/10.1201/9780203492598.ch7. doi:10.1201/9780203492598.ch7

Boland, W., & G. Müller, D. (1987). On the odor of the Mediterranean seaweed Dictyopteris membranacea; New C11 hydrocarbons from marine brown algae - III. *Tetrahedron Letters, 28*(3), 307-310. doi:10.1016/s0040-4039(00)95714-9

Buettner, A., & Schieberle, P. (1999). Characterization of the Most Odor-Active Volatiles in Fresh, Hand-Squeezed Juice of Grapefruit (Citrus paradisi Macfayden). *Journal of Agricultural and Food Chemistry, 47*(12), 5189-5193. doi:10.1021/jf990071l

Burdock, G. A. (2004). Fenaroli's Handbook of Flavor Ingredients, Fifth Edition. In *Fenaroli's Handbook of Flavor Ingredients, Fifth Edition*: CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420037876.fmatt. Retrieved 2012/05/07. doi:10.1201/9781420037876.fmatt

Campbell, S. J., & Burridge, T. R. (1998). Occurrence of Undaria pinnatifida (Phaeophyta : Laminariales) in Port Phillip Bay, Victoria, Australia. *Marine and Freshwater Research, 49*(5), 379-381. doi:10.1071/MF97010

Chatonnet, P., & Dubourdieu, D. (1998). Identification of substances responsible for the 'sawdust' aroma in oak wood. *Journal of the Science of Food and Agriculture, 76*(2), 179-188. doi:10.1002/(sici)1097-0010(199802)76:2<179::aid-jsfa924>3.0.co;2-6

Cho, D.-H., Kong, S.-H., & Oh, S.-G. (2003). Analysis of trihalomethanes in drinking water using headspace-SPME technique with gas chromatography. *Water Research, 37*(2), 402-408.

Choi, C. G., Oh, S. J., & Kang, I. J. (2009). Growth and morphological characteristics of Undaria pinnatifida in the cultivation ground at Busan, Korea. *Journal of the Faculty of Agriculture, Kyushu University, 54*(1), 47-51.

Dean, P. R., & Hurd, C. L. (2007). Seasonal growth, erosion rates, and nitrogen and photosynthetic ecophysiology of Undaria pinnatifida (Heterokontophyta) in southern New Zealand. *Journal of Phycology, 43*(6), 1138-1148.

Duflos, G., Leduc, F., N'Guessan, A., Krzewinski, F., Kol, O., & Malle, P. (2010). Freshness characterisation of whiting (Merlangius merlangus) using an SPME/GC/MS method and a statistical multivariate approach. *Journal of the Science of Food and Agriculture, 90*(15), 2568-2575. doi:10.1002/jsfa.4122

Food and Agriculture Organization of the United Nations. (2010). *World Review of Fisheries and Aquaculture*. Retrieved from http://www.fao.org/docrep/013/i1820e/i1820e01.pdf

Gomez, E., Ledbetter, C. A., & Hartsell, P. L. (1993). Volatile compounds in apricot, plum, and their interspecific hybrids. *Journal of Agricultural and Food Chemistry, 41*(10), 1669-1676. doi:10.1021/jf00034a029

Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochemistry, 34*(5), 1201-1218. doi:10.1016/0031-9422(91)80003-j

Hay, C. H. (1990). The dispersal of sporophytes of Undaria pinnatifida by coastal shipping in New Zealand, and implications for further dispersal of Undaria in France. *British Phycological Journal, 25*(4), 301-313. doi:10.1080/00071619000650331

Hay, C. H., & Luckens, P. A. (1987). The Asian kelp Undaria pinnatifida (Phaeophyta: Laminariales) found in a New Zealand harbour. *New Zealand Journal of Botany, 25*(2), 329-332. doi:10.1080/0028825x.1987.10410079

HunterLab. (2008). *Hunter L, a, b Color Scale* (Vol. 8)

Ikeda, K. (2002). New Seasonings. *Chemical Senses, 27*(9), 847-849. doi:10.1093/chemse/27.9.847

Isabel, S.-A., Marta, B., & Mercedes, C. (2009). Instrumental Texture. In L. M. L. Nollet & F. Toldrá (Eds.), *Handbook of Seafood and Seafood Products Analysis* (pp. 425-437): CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420046359-c23. doi:10.1201/9781420046359-c23

Jamieson, P. R. (2011). Sorbitol and Mannitol. In L. O. B. Nabors (Ed.), *Alternative Sweeteners* (4th ed., pp. 333-348): CRC Press. Retrieved from http://dx.doi.org/10.1201/b11242-23. doi:10.1201/b11242-23

Josephson, D. B., Lindsay, R. C., & Stuiber, D. A. (1983). Identification of compounds characterizing the aroma of fresh whitefish (Coregonus clupeaformis). *Journal of Agricultural and Food Chemistry, 31*(2), 326-330. doi:10.1021/jf00116a035

Jun, N.-Y., Kim, S.-O., & Han, J.-S., &. (2008). *The Quality Characteristics of Bacsulgi with Sea Mustard((Undaria pinnatifida) powder*: Korean Society of Food and Cookery Science.

Kajiwara, T., Hatanaka, A., Kawai, T., Ishihara, M., & Tsuneya, T. (1988). Study of Flavor Compounds of Essential Oil Extracts from Edible Japanese Kelps. *Journal of Food Science, 53*(3), 960-962. doi:10.1111/j.1365-2621.1988.tb08995.x

Kajiwara, T., Matsui, K., Akakabe, Y., Murakawa, T., & Arai, C. (2007). Antimicrobial browning-inhibitory effect of flavor compounds in seaweeds

Proceedings of the Eighteenth International Seaweed Symposium. In R. Anderson, J. Brodie, E. Onsøyen, & A. T. Critchley (Eds.), (Vol. 1, pp. 187-196): Springer Netherlands. Retrieved from http://dx.doi.org/10.1007/978-1-4020-5670-3_24. doi:10.1007/978-1-4020-5670-3_24

Kantono, K. (2011). *Undergraduate project on Consumer sensory testing of commercial and New Zealand Wakame (Undaria pinnatifida)*. Auckland: AUT University

Kennedy, J. (2010). Evaluation of replicated projective mapping of granola bars. *Journal of Sensory Studies, 25*(5), 672-684.

Kennedy, J., & Heymann, H. (2009). Projective mapping and descriptive analysis of milk and dark chocolates. *Journal of Sensory Studies, 24*(2), 220-233.

Lawless, H. T., & Heymann, H. (2010). *Sensory Evaluation of Food* (2nd ed.). New York: Springer. doi:10.1007/978-1-4419-6488-5
Le Pape, M.-A., Grua-Priol, J., Prost, C., & Demaimay, M. (2004). Optimization of Dynamic Headspace Extraction of the Edible Red Algae Palmaria palmata and Identification of the Volatile Components. *Journal of Agricultural and Food Chemistry, 52*(3), 550-556. doi:10.1021/jf030478x
León, K., Mery, D., Pedreschi, F., & León, J. (2006). Color measurement in L*a* b* units from RGB digital images. *Food Research International, 39*(10), 1084-1091. doi:10.1016/j.foodres.2006.03.006
Liu, T.-T., & Yang, T.-S. (2002). Optimization of Solid-Phase Microextraction Analysis for Studying Change of Headspace Flavor Compounds of Banana during Ripening. *Journal of Agricultural and Food Chemistry, 50*(4), 653-657. doi:10.1021/jf010891+
Marsili, R. T. (2006). Comparing sensory and analytical chemistry flavor analysis. In *Sensory-Directed Flavor Analysis* (pp. 1-22): CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420017045.ch1. Retrieved 2012/01/18. doi:10.1201/9781420017045.ch1
Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Introduction to Sensory Techniques. In *Sensory Evaluation Techniques, Third Edition*: CRC Press. Retrieved from http://dx.doi.org/10.1201/9781439832271.ch1. Retrieved 2012/01/18. doi:10.1201/9781439832271.ch1
Mester, Z., Sturgeon, R., & Pawliszyn, J. (2001). Solid phase microextraction as a tool for trace element speciation. *Spectrochimica Acta Part B: Atomic Spectroscopy, 56*(3), 233-260.
Miller, M. E., & Stuart, J. D. (1998). Comparison of Gas-Sampled and SPME-Sampled Static Headspace for the Determination of Volatile Flavor Components. *Analytical Chemistry, 71*(1), 23-27. doi:10.1021/ac980576v
Ministry of Agriculture and Forestry. (2010). *The commercial use of Undaria pinnatifida - an exotic Asian Seaweed*. Wellington: Ministry of Agriculture and Forestry.
Morales, M. T., Rios, J. J., & Aparicio, R. (1997). Changes in the Volatile Composition of Virgin Olive Oil during Oxidation: Flavors and Off-Flavors. *Journal of Agricultural and Food Chemistry, 45*(7), 2666-2673. doi:10.1021/jf960585+
Moshonas, M. G., & Shaw, P. E. (1973). Some newly found orange essence components including trans-2-pentenal. *Journal of Food Science, 38*(2), 360-361. doi:10.1111/j.1365-2621.1973.tb01429.x
Münch, P., Hofmann, T., & Schieberle, P. (1997). Comparison of Key Odorants Generated by Thermal Treatment of Commercial and Self-Prepared Yeast Extracts: Influence of the Amino Acid Composition on Odorant Formation. *Journal of Agricultural and Food Chemistry, 45*(4), 1338-1344. doi:10.1021/jf960658p
Nestrud, M. A., & Lawless, H. T. (2009). Perceptual mapping of apples and cheeses using projective mapping and sorting. *Journal of Sensory Studies, 25*(3), 390-405.
Nor Qhairul Izzreen, M. N., & Vijaya Ratnam, R. (2011). Volatile compound extraction using Solid phase micro extraction coupled with gas chromatography mass spectrometry (SPME-GCMS) in local seaweeds of Kappaphycus alvarezii, Caulerpa lentillifera and Sargassum polycystem. *International Food Research Journal, 18*(4), 1449-1456.
Pawliszyn, J., Pawliszyn, B., & Pawliszyn, M. (1997). Solid Phase Microextraction (SPME). *The Chemical Educator, 2*(4), 1-7. doi:10.1007/s00897970137a
Pelusio, F., Nilsson, T., Montanarella, L., Tilio, R., Larsen, B., Facchetti, S., & Madsen, J. (1995). Headspace Solid-Phase Microextraction Analysis of Volatile Organic Sulfur Compounds in Black and White Truffle Aroma. *Journal of Agricultural and Food Chemistry, 43*(8), 2138-2143. doi:10.1021/jf00056a034
Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., ... Miyashita, K. (2009). Edible Japanese seaweed, wakame (Undaria pinnatifida) as an

ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry, 115*(2), 501-508.
Punín Crespo, M. O., & Lage Yusty, M. A. (2006). Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of aliphatic hydrocarbons in seaweed samples. *Ecotoxicology and Environmental Safety, 64*(3), 400-405. doi:10.1016/j.ecoenv.2005.04.010
Riccioni, G., D'Orazio, N., Franceschelli, S., & Speranza, L. (2011). Marine Carotenoids and Cardiovascular Risk Markers. *Marine Drugs, 9*(7), 1166-1175.
Risvik, E., McEwan, J. A., Colwill, J. S., Rogers, R., & Lyon, D. H. (1994). Projective mapping: A tool for sensory analysis and consumer research. *Food Quality and Preference, 5*(4), 263-269.
Risvik, E., McEwan, J. A., & Rødbotten, M. (1997). Evaluation of sensory profiling and projective mapping data. *Food Quality and Preference, 8*(1), 63-71.
Rocha, S. l., Ramalheira, V. t., Barros, A. n., Delgadillo, I., & Coimbra, M. A. (2001). Headspace Solid Phase Microextraction (SPME) Analysis of Flavor Compounds in Wines. Effect of the Matrix Volatile Composition in the Relative Response Factors in a Wine Model. *Journal of Agricultural and Food Chemistry, 49*(11), 5142-5151. doi:10.1021/jf010566m
Rogerson, F. S. S., Castro, H., Fortunato, N., Azevedo, Z., Macedo, A., & De Freitas, V. A. P. (2000). Chemicals with Sweet Aroma Descriptors Found in Portuguese Wines from the Douro Region: 2,6,6-Trimethylcyclohex-2-ene-1,4-dione and Diacetyl. *Journal of Agricultural and Food Chemistry, 49*(1), 263-269. doi:10.1021/jf000948c
Rotzoll, N., Dunkel, A., & Hofmann, T. (2006). Quantitative Studies, Taste Reconstitution, and Omission Experiments on the Key Taste Compounds in Morel Mushrooms (Morchella deliciosa Fr.). *Journal of Agricultural and Food Chemistry, 54*(7), 2705-2711. doi:10.1021/jf053131y
Sartin, J. H., Halsall, C. J., Hayward, S., & Hewitt, C. N. (2002). Emission rates of C8–C15 VOCs from seaweed and sand in the inter-tidal zone at Mace Head, Ireland. *Atmospheric Environment, 36*(34), 5311-5321. doi:10.1016/s1352-2310(02)00639-8
Seppo, S., & Anja, H. (2001). Sweeteners. In J. H. T. III, S. Salminen, L. A. Branen, & M. P. Davidson (Eds.), *Food Additives*: CRC Press. Retrieved from http://dx.doi.org/10.1201/9780824741709.ch15. doi:10.1201/9780824741709.ch15
Simkin, A. J., Schwartz, S. H., Auldridge, M., Taylor, M. G., & Klee, H. J. (2004). The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β-ionone, pseudoionone, and geranylacetone. *The Plant Journal, 40*(6), 882-892. doi:10.1111/j.1365-313X.2004.02263.x
Stuart, M. D. (2004). *Review of research on Undaria pinnatifida in New Zealand and its potential impacts on the eastern coast of the South Island*. Wellington: Department of Conservation.
The Good Scents Company. Retrieved May 05, 2012, from http://www.thegoodscentscompany.com/index.html
Traiger, G. J., & Bruckner, J. V. (1976). The participation of 2-butanone in 2-butanol-induced potentiation of carbon tetrachloride hepatotoxicity. *Journal of Pharmacology and Experimental Therapeutics, 196*(2), 493-500.
Ullrich, F., & Grosch, W. (1988). Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room temperature. *Journal of the American Oil Chemists' Society, 65*(8), 1313-1317. doi:10.1007/bf02542413
Vejaphan, W., Hsieh, T. C. Y., & Williams, S. S. (1988). Volatile Flavor Components from Boiled Crayfish (Procambarus clarkii) Tail Meat. *Journal of Food Science, 53*(6), 1666-1670. doi:10.1111/j.1365-2621.1988.tb07811.x

Venugopal, V. (2008). Seaweed: Nutritional Value, Bioactive Properties, and Uses. In *Marine Products for Healthcare* (pp. 261-295): CRC Press. Retrieved from http://dx.doi.org/10.1201/9781420052640.ch9. doi:10.1201/9781420052640.ch9

Wang, F. (2009). Factor Analysis and Principal-Components Analysis. In K. Rob & T. Nigel (Eds.), *International Encyclopedia of Human Geography* (pp. 1-7). Oxford: Elsevier. Retrieved from http://www.sciencedirect.com/science/article/pii/B978008044910400434X

Wang, Y.-J. (2003). Saccharides. In P. Tomasik (Ed.), *Chemical and Functional Properties of Food Saccharides*: CRC Press. Retrieved from http://dx.doi.org/10.1201/9780203495728.ch3. doi:doi:10.1201/9780203495728.ch3

Watson, S. B., Brownlee, B., Satchwill, T., & Hargesheimer, E. E. (2000). Quantitative analysis of trace levels of geosmin and MIB in source and drinking water using headspace SPME. *Water Research, 34*(10), 2818-2828.

Whale, S. K., Singh, Z., Behboudian, M. H., Janes, J., & Dhaliwal, S. S. (2008). Fruit quality in "Cripp's Pink" apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon. *Scientia Horticulturae, 115*(4), 342-351.

White, W. L., Coveny, A. H., Robertson, J., & Clements, K. D. (2010). Utilisation of mannitol by temperate marine herbivorous fishes. *Journal of Experimental Marine Biology and Ecology, 391*(1–2), 50-56. doi:10.1016/j.jembe.2010.06.007

Yamanaka, R., & Akiyama, K. (1993). Cultivation and utilization of Undaria pinnatifida (wakame) as food. *Journal of Applied Phycology, 5*(2), 249-253.

Yang, X., & Peppard, T. (1994). Solid-Phase Microextraction for Flavor Analysis. *Journal of Agricultural and Food Chemistry, 42*(9), 1925-1930. doi:10.1021/jf00045a018

Young, W. F., Horth, H., Crane, R., Ogden, T., & Arnott, M. (1996). Taste and odour threshold concentrations of potential potable water contaminants. *Water Research, 30*(2), 331-340. doi:10.1016/0043-1354(95)00173-5

Zenkevich, I. G. (2005). Kovats' Retention Index System. In *Encyclopedia of Chromatography, Second Edition* (pp. 901-907): CRC Press. Retrieved from http://dx.doi.org/10.1201/NOE0824727857.ch193. Retrieved 2012/04/02. doi:doi:10.1201/NOE0824727857.ch193

Appendix A: Ethics Approval

MEMORANDUM

Auckland University of Technology Ethics Committee (AUTEC)

To:	Nazimah Hamid
From:	**Dr Rosemary Godbold and Madeline Banda** Executive Secretary, AUTEC
Date:	17 June 2011
Subject:	Ethics Application Number 11/113 **Sensory analysis of commercial and New Zealand Wakame (*Undaria pinnatifida*).**

Dear Nazimah

Thank you for providing written evidence as requested. We are pleased to advise that it satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC) at their meeting on 9 May 2011 and we have approved your ethics application. This delegated approval is made in accordance with section 5.3.2.3 of AUTEC's *Applying for Ethics Approval: Guidelines and Procedures* and is subject to endorsement at AUTEC's meeting on 11 July 2011.

Your ethics application is approved for a period of three years until 15 June 2014.

We advise that as part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/research/research-ethics/ethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 15 June 2014;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/research/research-ethics/ethics. This report is to be submitted either when the approval expires on 15 June 2014 or on completion of the project, whichever comes sooner;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are reminded that, as applicant, you are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this.

When communicating with us about this application, we ask that you use the application number and study title to enable us to provide you with prompt service. Should you have any further enquiries regarding this matter, you are welcome to contact Charles Grinter, Ethics Coordinator, by email at ethics@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of AUTEC and ourselves, we wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Dr Rosemary Godbold and Madeline Banda

Executive Secretary

Auckland University of Technology Ethics Committee

Cc: Jessica Marie Garcia Balbas jembalbas@gmail.com

Appendix B: Participant Information Sheet

Participant Information Sheet

Date Information Sheet Produced:

28 February 2011

Project Title

Sensory analysis of commercial and New Zealand wakame

An Invitation

Dear Sir/Madam,

My name is Jessica Balbas and I am a research student from the School of Applied Sciences at AUT University. I would like to invite you to participate in the tasting of wakame soup. The research is being funded by the AUT School of Applied Sciences and Wakatu, Inc. and will contribute to my Masters degree in Applied Sciences. Please read this information sheet carefully before deciding whether or not to participate. Your participation is voluntary and you may withdraw at any time prior to the completion of data collection without any adverse consequences. If there are potential conflict of interest issues, whether you choose to participate or not will neither advantage nor disadvantage you. I thank you for considering our request.

What is the purpose of this research?

The project aim is to recruit potential participants to carry out sensory analysis of commercial and New Zealand wakame. This project is being undertaken as part of the requirements for a Masters in Applied Science and will result in a thesis publication and may be published as a journal article.

How was I identified and why am I being invited to participate in this research?

We wish to include in this study men and women aged 18 years and above. If you wish to participate, you should be in good health with no allergies to seaweed, miso and/or products made from them, and be willing to complete the entire study.

You must be regular consumers of wakame, which is defined as consuming wakame “at least once every two weeks”.

What will happen in this research?

Should you agree to take part in this project, you will be asked to attend a sensory tasting session at the School of Applied Sciences, Auckland University of Technology on three occasions. The experiment will be run once a week, over 3 consecutive weeks.
This will involve the following:
You will be asked to taste samples of wakame. In order to help participants understand principles of the technique, the basis of Projective Mapping will be explained in the first sensory session. Samples will be presented at the same time, allowing direct comparison of the different samples during the session.
You will be asked to group the wakame samples on-screen, taking into account product sensory similarities and differences. The way the samples will be evaluated will be explained during the first session, stressing that odour, taste and texture should be considered at the time of judgment to give an overall assessment.

What are the discomforts and risks?

There will be no harm to participants. For the study, only commercially available foods, or foods manufactured entirely from food grade materials will be consumed.
Strict care will be taken to ensure that foods are prepared, stored and handled according to food hygiene standards.

What compensation is available for injury or negligence?

In the unlikely event of a physical injury as a result of your participation in this study, rehabilitation and compensation for injury by accident may be available from the Accident Compensation Corporation, providing the incident details satisfy the requirements of the law and the Corporation's regulations.

What are the benefits?

This study is expected to explore consumers' perception of the sensory quality of wakame products from New Zealand and South East Asia (South Korea, Japan, and China). Results from this study will provide a basis to determine whether the characteristics of New Zealand wakame are desirable in terms of taste and flavour as compared to the commercial South East Asian samples. This is important to determine how New Zealand wakame compares to commercial samples which will be important to support the development of a local product for sale in lucrative overseas markets.

How will my privacy be protected?

Any personal information that you provide will only be used to assist in explaining study results. Personal information will be published only as aggregate values, e.g. mean age. Only the

researchers directly involved in data collection will have access to the data. Results of this study may be published, but any data included will in no way be linked to any specific participant. The data collected will be securely stored in such a way that only those mentioned below will be able to gain access to it. At the end of the project any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.

What are the costs of participating in this research?

In return for attending the three 30-minute long sessions, you will be given a $10 petrol voucher as a token of appreciation for your participation.

What opportunity do I have to consider this invitation?

You have two working days to consider this invitation.

How do I agree to participate in this research?

You will need to obtain a consent form from the researcher if you agree to participate in this research. You may contact me at the e-mail address provided below. You may hand in the signed consent form when you are invited to attend the first sensory session at a specified place and time.

Will I receive feedback on the results of this research?

You are most welcome to request a summary of the study results, which can be e-mailed to you.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor,

Dr. Nazimah Hamid
nazimah.hamid@aut.ac/nz
09 9219999 ext 6453
Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, *madeline.banda@aut.ac.nz* , 921 9999 ext 8044.

Whom do I contact for further information about this research?

Researcher Contact Details:

Jessica Balbas
jembalbas@gmail.com
Mobile Number: 021508450

Project Supervisor Contact Details:

Dr Nazimah Hamid
nazimah.hamid@aut.ac.nz
09 9219999 ext 6453

Approved by the Auckland University of Technology Ethics Committee on *type the date final ethics approval was granted*, AUTEC Reference number *type the reference number*.

Appendix C: Consent Form

Consent Form

Project title: ***Sensory analysis of commercial and New Zealand wakame***

Project Supervisor: ***Dr Nazimah Hamid***

Researchers: ***Jessica Balbas***

- ○ I have read and understood the information provided about this research project in the Information Sheet dated 28 February 2011.
- ○ I have had an opportunity to ask questions and to have them answered.
- ○ I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- ○ I am not suffering from any allergies arising from consumption of seaweed and its products.
- ○ I agree to provide information on the sensory properties of the seaweed.
- ○ I agree to take part in this research.

Participant's signature:

.....

Participant's name:

.....

Participant's Contact Details (if appropriate):

……..
……..
……..
……..

Date:

Approved by the Auckland University of Technology Ethics Committee on type the date on which the final approval was granted AUTEC Reference number type the AUTEC reference number

Note: The Participant should retain a copy of this form.

Appendix D: Instructions

Projective Mapping of Commercial and New Zealand wakame

Date: July 2011

Time: You will need to come to three sessions over three consecutive days between 10 and 12am.

Location: Sensory Lab (WN Building)

Duration: Up to 30 minutes for each session.

Prior to Tasting: Please avoid drinking coffee and/or smoking cigarette one hour prior to wakame tasting.

What: Each tasting session will begin with a 5-minute presentation. 6 different cooked wakame products will be provided for you to taste and group (to be done using FIZZ software) in such a way that samples that are similar in taste are located near one another and samples different in taste are placed far apart. You will also be asked to write attributes of the seaweed samples to describe their tastes.

Please contact me if you are able to attend ALL three sessions.

Jessica Balbas.jembalbas@gmail.com ; 021 xxx xxxx

Appendix E: Mannitol Standard Curve