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Development of a Process Approach for Retaining Seaweed Sugar Kelp (Saccharina latissima) Nutrients

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DEVELOPMENT OF A PROCESS APPROACH FOR RETAINING SEAWEED SUGAR KELP (*Saccharina latissima*) NUTRIENTS

by

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ABSTRACT

Sugar kelp (*Saccharina latissima*) is a marine macroalgae that contains a rich source of fibers, vitamins, minerals and antioxidants. However, systematic studies on the effects of dehydration and drying on the kelp composition and quality attributes are limited. The aim of this research is to investigate the effects of process parameters of a convective air-oven on the quality and nutritional attributes of dried sugar kelp. The study evaluated the effects of hot air drying temperature, humidity and time on the physicochemical properties (water activity, moisture content, pH, color, water holding capacity, oil holding capacity, ash content, fat content and vitamin C) of sugar kelp during drying. The ash content of the samples was found to be in the range of 23.32% - 33.05% (dry basis) and is inversely correlated with the water holding capacity ($r = -0.84$) and oil holding capacity ($r = -0.84$), which suggest that the textural properties in kelp are highly dependent on the ash content irrespective of the drying temperature and humidity conditions. Further, the results indicated that the moisture content was dependent on the humidity and decreased with an increase of drying temperature. The water activity of dried kelp was below 0.66 for all samples, which was expected due to the free and bound water taken out during the drying process. Heat sensitive nutrients such as vitamin C showed a positive correlation with respect to the drying temperature (30°C to 70°C), which indicates that the drying time has a significant effect ($p<0.05$) on vitamin C.

To my mother… who has supported me and guided me through this journey

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INTRODUCTION

1.1 Background

Seaweed is a colloquial term for many species of plants and algae that grow in oceans, lakes, rivers and other bodies of water, and whose appearance resembles that of terrestrial plants. There are different types of seaweed depending on their color and family. Seaweeds are classified into three major groups; the green algae (Chlorophyta), the brown algae (Phaeophyta), and the red algae (Rhodophyta) (Hurd et al 2014). Seaweeds are placed into one of these groups based on their pigments and coloration (Hurd et al 2014). Other features used to classify algae are; cell wall composition, reproductive characteristics, and the chemical nature of their photosynthetic products (oil and starch) (Hurd et al 2014). Within each of the three major groups of algae, further classification is based on characteristics such as plant structure, form, and shape (Figure 1). *Saccharina latissima* is an edible species of seaweed that belongs to the brown algae family and grows in extreme low intertidal and shallow subtidal zones (Figure 2) (Hurd et al 2014). The frond of *Saccharina latissima* has a distinctive frilly undulating margin; it lives for 2 to 4 years and grows quickly from winter to April (Hurd et al 2014). It is known as sugar kelp because of its sweet-tasting powder and the its high content of mannitol (a sweet chemical) (Hurd et al 2014). Sugar kelp is closely related to *Saccharina Japonica*, the (farmed) seaweed basis of nearly all Japanese dashi, and can be used in similar ways – adding umami to soups, stews and stocks (Hurd et al 2014).

Fresh seaweeds collected or cultivated from the sea are usually dried before being used in any nutritional evaluation or industrial processing as they originally consist of 70 –90% water (Jensen, 1993). Drying, which is a process to reduce moisture content could be an important factor affecting the nutritional content of seaweeds. Algae (seaweed) generally has high moisture content (92% wet basis) and they are prone to microbial spoilage, similarly to land vegetables- which is why drying is important. In addition, drying reduces the enormous wet bulk before industrial processing. It has been reported that crude extracts from fresh wet seaweeds do not gel (Naylor, 1976). However, if they are dried properly, not only can the maximum yield of phycocolloids extraction be obtained, but the seaweeds can be stored for a number of years without appreciable loss of their gelling property (Naylor, 1976).

Oven-drying (Kaehler & Kennish, 1996; Robledo & Pelegrin, 1997) and freeze-drying (Norziah & Ching, 2000; Suzuki et al., 1996) are the two most widely used methods for seaweed drying. The basic mechanism of an oven-drying method is drying by hot air convection. The drying temperature is usually below 65ºC to avoid adverse thermal reactions (Anderson, 1996), and the usual drying time is 8–16 hours for seaweeds. In general, rapid drying under high temperature causes complex and physical degradative changes and losses of volatile compounds such as flavor and aroma in plant materials (Fellows, 1988). Freeze-drying is developed to overcome the problem of the loss of volatile compounds in food during conventional drying operations. In freeze-drying, seaweeds are frozen and dried by direct sublimation of the ice under reduced pressure (vacuum). This process minimizes the physical damage to the plant material, enhances reconstitution characteristics and minimizes the occurrence of oxidation and

thermal reactions. Drying could be an important factor affecting the nutritional value of seaweeds either through chemical modifications or direct losses of the nutrients. A previous report has shown that the nutritional composition of brown seaweed, S. hemiphyllum was greatly affected by different drying methods (Chan et al., 1997). However, information concerning the effect of different drying methods on the nutritional composition of different brown seaweeds including sugar kelp is limited.

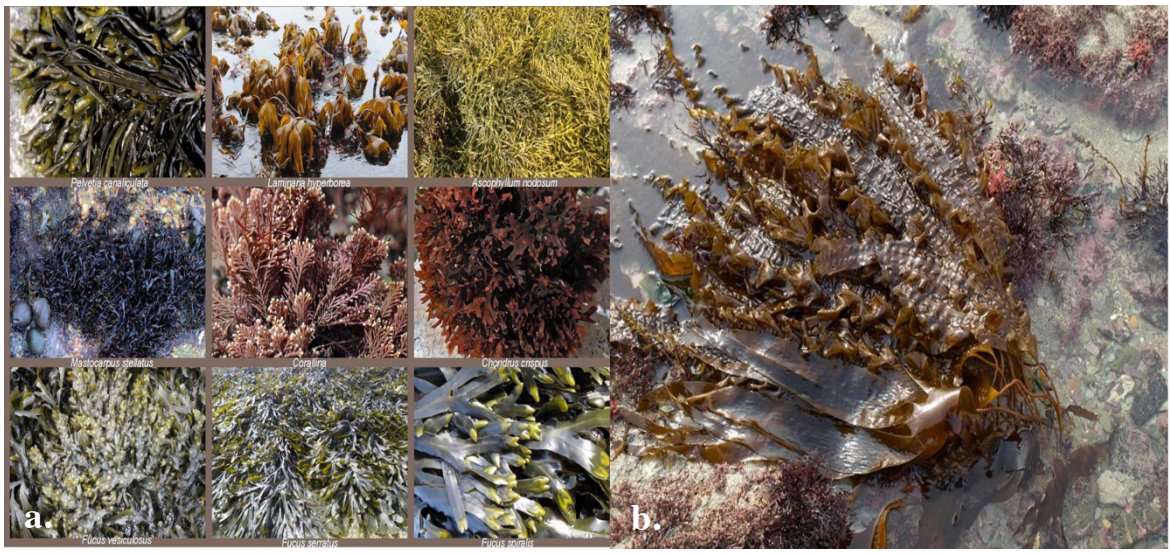

Figure 2: a. Some of the different types of seaweed and their species name (photo credit: Tabitha Pearman: Salty Scavenger (Rockpooling), British Marie Life Study Society) b. Sugar Kelp seaweed located in the base of the outside of North Pier, Newlyn Harbour, Newlyn, Cornwall (photo credit: David Ferwick, aphotomarine.com)

1.2 Benefits of Seaweed consumption

Seaweed does not only provide benefits to other marine creatures, but to the humans who consume it. Japan has been eating and using it for over a thousand years and it is eaten in other countries with raw fish, rice, and other ingredients. It contains a wide variety of minerals such as iodine --which is essential for the prevention of goiters,

vitamins, and fiber. Kelp has cancer-fighting agents for the treatment of tumors and leukemia (Darcy-Vrillon, 1993).

Moreover, recent interests among consumers in no added chemical additives/preservatives into food products, the potentials of seaweed as a source of natural and healthy food became widely recognized and studies on the nutritional values of seaweeds have become more widespread. Seaweeds are traditionally consumed in the Far East, while in the West they are used almost exclusively for the phycocolloid industry (Mabeau and Fleurence, 1993). In comparison with land vegetables, seaweeds are potentially good sources of polysaccharides, minerals, and certain vitamins (Darcy-Vrillon, 1993). Brown seaweed is one of the most abundant seaweed groups of economic importance and has been used as food and medicine in both China and Japan. It has also been collected and used as fertilizer and raw materials for the alginate-processing industry in the nearby region (Ho, 1988). Recently, in France, seaweeds have been authorized as vegetables and condiments (Mabeau, 1989). Therefore, seaweeds have become a valuable vegetable (fresh or dried) and an important food ingredient in the human diet.

Moreover, the potentials of seaweed as a source of natural and healthy food has become widely recognized as studies on the nutritional values of seaweeds have become widespread. Nutritional value of some brown seaweed species (i.e. S. hemiphyllum and Sargassum) from the sea regarding the composition of their protein and amino acids, lipid and fatty acids, dietary fibers, minerals, and vitamins has been investigated and reported before.

1.3 Objectives

Maine has various companies that harvest sugar kelp from the North Atlantic Ocean. However, sugar kelp needs to be dehydrated/dried and processed to preserve for off-season use or for various value-added products. The current practice of sugar kelp business in Maine does not include a standardized drying or processing method, and no study has been done on the effects of oven drying and freeze drying on their nutritional quality of dried sugar kelp

The objectives of this investigation were to study: (i) the nutritional composition of the dried sugar kelp; (ii) effects of drying/dehydration and processing effects on these nutritional attributes; (iii) the variations of the nutritional composition and drying between two seasons of sugar kelp (March 2016 and June 2016). The research has provided Maine Seaweed (sugar kelp) farmers/processors a method that allows better processing and preservation.

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MATERIALS AND METHODS

2.1 Sample Preparation

Sugar kelp (*Saccharina latissima)* was collected from the Center for Co-operative Aquaculture Research (CCAR), Franklin, ME and transported to the Food Process Engineering Laboratory at the University of Maine in the beginning of March 2016 for harvest one (also referred to as season one) (S1) and beginning of June 2016 (also referred as season two) for harvest two (S2). The holdfasts at the end were cut off and the blade and stipe parts were washed with running water to remove the attached biofouling and salts. The raw sugar kelp was divided into three groups (a, b and c) for physicochemical analysis. Fresh sugar kelp samples of approximately 450g were dried at an air temperature of 30°C, 40°C, 50°C, 60°C and 70°C with relative air humidity levels of 25% and 50% and air velocity of 10.0 m/s in the convective dryer (Cincinnati sub-zero, CSG, OH, USA). After drying process was over, dried seaweed was allowed to cool down in the desiccator for 2 hours. Dried Seaweed collected was weighted and distributed equally into air tight zip lock bags which were stored at -80°C in the Pilot Plant in the School of Food and Agriculture until further physicochemical analysis. The last group of dried seaweed was freeze dried – which was considered the control group. Approximately, 250 grams of freeze dried seaweed were dried in a freeze dryer (Virtis Ultra 35 EL, Stone Ridge, NY) for 20 hours. The freeze dried sugar kelp was pulverized

to a fine powder using a mortar and pestle and stored in an opaque brown container at -20°C until further analysis.

Each experiment was conducted for two harvest in triplicates (a, b and c).

2.2 Reagents

For Fat Content: 8.1N HCl was added (Fisher Scientific, Waltham, MA), ethanol (Fisher Scientific, Waltham, MA), ethyl ether (Fisher Scientific, Waltham, MA) and petroleum ether (Fisher Scientific, Waltham, MA).

For Vitamin C: glacial metaphosphoric acid (Fisher Scientific, Waltham, MA, glacial acetic acid (Fisher Scientific, Waltham, MA), ethylene diamine tetra acetic acid (EDTA) (Sigma-Aldrich, St. Louis, MO), ascorbic acid (Fisher Scientific, Waltham, MA), 2,6-dichlorophenolindophenol sodium salt (Fisher Scientific, Waltham, MA), sodium bicarbonate (Fisher Scientific, Waltham, MA)

2.3 Physicochemical Analysis

2.3.1 Moisture analysis

The moisture content of dried sugar kelp was determined gravimetrically according to the AOAC method (AOAC, 2005). One gram of seaweed was dried in a pre-weighed 20ml. disposable scintillation vials in triplicates in a forced air-oven at 105 ºC (VWR International, Radnor, PA). After 48 hours, the vials containing the dried seaweed were reweighed and the percent moisture was calculated by using the formula given below.

$$Moisture\ \left(\frac{g}{100g}\right)$$

$$= \frac{[pan\ wt.(g) +\ wet\ sample\ wt.(g)] - [pan\ wt.(g) + dried\ sample\ wt.(g)]}{[(pan\ weight\ (g) + wet\ sample) - pan\ weight\ (g)]}$$

$$\times 100$$

The moisture content was expressed as percentage by weight of dry sample or in dry basis.

2.3.2 Ash Content

The ash content was determined gravimetrically by heating the vials with the dried seaweed samples in the muffle furnace at 550°C for 7 hours (AOAC, 2005) (Thermolyne Model F-A1730, Dubuque, IA, USA). Vials containing the samples were reweighed and the percent ash on dry basis (d.b) was calculated using the formula given below.

$$Ash\ Content\ \left(\frac{g}{100g}\right) = \frac{[vial\ wt.(g) + ash\ wt\ (g)\ ] - vial\ wt\ (g)}{[dry\ sample\ weight\ (g)]} \times 100$$

2.3.3 Water Holding Capacity (WHC)

Water-holding capacity (WHC) of seaweed samples was measured in triplicates by the modified centrifugation method described by Suzuki, Ohsugi, Yoshie, Shirai and Hirano (1996). Twenty ml of de-ionized water was added to each centrifuge tube (CellTreat, Pepperell, MA) containing 0.2g of dried kelp. The tubes were shaken in a water bath (Julabo SW22, Allentown, PA) for 24 hours at room temperature ~ 22 ºC. After centrifugation (Beckman, Avanti J-25, Fullerton, California) at 14,000 X g for 30

minutes (min), the supernatant was collected and the water absorbed by the pellet was determined by measuring the weight of the supernatant. The WHC of seaweed was expressed as the weight of grams of water held by 1g of sample dry weight (DW).

$$WHC\ (\%) = \frac{water\ added\ (g) - water\ decant(g)}{dry\ sample\ weight\ (g)} \times 100$$

2.3.4 Oil-holding capacity (OHC)

Oil-holding capacity (OHC) of seaweed was determined by the method of Caprez, Arrigoni, Amado and Neukom (1986) with slight modifications. Three grams of dried sugar kelp were placed in each centrifuge tube (Celltreat, Pepperell, MA), to which 10.5g of corn oil (Mazola Corn Cooking Oil, Memphis, TN) were added. The tubes were mixed in a compact agitator (Thermo Scientific, Compact Digital Mini Rotator/Shaker, Pittsburgh PA) for 30 min at room temperature (22ºC). After that, the mixture was centrifuged (Beckman, Avanti J-25, Fullerton, California) at 2500 X g for 10 min. The excess oil supernatant was then removed and measured for its weight. The OHC of dried seaweed was expressed as the number of grams of oil held by 1g of sample (d.b). Density of the oil was found to be 0.92 g/ml at room temperature ~ 22ºC.

$$OHC\ (\%) = \frac{oil\ added\ (g) - oil\ decant(g)}{dry\ sample\ weight\ (g)} \times 100$$

2.3.5 Fat Content

Fat Content was determined using the protocol described in the standard NF V 03-713 (AOAC, 2005) with slight modifications. Approximately 2.5 g of the dried sugar kelp was weighed and placed in a French square bottle. Ten ml of 8.1N HCl was added to digest all the carbohydrates and proteins. For complete digestion, the dried sugar kelp was placed in a water bath (Julabo SW22, Allentown, PA) for 90 min at 85ºC. Three extractions were performed to each of the samples of kelp. For the first extraction only: 7ml of ethanol were added and then agitated vigorously for 30 seconds. After the ethanol was added, the second step was to add 25ml of ethyl ether (followed by 15 seconds of slow/moderate agitation and by rigorous agitation for 45 seconds). The third step in the process was to add 25ml of Petroleum Ether to the above mixture (followed by 15 seconds of slow/moderate agitation and by rigorous agitation for 45 seconds). Lastly, the fourth step was to allow the digested sediments to settle down for 30 minutes. The top layer (ether plus fat) was carefully extracted using a glass pipette and transferred to a pre-weighed flat-bottom beaker. Steps 2, 3 and 4 were repeated twice for all the dried seaweed samples. The pooled ether with lipid was allowed to dry overnight under the chemical hood followed by drying in an oven at 105 ºC for 15 min (VWR International, Radnor, PA). The fat content was calculated by reweighing the cooled beakers and using the following formula:

$$Crude\ Fat\left(\frac{g}{100g}\right) = \frac{[flask\ (g) + fat\ weight\ (g)] - [flask\ (g)]}{[sample\ weight\ (g)]} \times 100$$

Fat content was expressed as the percentage of lipids (fat) in the dry matter of the kelp.

2.3.6 pH

One gram of dried sugar kelp was weighed and placed in a centrifuge tube (Celltreat, Pepperell, MA) to which 15ml of de-ionized water was added. Contents were mixed utilizing a mechanical agitator (Thermo Scientific Compact Digital Mini Rotator/Shaker, Pittsburgh PA). The pH was measured with a pH meter (Benchtop pH / MV Meter – 860031, Scottsdale, AZ) based on the Antimony-Electrode Method (Horn et'al 2000).

2.2.7 Water Activity

The water activity meter (AquaLab Decagon, Pullman, WA) was turned on for 20 min. before performing the first reading. It was calibrated with the standard salts solutions with water activity of 0.500 and 0.250. The water activity was determined by weighing approximately 1g of dried sugar kelp in disposable water activity cups.

2.3.8. Color Analysis

Color was determined using a Hunter colorimeter (LabScan XE, Hunter Labs, Reston, VA) and expressed in L*a*b* values, in which L* values are based on a scale of dark (0) to light (100), a* values are based on a scale of green (-) to red (+), and b* values are based on a scale of blue (-) to yellow (+). Black and white ceramic standard plates were used to standardize the colorimeter before each use and the colorimeter was allowed to warm up for 20 min prior to color analysis. A port size of 50.5 mm, area view of 44.5 mm, and D65 illumination were used. The disc with 5.1 cm diameter hole was used. Approximately 1g of dried kelp was placed in colorimeter cups for analysis.

2.3.9. Vitamin C

Vitamin C was determined by titrating dried sugar kelp using 2,6-dichlorophenolindophenol dye method (AOAC methods 967.21 and 985.3, 2005). To perform vitamin C analysis, the following solutions were prepared: precipitant/extraction solution, ascorbic acid standard solution, and indophenol standard solution.

One gram of pulverized dried sugar kelp was homogenized for 2 min with 15ml cold precipitant solution using a polytron homogenizer (Brinkman Instruments, Westbury, NY). It was then centrifuged (Beckman J-25, Brea, CA) at 10,000 X g for 15 min at 25ºC. The pellet was re-suspended in 15ml precipitant solution and centrifuged again. The supernatants were pooled together and the final volume was recorded.

The precipitant solution was made by mixing equal amounts of two solutions. The first solution was made by dissolving 15g of glacial metaphosphoric acid in 40ml of glacial acetic acid and bringing it to 250ml with distilled water. The second solution was made by dissolving 0.9g of ethylene diamine tetra acetic acid (EDTA) in 200ml of de-ionized water and bringing it up to 250ml. The precipitant solution was made fresh on the day of use.

Ascorbic acid (1 mg/mL) was used as the standard solution and was prepared by diluting 50mg ascorbic acid to 50ml with the precipitant solution in a volumetric flask. For the dye, 0.0625 g of 2,6- dichlorophenolindophenol sodium salt and 0.0525g of sodium bicarbonate were brought up to 250ml with distilled water. The ascorbic acid standard plus 5 mL precipitant solution was titrated using the indophenol dye (25% DCIP and 21% $NaHCO_3$ in water) until rose pink color persisted for 10s. Fifteen mL aliquots of

sample extracts were poured in 50 mL Erlenmeyer flasks and titrated with the indophenol dye until the rose-pink endpoint lasted for 10s. For the sample blank, two 15ml aliquots of precipitant solution were added into separate 50ml. Erlenmeyer flasks and titrated with indophenol standard solution to obtain the same endpoint. The ascorbic acid concentration of the sample was calculated using the following formula:

$$\frac{\text{mg of ascorbic acid}}{g\ of\ dried\ sample} = \text{C}\times\text{VX}\frac{\text{DF}}{\text{WT}}$$

Where,

C = mg of ascorbic acid/mL of dye

V = mL of dye used for titration of diluted sample (subtract blank volume first)

DF = dilution factor

WT = sample weight (g)

2.3.10. Statistical analysis

Analyses for each experiment was performed in triplicates. All data are presented as mean values ± standard deviation. The effects of the different factors (temperature, humidity and season)were analyzed by multi-way ANOVA (SAS University Edition 2016) to detect significant differences among groups followed by Tukey's honest significant difference (HSD) test was performed at confidence level of 0.05 (i.e. ∞=0.05). Appendix shows these relations.

RESULTS

For all the experiments the average of the triplicates is shown and freeze dried served as control, all results are shown in dry basis (d.b)*.

*Except Moisture Content: shown in Wet Basis

3.1 Moisture Content

Table 3.1.1 shows the average variation of moisture content as a function of time for the temperatures and humidities in wet basis. Results revealed that moisture content of dried sugar kelp was, as expected, less than 15% for both harvest and humidities. There was a significant difference in the moisture content with different drying temperatures. As expected, when both seasons are compared, the moisture content decreases as the temperatures increases. When air oven humidities (25% and 50%) during drying are compared, the values of 50% air oven humidity for both seasons were substantially higher than 25% air oven humidity values. Therefore, there was a significant effect between humidities.

On the other hand, statistical analysis revealed that all individual, double and triple interaction factors had a significant effect. It also shows that of the dried kelp moisture content values for both seasons, freeze dried was substantially lower (3%) than any of the oven dried sugar kelp at 25% and 50% air oven humidity.

Table 3.1.1: Moisture Content (%) of Oven Dried Kelp at 25% and 50% Air Oven Humidities**

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	5.08 ±0.00	5.08 ±0.00	2.05 ±0.00	2.05 ±0.00
30*	10.73 ±0.00 aAx	13.78 ±0.00 aAy	11.36 ±0.00 aBx	14.33 ±0.00 aBy
40*	8.91 ±0.00 bAx	11.19 ±0.00 bAy	7.35 ±0.00 bBx	16.63 ±0.01 bBy
50*	8.15 ±0.00 bAx	13.73 ±0.01 bAy	9.89 ±0.00 bBx	12.82 ±0.01 bBy
60*	7.11 ±0.00 cAx	12.14 ±0.00 cAy	8.06 ±0.00 cBx	12.67 ±0.00 cBy
70*	6.06 ±0.00 dAx	11.27 ±0.00 dAy	3.09 ±0.00 dBx	14.62 ±0.00 dBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in MC with temperatures

Capital letter: denotes significant difference in MC between season 1 and season 2

x/y Denote significant difference between humidities

***Wet Basis*

3.2 Ash content

Table 3.2.1 show the average ash content of freeze dried and oven dried kelp (30-70ºC) at air oven humidities of 25% and 50%, respectively. The ash contents of all the seaweed samples were similar in that the sugar kelp had ash contents between 22-35% for both of the harvest's dry weight. This is expected because ash content measures the amount of minerals (which are not destroyed by heating). For both air oven humidities, ash content values of season two were slightly higher, as the values ranged from 22-35%; while for season one the values ranged from 25-28%

Air oven humidities and temperatures did not have a significant effect, while season as individual factor and triple factor (season*temperature*humidity) had a significant effect.

Table 3.2.1: Ash Content (%) of Oven Dried Kelp at 25% and 50% Air Oven Humidities

	Season 1		Season 2	
	Humidity			
Temperatures	25%	50%	25%	50%
FD	26.96 ± 3.7	26.96 ± 3.7	24.78 ± 0.3	24.78 ± 0.3
30*	25.47 ± 0.1 A	28.99 ± 0.5 A	33.93 ± 0.3 B	31.24 ± 2.8 B
40*	24.90 ± 0.5 A	25.58 ± 1.1 A	33.06 ± 2.4 B	34.23 ± 0.1 B
50*	27.23 ± 0.1 A	24.45 ± 0.5 A	26.26 ± 9.7 B	34.89 ± 0.2 B
60*	25.59 ± 0.2 A	24.11 ± 1.5 A	27.46 ± 0.7 B	30.89 ± 0.7 B
70*	25.72 ± 3.3 A	23.75 ± 0.5 A	35.39 ± 0.2 B	26.29 ± 0.4 B

FD: Freeze Dried *Air-Oven Humidity Samples

Small letter: denotes row-wise significant difference in AC with temperatures

Capital letter: denotes significant difference in AC between season 1 and season 2

x/y Denote significant difference between humidities

3.3 Water Holding Capacity (WHC)

WHC of the dried sugar kelp ranged from 857% to 2079% (8.57g and 20.79g of water per g of sugar kelp) as shown above (Table 3.3.1). With respect to 25% air oven humidity, the WHC of the kelp on season one was the highest with freeze-dried and the lowest at 30ºC. On the other hand, on season two (for 25% air oven humidity) the sugar kelp had the highest value at 30ºC and freeze dried had the lowest. The WHC of the kelp

was the highest at 70ºC and the lowest at 40ºC at 50% air oven humidity. In season two, 30ºC had the highest WHC while freeze dried had the lowest. At the same time, WHC values of the dried kelp were lower at 25% air oven humidity, when compared to 50% air oven humidity. Overall, season two dried kelp WHC values were higher than harvest one.

Seasons one and two, were significantly different. Air oven humidities of the sugar kelp at 25% and 50% were also significantly different. In this case, double and triple interaction factors of the sugar kelp between temperature*season, humidity*season, temperature*humidity, temperature*season*humidity and season had all a significant effect.

Table 3.3.1: WHC (%) of Oven Dried Kelp at 25% and 50% Air Oven Humidities

	Season 1		Season 2	
	Humidity			
Temperatures	25%	50%	25%	50%
FD	1356.08 ± 0.3	1356.08 ± 0.3	1310.9 ± 0.3	1310.9 ± 0.3
30*	857.33 ± 5.3 bAx	1269.4 ± 1.0 bAy	2279.66 ± 0.4 bBx	1875.06 ± 0.2 bBy
40*	990.19 ± 5.9 cAx	1082.04 ± 0.8 cAy	1481.24 ± 0.2 cBx	2051.52 ± 0.8 cBy
50*	1429.21 ± 14.1 bAx	1078.89 ± 0.7 bAy	1906.42 ± 0.7 bBx	1515.41 ± 0.4 bBy
60*	860.11 ± 11.8 cbAx	1493.48 ± 1.7 cbAy	1740.23 ± 0.3 cbBx	1655.85 ± 0.5 cbBy
70*	1299.45 ± 0.6 aAx	1470.48 ± 0.5 aAy	2106.31 ± 0.8 aBx	1754.85 ± 0.4 aBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in WHC with temperatures

Capital letter: denotes significant difference in WHC between harvest 1 and season 2

x/y Denote significant difference between humidities

3.4 *Oil Holding Capacity (OHC)*

Table 3.4.1 show the average oil holding capacity of dried sugar kelp. OHC of the kelp samples ranged from 75% to 253% (0.75 and 2.53 of oil per 1g of seaweed). Kelp OHC values were higher for season two at both 25% and 50% air oven humidities-- when compared to harvest one OHC values. There was also a decrease in OHC values of the kelp with oven temperature increase. On the other hand, OHC values for the dried seaweed on season one were the highest with freeze dried (150% d.b) at 50% air oven humidity and at 70ºC (225% d.b) for 25% air oven humidity. The lowest OHC of the season one kelp were at 60ºC for 50% air oven humidity and at 70ºC for 25% air oven humidity.

Freeze dried was the only sample that was significantly different for all the oven-dried temperatures in both air oven humidities and harvest. Moreover, both seasons and air oven humidity (25 and 50%) had significant differences. Statistical Analysis also revealed that interaction of double and triple factors of the sugar kelp between temperature*humidity, temperature*season, humidity*season and temperature*season*humidity had also significant effects.

Table 3.4.1: OHC (%) of Oven Dried Kelp at 25% and 50% Air Oven Humidities

	Season 1		Season 2	
	Humidity			
Temperatures	25%	50%	25%	50%
FD	168.52 ±4.7	168.52 ± 1.1	162.9 ± 4.5	162.9 ± 4.5
30*	118.46 ± 3.8 bAx	178.95 ± 6.7 bAy	202.01 ± 2.0 bBx	109.61 ± 8.8 bBy
40*	107.61 ± 1.2 abAx	189.52 ± 7.8 abAy	241.77 ± 3.4 abBx	150.33 ± 1.9 abBy
50*	158.17 ± 1.2 abAx	196.16 ± 0.98 abAy	237.58 ± 0.9 abBx	210.72 ± 1.1 abBy
60*	113.88 ± 0.8 aAx	173.09 ± 0.9 aAy	246.77 ± 2.5 aBx	191.7 ± 1.7 aBy
70*	81.05 ± 1.5 abAx	223.2 ± 0.9 abAy	237.77 ± 12.0 abBx	141.38 ± 1.7 abAy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in OHC with temperatures

Capital letter: denotes significant difference in OHC between season 1 and season 2

x/y Denote significant difference between humidities

3.5 *Fat Content*

In general, the fat contents of the two harvest of sugar kelp were low (0.62- 4.6%), but within the range (1.00±3.00%) reported previously and as expected (Mabeau & Fleurence, 1993). For season one, fat content of the kelp at 25% air oven humidity was the lowest values at 70º C (0.62%), while highest was freeze dried (3.6%). On the contrary, for season two at 25% air oven humidity, freeze dried was the lowest (1.1%) and 40ºC was the highest (1.9%). With respect to 50% humidity, the highest value for season one was at 40ºC while the lowest was at 30 ºC. Overall, season one and two values seemed to decrease with temperature increase. In comparison, values for season one were higher than for harvest two at both air oven humidities (25 and 50%); showing

that harvest had a significant effect. Humidity also had a significant effect. There was a significant difference between freeze dried and all of the other oven-dried samples.

The interaction of double and triple factors of temperature*humidity, temperature*season, humidity*season and temperature*season*humidity also had significant effects.

Table 3.5 Fat Content (%) of Oven Dried Kelp at 25% and 50% Air Oven Humidities

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	3.65 ± 0.0	3.65 ± 0.0	1.27 ± 0.0	1.27 ± 0.0
30*	2.90 ± 0.1 bAx	2.54 ± 0.2 bAy	1.61 ± 0.0 bBx	1.55 ± 0.0 bBy
40*	2.50 ± 0.4 aAx	4.60 ± 0.4 aAy	1.77 ± 0.1 aBx	1.52 ± 0.0 aBy
50*	3.03 ± 0.2 bAx	2.84 ± 0.4 bAy	1.46 ± 0.0 bBx	1.48 ± 0.0 bBy
60*	2.69 ± 0.2 bAx	2.32 ± 1.1 bAy	1.39 ± 0.0 bBx	1.55 ± 0.0 bBy
70*	0.62 ± 0.8 bAx	3.01 ± 0.2 bAy	1.46 ± 0.0 bBx	1.48 ± 0.0 bBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in FC with temperatures

Capital letter: denotes significant difference in FC between season 1 and season 2

x/y Denote significant difference between humidities

3.6 *pH*

Tables 3.6.1 show the values of pH for FD and oven-dried at various temperatures. Results revealed that 50% air oven humidity had higher pH values than 25% air oven humidity (for both harvests). The pH of harvest one sugar kelp ranged from 4.9-5.9. Sugar kelp from harvest two had, slightly, higher pH than harvest one, with a range of

6.1- 6.07. It is important to notice how freeze dried samples represented the lowest when compared to the other temperatures for both harvests and oven air humidities. Moreover, an increase in temperature resulted in an increase in pH for both seasons and humidities.

Season, temperature and humidity had significant effects. All interaction factors (individual, double and triple) had significant effect.

Table 3.6 pH of Oven Dried Kelp at 25% and 50% Air Oven Humidities

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	4.97 ± 0.0	5.7 ± 0.0	6.05 ± 0.0	6.05 ± 0.0
30*	5.52 ± 0.28 dAx	6.06 ± 0.0 dAy	6.03 ± 0.0 dBx	6.11 ± 0.0 dAy
40*	5.60 ± 0.0 cAx	6.20 ± 0.0 cAy	6.05 ± 0.0 cBx	6.20 ± 0.0 cBy
50*	5.51 ± 0.0 cbAx	6.23 ± 0.0 cbAy	6.07 ± 0.0 cbBx	6.21 ± 0.0 cbBy
60*	5.86 ± 0.1 aAx	6.15 ± 0.0 aAy	6.01 ± 0.0 aBx	6.31 ± 0.0 aBy
70*	5.96 ± 0.0 abAx	6.49 ± 0.0 abAy	6.07 ± 0.0 abBx	6.12 ± 0.0 abBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in pH with temperatures

Capital letter: denotes significant difference in pH between harvest 1 and season 2

x/y Denote significant difference between humidities

3.7 *Water activity*

The dried kelp had, as expected, a low water activity a_w (below 0.66 for all samples). For harvest one at 25% air oven humidity, the lowest value was freeze dried (a_w = 0.1) and the highest was at 30ºC (a_w = 0.40). On the other hand, the lowest value for season two at 25% humidity was at 40ºC (a_w = 0.20) and the highest was at 30ºC (a_w =

0.44). With respect to 50% air oven humidity season one dried kelp, the lowest value was freeze dried (a_w= 0.10) and the highest was 50ºC (a_w = 0.56). The lowest and highest value for harvest two sugar kelp at 50% air oven humidity were at 70ºC (a_w = 0.44) and 30ºC (a_w 0.66), respectively.

Moreover, the values for water activity at 50% air oven humidity were higher than 25% air oven humidity dried sugar kelp values. Overall then, water activity values for the dried kelp were higher for harvest two than for harvest one. On the other hand, all of the individual, double and triple factors (temperature, humidity and season) of the dried seaweed had all a significant effect.

Table 3.7 Water Activity of Oven Dried Kelp at 25% and 50% Air Oven Humidities

	Season 1		Season 2	
	Humidity			
Temperatures	25%	50%	25%	50%
FD	0.101 ± 0.0	0.101 ± 0.0	0.423 ± 0.0	0.569 ± 0.0
30*	0.404 ± 0.0 aA	0.503 ± 0.0 aA	0.473 ± 0.0 aB	0.645 ± 0.0 aB
40*	0.301 ± 0.0 dA	0.443 ± 0.0 dA	0.201 ± 0.0 dB	0.487 ± 0.0 dB
50*	0.362 ± 0.0 bA	0.568 ± 0.0 bA	0.389 ± 0.0 bB	0.517 ± 0.0 bB
60*	0.312 ± 0.0 cA	0.511 ± 0.0 cA	0.349 ± 0.0 cB	0.603 ± 0.0 cB
70*	0.266 ± 0.0 cA	0.490 ± 0.0 cA	0.338 ± 0.0 cB	0.423 ± 0.0 cB

FD: Freeze Dried *Air-Oven Humidity Samples

Small letter: denotes row-wise significant difference in WA with temperatures

Capital letter: denotes significant difference in WA between season 1 and season 2

x/y Denote significant difference between humidities

3.8 *Color Analysis*

3.8.1 L Values*

Table 3.8.1.1 shows the average color parameters for L* values of the dried sugar kelp. Colorimetric L* values are used to measure lightness and range from 0 to 100, where the values 0 and 100 corresponds to black and white, respectively. Results revealed that temperature, humidity and season significantly affected the L* values of the dried kelp for both seasons. Interestingly, temperature increase significantly increased L* values for both seasons. Overall, season two dried sugar kelp had higher L* values when compared to season one dried kelp. With respect to freeze dried kelp, its values were the highest for both seasons and air oven humidities; indicating that freeze drying caused the most fading of the samples. For L* values, air oven humidities (25 and 50%) and seasons had significant effects.

Table 3.8.1 L* Values of Oven Dried Kelp at 25% and 50% Air Oven Humidities

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	4.97 ± 0.0	5.7 ± 0.0	6.05 ± 0.0	6.05 ± 0.0
30*	5.52 ± 0.28 dAx	6.06 ± 0.0 dAy	6.03 ± 0.0 dBx	6.11 ± 0.0 dAy
40*	5.60 ± 0.0 cAx	6.20 ± 0.0 cAy	6.05 ± 0.0 cBx	6.20 ± 0.0 cBy
50*	5.51 ± 0.0 cbAx	6.23 ± 0.0 cbAy	6.07 ± 0.0 cbBx	6.21 ± 0.0 cbBy
60*	5.86 ± 0.1 aAx	6.15 ± 0.0 aAy	6.01 ± 0.0 aBx	6.31 ± 0.0 aBy
70*	5.96 ± 0.0 abAx	6.49 ± 0.0 abAy	6.07 ± 0.0 abBx	6.12 ± 0.0 abBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in L values with temperatures*

Capital letter: denotes significant difference in L values between season 1 and season 2*

x/y Denote significant difference between humidities

3.8.2 *a* Value*

a* values indicate the scale from (-) green to red (+) of the colorimeter values. Tables 3.8.2.1 show how the a* values of the dried sugar kelp ranged from 1.0 to 3.0. Overall, the a* values for season one seemed to decrease with a temperature increase for both air oven humidities (25% and 50%); indicates that higher temperature accelerated the loss of color in those samples. Similarly, the a* values of the dried kelp of season two significantly decreased over time indicating that the samples were also fading with respect to the red color. Moreover, season two had, overall, lower values when compared to season one dried kelp values, which could indicate a loss of redness.

Again, freeze dried sugar kelp showed significant variations for all oven-dried temperatures in both air oven humidities (25% and 50%). On the other hand, both temperature, season and humidity had a significant effects.

Tables 3.8.2.1 a* Values of Oven Dried Kelp at 25% and 50% Air Oven Humidities

	Season 1		Season 2	
	Humidity			
Temperatures	25%	50%	25%	50%
FD	2.54 ± 1.1	2.54 ± 1.1	2.35 ± 0.0	2.35 ± 0.0
30*	3.35 ± 0.2 aAx	4.7 ± 0.3 aAy	1.72 ± 0.9 aBx	1.02 ± 0.0 aBy
40*	3.05 ± 0.1 bAx	3.00 ± 0.2 bAy	1.03 ± 0.8 aBx	0.94 ± 0.0 bBy
50*	2.6 ± 0.0 bAx	2.77 ± 0.2 bAy	1.02 ± 1.1 aBx	1.82 ± 0.9 bBy
60*	2.66 ± 0.1 aAx	3.84 ± 0.1 aAy	2.75 ± 1.2 aBx	2.98 ± 0.5 aBy
70*	2.5 ± 0.2 aAx	1.69 ± 0.1 aAy	1.84 ± 1.2 aBx	3.71 ± 0.6 aBy

FD: Freeze Dried *Air-Oven Humidity Samples

Small letter: denotes row-wise significant difference in a* values with temperatures

Capital letter: denotes significant difference in a* values between season 1 and season 2

x/y Denote significant difference between humidities

8.3 b Values*

b* values measure yellowness of the samples on the scale from blue (-) to yellow (+). Colorimeter b* values seemed to increase with temperatures increase for both air oven humidities (25% and 50%) and seasons. Similarly, to L* values, freeze dried had the highest values for both seasons and air oven humidities. In this case, seasons and humidity did not have a significant effect. Interestingly, the yellowness increased for

sugar kelp harvested in season two over temperature; as b* values for season two increased when compared to season one. Moreover, most of the values increase with temperature increase which indicates that the dried sugar kelp was becoming more yellow. These results indicate that the higher drying temperature and sugar kelp harvested later in the season (season two) led to blue deterioration more quickly. Individual factors of temperature and humidity, double and triple factors of temperature*humidity, temperature*season, and temperature*season*humidity had, all, significant effects.

Table 3.8.3.1 b* Values of Oven Dried Kelp at 25% and 50% Air Oven

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	16.69 ± 1.4	16.69 ± 1.4	18.86 ± 1.3	18.86 ± 1.3
30*	15.45 ± 0.9 bc	14.6 ± 1.1 bc	12.86 ± 0.4 bc	11.24 ± 0.6 bc
40*	16.32± 0.8 ab	15.50 ± 1.6 ab	14.47 ± 1.4 ab	13.16 ± 1.8 ab
50*	10.10 ± 0.6 c	12.60 ± 1.7 c	13.82 ± 0.4 c	15.35 ± 1.5 c
60*	15.13 ± 1.9 a	14.13 ± 0.4 a	16.32 ± 1.7 a	16.72 ± 2.2 a
70*	14.12 ± 1.9 ab	12.00 ± 0.3 ab	15.91 ± 1.1 ab	15.71 ± 0.0 ab

Humidities

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference

Capital letter: denotes significant difference in season 1 and season 2

3.9 *Vitamin C*

Vitamin C, a heat sensitive nutrient, showed a positive correlation among the dried sugar kelp with respect to the drying temperature (30°C to 70°C) indicating drying time have significant effect and it increases from 0.098 mg to 0.203 mg of ascorbic acid per gram of sample and 0.128mg to 0.211mg of ascorbic acid per g of sample as the drying temperature increases-- corresponding to drying humidity of 25% and 50%, respectively. Freeze dried sugar kelp was the highest value for both seasons and air oven humidities. Overall, vitamin C content was higher for season one --for both air oven humidities (25% and 50%)-- when compared to the vitamin C content for season two dried kelp.

Vitamin C showed significant differences between seasons and humidities. Also, freeze dried and 50ºC were both significantly different from all of the other dried kelp samples; while 60ºC (at both air oven humidities) was the only dried kelp sample that was significantly different from all of the others. Based on the statistical analysis, individual, double and triple factors of the dried sugar kelp vitamin C values (temperature*humidity, humidity*season, temperature*season and temperature*humidity*season) had all significant effects on vitamin C content.

Table 3.9.1 Vitamin C of Oven Dried Kelp at 25% and 50% Air Oven Humidities

Temperatures	Season 1		Season 2	
	Humidity			
	25%	50%	25%	50%
FD	0.216 ± 0.0	0.216 ± 0.0	0.206 ± 0.0	0.206 ± 0.0
30*	0.159 ± 0.0 cAx	0.133 ± 0.0 cAy	0.098 ± 0.0 aBx	0.128 ± 0.0 aBy
40*	0.193 ± 0.0 cAx	0.132 ± 0.0 cAy	0.116 ± 0.0 cBx	0.137 ± 0.0 cBy
50*	0.263 ± 0.0 abAx	0.203 ± 0.0 abAy	0.194 ± 0.0 abBx	0.158 ± 0.0 abBy
60*	0.200 ± 0.0 bAx	0.210 ± 0.0 bAy	0.200 ± 0.0 bB	0.173 ± 0.0 bBy
70*	0.223 ± 0.0 aAx	0.233 ± 0.0 aAy	0.203 ± 0.0 aB	0.211 ± 0.0 aBy

FD: Freeze Dried **Air-Oven Humidity Samples*

Small letter: denotes row-wise significant difference in MC with temperatures

Capital letter: denotes significant difference in MC between season 1 and season 2

x/y Denote significant difference between humidities

Table 3.10: Summary of Values

Analysis	Influence	Trend	Season
Moisture Content	depend on air oven humidity	decrease with increase in temp.	S2 higher
Ash Content	it was related with WHC and OHC (protein/carbohydrate ratio)	decreased with temp increase	S2 higher
WHC	cell wall polysaccharides	decrease with temp.	S2 higher
OHC	different proportions of polar side chains on the surfaces	decrease with temp.	S2 higher
Fat Content	growth cycle and seasonal variations	decrease with temp increase	S1 higher
Water Activity	free and bound water taken out during the drying process	decrease with temp increase	S2 higher
pH	heavy metals uptake increase pH	increase with temp increase	S2 higher
Color	increased lightness; increased yellowness and decreased redness.	L* & b* increased with temp. increased a* decrease as temp increase	S2 higher S1 lower
Vitamin C	degradation due to heating time/exposure in drying	increase with temp. increase	S2 lower

DISCUSSION

4.1 Seasonal Variation

In New England, brown algae contribute to a significant percentage of total ocean primary productivity, providing coexisting understory flora and fauna with habitat structure and relief from stressors such as desiccation and predation (Bertness et al. 1999). In the Gulf of Maine, nutrient supply is influenced by a combination of biotic and abiotic factors. The temperate climate of the region combined with the bathymetry of the gulf produces pronounced seasonal variation in many natural processes. For example, towards the end of winter, coastal surface water becomes very cold and dense, causing it to sink to the ocean floor, driving deep water to the surface (Townsend 1998). This deep water is replete with sediment-derived nutrients (Townsend 1998). This annual process, known as seasonal overturn, creates an influx of nutrients in late winter and early spring, fueling increased primary production (Townsend 1998). Additionally, weather-related fluctuations such as increased wave action due to storms can alter nutrient availability for seaweed in Maine.

Many algae, especially in temperate waters, display patterns of growth that are closely linked to nutrient availability (Chapman and Cragie 1977, Hanisak 1979, Gagné et al. 1982). However, in areas where solar radiation is significantly reduced in the winter, such as in Maine, light has also been shown to limit algal growth, despite ample nutrients (Chapman and Lindley 1980). In their study, Chapman and Cragie (1977),

indicate that when the light is limiting growth, seaweed is able to store excess nutrients in tissues for use later in the year, when light conditions are optimal but ambient nutrients become scarce (Chapman and Cragie 1977). This storage ability decouples growth from nutrient availability, making it difficult to assess algal nutrient limitation (Fujita et al. 1989). Sugar kelp (*Saccharina latissima*), for instance, is a cold temperate group of marine macroalgae, able to take advantage of the increased nitrogen availability and reduced competition in the colder months of the season (Chapman and Cragie 1977). For these reasons, the growing season for kelp is from fall to spring in Maine, with a season that typically runs from October to May (Chapman and Cragie 1977).

This could be an explanation for why the sugar kelp from season two, harvested in early June (considered late in the season), had less vitamin C content when compared to season one kelp. Earlier in the season (March), there is still a larger storage of nutrients as opposed to later in the season (i.e. June) when there is more light (compared to March) and when temperatures are still cold in Maine. Because of this, when internal nutrient reserves are depleted, sugar kelp may reduce growth in the summer when ambient nutrients are sparse and environmental conditions are stressful (Cubit, 1984).

Moreover, environmental factors including light intensity, temperature, and availability of nutrients such as nitrates and phosphates determine the reproduction and growth rates of sugar kelp, with the favorable seasons being winter and spring (Parke 1948). Seasonal variation of sugar kelp in ash, crude protein, mannitol, laminarin and alginic acid contents was first reported by Black (1950). The author also reported that laminarin, a key component of the polysaccharide fraction of this species, is missing in the stipe portion and present in the fronds for only part of the year (Black, 1950). It is

important to note that the usual harvesting season for S. latissima begins in late winter to early summer, with the winter crop usually covered with epiphytes as summer progresses, rendering it unfit for human consumption. Epiphytes are small plant growths on sea vegetables that do not cause any harm to the host but may affect its acceptability as human food tremendously. Schiener et al., (2015) also looked at the seasonal variation in chemical constituents of sugar kelp harvested in Maine (Schiener et al., 2015). This study showed how on average, moisture and ash content of dried sugar kelp amounted to 85% (wet basis) and 31% (wet basis), respectively (Schiener et al., 2015). The study also showed how in kelp species there was a higher metal ion content, with potassium, calcium, magnesium and sodium found (Schiener et al., 2015). In our experiments, the moisture content of the dried kelp was less than 21%. The ash contents of the dried kelp were between 22-35% for both of the seasons. The value of moisture content was expected to be low since the samples were dried and the study from above (Schiener et al 2015) utilized fresh sugar kelp. On the other hand, the value for ash content of dried kelp are also closed to the values from the study mentioned.

4.2 Drying

Sugar kelp (*Saccharina latissima*) is a marine macroalgae and is a rich source of fibers, vitamins, minerals and antioxidants. Due to the high amount of moisture (~92%), it is highly susceptible to microbial attack and enzymatic deterioration and is either conventionally sun dried or hot air dried for extending its shelf life. Sun drying is one of the oldest techniques for food preservation, is very slow, and requires clear weather conditions which makes it an unreliable technique for Maine seaweed farmers.

Drying is a phase change process governed by simultaneous heat and mass transfer (Mujumdar, 2000). It removes free water and makes food less susceptible to microbial attack, lipid oxidation and enzymatic browning (Argyropoulos et al., 2011; Kurozawa et al., 2012; Zhang et al., 2006). The drying rate is highly dependent on several process parameters including drying temperature, moisture diffusion coefficient, the difference in partial pressure of water vapor in food and the surroundings, material thickness, surface area and phase transition (from glassy to a rubbery state) (Lewicki and Jakubczyk, 2004; Van Arsdel, 1973). According to FAO (1976), seaweeds that are dried properly can be stored for a number of years without appreciable loss of their gel content. The drying technique could be a factor that affects physico-chemical properties of the sugar kelp.

On the other hand, freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase (Lewicki and Jakubczyk, 2004; Van Arsdel, 1973). In addition, freeze-dried preserve by rapidly freezing it and then subjecting it to a high vacuum that removes ice by sublimation which causes the product to be stored for many years without spoilage (Lewicki and Jakubczyk, 2004; Van Arsdel, 1973). Preservation is possible because the greatly reduced water content inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance. Freeze-drying also causes less damage to the substance than other dehydration methods using higher temperatures. Freeze-drying does not usually cause shrinkage or toughening of the material being dried. In addition, flavors and nutritional content generally remain unchanged, making the process popular for preserving food. However, water is not the

only chemical capable of sublimation, and the loss of other volatile compounds such as acetic acid (vinegar) and alcohols can yield undesirable results. Moreover, due to longer drying time and energy consumption, the use is limited for high value-products.

Shelf-life of a food product depends on a number of intrinsic and extrinsic properties of the processed or stored product such as: water activity (available moisture), pH, available oxygen and nutrients, redox potential and glass transition temperature (Buera et al., 2011 and IFST 1993) and storage conditions such as temperature and relative humidity (Badii et al., 2014; Gonda et al., 2012). The temperature and humidity were evaluated as a two-way factor that could have affected many properties of the sugar kelp. Understanding these properties is important for retaining product quality and improving shelf life through optimized post-harvest processing.

4.3 Psychochemical Analysis

4.3.1 Moisture Content

In algae samples of the same species, the morphological and structural differences of the tissues, as well as age, size, collection site or seasonality, affect the total water and moisture content. The methods most commonly employed with these types of samples are drying methods (i.e. desiccation by water transfer and oven drying). In the oven-drying procedure, used in this study, the samples are heated to high temperatures (105 ºC) in an oven for a period of approximately 48 hours. This process is what gave the moisture content for the dried sugar kelp of our experiments (which was low). However, when it comes to brown algae, such as sugar kelp, this temperature range may cause the

decomposition of some components such as lipids, amino acids and carbohydrates, and the volatile substances formation and water, resulting in samples unsuitable for other analyses of interest. Although the determination of moisture content is one of the most commonly performed analyses on foodstuffs, it is not an easy task since foods are very complex matrices usually composed of a mixture of polar (proteins and carbohydrates) and apolar substances (lipids), thus requiring great care in the preparation of samples for analysis.

Moisture content performed in the dried sugar kelp revealed that all the drying curves showed a clear exponential tendency, and an increase in the temperature accelerated the drying process and lowered the moisture content. Overall, the lower drying temperature and relative air oven humidity increases the moisture content of dried sugar kelp and causes a slow-down of the drying time. In contrast to the higher drying temperatures and low relative air oven humidity, the moisture content will be rapidly reduced as shown in the results of the experiment (Tables 3.1.1) in which the higher temperature and lower humidity resulted in the samples with the least moisture content. Results also revealed that freeze dried kelp had the least moisture content (less than 3%). This is expected since freeze dried kelp does not depend on humidity while the oven-dried kelp does depend on the humidity in which it was dried.

4.3.2 Ash Content

Marine macroalgae absorb minerals and other nutrients from their surroundings. The presence of a cell wall filled with a polysaccharide matrix enables them to store this macro and microelements (Davis et al., 2003). The chemical composition of these walls

has a huge effect on absorption of these elements, resulting in varying amounts of minerals within sea vegetables of the same genus (Davis et al., 2003, Mišurcová et al., 2011). Sea vegetables, such as seaweed, have a greater ability to absorb rare earth elements in comparison to their terrestrial counterparts (Mišurcová et al., 2011). Various other factors such as physiological stress, pH, the salinity of water and other environmental changes have also been reported to influence mineral deposition in sea vegetables (Rao et al., 2007, Kumar et al., 2008, Mišurcová et al., 2011, Baghel et al., 2014, Astorga-España et al., 2015) and thus their ash content. Seaweeds are considered high in minerals and trace elements because sulfate and nitrate compounds are present in seaweeds.

Ash is the inorganic residue remaining after the water and organic matter has been removed by heating, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals can be distinguished from all the other components (the matrix) within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components.

In our study, the ash contents of the dried kelp were all similar. The sugar kelp had ash contents that ranged from 22-35% for both seasons (season 1 and 2) and air oven humidities (25 and 50%). This is consistent with the previous studies (see section 4.1). For both air oven humidities, season two ash content had, surprisingly, higher values that ranged from 22-35%; while for season one kelp the values ranged from 25-28%. In general, high level of ash was associated with a high number of mineral elements.

Therefore, season two kelp is suspected to have a higher amount of mineral elements than season one kelp. Moreover, previous studies reported that ash content of seaweed varies between 8% and 40% d.b (Mabeau and Fleurence, 1993) which is very similar to the values of our study. At the same time, the mean percentage of ash found in our study was comparable to those reported in other species i.e., Hypnea japonica (22.10% d.b). Several other studies showed that the variation in ash content depends on seaweed species, geographical origins and their method of mineralization (Nisizawa, 1987; Sanchez-Machado, 2004). Aji N, et al., (2003) reported there was an increased in ash content as well as increasing sulfate content. A reason for the difference in ash content between seasons could also be explained by the fact that there could have been a higher sulfate content and minerals in the water which the sugar kelp absorbed.

Also, the fluctuation of the levels of lipids (see 3.7 fat content section) and ash from March to July (Season 1 and 2) may be attributed to the seaweed growth's cycle. The sugar kelp growth cycle starts during the winter when sporulation occurs (Chapman, 1987). At this period, protein and lipid are synthesized, the level of ash increases as the winter progresses, and the content of laminarin decreases (Black and Dewar, 1949). During the winter and spring, a decrease in laminarin causes an increase in nutritive salts which results in a better growth and more protein and ash content. With the decline of nutritive salt in the water, the growth is reduced and laminarin accumulates in the fronds (Chapman and Craigie, 1977 and Chapman and Craigie, 1978). When the maximum laminarin level is attained in the fronds, the proportions of alginate, cellulose, and protein are minimal (Percival and McDowell, 1967) as well as the level of ash (Haug and Jensen, 1956). However, in our research, contrary to the previous research findings, the levels of

ash were higher for season two when it was expected to be lower because of the decrease nutritive salts and increase laminarin composition of the sugar kelp. However, more information on the laminarin and nutritive salts of the two seasons is needed in order to determine if those factors affected the ash composition of our study. Similarly, previous studies have seasonal differences in the iron content of seaweed species (Ulva spp., Sargassum spp., Porphyra spp., and Gracilariopsis spp.) in which there was a distinct seasonal cycle whereby iron content was highest in spring and summer and lowest in fall and winter (Garcia-Casal et al. (2007, 2009))

Moreover, when ash content values of season one kelp are compared, the values for 25% air oven humidity are higher for season one (when compared to 50% air oven humidity for the same season) and 50% humidity are higher (+2% d.b) for season two (when compared to 25% air oven humidity for the same season). However, statistical analysis on the ash content revealed that humidity did not have a significant effect. Air Oven Humidity was expected not to have a significant effect because the humidity of a sample does not affect or degrade the ash content as drying conditions do (some minerals are affected and degraded with drying).

On the other hand, in Wong et al. (2009) study, ash contents of freeze-dried *S. hemiphyllum and S patens* were significantly ($p < 0.05$) lower than those of the oven-dried samples. This is consistent with our findings for season two kelp in which the ash content for freeze dried was the lowest value (24.78%). However, for season one freeze dried kelp was among the highest in ash content (26.96%). Overall, the ash content for oven-dried temperatures seemed to be decreasing with an increase of temperature. This is consistent with other studies that revealed a loss of volatile minerals at high temperatures,

e.g., Cu, Fe, Pb, Hg, Ni, Zn (Kratochvil et'al., 1998). Sugar kelp contains Cu, Zn, and Fe in trace amounts (Kratochvil et'al., 1998).

4.3.3 Water Holding Capacity (WHC)

Water exists in fiber in three forms: it is bound to the hydrophilic polysaccharides; it is held within the fiber matrix or it is trapped within the cell wall lumen. WHC, determined by the centrifugation method used in this study, represented all three types of water associated with the fiber (Fleury et al., 1991). Apart from different water holding ability in fiber, the differences in WHC among the dried sugar kelp might be attributed to the different protein conformations and the variations in the number and nature of the water binding sites on the protein molecules. In addition to chemical compositions, some physical properties, such as structure, particle size, porosity, pH, temperature, ionic strength, types of ions in solutions and density were important to the understanding of the different behaviors of samples during hydration (Fleury N. et al., 1991).

In this study, WHC of dried kelp ranged from 857% to 2079% (8.57g and 20.79g of water per g of dried kelp (Figure 3.3.1 and 3.3.2). Furthermore, WHC of the oven-dried and freeze-dried kelp was much higher than comparable studies of the WHC of seaweeds. Lahaye et al. (1993) reported a WHC for the U. Lactuca alga at 25 °C of 7.5 g/g DW which is very close to the value of WHC for the sugar kelp dried at 30ºC 25% air oven humidity. Also, Wong et al. (2000) found that the WHC for the U. Lactuca seaweed at 37 °C was higher (9.71 g/g DW) than 40 °C, but also comparable to that of some

agricultural by-products (dietary fiber concentrates) (6.30–13.2 g/g DW) reported previously (Grigelmo-Miguel and Martin-Belloso, 1999).

A slight increase in the water holding capacity of sugar kelp harvested in season two was noticed. Such increase was probably related to the increase in the solubility of fibers and proteins (Fleury et al., 1991). It is well known that seaweeds are rich in dietary fiber (>50% d.w) and particularly in the soluble form (Darcy-Vrillon, 1993; Mabeau & Fleurence, 1993). Fleury and Lahaye (1991) reported that the physicochemical properties of seaweed powder could be assumed to reflect those of the dietary fiber present. Besides, since seaweed proteins are closely associated to the cell wall polysaccharides (Fleurence et al., 1995; Jordan and Vilter, 1991), seaweed proteins may also play a role in the physicochemical properties such as water holding (Chou and Morr, 1979).

In the present study, WHC values of dried sugar kelp decreased when dried at 25% air oven humidity when compared to 50% air oven humidity. This is expected since in 25% air oven humidity the water is removed at a much faster rate which collapses the structure more than 50% air oven humidity. The collapsing of the structure then decreases the WHC of the sugar kelp. The significantly higher WHC values of the freeze-dried for the sugar kelp in both air oven humidity in season one indicates that freeze-dried and 70ºC 25% air oven humidity for season one, and 30ºC 25% air oven humidity and 50% air oven humidity for season two would be more suitable for being as texturizing and bulking agents in making low calories food products because of their high WHC values.

Moreover, the high WHC of season one freeze-dried sugar kelp might be mainly related to the degree of damage in the seaweed cell wall polysaccharides. The degree of

damage to the cell wall would be greater in the oven-dried seaweed than that in the freeze-dried samples. This would result in a lower water holding ability of fibers in oven-dried seaweed samples, especially in a decrease of trapping water in the cell wall lumen. Although the cell wall bound protein in the oven-dried seaweeds might be more easily dissolved into the water to improve water holding, the effect of fibers on WHC would likely to be greater than that of the protein (Chou and Morr, 1979). Besides, the high temperature, oven-drying may cause the seaweed protein to denature, thus changing the protein conformations as well as the number and nature of the water binding sites on the protein molecules, resulting in lower WHC (Chou and Morr, 1979). Interestingly, the contrary happened with season two kelp in which freeze-dried had the lowest value for both air oven humidities, which would lead to think that for that there was more damage and denaturalization in that particular condition.

Furthermore, the WHC values, overall, suggested that oven and freeze-dried sugar kelp could be potentially used as a functional ingredient to reduce calories, avoid syneresis and modify the viscosity and texture of formulated food because the ranges in WHC were comparable to those used in the industry for those same uses (Chou and Morr, 1979).

4.3.4 Oil Holding Capacity

OHC is another functional property of food ingredients used in formulated food. Ingredients with a high OHC allow the stabilization of food emulsions and high-fat food products. The mechanism of OHC is mainly due to the physical entrapment of oil by capillary attraction (Kinsella, 1976). Moreover, the hydrophobicity of proteins also plays a major role in fat absorption (Voutsinas and Nakai, 1983) in sugar kelp. Therefore,

among the dried sugar kelp, the variations in OHC may be partially due to the different proportions of polar side chains of the amino acids on the surfaces of their protein molecules (Chau and Cheung, 1998). Furthermore, Fleury and Lahaye (1991) reported that the OHC of seaweed is also related to the particle size, overall charge density and hydrophilic nature of the individual particles. Similarly, the correlation between OHC and the total amount of protein has been studied in the past and has shown a very high (r=1.00). This implied that the OHC of seaweed might also depend on the total protein content (Grigelmo-Miguel et al., 1999).

In this experiment, the OHC of the sugar kelp samples ranged from 75% to 253% (0.75 and 2.53 g of oil per 1 g of dried seaweed). Moreover, among the sugar kelp samples analyzed, OHC values for season two were higher—for both 25% and 50% air oven humidities. This is consistent with the findings of WHC and ash content-- where the values for the dried kelp in season two were also higher. These could be because of the polysaccharide: protein ratio. However, no experiment on nitrogen content was performed in this study, Therefore, we can't conclude which season had the highest protein and the highest polysaccharide.

Surprisingly, decrease in the OHC with increasing temperature leads us to believe that highest temperatures affect the hydrophilic nature of the individual particles. In this study, the OHC of all freeze-dried kelp was higher than that of the oven-dried samples for season one and at 25% air oven humidity, while for season two and 50% air oven humidity the values for freeze-dried were among the lowest.

In this study, the significant differences on OHC between oven and freeze-dried sugar kelp would also be due to the damage in cell wall polysaccharides as well as the

denaturation of cell wall proteins as mentioned above. Studies have reported that three Sargassum species of freeze-dried seaweed have the exceptionally high OHC value (0.84g/g d.w) (Grigelmo-Miguel et al., 1999). However, OHC results for our experiment revealed that values were substantially higher for almost all of the samples (0.75g and 2.53g of oil per g of dried seaweed).

4.3.5 Fat content

Macroalgae are known for their low lipid content, making them appealing to certain health-conscious consumers. In general, the lipid fraction can be anywhere between 1- 3% of dry matter (Mabeau and Fleurence 1993, Bocanegra and 2009). One of the studies evaluating nutritional composition of nori (P. purpurea) and wakame (U. pinnatifida) found 1% and 2.7% (dwb) lipid content, respectively, falling within the previously reported range for sea vegetables (Taboada and others 2013). This is consistent with our findings in which the fat content of the sugar kelp samples was relatively low (0.62- 4.6%) but within the range (1.00±3.00% DW), though slightly higher than the range (1–3% d.w) for brown seaweeds reported by Mabeau and Fleurence (1993).

In general, for the first and second season, the fat content seemed to be slightly decreasing with drying temperature. Well M.L et'al (2016) in his research found that seaweed contains a low amount of fat content which consist of long-chain polyunsaturated fatty acids (PUFAs) and carotenoids (Well M.L et'al 2016). Moreover, the degree of unsaturation in fatty acids has usually a negative relationship with temperature as reported in the past (Olofsson M., et'al, 2012). Olofsson M., et'al also

reports that hydroperoxides of PUFAs are easily decomposed into a very complex mixture of secondary products with the decrease in unsaturation. His study on microalgae also revealed that temperature affects the fatty acid composition where at higher temperature, PUFAs in various microalgae decreased, while SAFAs increased probably due to the fluidity of cell membranes. Most reports including the present study show a tendency for microalgae to form a greater deal of PUFAs in response to low temperature (Olofsson M., et'al, 2012).

When compared values for dried kelp on first season were significantly higher than the second season for both air oven humidities. In Laurie-Eve, et.al study (2009), the brown seaweed composition (protein, lipid, and ash) was determined for each harvest period: May 2005 (M05), August 2005 (A05), November 2005 (N05) and June 2006 (J06). Seasonal trends in that study were observed with respect to the concentration of proteins, lipids, and ashes: higher amounts of protein (12.2%), lipid (0.8%) and ash (26.3%) were found in M05, whereas the amounts of each component decreased, respectively, by 5.0%, 0.3% and 22.6% by the end of the summer (A05).

For our study, the fluctuation of the levels of lipids from March to July (seasons one and two) may be attributed to the seaweed growth cycle. The sugar kelp growth cycle starts during the winter when sporulation occurs (Chapman, 1987). At this period, protein and lipid are synthesized, the level of ash increases as the summer progresses (after July), the content of laminarin decreases (Black and Dewar, 1949). With the decline of nutritive salt in the water, the growth is reduced and laminarin accumulates in the fronds (Chapman and Craigie, 1977 and Chapman and Craigie, 1978). When the maximum laminarin level is attained in the fronds, the proportions of alginate, cellulose, and protein

are minimal (Percival and McDowell, 1967) as well as the level of ash (Haug and Jensen, 1956). This is consistent with the findings in our study where the fat content was higher for season one when lipids are synthesized.

4.3.6 pH

The acidity of foods has been used for centuries to preserve foods. Acidity plays a primary role in the preservation of fermented foods and combined with other factors such as heat, water activity, and chemical preservatives act to prevent food deterioration and spoilage. The intensity of acidity of a food is expressed by its pH value. The pH of a food is one of the several important factors that determine the survival and growth of microorganisms during processing, storage, and distribution. pH stands for the power of hydrogen, which is a measurement of the hydrogen ion concentration in the body. The total pH scale ranges from 1 to 14, with 7 considered to be neutral. A pH less than 7 is said to be acidic and solutions with a pH greater than 7 are basic or alkaline.

Since the early studies of the biosorption phenomenon, it has been known that the uptakes of heavy-metal cations by most biomass types decreases dramatically as the pH of the metal solutions decreases from pH 6 to 2.5. Because most of the heavy metals precipitate at pH 5.5, it is thought that, at higher pH values, the metals might accumulate inside the cells and/or the cell walls by a combined sorption–microprecipitation (Kratochvil et'al., 1998).

Because heavy metals decrease pH, it is expected that season two which had the highest ash content will also have the lowest pH when compared to season one. Results in showed the values of pH for the dried sugar kelp. With respect to 25% air oven humidity,

season one had ranged from 4.9-5.9, while season two had higher pH with a range of 6.1-6.07. This would lead us to think that there is a higher uptake of heavy metals during season two --which is consistent with the increase of ash content values from season one to season two.

Freeze-dried was the lowest of all samples in both humidities and seasons because you are not exposing the sugar kelp to high temperatures. In case of 25% air oven humidity, the exposure time at particular temperature is less; 50% air oven humidity the exposure time is longer at particular temperature. Because of this, is suspected that the exposure time is degrading acidic compounds in 50% air oven humidity kelp. Acidic compounds that could be degrading are alginic acid which is an anionic polysaccharide distributed widely in the cell walls of brown algae, where through binding with water it forms a viscous gum (ChEBI, 2010)

4.3.7 Water Activity

The water activity (a_w) of a food is the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions. A water activity of 0.80 means the vapor pressure is 80% of that of pure water. The water activity (a_w) is determined by measuring the lowering of the freezing or fusion point of the water (cryoscopic depression), which is a colligative property and, as such, depends exclusively on the number of particles present in the solvent, and not on the nature of those particles The a_w is an intrinsic property of the sample, while the moisture depends on the atmosphere in equilibrium with it; however, it should be noted that most of the methods

for determining a_w depend on the moisture content. The most common methods for determining a_w involve measures based on different principles: psychrometry, cryoscopy, dew-point hygrometry and isopiestic equilibration. In this study, water activity was measured with a water activity meter that employed a simple and precise method to determine the a_w from the samples thermogravimetry. The results of these analyses provide a good biomass indicator to estimate the quantity of sample to be collected as a function of the mass required for a given analysis and to estimate the stability and microbiological safety of the algae.

Water activity (a_w) has its most useful application in predicting the growth of bacteria, yeasts, and molds. For a food to have a useful shelf life without relying on refrigerated storage, it is necessary to control either its acidity level (pH) or the level of water activity (a_w) or a suitable combination of the two. This can effectively increase the product's stability and make it possible to predict its shelf life under known ambient storage conditions. Oven and freeze drying decreases the water activity which eventually retards microbial growth, helps conserve the desirable qualities and reduces the storage volume (Gupta et al. 2011). The results of our study revealed that the sugar kelp samples had a low water activity (below aw 0.66). It is known that water activity below 0.6 will not support the growth of osmophilic yeasts and other pathogens. Therefore, the dried sugar kelp samples for both air oven humidities, seasons and temperatures are within the range of water activity that does not support pathogen growth.

Moreover, 50% air oven humidity resulted in an increase of water activity when compared to 25% air oven humidity. These results are expected: humidity increases because of the higher equilibrium in the moisture content of the sugar kelp. As we have

observed, the WHC of season two kelp were higher as compared to season one kelp. Therefore, this suggest that season two dried kelp require a longer drying time. This might be due to the available water or free water in season two dried kelp are higher. For removing the excess amount of water, more energy or more time is needed.

4.3.8 Color

The color is an important quality attribute in the food and bioprocess industries, and it influences consumer's choice and preferences. Food color is governed by the chemical, biochemical, microbial and physical changes which occur during growth, maturation, postharvest handling, and processing. Color measurement of food products has been used as an indirect measure of other quality attributes such as flavor and contents of pigments because it is simpler, faster and correlates well with other physico-chemical properties (Pathare et'al. 2012). A colorimeter works by shining a light onto the sample and measuring how many photons of light leave; this figure is then compared to a standard control amount. A known reagent is introduced to the sample and the color that develops as a result of the reagent is used to determine the sample's transmission of light.

Moreover, during drying conditions, such as the ones that the sugar kelp was exposed to in our experiments, the solid material can undergo several processes that modify the physical (rehydration, color loss), chemical (browning reaction, lipid oxidation) and also nutritional (vitamin and protein loss) properties (Bonazzi and Dumoulin 2011). Particularly, color is the main attribute with respect to the quality of dried materials and can change during drying due to chemical and biochemical reactions. Consequently, color characteristics, as a measure of the processes promoted during drying, could be related to the properties of the extracts. Some researchers have studied

the convective air drying effect on the antioxidant activity of different marine algae species (Tello-Ireland et al. 2011; Jiménez-Escrig et al. 2001; Kuda et al. 2005a; Kuda et al. 2005b; Le Lann et al. 2008) but no studies on sugar kelp were found. The color differences we found may be characteristics of dried sugar kelp or be representative of their chemical composition.

Sugar kelp samples were exhibited in all cases red ($a^* > 0$) and yellowness ($b^* > 0$) predominance. Overall, the L*, a*, b* color values provided crucial information on how the color quality deteriorated over time. The fading, which was likely due to loss of pigments such as fucoxanthin and chlorophyll c, was captured by increased lightness and yellowness and decreased redness values. Then, based on the results of season two dried sugar kelp (L*, b*values increased, and a* decreased) there was more chlorophyll present in those samples as opposed to season one.

4.3.8.1: Brightness/Darkness (L Value)*

Colorimetric L* values are used to measure lightness and range from 0 to 100, where 0 is black and 100 is white. Interestingly, time and temperature significantly increased L* values for season two, with increased fading for samples stored at a higher temperature over time; increased the L* values, indicating fading over time. Regarding color properties of size fractions, increase L* and b* decreased significantly for all systems as temperature increased. Both trends might be related to the presence of still structurally undamaged parts of the alga in the biggest particles.

4.3.8.2 Redness/Green (a Value)*

Regarding parameter a*, values indicate the scale from green (-) to red (+) of the colorimeter. Overall, the a* values seemed to decrease for season one dried kelp as temperature increased; indicating that the dried kelp was fading with respect to the red color. Season two, which had lowest values, indicates that higher temperature accelerated the loss of color in those samples. This could be explained by the process of drying/dehydration. During dehydration, the tonoplast, the plasmalemma, and the chloroplast membrane may suffer structural damage, and as result, a solute loss of chlorophyll and carotenoids, among others components, can occur (Burritt et al. 2002; Oliver et al. 1998). This damage of cell integrity could be related to a loss of antioxidant capacity due to membrane damage which could be enhanced by an increased reactive oxygen species (ROS) production induced by stress conditions (Burritt et al. 2002). Fucoxanthin is an important component of brown algae coloration, and in raw seaweed, it covers the pigmentation of chlorophyll. However, the chlorophyll leaching during drying may expose its color, and consequently, the parameter a* drastically decreases. During drying at higher temperatures (>60 °C), the released chlorophyll undergoes degradation reactions (Drążkiewicz & Krupa 1991). Chlorophylls are easily degraded in the presence of dilute acids, heat, light and oxygen. Along with degradation produced by external agents, chlorophyll is also degraded by chlorophyllase enzyme (Erge et al. 2008). Degradation of the chlorophyll is manifested as yellowing, as it allows the preponderance of carotenoid coloration (Drążkiewicz and Krupa 1991). With respect to freeze dried, its value was the lowest for both season and air oven humidities, thus it has a higher chlorophyll value.

4.3.8.3: Yellow/Blue (b Values)*

b* values measure yellowness of the samples on the scale from blue (-) to yellow (+). Colorimeter b* values results show that there was an increase in temperatures for both air oven humidities and seasons; indicating an increase in yellowness. Interestingly, the yellowness increased for dried sugar kelp harvested in season two as values for b* increased (when compared to season one dried kelp). Similarly, freeze-dried had the highest values for all seasons and air oven humidities: it was the sample with the bluest discoloration. These results may be linked to the reactions of carotenoids or other pigments, which could result in their degradation, or in the formation of alternative colored substances or volatile compounds (Landrum, 2009).

These findings (b* values) were similar to a* values results in that season two had lower values than season one, and it increased values with temperature increase. This indicates that the higher drying temperature and dried sugar kelp harvested later in the season led to color deterioration more quickly. Therefore, an increased temperature affects the chlorophyll content which makes the sample darker.

4.3.9 Vitamin C

Sea vegetables are also well recognized for their vitamin content. They contain water soluble vitamins such as vitamin C, (Mabeau and Fleurence 1993, MacArtain and others 2007, Miyamoto and others 2009) (MacArtain and others 2007, Mouritsen and others 2013b) which plays an antioxidants role and may be present due to exposure to physiological and/or environmental stress (MacArtain and others 2007). Selected sea vegetables are considered good sources of vitamin C.

The vitamin C content, in this study, of dried sugar kelp were among 0.098 mg ascorbic acid per gram of sample to 0.203 mg ascorbic acid per gram of sample and 0.128 mg ascorbic acid per gram of sample to 0.211 mg ascorbic acid per gram of sample as for 25% and 50% air oven humidity, respectively. McDermid and others (2003) reported that no vitamin C was detected in the two brown sea vegetables they assessed. However, MacArtin and others (2007) reported vitamin C content of Laminaria app. to be 35 g/100g fresh weight, which is higher but closer to the vitamin C content of the dried sugar kelp found in our study. Moreover, the differences in the vitamin C content, among our samples, could be due to differences in the harvest season, location, and species.

As reported by Katsube, higher drying temperatures (50-80°C) induces faster drying rate, but also leads to reduction in heat sensitive nutrients including vitamin C, antioxidants, phytochemicals, total flavonoid content and total phenolic content (Katsube et al., 2009; Sablani et al., 2011; Shi et al., 1999; Yang et al., 2010) and alterations in textural quality due to case hardening, undesirable color change and predominantly material shrinkage (Kurozawa et al., 2012; Russo et al., 2012). Removal of free water attached to the solid matrix of food creates void space and stress at the cellular level, leading to material shrinkage. Because of this, it was expected that higher drying temperatures would result in the most vitamin C lost in the study. However, the higher the temperature the higher the vitamin C; it increases from (0.098 mg to 0.203 mg) and (0.128 mg to 0.211 mg) as the drying temperature increases. This could be explained by the fact that higher temperature results in less time drying which might have caused less vitamin C lost --it is important to note that vitamin C is a heat sensitive nutrient.

In addition, the differences mentioned below, between seasons, can also be explained by environmental factors. For instance, there are notable variations in the levels of β-carotene and vitamin C between samples of Ulva fasciata collected from different sites in a study by McDemid and Stuerke (McDermid and Stuercke 2003). Similarly, studies have revealed that levels of Vitamin C were lowest in the summer months (June, July, August) and reached the highest concentrations in April/September (Hernandez-Carmona et al. 2009).

Some reports suggested that freeze drying is the most appropriate drying technique in retaining the nutritional composition (Chan et al. 1997) and anti-inflammatory activities of polysaccharides fraction (Hammed et al. 2013) of dried seaweeds. Another similar study revealed that freeze dried and oven dried samples of Sargassum hemiphyllum yielded substantially different vitamin C contents (Chan et al. 1997). This study confirmed those assumptions: as the freeze dried sugar kelp had the highest vitamin C content for both seasons and air oven humidities.

CONCLUSION

The effect oven-drying and freeze-drying methods have on the nutritional composition of the sugar kelp (*Saccharina Latissima)* was investigated. Nutrient compositions and physicochemical properties (moisture content, ash content, WHC, OHC, fat content, pH, water activity, color analysis and vitamin C content) of the dried sugar kelp samples were determined. The results indicated that moisture content was depended on air oven humidity and decreased with an increase of temperature. Ash content results indicated that season two dried kelp had higher values and that it was related with WHC and OHC --which also had high values for season two. Moreover, since sugar kelp proteins are closely related to the cell wall polysaccharides (Fleurence, 1999), they may also play a role in the physicochemical properties, such as WHC. On the other hand, OHC increased with temperature for both seasons and humidities and also revealed that it was higher for season two. This was attributed to the different proportions of polar side chains of the amino acids on the surfaces of their protein molecules (Chau & Cheung, 1998).

Season one had a higher fat content and it seemed to be decreasing with temperature; which could be attributed to seaweed growth cycle variations. Moreover, significant variations in the amounts of fat and ash observed between the seasons could be attributed to the level of nutritive salts, the growth cycle, and the frond age. Other factors, which could not be verified, such as sea current, waves, the thickness of ice

cover, and intertidal environment could also have an influence on the seaweed constituents. Each factor could impact either individually or simultaneously the growth, and thus of the accumulation of polysaccharides, proteins, lipids and ash.

On the other hand, water activity was below 0.66 for all samples which were expected due to the free and bound water taken out during the drying process. Because heavy metals decrease pH, it is expected that season two which had the highest ash content will also have the lowest pH when compared to season one. At the same time, the color analysis revealed that L* and b* values increased as temperatures increased while a values decreased. This indicated an increased lightness in the dried sugar kelp: increased yellowness and loss of redness.

Freeze-dried sugar kelp had the highest content of vitamin C when compared with oven-dried seaweed. However, freeze-dried sugar kelp seaweed has the lowest values of ash content and WHC. Although oven-dried sugar kelp had the greatest nutrient losses, probably due mainly to the effect of high temperature during drying, it contained the highest ash content.

The faster drying rate in the oven-drying preserved the ash content, but the use of high temperature during drying caused greater nutrient loss and lower physicochemical properties (WHC, OHC) than those of freeze-drying. One of the goals of this study was to find an oven-dried temperature that was comparable to freeze dried and retained the same favorable qualities. We can conclude that temperatures 40ºC and 60ºC oven-dried are both comparable to freeze dried in terms of vitamin C content, WHC, OHC, ash content and fat content (See Table 5.1). For further conclusions, about these two oven

temperatures, energy analysis would need to be conducted to decide which one is the most energy efficient.

Thus, it can be concluded that the nutritional composition of sugar kelp is greatly affected by different drying methods. Moreover, the equipment and operation cost for freeze-drying are higher and its drying capacity is much lower than that of oven-drying--hence one of the oven-dried temperatures, 40ºC or 60ºC, should be used. However, in choosing the most appropriate drying method for sugar kelp, one needs to consider the economic factors and the way that the seaweeds will eventually be used as independent factors.

Moreover, drying is an important step in seaweed production (semi-dried seaweed product), but it can negatively impact the phytochemical constituents in the seaweed. Thus, new research into protecting phytochemical components of sugar kelp upon processing would be needed. As a robust natural source of important bioactive compounds, having knowledge of the optimum post-harvest drying treatment for sugar kelp (*Saccharina latissima*) would be commercially advantageous.

Table 5.1: Freeze Dried and Oven-dried Kelp Comparison

Analysis	Significant Different to FD	Not Significant Different	Highest and Lowest Value	Comparable Temperature
Moisture Content	All Temp.	n/a	Highest 30℃ Lowest 70℃	N/A Everything is below 15%
Ash Content	All	---	Highest: 30℃ Lowest: 60℃	Comparable: 40-60℃ (freeze dried among the lowest for ash content)
WHC	30, 40, 70℃	50 and 60℃	Highest: 70℃ Lowest: 50℃	Comparable/Best: 60℃
OHC	30℃, 60℃	40℃, 50℃, 70℃	60℃: highest 50℃: lowest	FD: among highest Comparable/Best: 70°
Fat Content	All	n/a	60℃: among lowest 30,40, 70℃: among highest	FD: lowest Comparable/Best: 60℃
Water Activity	30, 40, 50, 70℃	60℃	Highest: 30℃ Lowest: 40℃	FD: among lowest Comparable: 60℃ (low) Best: 40℃
pH	40-60℃	30, 70℃	Highest: 60℃ Lowest: 70℃	FD: lowest Comparable/best: 70℃
Color Values L* a* b*	 All All 30-50℃	 n/a n/a 60, 70 ℃	 Highest:40℃ Lowest: 30℃ Highest:60℃ Lowest: 40℃ Highest:70℃ Lowest: 30℃	 Comparable/best: 40, 60 ℃ Comparable/best: 60-70℃ Comparable/best: 60, 70 ℃
Vitamin C	30, 40m 60, 70 ℃	50 ℃	High: 70℃ Lowest: 30℃	Comparable/best: 60, 70 ℃

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APPENDIX

7.1 *Moisture Content*

Table 7.1.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for Moisture Content Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	126.3839921	31.5959980	116.97	<.0001
HUMIDITY	1	634.1855748	634.1855748	2347.77	<.0001
SEASON	1	16.0984040	16.0984040	59.60	<.0001
TEMPERATURE*HUMIDITY	4	61.6152661	15.4038165	57.03	<.0001
TEMPERATURE*SEASON	4	15.0278566	3.7569641	13.91	<.0001
HUMIDITY*SEASON	1	27.8951654	27.8951654	103.27	<.0001
TEMPER*HUMIDI*SEASON	4	88.7599726	22.1899931	82.15	<.0001

Figure 7.1.1: Moisture Content (%) of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

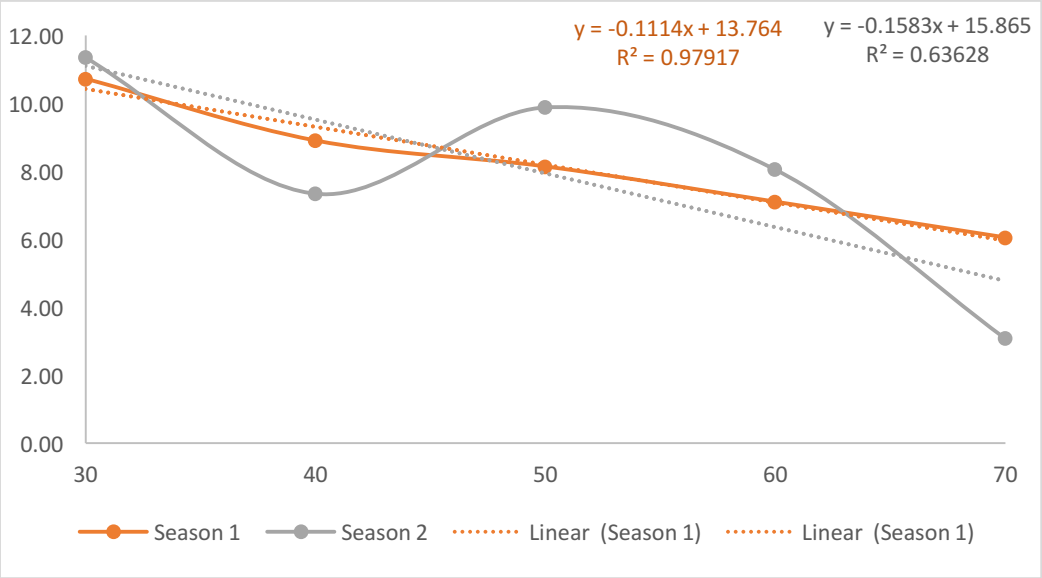
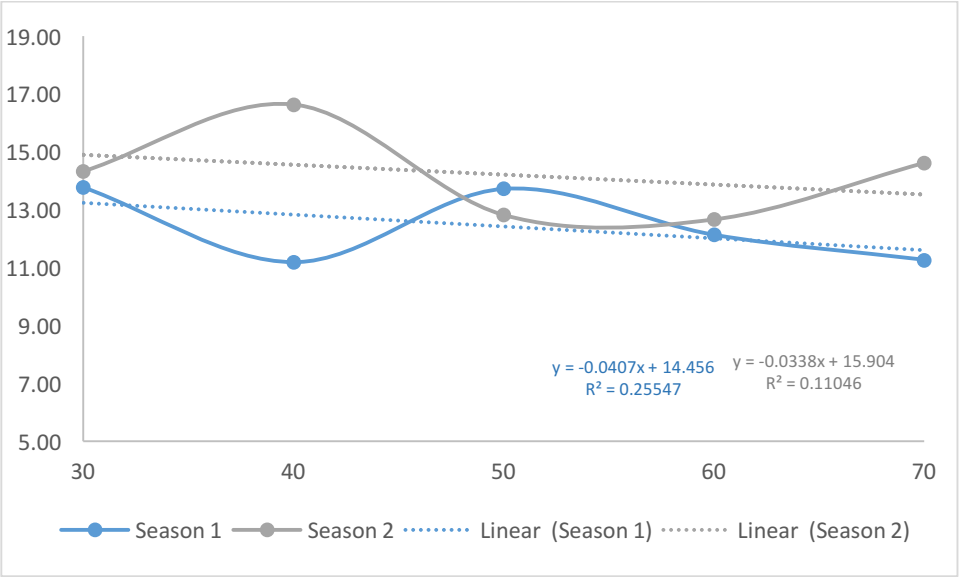


Figure 7.1.2: Moisture Content (%) of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.2 *Ash Content*

Table 7.2.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for Ash Content Oven Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	60.9037227	15.2259307	1.23	0.3149
HUMIDITY	1	0.4105228	0.4105228	0.03	0.8566
SEASON	1	475.1439004	475.1439004	38.28	<.0001
TEMPERATURE*HUMIDITY	4	121.0267236	30.2566809	2.44	0.0627
TEMPERATURE*SEASON	4	24.9701893	6.2425473	0.50	0.7338
HUMIDITY*SEASON	1	0.4072208	0.4072208	0.03	0.8572
TEMPER*HUMIDI*SEASON	4	185.1941289	46.2985322	3.73	0.0114

Figure 7.2.2: Ash Content (%) of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

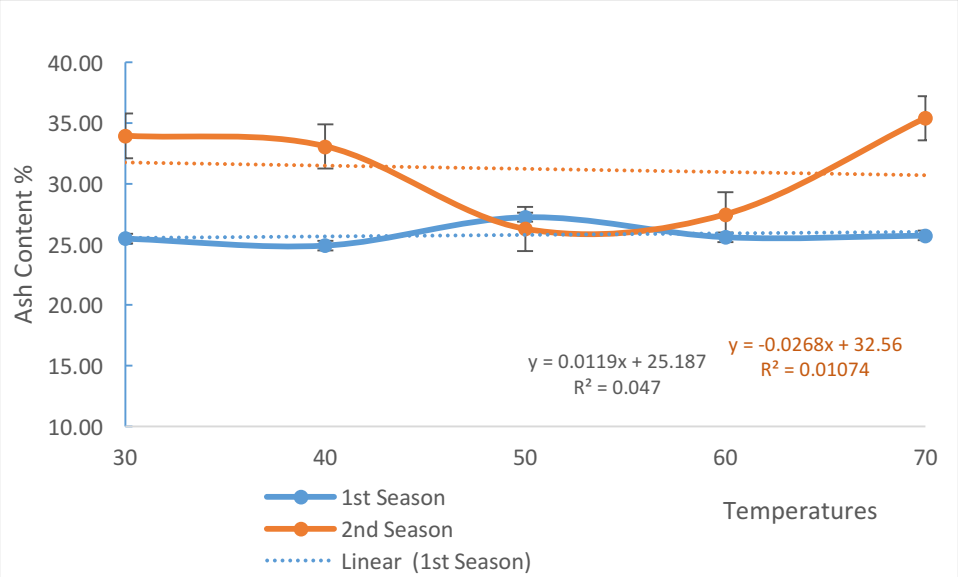
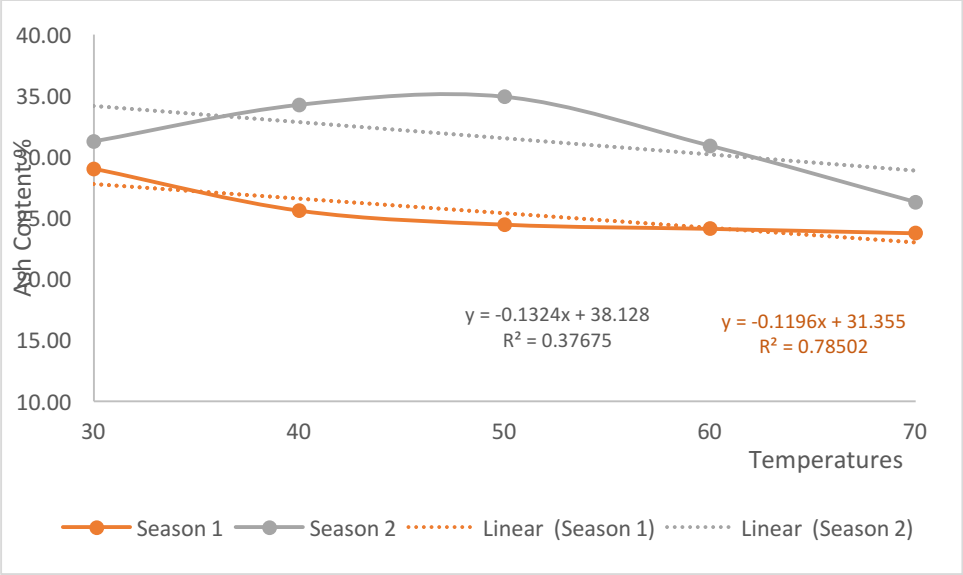


Figure 7.2.2: Ash Content (%) of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.3 *WHC*

Table 7.3.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for WHC

Oven Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	526954.855	131738.714	29.16	<.0001
HUMIDITY	1	94837.938	94837.938	20.99	<.0001
SEASON	1	4663114.031	4663114.031	1032.21	<.0001
TEMPERATURE*HUMIDITY	4	767971.349	191992.837	42.50	<.0001
TEMPERATURE*SEASON	4	409759.620	102439.905	22.68	<.0001
HUMIDITY*SEASON	1	528520.030	528520.030	116.99	<.0001
TEMPER*HUMIDI*SEASON	4	604954.575	151238.644	33.48	<.0001

Figure 7.3.1: WHC (%) of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

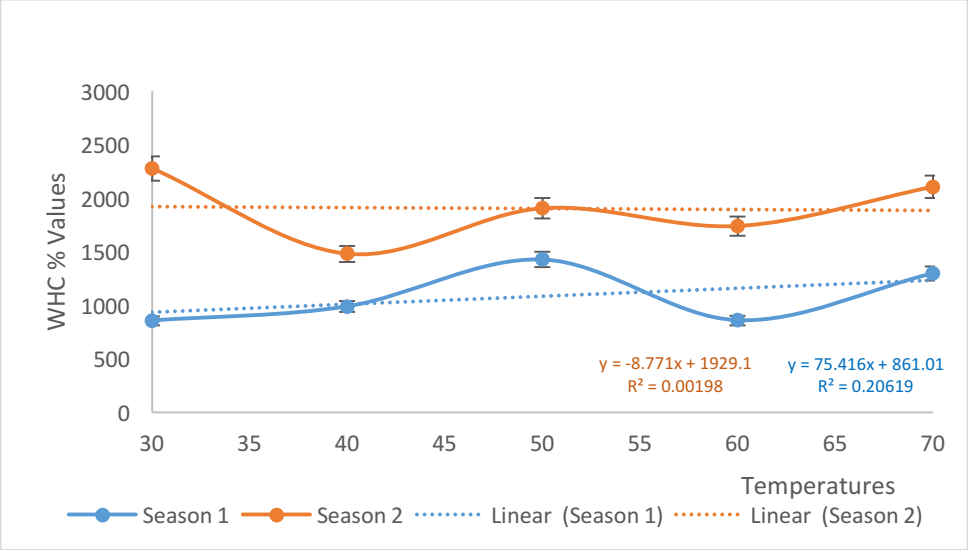
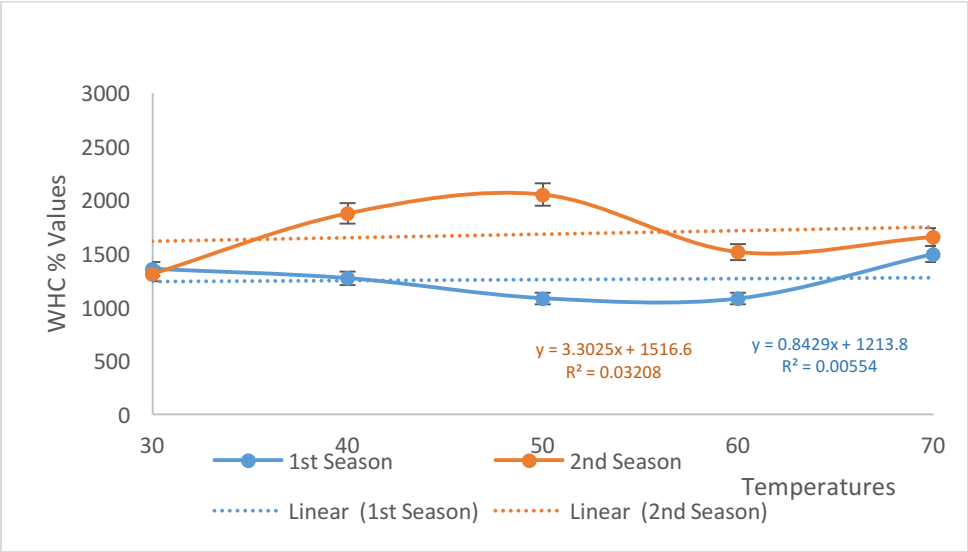


Figure 7.3.2: WHC (%) of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.4 *OHC*

Table 7.4.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for OHC

Oven Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	5552.09643	1388.02411	2.55	0.0538
HUMIDITY	1	2640.70351	2640.70351	4.86	0.0334
SEASON	1	16143.11087	16143.11087	29.68	<.0001
TEMPERATURE*HUMIDITY	4	9435.00838	2358.75209	4.34	0.0052
TEMPERATURE*SEASON	4	15786.84736	3946.71184	7.26	0.0002
HUMIDITY*SEASON	1	62480.65768	62480.65768	114.88	<.0001
TEMPER*HUMIDI*SEASON	4	40055.14601	10013.78650	18.41	<.0001

Figure 7.4.1: OHC (%) of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

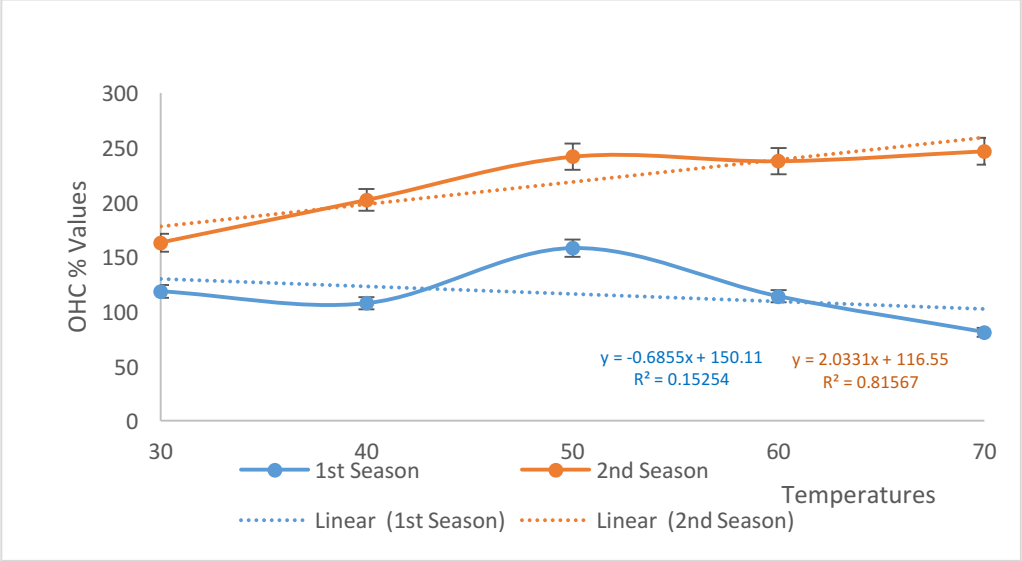
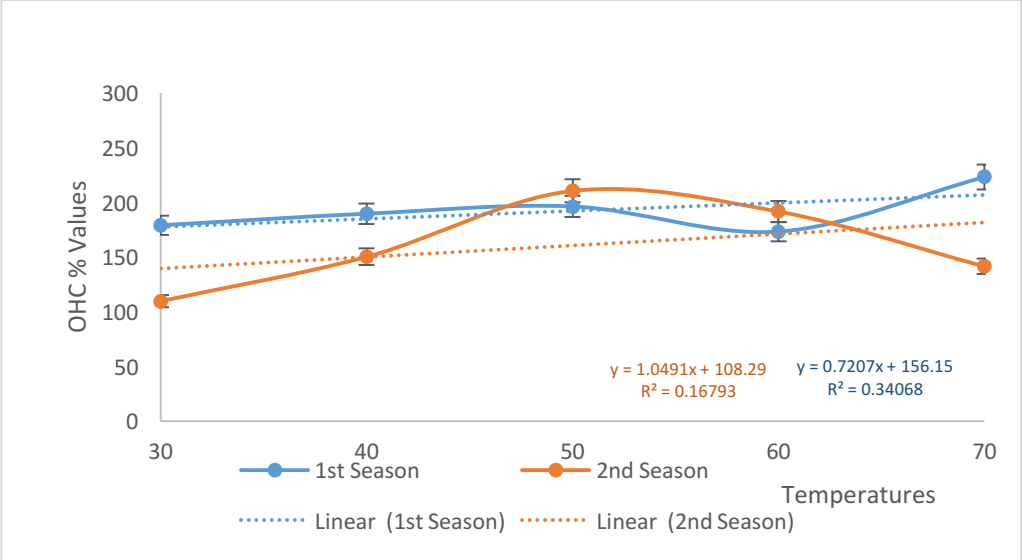


Figure 7.4.2: OHC (%) of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.5 *Fat Content*

Table 7.5.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for Fat Content Oven Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	1.68995540	0.42248885	11.74	<.0001
HUMIDITY	1	0.49777042	0.49777042	13.84	0.0006
SEASON	1	32.67716402	32.67716402	908.31	<.0001
TEMPERATURE*HUMIDITY	4	2.40211033	0.60052758	16.69	<.0001
TEMPERATURE*SEASON	4	0.91587407	0.22896852	6.36	0.0005
HUMIDITY*SEASON	1	0.63099015	0.63099015	17.54	0.0002
TEMPER*HUMIDI*SEASON	4	3.60048927	0.90012232	25.02	<.0001

Figure 7.5.1: Fat Content (%) of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

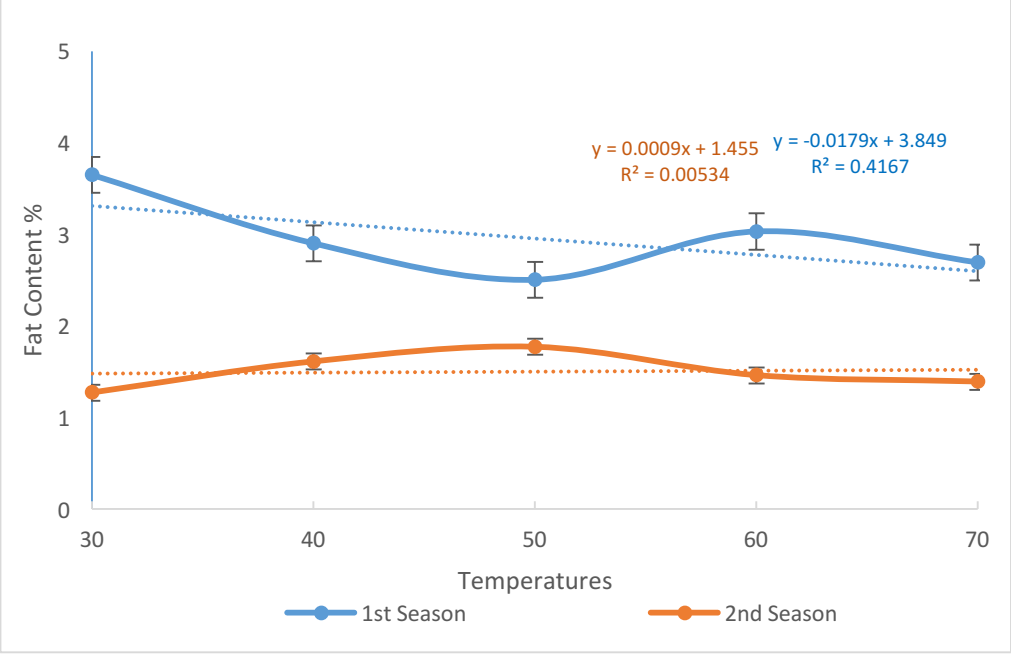
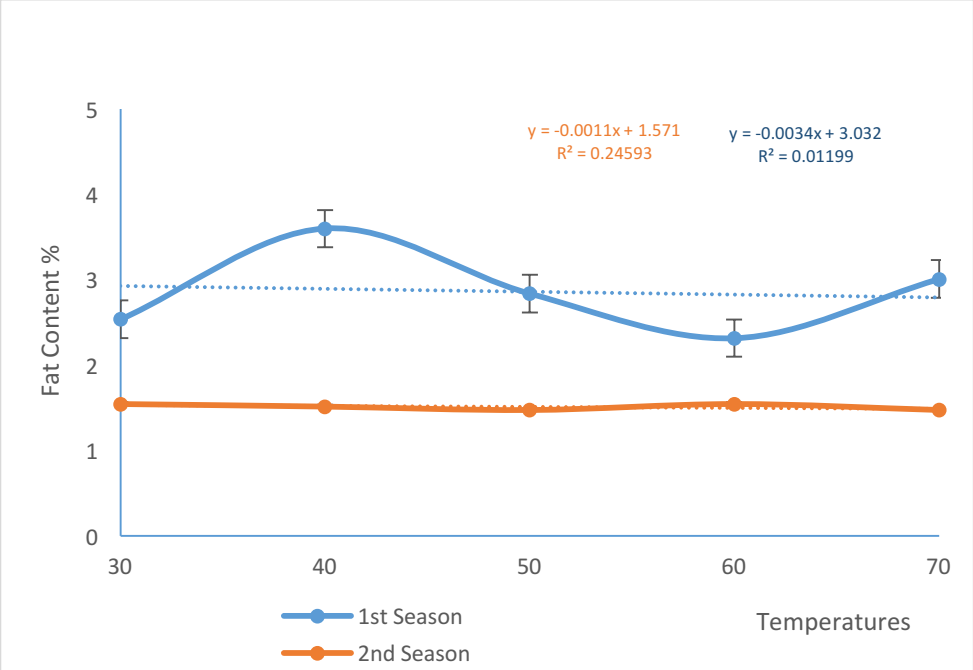


Figure 7.5.2: Fat Content (%) of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.6 *pH*

Table 7.6.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for pH

Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	0.51701667	0.12925417	19.23	<.0001
HUMIDITY	1	1.01920667	1.01920667	151.67	<.0001
SEASON	1	0.86400000	0.86400000	128.57	<.0001
TEMPERATURE*HUMIDITY	4	0.20824333	0.05206083	7.75	0.0001
TEMPERATURE*SEASON	4	0.31531667	0.07882917	11.73	<.0001
HUMIDITY*SEASON	1	0.20650667	0.20650667	30.73	<.0001
TEMPER*HUMIDI*SEASON	4	0.11514333	0.02878583	4.28	0.0056

Figure 7.6.1: pH of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

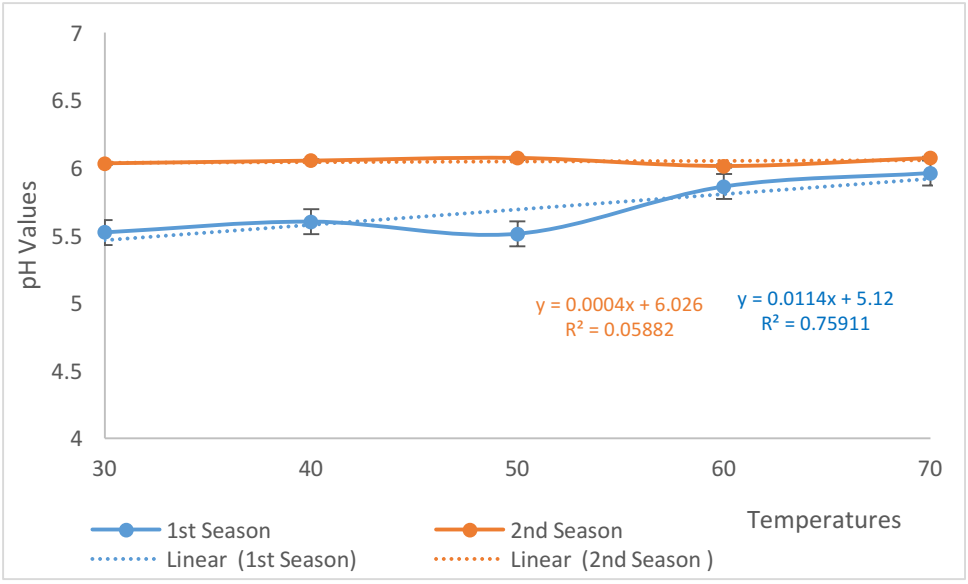
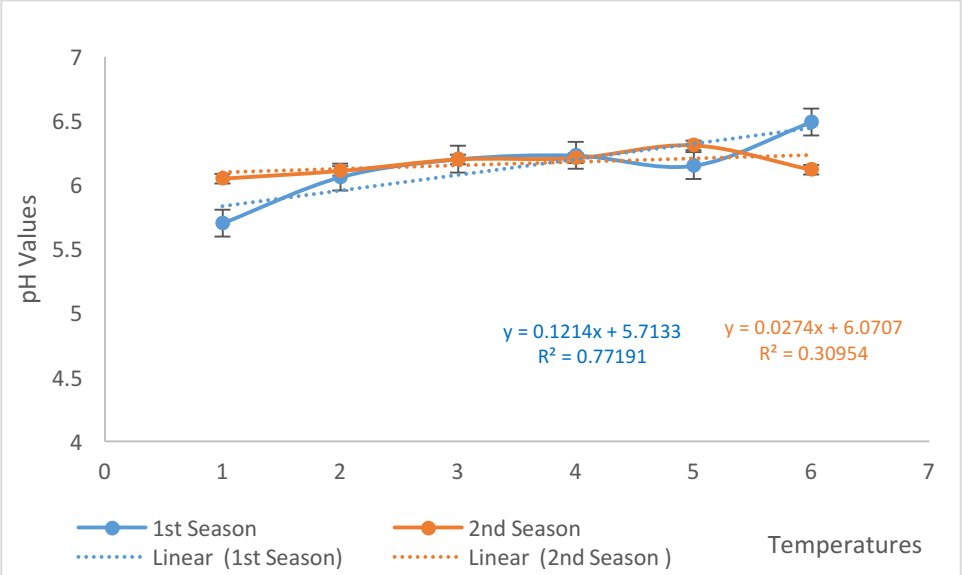


Figure 7.6.2: pH of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.7 *Water Activity*

Table 7.1.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for Water Activity of Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	0.05605510	0.01401378	85.82	<.0001
HUMIDITY	1	0.56454000	0.56454000	3457.43	<.0001
SEASON	1	0.02546160	0.02546160	155.94	<.0001
TEMPERATURE*HUMIDITY	4	0.07071883	0.01767971	108.28	<.0001
TEMPERATURE*SEASON	4	0.02556890	0.00639222	39.15	<.0001
HUMIDITY*SEASON	1	0.00600000	0.00600000	36.75	<.0001
TEMPER*HUMIDI*SEASON	4	0.07285717	0.01821429	111.55	<.0001

Figure 7.8.1: Water Activity of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

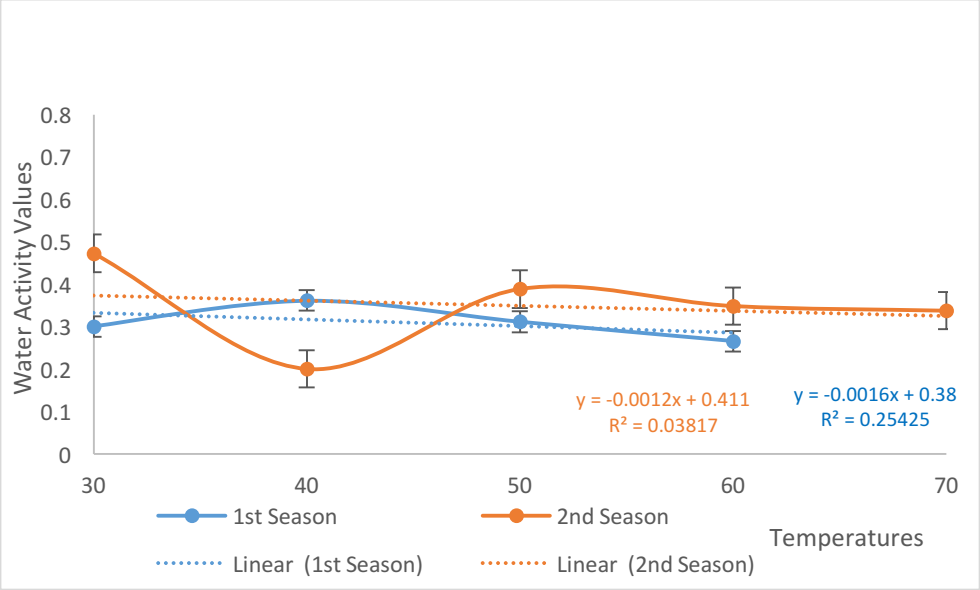
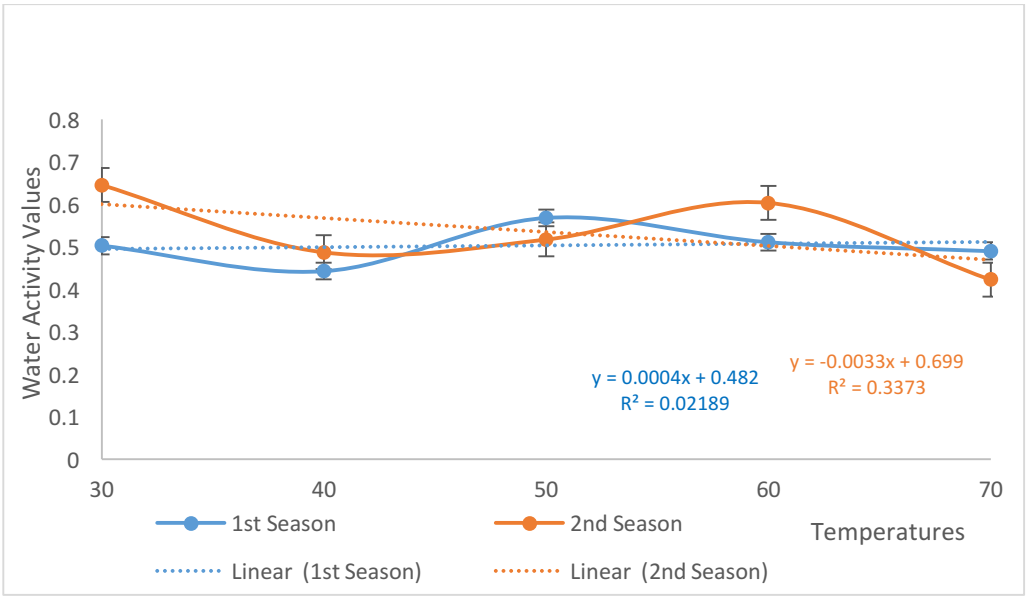


Figure 7.8.2: Water Activity of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.8 *Color Analysis*

7.8.1 L Values*

Table 7.8.1.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for L* Values of Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	699.5847767	174.8961942	15.71	<.0001
HUMIDITY	1	70.0920417	70.0920417	6.30	0.0162
SEASON	1	176.9196817	176.9196817	15.89	0.0003
TEMPERATURE*HUMIDITY	4	87.1689833	21.7922458	1.96	0.1197
TEMPERATURE*SEASON	4	124.4044433	31.1011108	2.79	0.0389
HUMIDITY*SEASON	1	0.1334817	0.1334817	0.01	0.9134
TEMPER*HUMIDI*SEASON	4	111.8533433	27.9633358	2.51	0.0567

Figure 7.8.1.1: L* Values of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

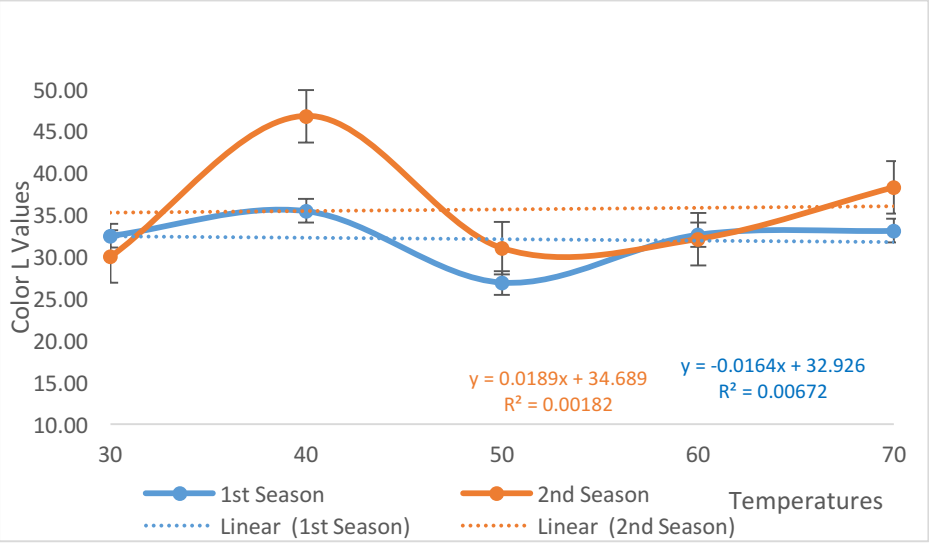
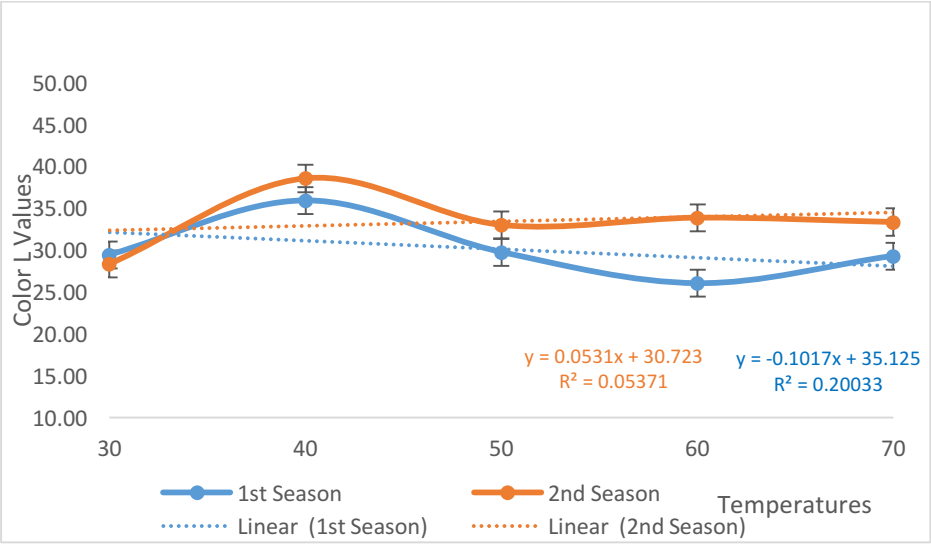


Figure 7.8.1.2: L* Values of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.8.2.1 a Values*

Table 7.8.2.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for L* Values of Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	6.38557667	1.59639417	15.39	<.0001
HUMIDITY	1	1.30537500	1.30537500	12.58	0.0010
SEASON	1	15.94441500	15.94441500	153.67	<.0001
TEMPERATURE*HUMIDITY	4	0.70661667	0.17665417	1.70	0.1685
TEMPERATURE*SEASON	4	23.90001000	5.97500250	57.59	<.0001
HUMIDITY*SEASON	1	0.24448167	0.24448167	2.36	0.1327
TEMPER*HUMIDI*SEASON	4	8.61214333	2.15303583	20.75	<.0001

Figure 7.8.2.1: a* Values of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

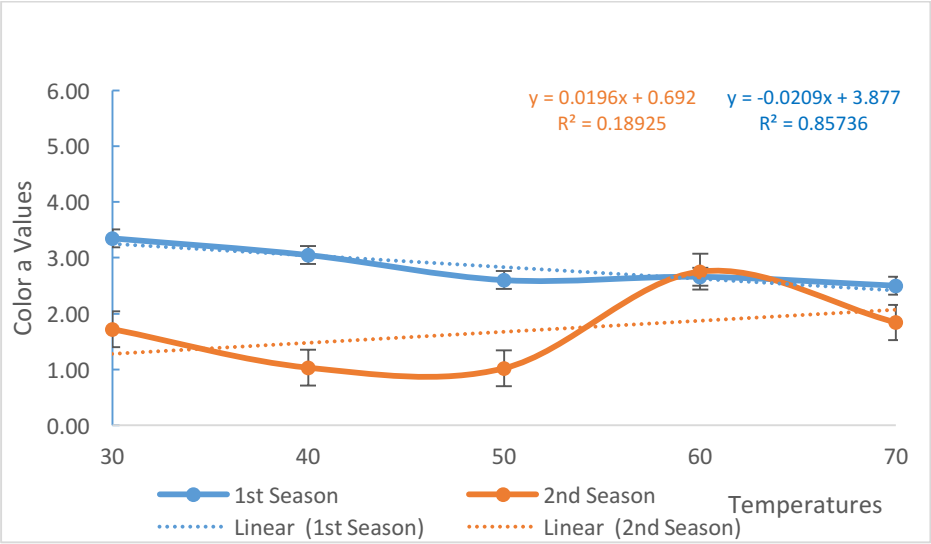
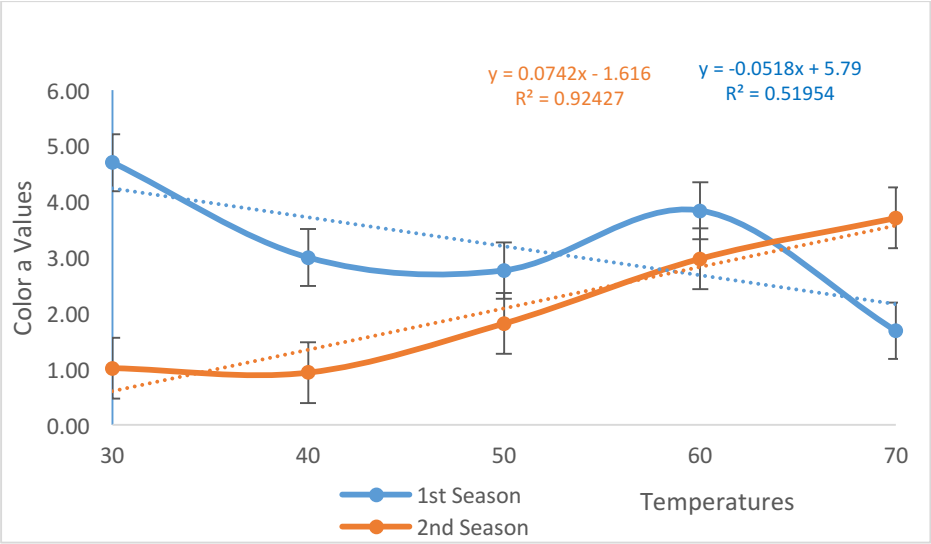


Figure 7.8.2.2: a* Values of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.8.3 b Values*

Table 7.8.3.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for L* Values of Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	89.3162067	22.3290517	12.76	<.0001
HUMIDITY	1	0.2148017	0.2148017	0.12	0.7279
SEASON	1	1.6633350	1.6633350	0.95	0.3354
TEMPERATURE*HUMIDITY	4	4.9195733	1.2298933	0.70	0.5946
TEMPERATURE*SEASON	4	126.9364733	31.7341183	18.14	<.0001
HUMIDITY*SEASON	1	0.2148017	0.2148017	0.12	0.7279
TEMPER*HUMIDI*SEASON	4	4.9195733	1.2298933	0.70	0.5946

Figure 7.8.3.1 b* Values of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

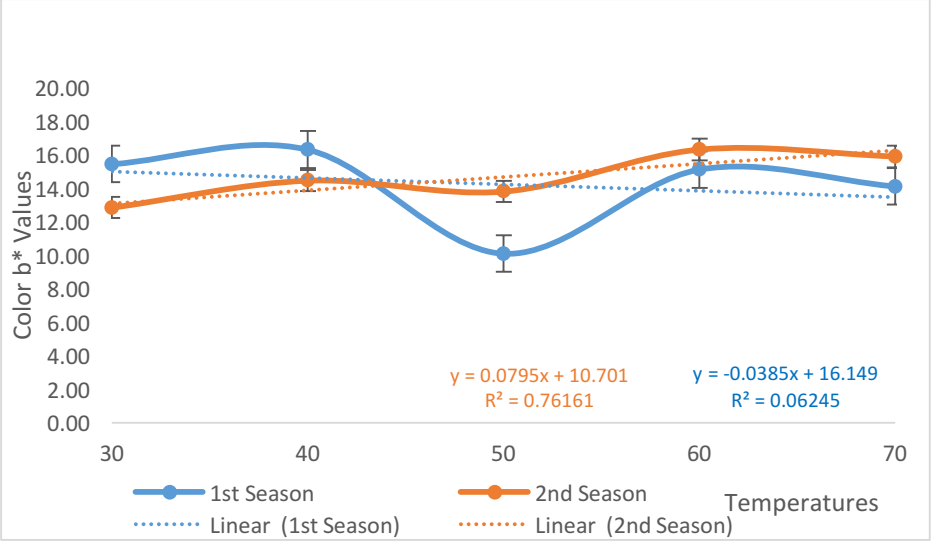
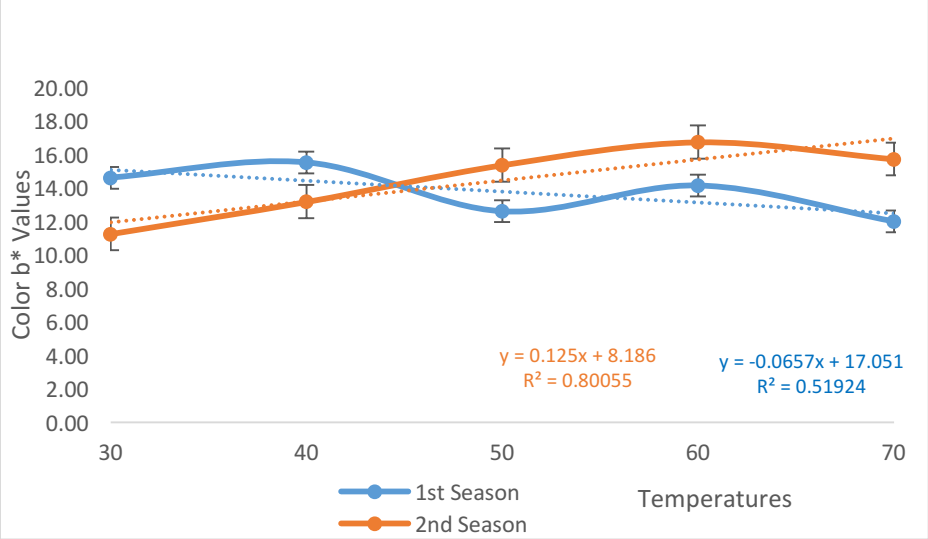


Figure 7.8.3.2: b* Values of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



7.9 *Vitamin C*

Table 7.9.1: Anova Thesis Statistics GLM Procedure Dependent Variable Data for Vitamin C of Oven-Dried Kelp

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TEMPERATURE	4	0.07205077	0.01801269	96.63	<.0001
HUMIDITY	1	0.00261360	0.00261360	14.02	0.0006
SEASON	1	0.01626907	0.01626907	87.28	<.0001
TEMPERATURE*HUMIDITY	4	0.00596690	0.00149173	8.00	<.0001
TEMPERATURE*SEASON	4	0.00280477	0.00070119	3.76	0.0109
HUMIDITY*SEASON	1	0.00233127	0.00233127	12.51	0.0010
TEMPER*HUMIDI*SEASON	4	0.00652823	0.00163206	8.76	<.0001

Figure 7.9.1: Vitamin C Content Values of Oven Dried Kelp at 25% Air Oven Humidity Linear Relationship

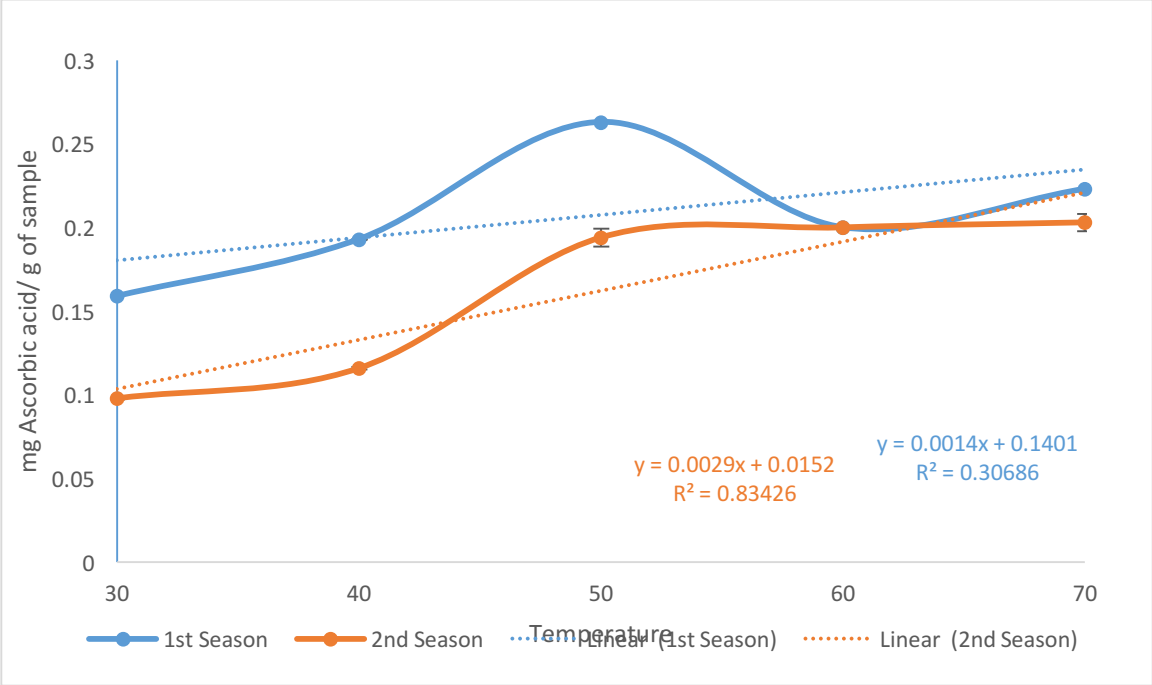
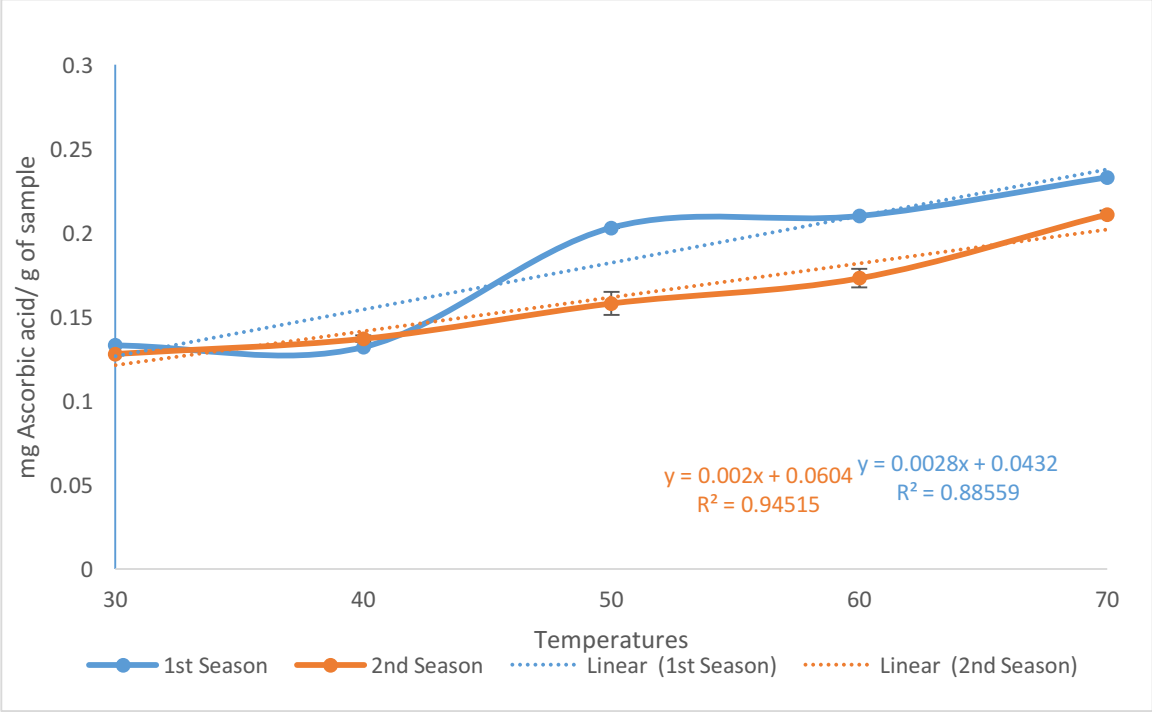


Figure 7.9.2: Vitamin C Content Values of Oven Dried Kelp at 50% Air Oven Humidity Linear Relationship



AUTHOR'S BIOGRAPHY

Emily Duran-Frontera was born Moca, Puerto Rico on December 12, 1995. She was raised in Las Marias, Puerto Rico where she graduated from Escuela Superior Eva y Patria Custodio in 2013. She moved to the United States to attend the University of Maine majoring in Food Science and Human Nutrition. She is a member of Phi-Mu Sorority and Co-Founder and President of the Caribbean Club and Women Empowering Women Forum on campus. She served as secretary of National Society of Collegiate Scholars from 2015-2016.

Upon graduation, Emily plans to go to Graduate School, with hopes of one day of returning to a free Puerto Rico.