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Physicochemical Properties and Sensory Evaluation of Cracker Incorporated with Green Spinach (*Spinacia oleracea*) and Red Spinach (*Amaranthus dubius*) during Storage

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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ABSTRACT

Incorporating spinach powder can be seen as a commodity to increase the nutritional value, physical properties and creating new flavour of cracker. The purpose of this study was to develop a new food product and determine the physicochemical properties and sensory acceptability of cracker incorporated with various concentrations of green spinach and red spinach powder. Shelf life study was conducted by observing the texture and colour properties during four weeks of storage. Overall texture analysis that was conducted by using texture analyzer can be concluded by saying that hardness and fracturability of cracker showed increasing progression with increasing concentration of spinach powder but showed decreasing trend with time. Chromatic parameters (L^*, a^*, b^*) of colour properties that was conducted using chromameter showed decreasing progression with increasing percentage of flour substitution and showed decreasing trend with time. For proximate analysis, the result indicated that with the increasing concentration of spinach powder, the values for proximate analysis decreases for moisture but increases for ash, fat and protein content. The sensory evaluation conducted among 40 panelists showed higher interest in red spinach cracker with 5% flour substitution. The effect of flour substitution with spinach powder was positive in terms of decreasing moisture content and increasing ash, fat and protein content. Along with the higher acceptance of the cracker incorporated with spinach powder, the objectives of the study were achieved.

Keywords: Cracker, Proximate value, Sensory acceptance, Shelf life, Spinach

ABSTRAK

Penggunaan serbuk bayam dapat dilihat sebagai komoditi untuk meningkatkan nilai pemakanan, sifat fizikal dan mencipta perisa baharu. Tujuan kajian ini adalah untuk membangunkan produk makanan baharu dan mengenal pasti sifat fizikokimia dan kebolehterimaan sensori kraker yang digabungkan dengan serbuk bayam hijau dan bayam merah dengan formulasi yang berbeza. Kajian jangka hayat dijalankan dengan memerhati sifat tekstur dan warna selama empat minggu penyimpanan. Analisis tekstur keseluruhan yang dilakukan dengan menggunakan penganalisis tekstur mendapati bahawa kekerasan dan keretakan fraktur dapat menunjukkan perkembangan yang semakin meningkat dengan peningkatan konsentrasi serbuk bayam tetapi menunjukkan penurunan trend dengan masa. Kromatik paramater (*L *, a *, b **) untuk sifat warna yang dijalankan menggunakan kromameter menunjukkan penurunan dengan peningkatan peratusan penggantian tepung dan menunjukkan penurunan trend dengan masa. Untuk analisis proksimat, hasilnya menunjukkan bahawa dengan peningkatan konsentrasi serbuk bayam, nilai untuk analisis proksimat berkurangan untuk kelembapan tetapi peningkatan untuk kandungan abu, lemak dan protein. Evaluasi deria yang dilakukan di kalangan 40 panelis menunjukkan minat yang lebih tinggi untuk kraker bayam merah dengan penggantian tepung 5%. Kesan penggantian tepung dengan serbuk bayam adalah positif dari segi penurunan kandungan kelembapan dan peningkatan kadar abu, lemak dan protein. Seiring dengan penerimaan yang tinggi untuk kraker yang digabungkan dengan serbuk bayam, objektif kajian telah dicapai.

Kata kunci: Analisis proksimat, Bayam, Jangka hayat, Kraker, Penerimaan deria

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Food as a basic necessity should be consumed meticulously based on our bodily requirements and not based on price or taste. The product that is healthier is a product that contains or provides necessary nutrients for humans. However, food product that is being claimed as rich in nutritional value or natural nowadays may have additional ingredients such as flavour enhancer or preservatives (World Health Organization, 2009). The decreasing rate of fruit and vegetable consumption with unhealthy eating lifestyle in some people especially in this modern era, will increase the chances of them getting various type of diseases or illnesses as they lack essential nutrients that are needed for the body to work at their optimum level. Therefore, food product development is important in improving food which can increase the nutritional value in said product and improve the quality given to the consumers for better health (Hasler-lewis, n.d.).

According to The Star (2017), it was found that Malaysian market has seen positive growth of baked goods as people tend to opt for other food option than rice and noodles during meal times. In addition, the rise of coffee culture has encouraged teatime seekers to have a bakery product or two with their drink. According to Malaysian-German Chamber of Commerce and Industry (2016), baked goods offered in the Malaysian market can be characterized as extremely versatile and come in a lot of varieties. One of the most common and important ingredient in baked-goods is dietary fibre. Important sources that are high in dietary fibre come from fruits and vegetables as well as in cereals. Spinach is one of the vegetables that rich in dietary fibre content and is commonly consume in fresh.

Spinach is a leafy green vegetable which belongs to the Amaranthaceae family and is related to beets and quinoa. It is thought to have originated from Persia but has now produced mostly in the United States and China (Gunnars, 2015). In Malaysia, there are two species of spinach that are commonly consumed namely green spinach (*Spinacia oleracea*) and red spinach (*Amaranthus dubius*). Both green spinach and red spinach are rich in vitamin C. However, green spinach is richer in vitamin A while red spinach contains more iron. Spinach's leaves contain vitamin A, vitamin B6, vitamin C, chlorophyll, beta carotene, and riboflavin as well as multiple secondary metabolites such as alkaloid, flavonoid, tannin, glycoside and many more (Muliani *et al.*, 2017).

Cookies and crackers are among the most popular foods that are low in moisture and is made with wheat flour as the main ingredients. In crackers, wheat flour is usually used because it contains high gluten protein strength due to gluten development in the dough of the cracker during mixing and sheeting, which is one of the important factors in maintaining the quality of the finished product. The most important ingredients in the cracker that makes it different from other baked goods such as cookies, is the sugar concentration. Cracker generally has lower concentration of sugar with lower than 30% in a typical cracker formula (Kweon *et al.*, 2013).

The objectives of this research is to determine the physicochemical properties of cracker with different concentration level of green and red spinach powder which involves proximate analysis, texture profile analysis and colour analysis. The shelf life of the cracker will also be determined by emphasizing on its physical properties up to three weeks of storage. Sensory acceptability among consumers on different concentrations of spinach powder, and comparison between green spinach and red spinach crackers will be determined by conducting sensory evaluation.

1.2 Problem Statement

This research investigates the addition of different percentage of green spinach and red spinach powder in the cracker. Spinach can be found easily in Malaysia local market. However, spinach was found to have limited usage in development of food products especially in bakery products and is usually being consumed in fresh. Some food developers or Small and Medium Enterprise (SME) companies may have the thought of incorporating spinach in food development. Due to lack of information and research done, they may not know how much spinach powder to add and what species of spinach is suitable for the baked goods.

Some people tend to avoid eating fruit and vegetable in fresh thus, causes them to have vitamin or mineral deficiency. Multiple micronutrient deficiency is more common than single deficiency in developing countries due to poor food consumption and bioavailability of micronutrients (Gupta & Prakash, 2011). In addition, most of the products that being marketed contains high amount of sugar with additional chemical preservatives, flavour enhancer and colouring agent which can cause negative effects to the consumer's health especially for those who had already have health problems.

The main problem in purchasing fresh fruit and vegetable is their short life cycle. Maximum shelf life for spinach was observed is 3 days at ambient condition (Kakade *et al.*, 2015). A short shelf life does not only limit the product to be stored in a short duration but it will also cause economic loss as they have to throw away the products that are spoiled which are not managed to be sold to consumers. Hence, the development of fruit and vegetable based products will lower the deterioration rate of the fresh products and also decrease the amount of commodity being wasted as the shelf life of product is prolong.

Cracker or cookie that has been incorporated with spinach will be able to attract anaemia patients' attention easily especially children who generally dislikes eating fresh vegetable as it does not look palatable. Children will find crackers a fun snack to consume and it ensures that they get the necessary nutrients they need.

1.3 Objectives

1. To determine the best formulations of spinach cracker compared to commercial vegetable cracker through sensory tests.
2. To determine the proximate composition of cracker incorporated with different concentration of spinach powder.
3. To determine the texture and colour properties of cracker incorporated with different concentration of spinach powder during storage.

1.4 Hypothesis

H_0 : Incorporation of green spinach and red spinach powder in cracker has no effect to the colour and texture.

H_1 : Incorporation of green spinach and red spinach powder in cracker has effects to the colour and texture.

H_0 : Incorporation of green spinach and red spinach in cracker has no effect to the shelf life.

H_1 : Incorporation of green spinach and red spinach powder in cracker has effects to the shelf life.

H_0 : Sensory properties of incorporation of green spinach and red spinach powder in cracker received lower acceptability by consumers.

H_1 : Sensory properties of incorporation of green spinach and red spinach in cracker received higher acceptability by consumers.

1.5 Scope of Study

The scope of this study focused on production of cracker based on spinach. Two types of spinach added to the cracker were green spinach (*Spinacia oleracea*) and red spinach (*Amaranthus dubius*). The spinach was developed into ready-to-eat (RTE) food product by converting fresh spinach into powder form and incorporated it into cracker.

The study was focused on physicochemical properties and sensory acceptability of the cracker when added with green spinach and red spinach powder. A normal sensory evaluation was carried out to know the acceptability of consumers towards the cracker. Total of 40 undergraduate students of University Malaysia Kelantan (UMK) Jeli campus were given the opportunity to take part in sensory evaluation of the spinach cracker which consisted of colour, flavour, taste, crispiness, hardness, and overall acceptability of the cracker. A comparison between green spinach and red spinach cracker was conducted to determine which species of spinach is suitable to be used for product development.

Shelf life of spinach cracker was determined by studying on its physical properties during different period of storage up to four weeks. Physical analysis of the cracker added with green and red spinach powder was determined in this study which included texture and colour analysis. The physical analysis was conducted to determine the shelf life and quality of the cracker products after every seven days.

Proximate analysis was conducted to determine the moisture, protein, fat, ash and carbohydrate content. The purpose of conducting proximate analysis is to determine how much of the major food components which are moisture, fat, protein, ash and carbohydrate exist in a given food. These food components may be of interest in food industry to ensure that their products meet the appropriate laws and legal declaration requirements as well as safety aspects of the final products when being released to end consumers. Those values obtained are being declared as nutritional facts shown on the labels of the final products. It can also be used as means for fair market competition between food companies. The analysis was conducted to determine the distribution of products when samples are heated under specified conditions.

1.6 Significance of Study

The significance of this study was to identify whether spinach is suitable to be incorporated into cracker and which types of spinach has the potential to be used for the development of food product. From this study, the abundance of local plant can be utilized and benefitted to the future via development of food product. The usage of this plant in food product especially in baked goods can increase the nutritional value, texture and flavour. Data obtain from this research will allow food developer to determine the right composition of ingredients and which species of spinach is suitable for development of baked goods. Spinach is commonly consumed fresh and is an important vegetable to prevent or control anaemia as it is rich in iron. Incorporating spinach into crackers will help anaemia patients especially children to find it as a fun snack to consume and ensures that they get the necessary nutrients they need.

1.7 Limitation of Study

In this research, there were some limitations on the sensory evaluation. Since the experiment and sensory evaluation were carried out in University Malaysia Kelantan (UMK) Jeli campus, feedback from a wide range of people and of all ages were unable to achieve. Besides that, the experiment required a huge amount of crackers to be produced to conduct physicochemical analysis and sensory evaluation. Due to the limitation in terms of time and cost, the experiment was unable to cover a wider scope.

CHAPTER 2

LITERATURE REVIEW

2.1 Green Spinach (*Spinacia oleracea*)

Green spinach (Figure 2.1) is known as Amaranthaceae with the genus *Spinacia*. For years, it was called "the Spanish vegetable" in England which eventually shortened to spinach which is commonly used by people from around the world. The cultivation of spinach is believed to have originated from ancient Persia (Mercola, 2016). Spinach is a temperate climate plant, and best grown in cooler places. According to Asia Farming (2019), five major countries that produce green spinach are China, United States, Japan, Turkey, and Indonesia. Green spinach is currently becoming more easily accessible from all parts of the world and is consumed by many people on a daily basis.

In Malaysia, green spinach has gained massive popularity alongside trendy health food. Green spinach can be prepared in various ways because it is one of those vegetables that can be consumed both raw and cooked. It is a vegetable that can be easily added into many dishes such as smoothies, pasta sauces, salads, soups, stir-fries or mix with burger patties or meatballs. The leaves of green spinach are alternate, simple, ovate to triangular, and variable in size.

Figure 2.1: Picture of green spinach (*Spinacia oleracea*)

Source: Szalay, (2015)

2.2 Nutritional Composition of Green Spinach

Green spinach is one of the most nutritious vegetable that can be eaten raw or cooked. It provides a very good amount of antioxidant compound, dietary fibre, minerals, vitamins, and iron compound which is beneficial to prevent anaemia (Miano, 2016). It has substantial health-promoting activities that are attributed to functional properties of their nutrients and non-essential compounds. It is regarded as a functional food due to its diverse nutritional composition, phytochemicals, and bioactives that promote health beyond basic nutrition (Robert & Moreau, 2016). It contains antioxidant known as alpha-lipoic acid which are able to increase insulin sensitivity, lower glucose level, and prevent oxidative, stress-induced changes in patients with diabetes.

Apart from that, spinach-derived phytochemicals and bioactives are able to scavenge reactive oxygen species and prevent macromolecular oxidative damage, modulate expression and gene activity, and curb food intake by inducing the secretion of satiety hormones (Robert & Moreau, 2016). These biological activities contributed by green spinach will be able to lower the risk or prevent cancer, obesity, and have hypoglycemic and hypolipidemic properties.

2.3 Red Spinach (*Amaranthus dubius*)

Red spinach (Figure 2.2) belongs to the same family as green spinach which is *Amaranthaceae,* with the genus *Amaranthus.* The difference between green and red spinach lies in their genus where the genus of red spinach is *Amaranthus* while the genus of green spinach is *Spinacia*. The noticeable difference on its characteristics is the colour of the leaves where Amaranthus has red pigment in its stem. Red spinach has been widely cultivated in the mountain areas throughout Indonesia (Muliani *et al.*, 2017).

The leaves of red spinach can vary from oval to slightly oblong and rounded arrowhead tip. It has a deep green colour with maroon veins and beet-red stems. The tip of the leaf is tapered, soft and wide. The stem is soft and white reddish (Maljeti *et al.*, 2017). Red spinach has red liquid in its stem which is responsible for the red colour of stems and leaves (Amritha, 2018).

Figure 2.2: Picture of red spinach (*Amaranthus dubius*)

Source: Wilsonbuy, (n.d.)

2.4 Nutritional Composition of Red Spinach

Both green spinach and red spinach are rich in vitamin C. However, green spinach is richer in vitamin A while red spinach has higher iron content. It is known that the leaves of red spinach are the storehouse for many phytonutrients, antioxidants, minerals and vitamins which contribute greatly to health and wellness. The leaves of red spinach contain vitamin A, vitamin B6, vitamin C, chlorophyll, beta carotene, and riboflavin. In addition, it also contains multiple secondary metabolites such as alkaloid, flavonoid, tannin, glycoside and many more (Muliani *et al*., 2017).

Besides iron, red spinach has higher concentration of other minerals than green spinach such as calcium, manganese, magnesium, copper and zinc. Manganese and copper act as a co-factor for the antioxidant enzyme, *superoxide dismutase* in the human body. In addition, copper is also required for the production of red blood cells which is found in red spinach. Zinc acts as a co-factor for many enzymes that regulate growth and development, digestion and nucleic acid synthesis (Rudrappa, 2019).

The leaves and stems of red spinach carry a good amount of soluble and insoluble dietary fibers. The leaves contain only 23 calories per 100g, and contain only traces of fats and no cholesterol (Rudrappa, 2019). Hence, red spinach is often recommended by dieticians for patients in the cholesterol controlling and weight reduction programmes. Nutritional benefits of red spinach are extremely beneficial for health, skin and hair. It is commonly used as a herbal remedy to cure gastric problems, improves digestion, treats cancer, aids in weight loss, treats anaemia, improves kidney function, improve immune system and boosts bone strength (Amritha, 2018).

2.5 Iron

Iron is an essential mineral that is required for the body to function normally and can only be obtained from dietary source. It is used in haemoglobin to transport oxygen to the cells throughout the body. Among the many essential minerals like calcium, sodium and zinc, iron is unique as it is very difficult for the body to regulate its consumption. This shows that unlike calcium which is excreted in the urine, excess iron may remain in the body. This finding makes iron to be a double-edged sword ie;

i. insufficient absorption of iron cause anaemia, which can lead to a range of problems from fatigue to death

ii. excessive absorption of iron increase risk of hemochromatosis, colorectal cancer, heart disease, infection, neurodegenerative disorders and inflammatory conditions (Nutrino, 2016).

Iron deficiency is the most widespread nutritional deficiency in the world. World Health Organization (WHO) has estimates that two billion people over 30% of the world's population are anemic, most of which due to iron deficiency (Nutrino, 2016). According to Ancuceanu *et al.* (2015), anaemia is defined as "haemoglobin concentration below established cut-off levels". It is not a disease but a state of reflecting nutrient deficiency or may sometimes an underlying disorder. To prevent iron deficiency, a balanced diet should be taken that includes good sources of iron and the effective way is to combine vegetarian sources of iron with vitamin C such as spinach with lemon juice (Kaufman, 2018).

2.6 Dietary Fibre

The demand for food that is rich in dietary fibre has increased in the past decade and lead to the development of many fibre rich products and ingredients (Drzikova *et al.*, 2005). Specific properties of dietary fibre has been reported to play an important role in preventing and treating various gastrointestinal disorders such as constipation, obesity, coronary heart disease, colorectal cancer and diabetes (Bingham, Day, Luben & Ferrari, 2003). According to Han *et al.* (2017), dietary fibres are often characterized by high nutritional quality because they are able to cure many chronic diseases and improve texture, sensory characteristics, and shelf life of foods.

Dietary fibre is composed of total dietary fibre (TDF) that includes both soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). SDF include pectin, oligosaccharides, guar, and gums, most of which are dietary and healthy additives (Han *et al.*, 2017). SDF can be found in fresh and dried fruit, vegetables, oats, legumes and seeds. It has been found that the viscosity of the intestinal contents can be increased and cholesterol absorption being reduced using some of SDF (Redondo-cuenca *et al.*, 2014). Some other SDF are fermented by the bacteria in the large intestine for maintaining good colon health and increasing the mineral absorption. Production of short chain fatty acids such as propionate and butyrate was resulted from the fermentation of SDF. Fatty acid called butyrate found acted as protective agent against experimental tumor genesis of the cells while propionate could be related to hypocholestrolemic effects (Redondo-cuenca *et al.*, 2014).

IDF can be found in plant cell walls of whole grain bread, whole grain cereals, fruits and vegetables. Examples of IDF are cellulose, lignin and hemicellulose. Difference between SDF and IDF is that many IDF are not fermentable. The characteristics of IDF are high water holding capacity, increase fecal bulk and reduce gastrointestinal transit time. This effect may be related to the treatment and prevention of some of the intestinal disorders (Redondo-cuenca, Goni & Villanueva-suarez, 2014).

2.7 Crackers and Physical Properties

Physical property is a characteristic of a substance that can be observed without changing the substance into another substance. Crackers are a wide range of products that have characteristics of crispy, open texture, less sweet and savoury flavours

(Davidson, 2016). Physical properties of fruit and vegetable cracker can be evaluated for various parameters such as thickness, diameter, weight, spread ratio, and texture profile analysis. By using texture profile analysis, various aspects can be determined such as hardness, springiness, cohesiveness, adhesiveness, and stickiness (Adeola & Ohizua, 2018). The cell structure, size and form of the products also an important influence on the properties of the texture analyses (Winopal, Drobny & Schneider-Hader, 2015).

Previous research has been done on crackers that has been incorporated with pumpkin pomace and it is found that the cracker has high content of total dietary fibre (51.77%), high ash concentration (6.65%) and low fat content (1.63%) (Kuchtova *et al.*, 2016). Cracker made from sprirullina powder had been found to improve textural and sensory properties as it increases protein content, levels of vitamin and essential amino acids of crackers (Amira, Morsy & Mawla, 2017).

Green gram flour was found to be a potential alternative to wheat flour in bakery products especially crackers as it exhibited improved nutritive and functional properties. However, sensory acceptance was slightly lower than the baseline of wheat flour based crackers (Venkatachalam & Nagarajan, 2017). Previous research was also conducted on wheat flour where 5% of wheat flour was substituted with pumpkin pulp flour and unripe banana pulp flour, and results shows that it was the most suitable with an added value of fibre (Noor Aziah & Komathi, 2009).

2.8 Textural Properties of Cracker

Texture analysis is the study of the properties of food product which can be analysed by the eyes and muscle senses in the mouth which are graded by smoothness, roughness, graininess and others. It is perceived by the senses of touch, sight and hearing. According to Winopal, Drobny and Schneider-Haider (2015), texture testing is a sub-area of rheology which is science that deals with the flow and deformation behaviour of solid and fluid bodies under influences of mechanical forces that being set.

Texture profile analysis (TPA) is an instrumental test that was originally developed at General Foods Corporation Technical Centre to provide objective measurements of texture parameters, which can be a major factor of food acceptability (Tuoc & Glasgow, n.d.). The original TPA parameters defined by the General Foods Corporation group are as shown in Table 2.1. Tuoc and Glasgow had done a texture profile analysis research on Colby cheese to fully understand the concept. Results obtained from the research is shown in Figure 2.3.

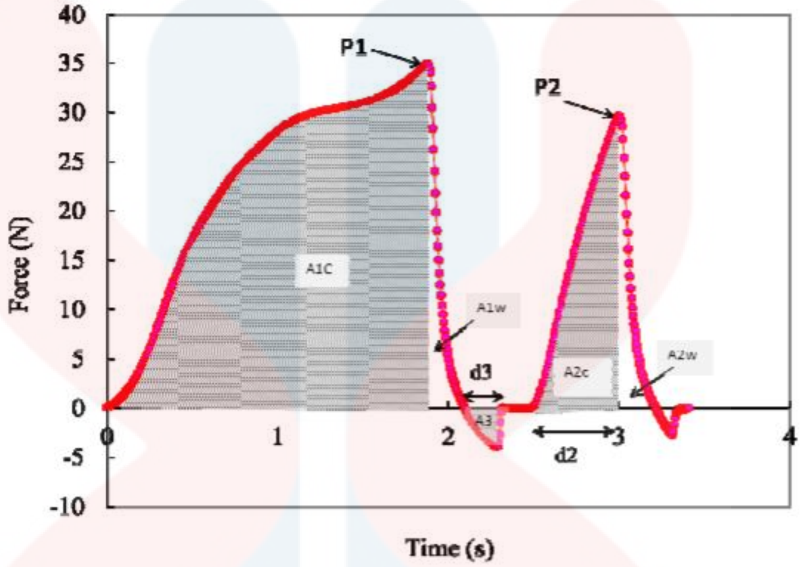

Figure 2.3: Force-time curves for a two bite test on Colby cheese

Table 2.1: Definition of different textural parameters in the TPA

TPA parameter (SI unit)	Definition	How measured
Hardness (N)	Force required for a pre-determined deformation	Force at P1
Fracturability (N)	Force at the first significant break in the curve	Force at F1
Cohesiveness (no unit)	Strength of internal bonds in the sample	A_2/A_1
Adhesiveness (J)	Work required to overcome the sticky forces between	A_3

	the sample and the probe	
Gumminess (N)	Energy needed to disintegrate a semisolid food until it is ready to swallow	Hardness x Cohesiveness
Chewiness (J)	Energy needed to chew a solid food until it is ready for swallowing	Hardness x Cohesiveness x Springiness
Springiness (m)	Originally refer as “Elasticity”; rate at which a deformed sample returns to its original size and shape	d_2
Stringiness (m)	Distance travelled by the probe during the negative force area A_3	d_3

TPA is commonly used among food developer to accurately describe the textural characteristics of food. This technique is able to provide a universal language for food scientists, vendor, sales staff, and customers. By doing this analysis, scientific data of the particular food will be able to be obtained and detailed characteristics of the food is able to be recorded for further research. Being able to know the texture profile of a particular product can determine the attributes that increase consumer liking. People will be able to find out certain attributes of the food product that can increase the value of the food and emphasize more on it. Figure 2.4 shows the texture analyzer that was used to conduct texture profile analysis which is found available at University Malaysia Kelantan (UMK) Jeli campus.

2.9 Colour analysis

Colour is one of the most important quality attributes in the food and bioprocess industries. It can highly influence consumer's choice and preferences on that particular food. The colour on the food is governed by the chemical, biochemical, microbial and physical changes which occur during growth, maturation, postharvest handling and processing. To determine the quality attributes such as flavour and contents of pigments in food products, colour measurement is usually conducted as an indirect measure of that quality attributes due to the fact that it is simple, fast and correlates well with other physicochemical properties (Pathare *et al.*, 2012).

Visual colour is closely related to people's perceptions. Consumer perception or purchase decision is made even prior to tasting food which makes it an important attribute that should be emphasized on. Colour is defined as the impact of wavelength in the visual spectrum from 390 to 760 nanometers (nm) of the human retina. Hence, reflected light is perceived as colour. To be able to detect the colour, the human eye or instrument used must be capable of recognizing the object and translate the stimuli into a perception of colour (Veeramuthu, 2014).

In food, the most frequently used colour measurement is the CIE *L* a* b** colour space. This colour space is widely used due to its uniform colour distribution and its perception of colour is the closest to the human eyes (Markovic *et al.*, n.d.). Figure 2.5 shows the colourimeter that was used to conduct analysis that is found available at UMK Jeli campus.

2.10 Crackers and Sensory Testing

Sensory evaluation is a scientific disciplines that analyses and measures human response to the composition and nature of foods. During the sensory evaluation, crackers of each experimental type will be placed separately and labelled randomly with three-digit codes. Prior to each tasting a sample, lemon water can be served to neutralize their mouth feel to maintain comparable testing over a sequence of samples (Venkatachalam & Nagarajan, 2017). According to Kohajdova, Karovicova and Magala (2013), a glass of water can be provided to cleanse the palate between samples. The environment in which the sensory test is conducted should be carefully controlled, and samples must be prepared and presented in a uniform fashion so as not to influence panellists' perception of the food's quality (Krumbein, 2014).

Previous research conducted on cracker incorporated with pumpkin pomace showed that higher amounts of pumpkin pomace negative affected taste, odour, consistency and overall appearance of crackers (Kuchtova *et al.*, 2016). For crackers incorporated with pea flour, high level of pea flour (20% and 30%) showed significant reduction in taste, odour, and overall acceptance of final products (Zlatica *et al.*, 2013).

Traditional products incorporated with green leafy vegetables were found that products incorporated with 4% dehydrated greens were similar to control in texture, taste and overall quality. However, acceptability scores reduced with increasing concentration of greens (Gupta & Prakash, 2011). According to Akonor *et al.* (2017), cracker made from 55% high quality cassava flour, 5% prawn powder and 40% cassava starch were found to have high acceptability in terms of taste, crispiness, puffiness and overall acceptability.

2.11 Incorporation of Spinach into Food Product

In the market, there is limited development of food products that incorporate spinach powder because there is a lack of information. However, as people start to be aware of the nutritional benefits of spinach, they research on spinach powder and how incorporation of spinach powder can be done on a variety of food products.

According to Syuhairah *et al.* (2016), research has been done on the incorporation of vegetable in chicken sausage. The vegetables used in chicken sausages were capsicum, carrot, spinach, purple cabbage and grey oyster mushroom. The results show that samples with higher vegetable levels demonstrated significantly lower values in hardness and Warner-bratzler. Colour of sausages varied significantly among samples due to the differences in original colour of sausages. Among all the vegetables used, capsicum, carrot and oyster mushroom gave significant overall acceptability as compared to control. The finding from this research suggested that vegetables can be valuable to the modification of sausage formulation.

Noodles with incorporation of spinach puree is another research with incorporated spinach. The results show that spinach puree can be added successfully in wheat flour up to 40 g/100 g of wheat flour. Sensorial qualities with respect to colour, flavour, and texture were greatly improved with addition of spinach puree. Proximate composition results showed that increase in level of spinach puree in noodle, there was progressive increase in moisture, fibre and ash content whereas carbohydrate content decreased significantly as compared to control noodles (Shere *et al.*, 2018).

In addition, chapaties were also part of these research. Chapati is a flat unleavened, hot plate baked product prepared from whole wheat flour. Different concentrations of spinach paste was used and it was found that chemical changes and overall acceptability scores were found to be negatively correlated during storage due to decrease in overall acceptability. Significant correlations were observed between textural properties like hardness, springiness, stiffness and chewiness. Total carotenoids and total chlorophyll contents played significant effect and exhibit significant positive correlation with sensory scores during storage (Khan *et al.*, 2013).

2.12 Proximate Analysis

Proximate analysis is defined in the Concise Chemical and Technical Dictionary as the "determination of a group of closely related components together such as total protein, fat etc." (Hart & Fisher, 1971). It is a method which is used to determine the values of the macronutrients in food samples. Those values are determined during the production process and are being declared as nutritional facts which are commonly shown on the labels of the final food products. According to Food Act 1983 and Food Regulations 1985, it is mandatory for almost all food products to have standardized nutritional labels. The nutritional label serves as communication tool between food manufacturers and consumers to ensure that consumers are well aware of the nutritional composition of foods so that they can make informed and knowledgeable decisions about their diet. In addition, it can be used as a strategic tool to compete with competitors in terms of nutritional content such as low fat, higher fiber, fat free or many more.

Five constituents that are mandatory to be declared to consumers are protein, fat, moisture, ash and carbohydrates (Food Act Malaysia 1983). Due to the fact that the constituents are known as proximates, hence the process to determine the contents are known as proximate analysis (Buchi Labortechnik AG, 2017). These food components may be of interest in the food industry for product development, quality control or regulatory purposes (Thangaraj, 2015).

Association of Official Analytical Chemists (AOAC) is an association that publishes standardized, chemical analysis methods designed to increase accuracy in the results of chemical and microbiologic analyses. Official AOAC methods are often used in conducting proximate analysis as the procedure is highly reliable. The methods are used by government agencies concerned with the analysis of fertilizers, foods. Feeds, pesticides, drugs, cosmetics, and other materials related to agriculture, health and welfare, and the environment. Besides that, AOAC methods are used by industry to check compliance of their products (Baur & Ensminger, 1977).

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Raw Materials and Chemicals

This study was conducted at the Food Laboratory of Faculty of Agro-Based Industry, Biology Laboratory and Husbandry Laboratory at University Malaysia Kelantan (UMK) Jeli campus which is located at Kelantan, Malaysia. Raw materials that were used in this study were green spinach, red spinach, wheat flour, sugar, salt and butter which were purchased from the local market. Chemicals used were 0.1 M hydrochloric acid, sulphuric acid, 4% boric acid, Kjedahl tablet, 40% sodium hydroxide, methyl red, bromocresol green and hexane.

3.1.2 Equipment

The equipment used in this study were colourimeter (Konica Minolta CR-400, Minolta model 3500, Minolta Camera Co., Ltd., Osaka, Japan), Texture Analyzer TA-XT2 (Brookfield CT3, USA), dehydrator machine, oven, electronic balance, hand mixer, measuring cylinder, spoon, cutting board, tray, wire rack, plastic bowl, rolling pin, flour siever and plastic glove, which can be found in Food Laboratory of Faculty of Agro-Based Industry. Other equipment such as crucible, muffle furnace, burette, conical flask, Buchner funnel, beaker, filter paper, Kjedahl auto distillation analyzer (Kjeltec 8200) and weighing dish can be found at Husbandry Laboratory. Additional equipment such as sealed plastic bag and cookie cutter were purchased from the local market.

3.2 Methodology

3.2.1 Preparation of Green and Red Spinach Powder

The total ingredients used were 360 g of commercial all-purpose wheat flour, 140 g of spinach powder, 50 g of sugar, 30 g of salt, 30 g of butter and 225 ml of water for making the dough of spinach cracker for each species of spinach. For the preparation of green and red spinach powder, the leaves and stems of the vegetables were rinsed under a running water to remove dirt and soil while the roots of the vegetables were removed because the roots are inedible. The vegetables were patted dry with a clean towel to remove excess water. The vegetables were cut into small pieces and dried in dehydrator for 24 hours at 60°C. The weight of the vegetables were measured before drying to obtain the initial weight and measured once again after drying to obtain the final weight. The drying and weighing process were repeated until a constant weigh were obtained and the moisture content of the vegetable was less than 3%. The dried spinach was ground and sieved to obtain the powder form. The moisture content was determined using the formula as shown below:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)- final weight (g)}}{\text{initial weight}}\ x\ 100\% \tag{3.1}$$

3.2.2 Formulation of Green and Red Spinach Cracker

Cracker samples were prepared in a straight dough process according to the recipe which was 100 g of commercial all-purpose wheat flour, 10 g of sugar, 1 g of salt, 6 g of butter and 45 ml of water. Spinach cracker was made at different percentage of wheat flour and spinach powder as shown in Table 3.1. All of the ingredients were mixed using an electrical hand mixer to form a cohesive dough. The dough was then shaped using a cookie cutter of square shape with dimensions of 3 cm x 3 cm. The thickness of the dough was measured by using rolling pin and ruler to have thickness of 0.3 cm. The crackers were baked in an oven at 180°C for 15 minutes. Baked crackers were allowed to cool at room temperature. All the steps were done using green spinach first then followed by red spinach using the same steps. Dependent variable is the amount by weight of spinach powder while amount of wheat flour by weight is independent variable. Commercialized vegetable cracker was used as positive control while standard cracker formulation with 0% of spinach powder was used as negative control of the study (refer to Table 3.2). Figure 3.1 and Figure 3.2 show the actual appearance of the samples for green spinach cracker and red spinach cracker, respectively.

Table 3.1: Flour substitution with spinach powder

Ingredients	Flour substitution			
	0%	5%	10%	15%
Wheat flour (g)	100	95	90	85
Spinach powder (g)	0	5	10	15
Sugar (g)	10	10	10	10
Salt (g)	1	1	1	1
Butter (g)	6	6	6	6
Water (mL)	45	45	45	45
Total (g)	162	162	162	162

Table 3.2: Ingredients for standard cracker formulation (negative control)

Ingredients	Weight (g)	Percentage (%)
Wheat flour	100	62
Spinach powder	0	0
Sugar	10	6
Salt	1	1
Butter	6	3
Water	45	28
Total	162	100

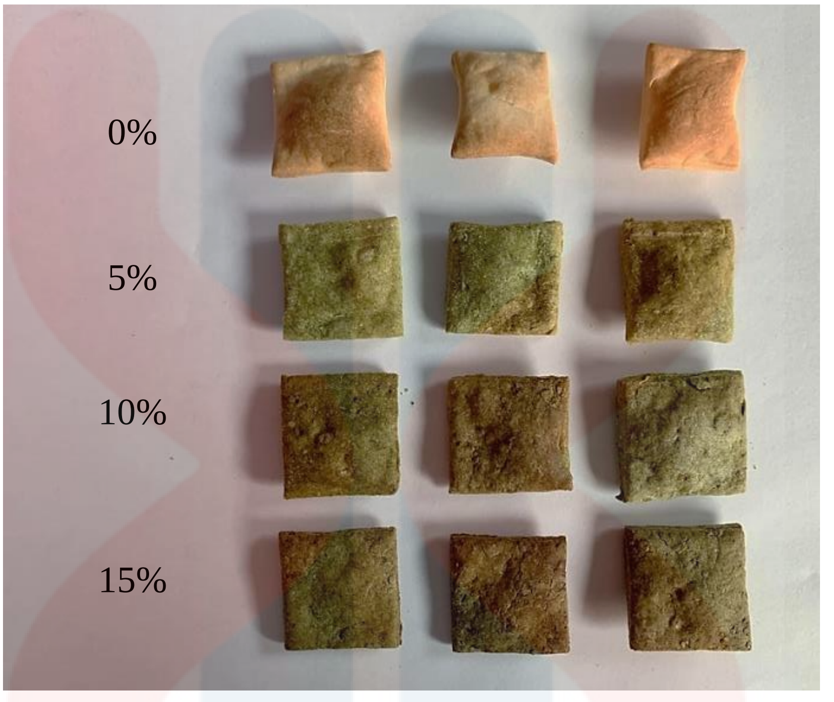

Figure 3.1: Green spinach cracker with different percentages of flour substitution

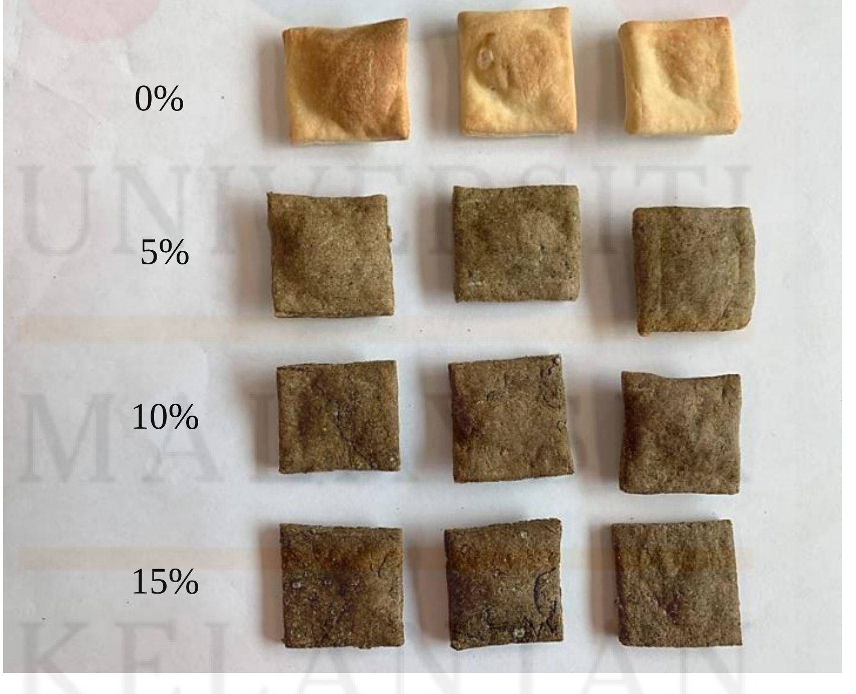

Figure 3.2: Red spinach cracker with different percentages of flour substitution

3.2.3 Determination of Colour Attributes of Green and Red Spinach Cracker

Determination of colour attributes of spinach cracker was carried out by using colourimeter (Konica Minolta CR-400). The instrument was first calibrated with white CR-A47Y standard tile. All samples of the spinach cracker with different percentage were tested during four weeks of storage at 0 days, 7 days, 14 days, 21 days and 28 days. The results of colour attributes of the spinach cracker were conveyed in the CIE *L* a* b** colour space to determine the lightness (*L**), redness (*a**) and yellowness (*b**). All data was recorded in triplicate.

3.2.4 Determination of Texture Attributes of Green and Red Spinach Cracker

Determination of texture attributes of spinach crackers was carried out by using Texture Analyzer (Brookfield, CT3, USA) which consisted of TPA test type, probe TA7, trigger load 5 g in a speed of 10.00 mm/s. All samples of the spinach cracker with different percentage were tested during four weeks of storage at 0 days, 7 days, 14 days, 21 days and 28 days. The texture analyzer was focusing on hardness and fracturability of the spinach cracker. All the data was recorded in triplicate.

3.2.5 Shelf Life of Green and Red Spinach Cracker during Storage

Shelf life of green and red spinach cracker was determined by studying its physical properties which were texture and colour analysis after different period of storage. After the crackers have been baked and allowed to cool at room temperature, texture profile and colour analysis will be carried out. The data was used as the control experiment. The crackers were placed in a sealed plastic bag and analysis on the texture and colour was carried out up to four weeks of storage at 0 day, 7 days, 14 days, 21 days and 28 days. After four weeks, all the data collected was analyzed to determine the changes in its physical properties during storage.

3.2.6 Proximate Analysis

Proximate composition analysis was conducted to determine moisture, protein, fat, ash and carbohydrate content of the spinach cracker according to Association of Official Analytical Chemists (AOAC) method (A.O.A.C, 1990). Moisture content was determined after drying the samples in 100°C for 24 hours. The protein and lipid content were determined by Kjedahl and filtration method, respectively while the ash content was calculated after the sample was fully dried in the muffle furnace at 600°C for 6 hours. The carbohydrate content was calculated by subtracting the percentage of moisture, protein, fat and ash content.

3.2.6.1 Moisture Content

All samples were taken to perform moisture content using AOAC method. Triplicate samples of spinach cracker were weighed about 1 g each before drying to obtain the initial weight and were dried in conventional oven at 100°C for 24 hours. The drying and weighing process were repeated until constant weigh was obtained. The moisture content was determined using the formula as shown below:

$$\text{Moisture (\%)} = \frac{\text{initial weight (g)- final weight (g)}}{\text{initial weight}}\ x\ 100\% \qquad (3.2)$$

3.2.6.2 Protein Content

Protein composition in spinach cracker was determined at laboratory level by using chemical analysis method known as Kjedahl Nitrogen Method. The 1 g of spinach cracker sample was digested using 12 ml of concentrated sulphuric acid (1 mg/L) with 1 tablet of Kjeldahl tablet as the catalyst under direct heating. Heat side shields were attached to the tube rack. During the digestion, fume manifold was placed tightly on the tubes and the H_2O aspirator was turned on completely. Digestion converts any nitrogen in the food in the form of nitrates or nitrile into ammonium and other organic matter to CO_2 and H_2O. The mixture was boiled and digested for 60 minutes at 420°C.

The digestion block was then turned off. The rack of tubes wilt exhaust still was removed and allowed to cool for 10 to 20 minutes. The system was cleaned by using 80 ml of distilled water into each tube and followed by sample distillation. Each sample was distillated by using Kjedahl auto distillation analyser. 50 ml of 40% NaOH was added into the alkali tank of distillation unit. 30 ml of solution containing 4% boric acid, 1.75 ml of methyl red and 2.5 ml of bromocresol green was placed in the receiving flask.

The solution was made alkaline by addition of sodium hydroxide (0.1 mg/mL) to convert ammonium sulphate to ammonium gas. The solution in the receiving flask after distillation was titrated using 0.1 M hydrochloric acid. The protein content was determined using the formula as shown below:

$$\text{Nitrogen (\%)} = \frac{[\text{sample (ml)- blank (ml)}] \times \text{n} \times 14.01}{\text{sample (mg)}} \; x\ 100\% \tag{3.3}$$

Where n is the molarity of HCl used for titration and 14.01 is the molecular weight of nitrogen. A blank sample was allowed to run at the same time. Once the nitrogen content is determined, it was converted to percentage of protein content using the appropriate conversion factor of 6.25. The procedure was repeated for each formulation prepared.

$$\text{Protein (\%)} = \text{N (\%)} \text{ x } 6.25 \tag{3.4}$$

3.2.6.3 Fat Content

Total of 5 g of dried sample undergo fat extraction using 40 ml of hexane and was homogenized at 2000 rpm for 2 minutes. After homogenization, 20 ml of distilled water was added to separate hexane. The sample was filtered through a Buchner funnel to remove solid sample. The filtrate was put into a separating funnel and allowed to form a layer. The bottom layer was put into a weighing dish which weight was recorded beforehand and put under fume chamber to allow the hexane to evaporate. The weighing dish with excess fat was weighed and recorded. The fat content was determined using the formula as shown below:

$$\text{Fat (\%)} = \frac{[(\text{weighing dish} + \text{fat})(\text{g}) - (\text{weighing dish})(\text{g})]}{\text{initial sample weight (g)}} \; X\ 100\% \tag{3.5}$$

Weight of initial sample = (Weight of beaker + Filter paper + Dried sample) – (Weight of beaker + Filter paper)

(3.6)

3.2.6.4 Ash Content

Total of 0.5 g of dried sample was placed in a constant weight porcelain crucible with cover and was placed in a muffle furnace. The weight of dried samples and crucible with lid was measured. The samples were hot-plated at 60°C for 1 to 2 hours. After this procedure, the sample turned into black colour. The samples were ignited at

600°C for 6 hours. After ignition, the crucibles were placed in the oven to bring down the temperature for about 30 minutes and were cooled to room temperature for another 30 minutes. The final sample was whitish in colour. The samples were weighed to obtain a constant weight. The ash content was determined using the formula as shown below:

$$\text{Ash (\%)} = \frac{[\text{weight of crucible} + \text{ash}](\text{g}) - \text{weight of crucible (g)}}{\text{weight of sample (g)}} \; x\ 100\% \qquad (3.7)$$

3.2.6.5 Carbohydrate Content

Available carbohydrate content was calculated by subtracting the sum of moisture, protein, lipid, and ash from 100%. The carbohydrate content was determined using the formula as shown below:

$$\text{Carbohydrate (\%)} = 100\% - [\text{Moisture (\%)} + \text{Protein (\%)} + \text{Lipid (\%)} + \text{Ash (\%)}] \qquad (3.8)$$

3.2.7 Sensory Evaluation

Sensory evaluation is a common tool to evaluate the general acceptability and quality attributes of the products. Sensory evaluation was carried out with 40 panelists which involve undergraduate students from University Malaysia Kelantan (UMK) Jeli campus. The testing was done in Food Laboratory of Faculty of Agro-Based Industry in UMK Jeli campus. Each panelist was served with one sample of negative control, three samples of green spinach crackers and three samples of red spinach crackers. The coded samples were served in a clean tray with adequate florescent lights. Sample presentation to the panelists was at random and one at a time. As the test was conducted by using taste buds, plain water was provided for mouth rinsing between samples to avoid any confusion regarding the taste. The parameters evaluated were colour, flavour, taste, crispiness, hardness, and overall acceptance. This test was done in order to analyze consumer acceptance on spinach cracker compared to commercial vegetable cracker. The evaluation forms were as listed in (Appendix A).

Evaluation of the attributes of spinach cracker was evaluated by using 7-points hedonic scale depicted on the score sheet as shown in Table 3.3. The data was recorded from the panelists and was analyzed using Statistical Package of Social Sciences (SPSS) version 20.0 using One-Way-Analysis of Variance (ANOVA).

Table 3.3: Hedonic scale rating for sensory evaluation

Likeness	Sensory Score
Like very much	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike very much	1

3.2.8 Statistical analysis

Proximate analysis and shelf life study of cracker were carried out in triplicate and mean values with standard deviation (SD) were computed by using Microsoft Excel, 2010. Microsoft Excel was used as database to optimize the data collected from various tests that were conducted. The data obtained from sensory evaluation was recorded from the panelists and was analyzed using Statistical Package of Social Sciences (SPSS) version 20.0 using One-Way-Analysis of Variance (ANOVA). The difference among means was analyzed using a Tukey HSD test ($p < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Shelf Life of Spinach Cracker during Four Weeks of Storage

4.1.1 Colour Analysis

The shelf life of spinach cracker was determined by studying its physical properties which were colour analysis and texture profiling after different period of storage up to 4 weeks. Changes of colour for cracker incorporated with spinach powder are shown by the chromatic parameters ($L*$, $a*$, $b*$) where $L*$ is for lightness, $a*$ is for redness whereas $b*$ is for yellowness (refer Figure 4.1, Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5 and Figure 4.6).

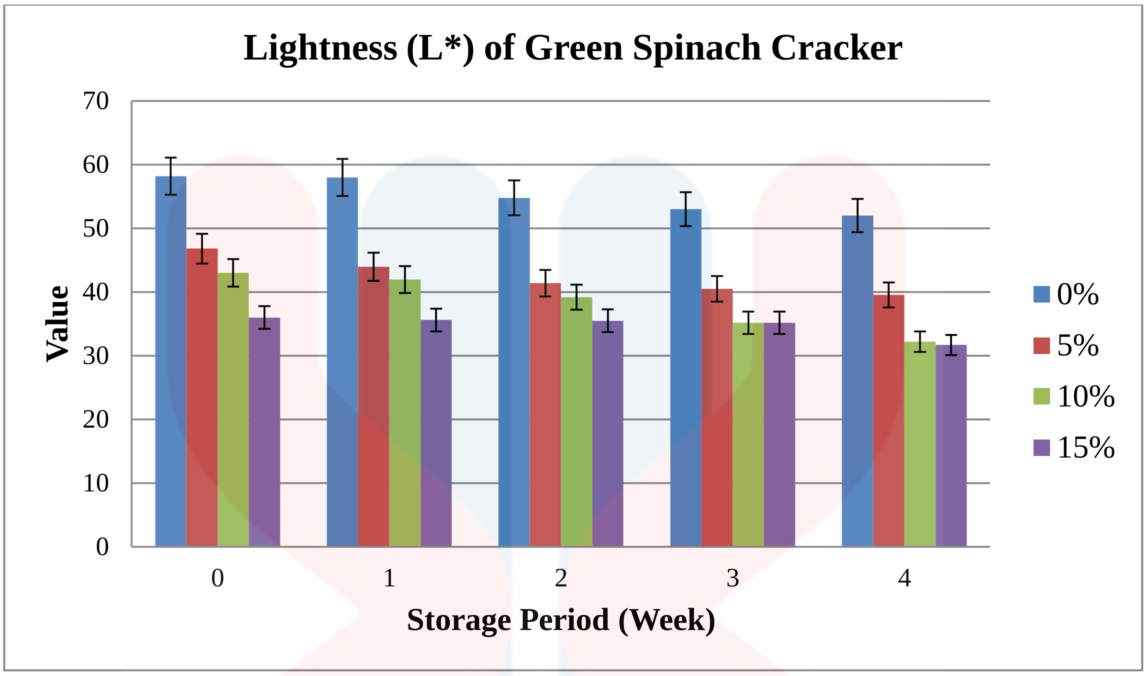

Figure 4.1: Value (mean ± SE) for lightness (*L**) of green spinach cracker of different percentages of spinach powder substitution during four weeks of storage

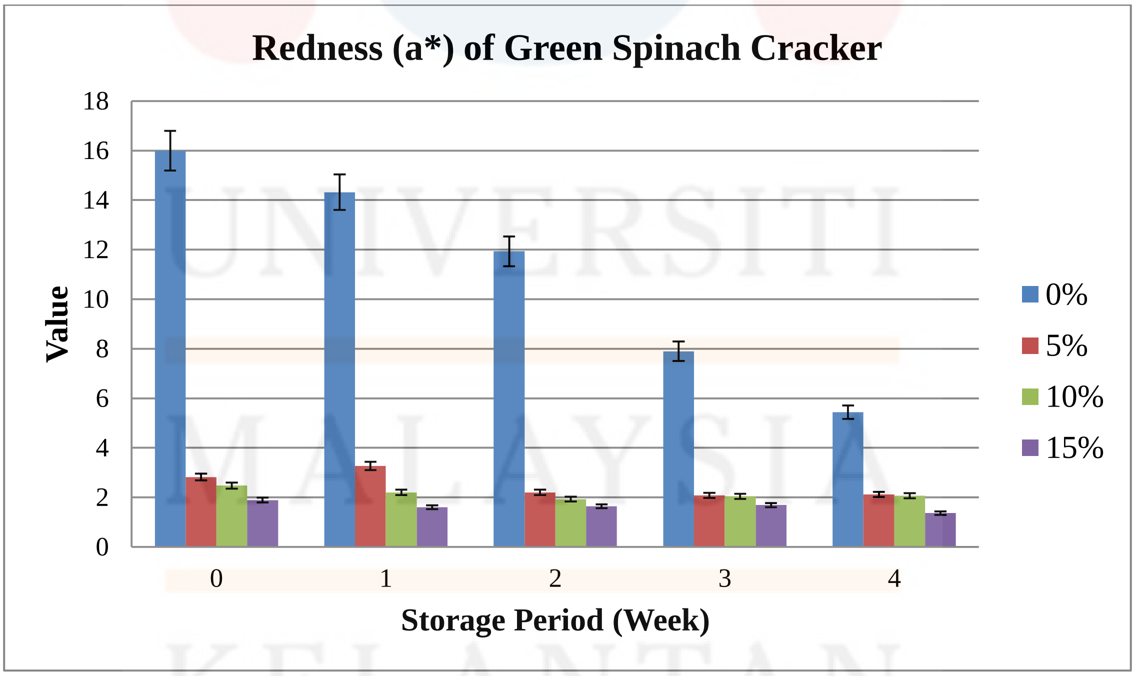

Figure 4.2: Value (mean ± SE) for redness (*a**) of green spinach cracker of different percentages of spinach powder substitution during four weeks of storage

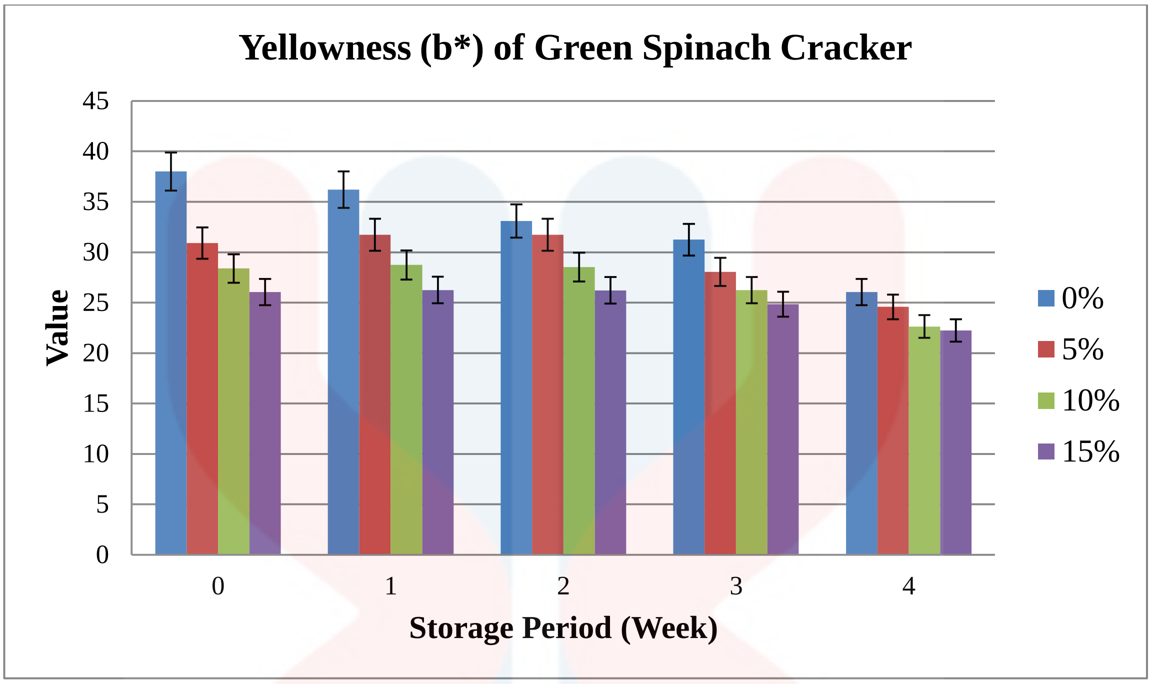

Figure 4.3: Value (mean ± SE) for yellowness (*b**) of green spinach cracker of different percentages of spinach powder substitution during four weeks of storage

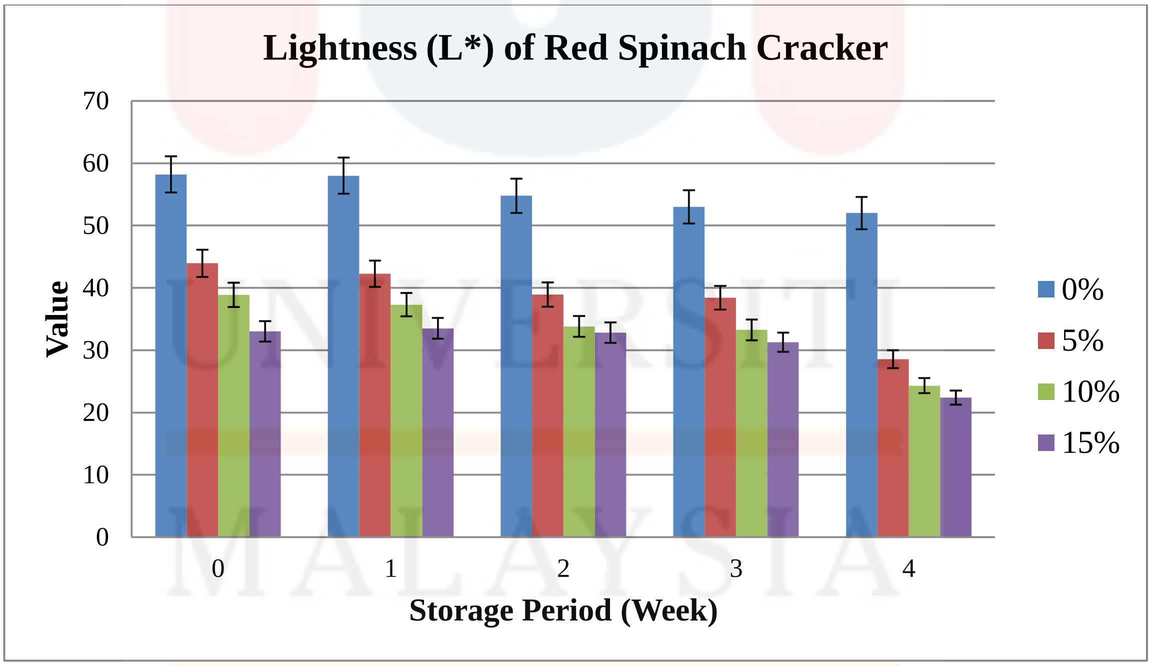

Figure 4.4: Value (mean ± SE) for lightness (*L**) of red spinach cracker of different percentages of spinach powder substitution during four weeks of storage

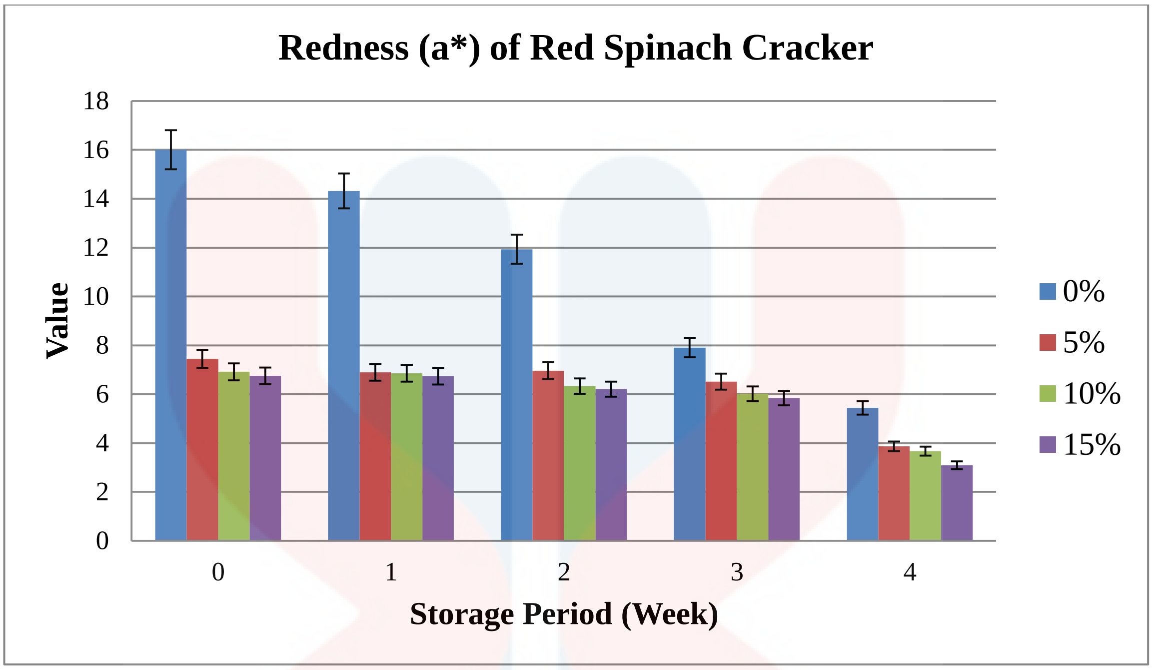

Figure 4.5: Value (mean ± SE) for redness (*a**) of red spinach cracker of different percentages of spinach powder substitution during four weeks of storage

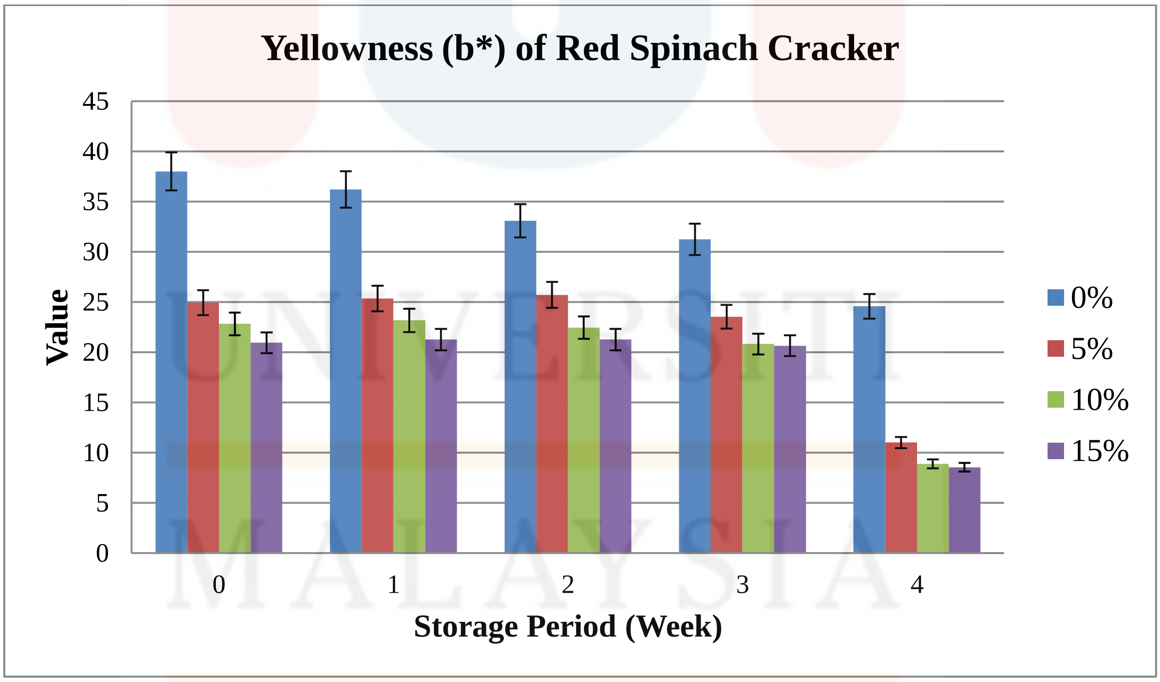

Figure 4.6: Value (mean ± SE) for yellowness (*b**) of red spinach cracker of different percentages of spinach powder substitution during four weeks of storage

Appearance and colour of foods are prime factors commonly used in judging food products quality and become consumer's first impression about the foods. Vision has the advantage over the other senses in that an observer's appreciation of an object's appearance can be recorded pictorially (Piggott, 1988). Most used colour spaces in the measuring of colour in food is the *L* a* b** colour space due its uniform colour distribution (Markovic, Ilic, Markovic *et al.*, 2013).

Lightness (*L**) value ranges from 0 to 100 indicating luminance or lightness. It is the perception by which white objects are distinguished from grey whereas light coloured objects from dark coloured. It was observed that the colour of spinach cracker had changed from brighter colour to brownish colour as higher level of spinach powder being substituted into the cracker. Food products with higher concentrations of sugar will tend to react with amino acid or become parts of the protein chains to form dark compounds (Sabeera, Baba, Nazir *et al.*, 2016).

From the results obtained, intensity of lightness showed a decreasing progression during four weeks of storage (refer Figure 4.1 & Figure 4.4). After four weeks, changes in *L** value for control was ΔL^*= -6.2. As for green spinach cracker, changes in *L** value was ΔL^*= -7.28 for 5% flour substitution, ΔL^*= -10.78 for 10% flour substitution, and ΔL^*= -4.30 for 15% flour substitution. While for red spinach cracker, changes in *L** value was ΔL^*= -15.39 for 5% flour substitution, ΔL^*= -14.58 for 10% flour substitution, and ΔL^*= -10.64 for 15% flour substitution. These changes might be occurred due to non-enzymatic browning or Maillard reaction of spinach. Maillard reaction creates brown pigments in cooked food by rearranging amino acids and certain simple sugars in collections of rings that reflect light in a way to give brown colour (Nathan, 2013). This reaction which caused browning may be due to browning of spinach carbohydrates during baking process and also during storage for extended period of time (Galla *et al.*, 2017).

Redness of spinach cracker is shown by *a** value. From the results obtained, intensity of redness of spinach cracker showed a decreasing progression during four weeks of storage (refer Figure 4.2 & Figure 4.5). After four weeks, changes in *a** value for control was Δa^*= -10.56. As for green spinach cracker, changes in *a** value was Δa^*= -0.70 for 5% flour substitution, Δa^*= -0.40 for 10% flour substitution, and Δa^*= -0.53 for 15% flour substitution. While for red spinach cracker, changes in *a** value was Δa^*= -3.58 for 5% flour substitution, Δa^*= -3.25 for 10% flour substitution, and Δa^*= -3.66 for 15% flour substitution. Within the storage time of four weeks, *a** value of spinach cracker was observed to decrease in slow rate.

Red spinach cracker generally had higher *a** value compared to green spinach cracker due to the presence of red pigment called betacyanins in the stem of red spinach which is responsible for the red colour of stems and leaves (Amritha, 2018). Natural pigments such as betacyanins have been commercially used as food colorants (Von Elbe, Schwartz & Attoe, 1983). It was observed that cracker with lower percentage of flour substitution had higher *a** value compared with cracker with higher flour substitution. The change of *a** value was attributed by the occurrence of Maillard reactions when being exposed at high temperature during baking process (Jagadeesh, Basavaraj, Reddy & Swamy, 2007). However, according to Krokida, Oreopoulou, Maroulis and Marinos-Kouris (2001), redness in food is an undesirable quality factor especially for fried foods as it indicates increased crust development which can results in lower acceptability of consumers.

Based on Figure 4.3 and Figure 4.6, the yellowness (b^*) value of spinach cracker showed a decreasing trend in increasing level of spinach powder in the cracker. Intensity of b^* value showed a decreasing progression during four weeks of storage. After four weeks, changes in b^* value for control was $\Delta b^* = -11.94$. As for green spinach cracker, changes in b^* value was Δb^* *= -6.34 for 5% flour substitution, $\Delta b^* = -5.75$ for 10% flour substitution, and $\Delta b^* = -3.80$ for 15% flour substitution. While for red spinach cracker, changes in b^* value was $\Delta b^* = -13.93$ for 5% flour substitution, $\Delta b^* = -13.93$ for 10% flour substitution, and $\Delta b^* = -12.39$ for 15% flour substitution.

The decrease in trend of b^* value for spinach cracker can be due to the degradation of carotenoids. Spinach contains few coloured pigments which fall into two categories which are chlorophylls and carotenoids. Chlorophylls high conjugated compounds capture light energy used in photosynthesis while carotenoids are part of a larger collection of plant-derived compounds known as terpenes (Pavia, Lampman, Kriz & Engel, 1999). Spinach was found to contain different carotenoids such as lutein and beta-carotene (Riso, Brusamolino, Scalfi & Porrini, 2004). Carotenoids have a double bond in carbon chain that make it capable to certain reaction during food storage and processing that can cause degradation of colour such as oxidation and isomerization. In addition, occurrence of non-enzymatic browning reactions that took place together with oxidation and isomerization of beta-carotene change the colour parameters of cracker (Sabeera *et al.*, 2016).

Spinach powder can be used as natural food dye due to its ability to retain its natural colour after dehydration and grinding of spinach into powder. According to Galla *et al.* (2017), research study was done on dehydrated spinach and it was found that colour values of dehydrated spinach showed greenness indicated by negative value for a^* (-3.36). However, biscuits incorporated with dehydrated spinach could not retain the greenness during baking due to the browning of spinach carbohydrates.

4.1.2 Texture Profile Analysis

Texture profile analysis (TPA) was conducted by focusing on two parameters which were hardness and fracturability of spinach cracker during four weeks of storage period (refer Figure 4.7, Figure 4.8, Figure 4.9, Figure 4.10).

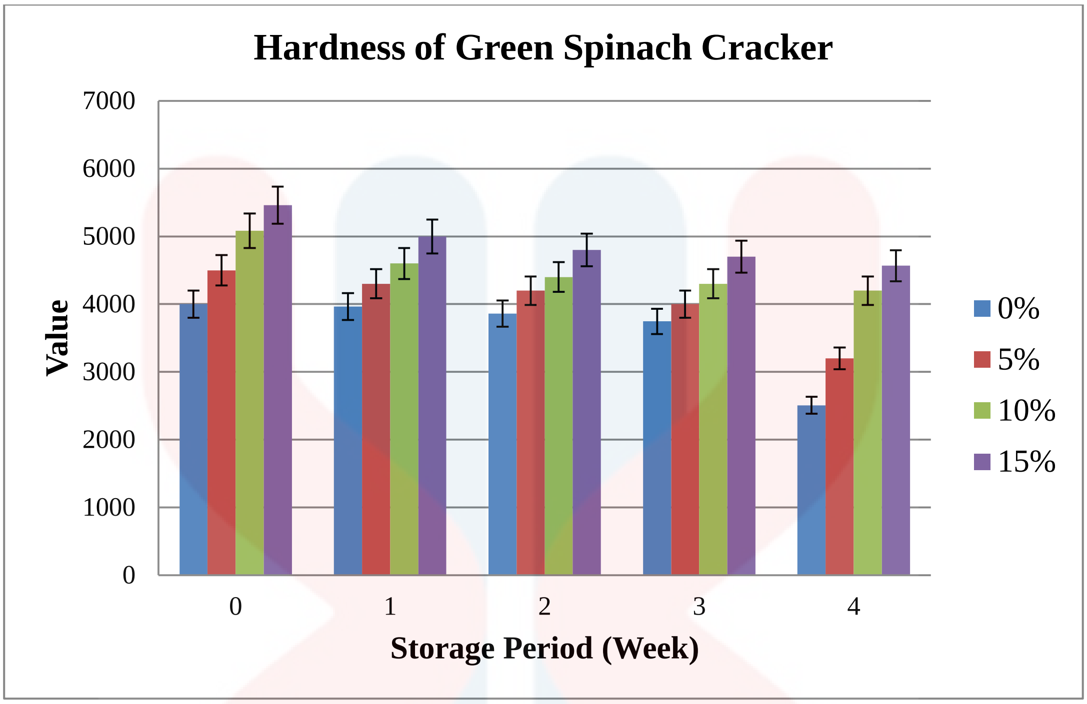

Figure 4.7: Value (mean ± SE) of hardness attribute of green spinach cracker of different percentages of spinach powder substitution during four weeks of storage

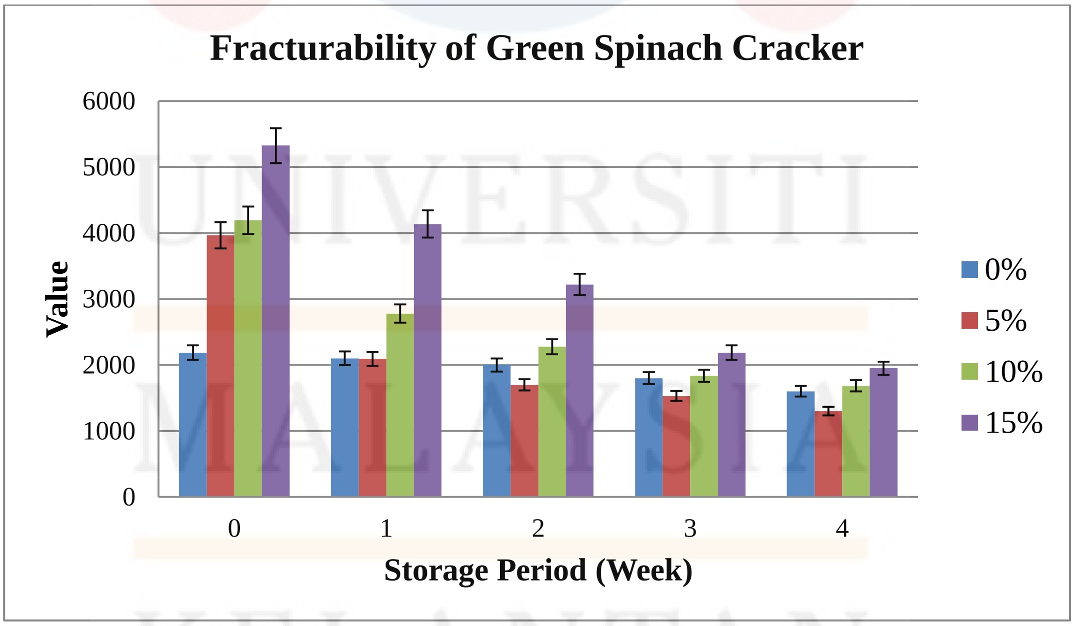

Figure 4.8: Value (mean ± SE) of fracturability attribute of green spinach cracker of different percentages of spinach powder substitution during four weeks of storage

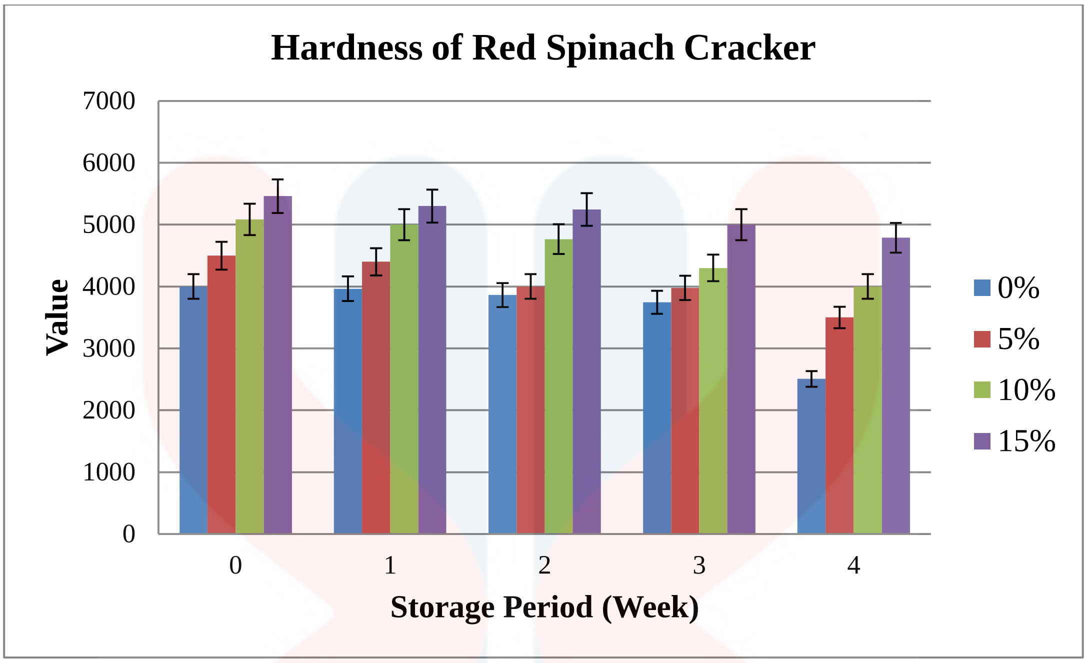

Figure 4.9: Value (mean ± SE) of hardness attribute of red spinach cracker of different percentages of spinach powder substitution during four weeks of storage

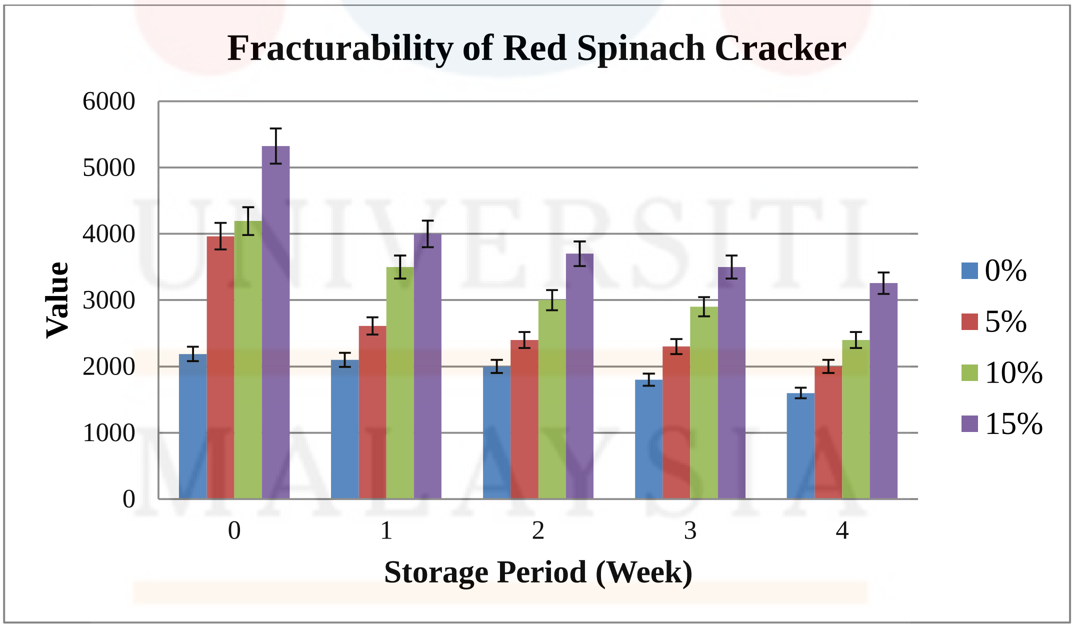

Figure 4.10: Value (mean ± SE) of fracturability attribute of red spinach cracker of different percentages of spinach powder substitution during four weeks of storage

Texture profiling was conducted based on two main texture criteria; hardness and fracturability. Each profiling proposes different meanings whereby hardness relates to the force applied by the molar teeth to compress the food (for solids) or between the tongue and palate (for semi-solids) to a given deformation or penetration. On the other hand, fracturability relates to tendency to fracture, crumble, crack or fall upon the application of a relatively small amount of force or impact where horizontal force with which the fragments move away from the point where the vertical force is applied. Fracturability is the result of a high degree of hardness and low degree of adhesiveness (Piggott, 1988).

Hardness is a mechanical textural attribute relating to the force required to compress the sample (Di Monaco, Cavella & Masi, 2007). Based on Figure 4.7 and Figure 4.9, the hardness of spinach cracker showed an increasing trend (4000-5461) as spinach flour substitution increased. The highest value for the hardness attribute was spinach cracker with 15% flour substitution for both green spinach and red spinach cracker. A similar trend was noticed in cookies where hardness increased with increased addition of dehydrated *Murraya koenigii* powder. The higher levels of spinach powder might have added higher fibre resulting in lower binding of carbohydrates and further lesser breaking strengths (Drisya *et al.,* 2015).

Fracturability is the ability to break food into pieces when it is bitten using incisors (Paula & Conti-Silva, 2014). The results of fracturability showed similar trend where there was an increasing trend when concentration of spinach powder increased. Spinach cracker with 15% flour substitution had the highest results which were 5323 g for green spinach cracker and 5350 g for red spinach cracker.

Decreasing trend of hardness and fracturability of spinach cracker during storage can be further supported by moisture content analysis for proximate analysis testing where spinach cracker with 15% flour substitution had the least amount of moisture content. According to Mihiranie, Jayasundera and Perera (2017), moisture content is a critical factor which can be affected by sensory attributes such as texture and crispness where moisture content was found to be increasing during storage. This can be concluded that amount of moisture content can directly influence its texture properties. As storage time increasing, results showed a decreasing trend in hardness and fracturability for each of the spinach cracker. This can be due to the increase in moisture content in cracker after an extended period of storage. This observation can be due to the storage method of cracker. In this research, the cracker was placed in a sealed plastic bag and was taken out every week for testing up to four weeks of storage. This allowed the absorption of moisture and air from the atmosphere when the plastic bag containing the crackers were being opened. Therefore, proper container is recommended to ensure that the texture of cracker can be preserved for an extended period of time.

Starch is the main storage of carbohydrate in the early stages of fruit development that degraded with the onset of ripening of fruit (Sharma & Rao, 2013). In baking, little to no presence of gluten is required as gluten is used in leading to tougher products without shortness. The presence of starch in spinach powder is useful in gelatinization and pasting characteristics of cracker which can improve the quality and texture of the cracker. The starch granule swells and absorbs water to become functional (Saeleaw & Schleining, n. d.) When spinach powder was incorporated into the dough, the dough became thick and viscous. As amount of spinach powder increased, the texture of dough became thicker and more viscous.

This can be supported by the findings in research on chapatti premixes where it was observed that the time for dough development increased and stability decreased which may be attributed to the decrease in strength of dough due to increase in fibre content owning to disruption of continuity of gluten (Khan, Mahesh, Semwal & Sharma, 2013). According to Gomez *et al.* (2003), time required for dough development increases as a consequent of fibre addition to wheat flour. Similar finding was found by Dachana *et al.* (2010) where it was observed in preparation of cookies that increasing amount of dehydrated moringa leaves in wheat flour will cause an increase in time for dough development. Therefore, hardness and fracturability attributes are mainly affected by the presence of starch in cracker.

4.2 Proximate Analysis

All samples were analysed by using proximate analysis to determine moisture, protein, fat, ash and carbohydrate of the spinach cracker according to Association of Official Analytical Chemists (AOAC) method (A.O.A.C, 1990).

4.2.1 Moisture Content

Total of 1 g of each cracker sample was dried in a conventional oven with temperature 100°C for 24 hours. The drying and weighing process was repeated until constant weigh was obtained. The green spinach cracker with 5% flour substitution had the most moisture content (10.76 ± 1.248) while green spinach cracker with 15% flour substitution had the least moisture content (3.35 ± 0.832) out of all samples. Negative control which consisted of 0% flour substitution had higher moisture content (9.04 ± 3.812) than positive control (4.74 ± 3.140) (refer Figure 4.11).

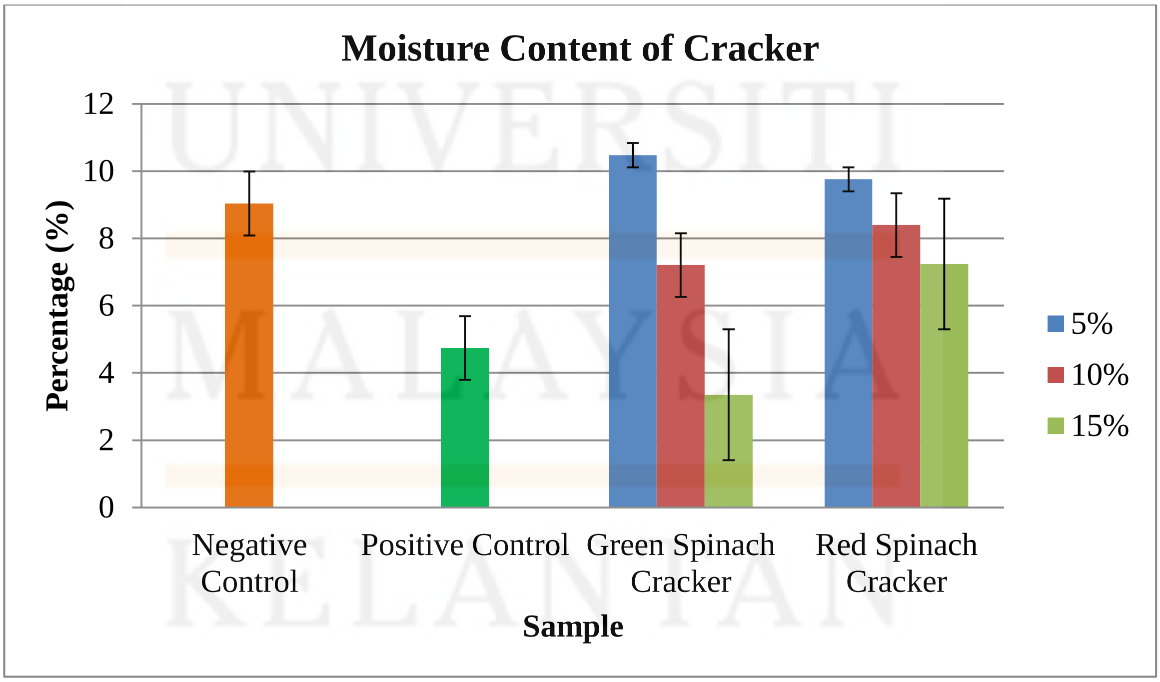

Figure 4.11: Moisture content of cracker (mean ± SE)

For green spinach cracker, 5% flour substitution had the most moisture content (10.48 ± 1.555), 10% (7.21 ± 0.854) and followed by 15% (3.35 ± 0.832). Similar to red spinach cracker, 5% flour substitution had the most moisture content (9.76 ± 1.114), 10% (8.40 ± 3.175) and followed by 15% (7.24 ± 0.367). The moisture content of spinach cracker showed a decreasing progression with 5% being the highest, followed by 10% and lastly 15%. It can be concluded that as the percentage of flour substitution increases, the moisture content of the sample decreases. By comparing green and red spinach cracker, red spinach cracker generally had higher moisture content compared to green spinach cracker (refer Figure 4.11).

The decrease in moisture content of spinach cracker can be supported by the research findings on chapatti premixes where it was observed that water absorption increased with the increase in spinach powder concentration. The increase in water absorption may be attributed to the presence of hydroxyl groups in fibre structure which allows more water interaction through hydrogen bonding (Khan, Mahesh, Semwal & Sharma, 2013). The increasing water absorption capacity of spinach powder will decrease the overall moisture content of spinach cracker.

Similar trends and values were observed by Olubukola *et al.* (2017) for vegetable enriched with *Chinchin* where there was a reduction in moisture content as the quantity of vegetable incorporation increased. Besides that, decrease in moisture content was also reported by Adeyeye and Akingbala (2014) for cookies produced from sweet potato-maize flour blends (5.0-6.1%). In addition, similar results for soybean fortified Tapioca was also reported (Kolapo & Sanni, 2005). According to Sanni, Adebowale and Tafa (2006), lower moisture content of a product is to be stored better as it has better shelf stability. Hence, low moisture content ensures higher shelf stability of dried product.

4.2.2 Fat Content

The fat content of spinach cracker showed an increasing progression for both green and red spinach cracker with 15% flour substitution being the highest, followed by 10% and lastly 5%. Positive control had the highest fat content (11.53 ± 1.33) while negative control had the least fat content (5.87 ± 3.06) out of all samples tested.

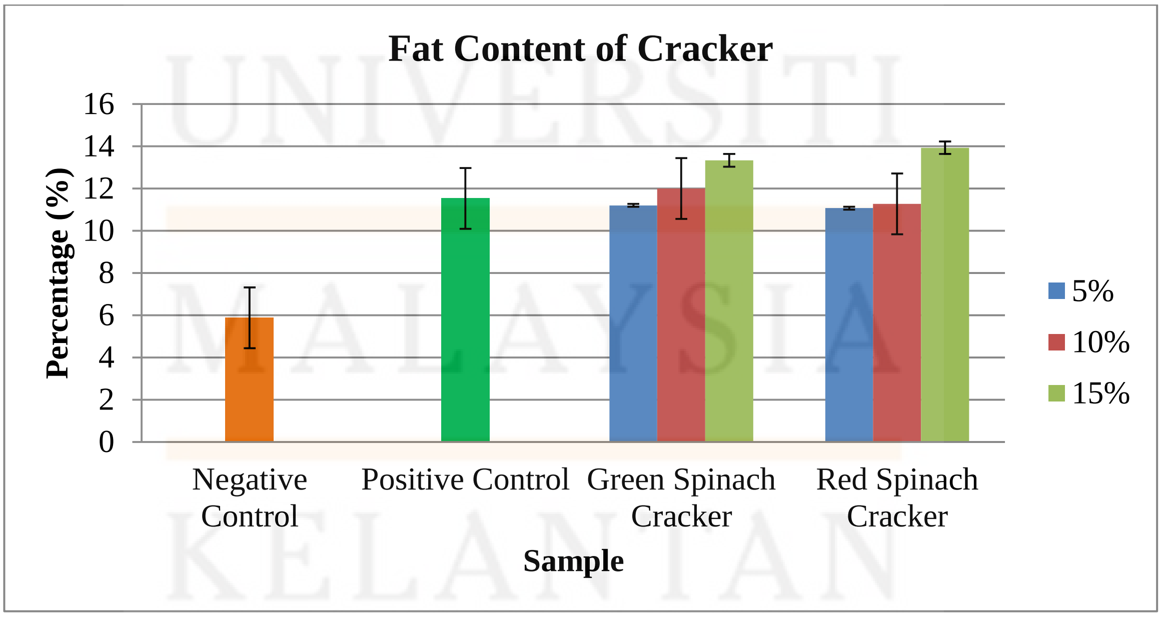

Figure 4.12: Fat content of cracker (mean ± SE)

For green spinach cracker, 15% flour substitution had the highest fat content (13.33 ± 8.57), 10% (12 ± 3.33) and followed by 5% (11.2 ± 3.30). Similar to red spinach cracker, 15% flour substitution had the highest fat content (13.93 ± 1.10), 10% (11.27 ± 0.61) and followed by 5% (11.07 ± 2.37). It can be concluded that as the percentage of flour substitution increases, the fat content of the sample increases (refer Figure 4.12).

Increasing trend in fat content in this research can be supported by research done by Olubukola *et al.* (2017) for *Chinchin* enriched with *Ugu* and Indian Spinach Vegetables where fat content was found to be increasing as the level of incorporated vegetable increased. In addition, similar trend was also reported for the development of snack crackers incorporated with defatted coconut flour (Mihiranie, Jayasundera & Perera, 2017). The increase of fat content may be due to high oil absorption capacity of vegetable enriched flour. According to Fasasi (2009), it was reported that low fat content in dry product will help in increasing the shelf life of food product by decreasing the chances of rancidity and also contribute to low energy value of food whereas high fat content product will have high energy value and promotes lipid oxidation.

4.2.3 Ash Content

The ash content of spinach cracker showed an increasing progression for both green and red spinach cracker with 15% flour substitution being the highest, followed by 10% and lastly 5%. Positive control was found to have the same amount of ash content with 15% flour substitution of both spinach cracker while negative control had the least ash content (1.03 ± 0.88) out of all samples tested.

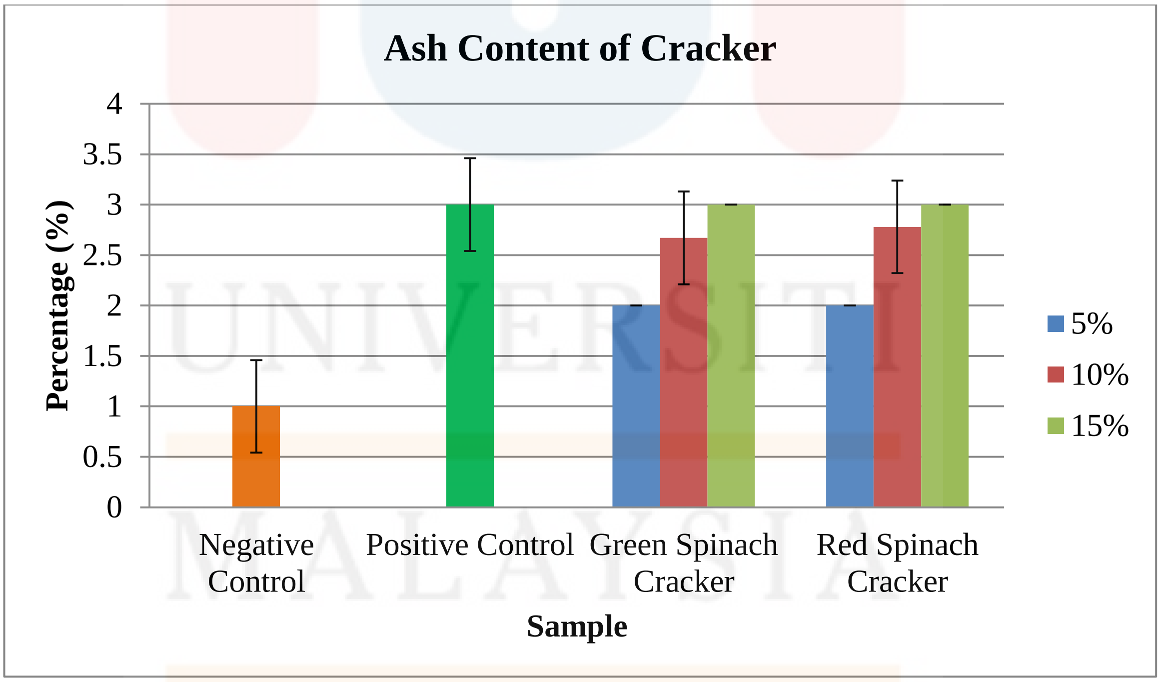

Figure 4.13: Ash content of cracker (mean ± SE)

For green spinach cracker, 15% flour substitution had the highest ash content (13.33 ± 8.57), 10% (12 ± 3.33) and followed by 5% (11.2 ± 3.30). Similar to red spinach cracker, 15% flour substitution had the highest ash content (13.93 ± 1.10), 10% (11.27 ± 0.61) and followed by 5% (11.07 ± 2.37). It can be concluded that as the percentage of flour substitution increases, the ash content of the sample increases (refer Figure 4.13)

Increasing trend obtained for ash content of spinach cracker was found to have similar results reported by Olubukola *et al.* (2017) where there was an increase in ash content of *Chinchin* enriched with *Ugu* and Indian Spinach Vegetables as the amount of vegetable incorporation was increased. Similar results were obtained on the incorporation of carrot pomace powder and germinated chickpea flour into biscuit (Baljeet, Ritika & Reena, 2014).

Ash content is an index of inorganic mineral elements in food (Onyeka & Dibia, 2002). Higher concentration of spinach powder was found to have higher ash content due to high value of mineral content of the dried vegetables (Aletor, Oshodi & Ipinmoroti, 2002). Spinach is regarded as a functional food due to its diverse nutritional composition, phytochemicals, and bioactives that promote health beyond basic nutrition (Robert & Moreau, 2016). It has substantial health-promoting activities that are attributed to functional properties of their nutrients and non-essential compounds. In comparison between green spinach and red spinach, both green spinach and red spinach are rich in vitamin C. However, green spinach is richer in vitamin A while red spinach has higher iron and other minerals content such as calcium, manganese, magnesium, copper and zinc (Muliani *et al*., 2017).

4.2.4 Protein Content

The protein content of spinach cracker showed an increasing progression for both green and red spinach cracker with 15% flour substitution being the highest, followed by 10% and lastly 5%. Green spinach cracker with 15% flour substitution had the highest protein content (14.01 ± 8.07) while positive control had the least protein content (4.76 ± 0.19) out of all samples tested.

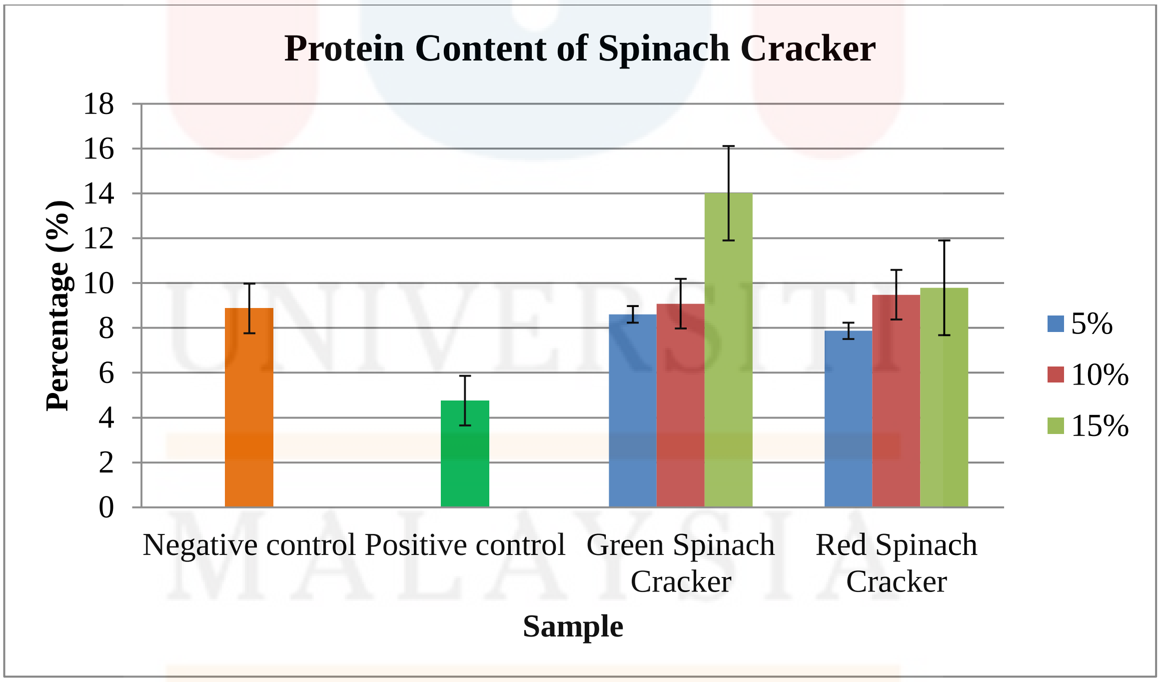

Figure 4.14: Protein content of spinach cracker (mean ± SE)

For green spinach cracker, 15% flour substitution had the highest protein content (14.01 ± 8.07), 10% (9.08 ± 1.47) and followed by 5% (8.61 ± 1.29). Similar to red spinach cracker, 15% flour substitution had the highest protein content (9.79 ± 0.86), 10% (9.48 ± 1.17) and followed by 5% (7.87 ± 1.86). Hence, it can be concluded that as the percentage of flour substitution increases, the protein content of the sample increases (refer Figure 4.14)

The protein content of spinach cracker was noted to have the same trend as that of fat and ash content of spinach cracker. Increasing trend in protein content in this research can be supported by research done by Olubukola *et al.* (2017) for *Chinchin* enriched with *Ugu* and Indian Spinach Vegetables where protein content was found to be increasing as the level of incorporated vegetable increased. Besides, similar results was reported by Oluwole and Karim (2005) which showed an increase in protein content with increasing incorporation of bambara flour supplementation in biscuit production from cassava-wheat-bambara flour blends. Green spinach cracker with 15% flour substitution was found to have the highest protein content (14.01 ± 8.07) which can be due to high protein content of green spinach.

4.2.5 Carbohydrate Content

Carbohydrate content of spinach cracker was calculated by subtracting the sum of moisture, protein, lipid, and ash from 100%. Carbohydrate content of each spinach cracker is presented in Figure 4.15 shown below.

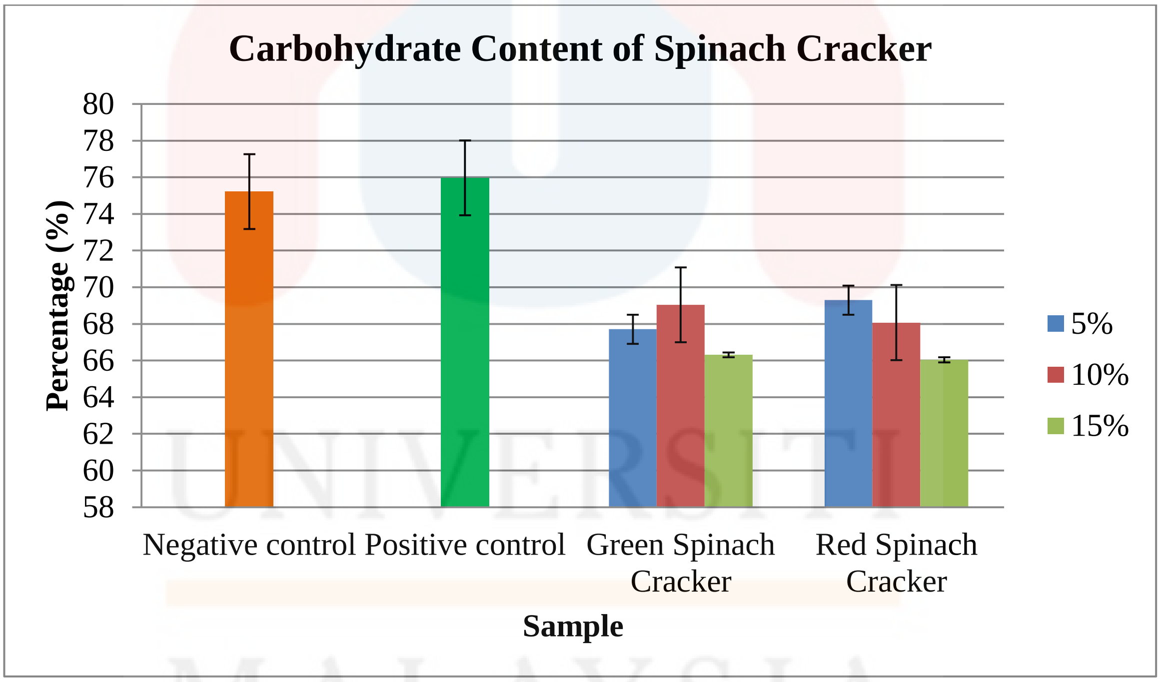

Figure 4.15: Carbohydrate content of spinach cracker (mean ± SE)

Carbohydrate content of spinach cracker ranged from 66.04 to 75.97%. Positive control had the highest carbohydrate content (75.97%) while red spinach cracker with 15% flour substitution had the least amount of carbohydrate content (66.04%) out of all samples tested. For green spinach cracker, 10% flour substitution had the highest carbohydrate content (69.04%), 5% (67.71%) and followed by 15% (66.31%). As for red spinach cracker, there was a decreasing trend where 5% flour substitution had the highest carbohydrate content (69.30%), 10% (68.07%) and followed by 5% (66.04%). Carbohydrate content of spinach cracker varies significantly between samples (refer Figure 4.15).

Carbohydrate content of negative control and positive control were found to be significantly higher than spinach cracker due to the use of high amount of wheat flour during the baking of cracker. Addition of spinach powder will decrease the amount of wheat flour used because it is being substituted with spinach powder which contained more fibre and minerals than carbohydrate. Spinach cracker was noted to have higher concentration of moisture, protein, fat, and ash content which consequently caused the lesser amount of carbohydrate content.

The decrease in value of carbohydrate content in spinach cracker was found to have similar results with research study reported by Olubukola *et al.* (2017) for *Chinchin* enriched with *Ugu* and Indian Spinach Vegetables where addition of vegetable was found to decrease carbohydrate content. Decreasing amount of carbohydrate content can be due to low carbohydrate content of the vegetables added itself. This can be further supported by the findings by Olatidoye and Sobowale (2011) where carbohydrate content of cookies decreased with increase proportion of cucurbita seed flour added.

4.2.6 Overall Chemical Composition of Spinach Cracker

Formulations of spinach cracker that were analyzed include negative control, positive control, green spinach cracker with 5% flour substitution (G5), green spinach cracker with 10% flour substitution (G10), green spinach cracker with 15% flour substitution (G15), red spinach cracker with 5% flour substitution (R5), red spinach cracker with 10% flour substitution (R10), and red spinach cracker with 15% flour substitution (R15).

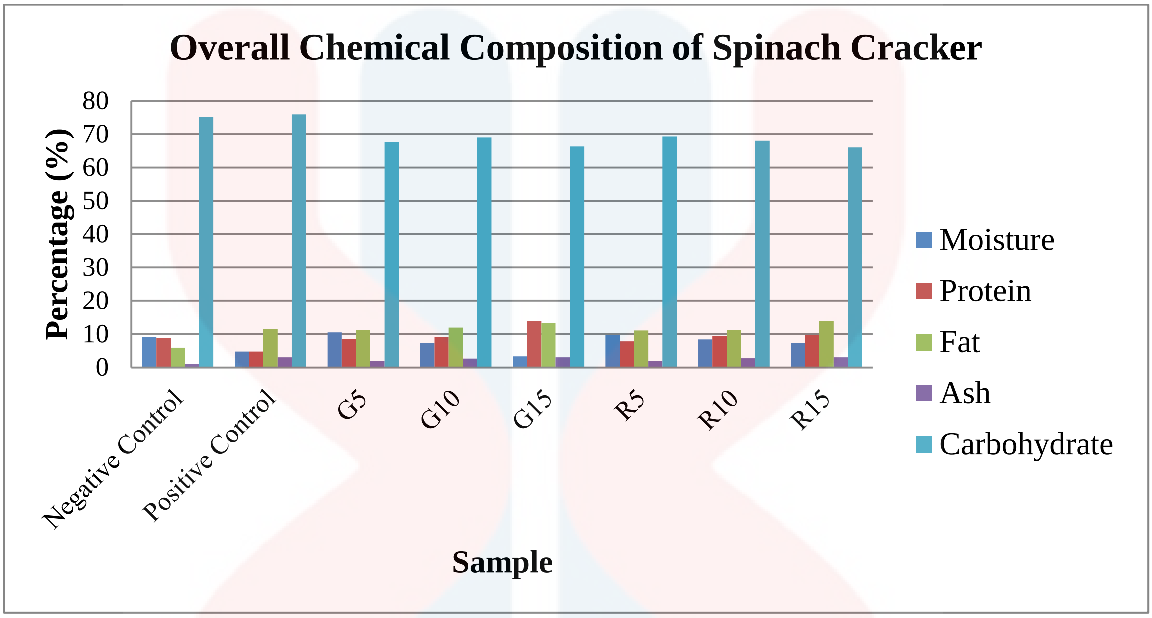

Figure 4.16: Overall chemical composition of spinach cracker

Based on Figure 4.16, it was observed that carbohydrate content of spinach cracker was the highest out of other chemical content. It was found that spinach cracker generally contain high amount of moisture, protein, fat and carbohydrate content. Ash content was the lowest for all samples. There was a noticeable difference for positive control sample where only fat content was the highest besides carbohydrate content, thus indicating that positive control sample that has been commercialized contained high amount of fats.

Proximate analysis was conducted to determine the distribution of chemical composition of food product when samples are heated under specified conditions. These food components may be of interest in food industry to ensure that their products meet the appropriate laws and legal declaration requirements as well as safety aspects of the final products when being released to end consumers. Those values obtained are being declared as nutritional facts shown on the labels of the final products.

4.3 Sensory Acceptability of Cracker Incorporated with Spinach Powder

A newly developed food product needs a correct measurement of the sensory evaluation and good in understanding of an acceptance pattern of the consumers regarding the product (Yang & Moon, 2011). Before a certain product is being sold to the general consumer, it needs to be tested to ensure that it will be accepted by the consumers in order to avoid failure of the product in the market. Sensory evaluation can be described as a method or test that is used to evoke, measure, analyze and interpret responses through the five senses of human which are sight, smell, touch, taste and hearing.

Consumer acceptance for green spinach cracker was conducted using sensory evaluation to identify which formulation of cracker is the best and the most suitable type of spinach in the development of spinach cracker. The attribute that was observed were colour, texture, flavour, taste and overall acceptance of the green spinach cracker. Specific parameters for texture that were evaluated were crispiness and hardness. Formulations of spinach cracker that were analyzed include green spinach cracker with 5% flour substitution (G5), green spinach cracker with 10% flour substitution (G10), green spinach cracker with 15% flour substitution (G15), red spinach cracker with 5% flour substitution (R5), red spinach cracker with 10% flour substitution (R10), red spinach cracker with 15% flour substitution (R15), and control sample (C).

Table 4.1: Score values from sensory evaluation in mean and standard deviation for negative control and green spinach cracker

		Attributes					
Formulation of spinach cracker (%)		Flavour	Colour	Taste	Crispiness	Hardness	Overall acceptability
Negative control	0	4.28 ± 1.47	4.75 ± 1.53	4.75 ± 1.60	3.60 ± 1.60	3.63 ± 1.69	4.13 ± 1.20
Green spinach cracker	5	5.05 ± 1.41	5.13 ± 1.42	5.03 ± 1.37	5.43 ± 1.15	5.03 ± 1.05	5.13 ± 1.18
	10	5.05 ± 1.20	5.00 ± 1.20	5.08 ± 1.00	4.88 ± 1.47	4.73 ± 1.20	5.10 ± 1.06
	15	4.80 ± 1.42	4.65 ± 1.33	4.33 ± 1.51	5.20 ± 1.18	4.95 ± 1.13	4.70 ± 1.45
Red spinach cracker	**5**	**5.23 ± 1.56**	**4.55 ± 1.66**	**5.35 ± 1.46**	**5.58 ± 1.32**	**5.38 ± 1.28**	**5.33 ± 1.23**
	10	4.48 ± 1.43	4.40 ± 1.37	4.55 ± 1.41	3.23 ± 1.69	3.23 ± 1.37	3.85 ± 1.31
	15	4.28 ±	3.83 ±	4.15 ±	5.30 ±	5.03 ±	4.55 ± 1.55

	1.58	1.30	1.79	1.21	1.07

From sensory evaluation test, texture of cracker particularly hardness and crispiness of cracker incorporated with spinach powder is more preferable compared to control. The hardness of spinach increased as spinach flour substitution increased. This showed that texture attribute of food product plays an important role in increasing consumer's preference towards a certain food product. Unlike colour and flavour, texture is used by consumers not only as an indicator of food safety but also an indicator of food quality (Lawless & Heymann, 1999). According to Szczesniak and Kleyn (1963), employee working in major food company placed relatively more emphasis on texture than the general population. A defect in perceived texture would have an extremely negative impact on consumers' hedonic responses to the product.

For hardness, the most preferred cracker was red spinach cracker with 5% flour substitution (R5) with mean score of 5.38 ± 1.28. R5 was perceived to have the right level of hardness for a cracker. Based on texture profile analysis (TPA) of R5, the amount of hardness was 4500 g. For crispness, the most preferred cracker was only red spinach cracker with 5% flour substitution (R5) with mean score of 5.58 ± 1.32. This can be concluded that R5 was perceived to have the most preferred texture.

Besides that, taste of spinach cracker with lower concentration of spinach powder is more preferable than spinach cracker with higher concentration of spinach powder. Taste of spinach cracker was observed to be bitter due to the presence of antioxidants compounds in spinach powder (Vecchio, Cavallo, Cicia & Giudice, 2019). Plants tend to produce toxic substances which are bitter as an act of defense strategy from herbivores. This is the reason why during evolution, humans learnt to avoid this sensory property in food as it was perceived as a danger signal (Drewnowski & Gomez-Carneros, 2000). Nevertheless, there can be some exceptions to this general tendency which can be represented by individual preference and differences among consumers. Level of bitterness detected by humans in food varies according to individuals due to genetic (Tepper, 2008). This can observed by the data obtained through this sensory evaluation where preference of taste of spinach cracker varies significantly.

As for flavour attribute, similar observation was obtained with taste and colour attributes where spinach cracker with lower concentration of spinach powder is more preferable than spinach cracker with higher concentration of spinach powder. The difference between flavour and taste is that flavour is the sense of taste and smell combined whereas taste is the sensation of saltiness, sweetness, sourness, bitterness or umami (savoriness). Similar trend for flavour attribute was observed due to the correlation between flavour and taste attribute.

On the other hand, as for colour attributes, colour of spinach cracker with lower concentration of spinach powder is more preferable than spinach cracker with higher concentration of spinach powder. Preference in colour for spinach cracker was found to be decreasing as spinach powder concentration increased. The colour of green spinach cracker was observed to be greenish brownish while red spinach cracker was brownish in colour. It was observed that the colour of spinach cracker had changed from brighter colour to brownish colour as higher level of spinach powder being substituted into the cracker. Food products with higher concentrations of sugar will tend to react with amino acid or become parts of the protein chains to form dark compounds (Sabeera, Baba, Nazir *et al.*, 2016). The dark colour of spinach cracker was also due to occurrence of non-enzymatic browning or Maillard reaction of spinach that creates brown pigments in cooked food (Nathan, 2013).

Therefore, addition of spinach powder into food products are able to enhance the product's texture, taste, colour and flavour which can be an added value for a food product. The incorporation of spinach powder was found to be generally accepted by consumers based on sensory evaluation test among 40 panellists.

The results obtained from sensory evaluation for overall acceptance were further analyzed using Statistical Package of Social Sciences (SPSS) version 20.0 using One-Way-Analysis of Variance (ANOVA). From the scores collected from 40 panellists, R5 had the highest mean score (5.33 ± 1.228) followed by G5 (5.13 ± 1.181), G10 (5.10 ± 1.057), G15 (4.70 ± 1.454), R15 (4.55 ± 1.552), C (4.13 ± 1.202) and lastly R10 (3.85 ± 1.312). Based on Table 4.1, green spinach cracker generally had higher mean score compared to red spinach cracker. However, red spinach cracker with 5% flour substitution showed the highest mean score out of all formulations.

It was found that the significance value, p 0.000, which is smaller than 0.05. When the p-value is less than 0.05, the null hypothesis is rejected. It can be concluded that there is a significant difference in the overall acceptance level of at least two of the three formulations for spinach cracker. In order to determine which formulations of spinach cracker had significant difference, post hoc test was conducted by comparing the data for each formulation.

Table 4.2: Homogeneous subsets table of Post hoc test results

Overall acceptance of spinach cracker

Tukey HSD

Formulation of spinach cracker	N	Subset for alpha = 0.05	
		1	2
R10	40	3.85	
C	40	4.13	
R15	40	4.55	4.55
G15	40	4.70	4.70
G10	40		5.10
G5	40		5.13
R5	40		5.33
Sig.		.054	.107

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 40.000.

From Table 4.2, R10, C, R15 and G15 differed significantly in the overall acceptance with G10, G5 and R5. However, there is no significant difference in the overall acceptance between R10, C, R15 and G15. It was found that panellists preferred R5 because the score for R5 (5.33) is higher than other formulations. Hence, according to hedonic scale, the panellists like R5 slightly. Therefore, it can be concluded that red spinach cracker with 5% flour substitution was the most preferred and accepted by the panels during sensory evaluation.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Throughout the whole study of spinach cracker incorporated with green spinach and red spinach powder with different concentrations, it can be concluded that values of chromatic parameters which are lightness, redness and yellowness of spinach cracker decreased with increasing percentage of flour substitution. It was observed that the colour of spinach cracker had changed from brighter colour to brownish colour as higher level of spinach powder being substituted into the cracker due to the reaction of amino acid and protein chains that form dark compounds. Similar decreasing trend was observed for shelf life of spinach cracker during four weeks of storage where lightness, redness and yellowness of spinach cracker decreased with time. The main factor that affected the colour of spinach cracker was due to non-enzymatic browning or Maillard reaction of spinach carbohydrates during baking process and also during storage for extended period of time. Besides that, presence of pigment such as chlorophylls, carotenoids and betacyanins make it suitable for spinach powder to act as a natural colouring for food product.

The texture of spinach cracker for hardness and fracturability showed increasing progression with increasing concentration of spinach powder but showed decreasing progression during four weeks of storage. Factors contributing to the texture of spinach cracker are mainly affected by fibre, carbohydrates and starch composition present in spinach powder. The higher levels of spinach powder might have added higher mineral composition resulting in lower binding of carbohydrates and further lesser breaking strengths.

From the proximate analysis results, red spinach cracker with 5% flour substitution showed higher percentage of moisture and carbohydrate but lower percentage of protein, fat and ash content. Due to high percentage of carbohydrate, the spinach cracker is suitable to be consumed for energy. Red spinach cracker with 5% flour substitution showed promising results in all of the procedure and also being the most accepted or preferred among other spinach cracker formulations.

It can be recommended that red spinach cracker with 5% flour substitution would be the most suitable level of substitution in cracker formulation which could be commercialized. The composition of the spinach cracker proved to be most favorable in terms of colour, texture profile, proximate values and lastly sensory acceptance of 40 random consumers.

It could be suggested that the study increases its prospect to other type of bakery product such as muffin, bread or cookies with the same percentage of substitution. Shelf life study of spinach cracker could also be extended with microbiological analysis to determine presence of microbial activity during storage. In addition, the study could be extended for research on economic feasibility and nutritional analysis of spinach cracker.

Cost of production per 100 g of red spinach cracker with 5% flour substitution was calculated based on raw materials and operation cost (refer Appendix Table E.1).

On the other hand, packaging to store the spinach cracker was designed as shown in Appendix Figure F.1 and Figure F.2. The designs of the packaging are simple, bold and clear. The red packaging indicates the usage of red spinach as one of the main ingredients. Simple design of packaging is important as consumers are busy and they do not have time to analyze the details of the product. Therefore, simple design that can give details of the product such as flavour on the first glance is important. Besides that, bright colour of the packaging is able to attract the attention of consumers as it is able to stand out among other brands. Other than that, the front of packaging has ingenious die cut which acts as a window to show the product content in a meaningful way that is able to make a buyer to stop and take notice.

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APPENDIX A

HEDONIC TEST

No. of Panel: ____________ Date: _____________

Gender : Male ☐ Female ☐

Sample: <u>Spinach Cracker</u>

You are given seven samples. Please taste and evaluate each sample. Give your score (1 to 7) based on your liking on the space below according to the scale given. Do rinse your mouth before and after testing each sample.

Scale 1 to 7 points

7 – Like very much
6 – Like moderately
5 – Like slightly
4 – Neither like nor dislike
3 – Dislike slightly
2 – Dislike moderately
1 – Dislike very much

Please write down the code labelled on the sample you received.

Sample Code: ____________

Aspect	Rating						
	1	2	3	4	5	6	7
Aroma							
Colour							
Taste							
Texture							
Overall acceptance							

Comment (if any):

APPENDIX B

UNIVERSITI MALAYSIA KELANTAN RESEARCH ETHICS COMMITTEE FOR HUMAN - UMK REC (HUMAN)

RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE :

Physicochemical and Sensory Properties of Cracker Incorporated with Green Spinach (*Spinacia oleracea*) and Red Spinach (*Amaranthus dubius*) during Storage

2. INTRODUCTION:

This research investigates the addition of different percentage of green spinach and red spinach powder in the cracker. Spinach can be found easily in Malaysia local market. However, spinach was found neglected due to limited usage in development of products especially in bakery products and is usually being consumed in fresh. Besides that, increase in awareness of consuming nutritious foods especially fruits and vegetables. and issue with short shelf life of vegetables are what this research aims to dissolve. Hence, this research is proposed to create a healthy snack that is ready-made and contain high nutritional value. The consumer's preference and degree of likeness of the spinach cracker will be determined by this sensory evaluation.

This research is;

(i) To produce cracker with different concentration of spinach powder from two variety of spinach species.

(ii) To determine the best formulations of spinach cracker through sensory tests.

(iii) To determine the proximate composition of cracker incorporated with different concentration of spinach powder.

(iv) To determine the texture and color properties of cracker incorporated with different concentration of spinach powder during storage.

3. WHAT WILL YOU HAVE TO DO?

Testing will be conducted in Food Laboratory of University Malaysia Kelantan (UMK) Jeli campus and will take approximately half an hour. Participation is voluntary, and that participant may withdraw anytime without penalty or loss of benefit to which the participant is entitled. Total of 7 samples will be given to each panel to evaluate the organoleptic characteristics of the given samples. All samples are prepared in Food Laboratory of UMK Jeli campus using food grade ingredients and are freshly prepared. Each panel will have to read and understand the respondent's information sheet and sign the consent form, followed by filling in sensory evaluation form after eating each sample. Plain water will be provided for mouth rinsing between samples to avoid any confusion regarding the taste. Results obtained from this sensory evaluation will be used to determine the best formulation of spinach cracker through sensory tests.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Individuals with allergy to wheat or gluten are refrain from participating. Individuals who dislike consuming spinach and/or cracker in general are refrain from participating as well. Participation of individuals with allergy reaction to wheat or gluten and dislike consuming spinach and/or cracker in general will be terminated. Individuals below the age of 18 years old are not allowed to participate without consent from guardian or parent.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

You, as the subject, will be able to participate as a sensory panel for the development of a new product which is spinach cracker.

(b) TO THE INVESTIGATOR?

The investigator is able to use the data obtained from sensory panels for analysis in choosing the best formulation of spinach cracker which can be used to develop a new product. The investigator is also able to use the spinach powder as natural colorant to improve appearance of cracker.

6. WHAT ARE THE POSSIBLE RISKS?

There will be no risks because all ingredients are food grade but has the potential for allergy reaction to occur for those who have allergy to wheat or gluten. The spinach cracker will be prepared and served under sanitary condition and are freshly prepared. No shelf life study conducted to determine microbial quality.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

Any information obtained in connection with this study and that could be identified with you will be kept confidential and will not be made publicly available, to the extent permitted by law. Summary results and statistical data may be reported in scientific journals or presented at scientific meetings; however, individual panellist responses will remain confidential.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

For any enquiry, please do not hesitate to approach or contact the investigator, **Daphane Teo Wen Xin** with contact number **019-2259163** or email address **daphanetwx@gmail.com**. UMKREC (HUMAN) has approved the study and can be reached through **09-9477000/09-9477200** regarding rights of study participants, including grievances and complaints.

Please initial here if you have read and understood the contents thus far ________________

9. CONSENT

I ………………………………… Identity Card No. …………………………… address………………………………………………………………………………………………….. . …………………………………………………..hereby voluntarily agree to take part in the research stated above *(video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse
effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ……..………………………… (Respondent)

Signature ……..………………………… (Witness)

Date :……………………………………..

Name :……………………………………..

I/C No. :……………………………………..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ……..…………………………

Signature ……..………………………… (Researcher)

APPENDIX C

Table C.1: Moisture content of spinach cracker

Cracker	Sample (%)	Fresh weight (g)	Dried weight (g)	Moisture (%)
Positive control		1.19 ± 0.006	1.14 ± 0.032	4.74 ± 3.140
Negative control	0	1.14 ± 0.032	1.03 ± 0.031	9.04 ± 3.812
Green spinach	5	1.21 ± 0.021	1.08 ± 0.006	10.48 ± 1.555
	10	1.20 ± 0.017	1.11 ± 0.006	7.21 ± 0.854
	15	1.19 ± 0.042	1.15 ± 0.047	3.35 ± 0.832
Red spinach	5	1.19 ± 0.021	1.08 ± 0.006	9.76 ± 1.114
	10	1.18 ± 0.031	1.08 ± 0.012	8.40 ± 3.175
	15	1.20 ± 0.031	1.09 ± 0.029	7.24 ± 0.367

Table C.2: Fat content of spinach cracker

Cracker	Sample (%)	Evaporating dish (g)	Dish + fat (g)	Fat (g)	Fat (%)
Positive control		105.80 ± 15.53	106.38 ± 15.56	0.58 ± 0.07	11.53 ± 1.33
Negative	0	107.35 ± 16.74	107.64 ± 16.88	0.29 ± 0.15	5.87 ± 3.06

control					
Green spinach	5	107.25 ± 16.88	107.81 ± 16.78	0.56 ± 0.17	11.2 ± 3.30
	10	106.72 ± 14.79	107.32 ± 14.69	0.6 ± 0.17	12 ± 3.33
	15	107.59 ± 16.29	108.25 ± 16.72	0.67 ± 0.43	13.33 ± 8.57
Red spinach	5	108.20 ± 15.64	108.75 ± 15.57	0.55 ± 0.12	11.07 ± 2.37
	10	98.34 ± 0.72	98.90 ± 0.72	0.56 ± 0.03	11.27 ± 0.61
	15	98.18 ± 1.17	98.87 ± 1.12	0.70 ± 0.06	13.93 ± 1.10

Table C.3: Ash content of spinach cracker

Cracker	Sample (%)	Fresh weight (g)	Ash (g)	Ash (%)
Positive control		0.50 ± 0.01	0.015 ± 0.01	3.02 ± 0.61
Negative control	0	0.50 0.01	0.005 ± 0.01	1.00 ± 3.56
Green spinach	5	0.50 ± 0.01	0.012 ± 0.03	2.34 ± 1.23
	10	0.50 ± 0.01	0.013 ± 2.45	2.67 ± 0.56
	15	0.50 ± 0.01	0.015 ± 0.01	3.02 ± 0.56
Red spinach	5	0.50 ± 0.01	0.012 ± 0.01	2.33 ± 0.61
	10	0.50 ± 0.01	0.014 ± 0.03	2.78 ± 0.56
	15	0.50 ± 0.01	0.016 ± 0.01	3.23 ± 0.45

Table C.4: Protein content of spinach cracker

Type of Spinach Cracker	Sample (%)	Fresh weight (g)	HCl (mL)	N (%)	Protein (%)
Positive control		1.002 ± 0.0011	6.35 ± 0.21	0.76 ± 0.03	4.76 ± 0.19
Negative control	0	1.0016 ± 0.0002	11.05 ± 1.20	1.42 ± 0.17	8.87 ± 1.05
Green spinach	5	1.0021 ± 0.0010	11.05 ± 1.48	1.38 ± 0.21	8.61 ± 1.29
	10	1.0024 ± 0.0014	11.20 ± 1.70	1.45 ± 0.24	9.08 ± 1.47
	15	1.0024 ± 0.0014	17.05 ± 9.26	2.24 ± 1.29	14.01 ± 8.07
Red spinach	5	1.0013 ± 0.0001	10.20 ± 2.12	1.26 ± 0.30	7.87 ± 1.86
	10	1.0021 ± 0.0001	11.65 ± 1.34	1.52 ± 0.19	9.48 ± 1.17
	15	1.0017 ± 0.0006	12.00 ± 0.99	1.57 ± 0.14	9.79 ± 0.86

Table C.5: Carbohydrate content of cracker

Type of Spinach Cracker	Sample (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)

Positive control		4.74 ± 3.140	4.76 ± 0.19	11.53 ± 1.33	3.00 ± 1.47	75.97
Negative control	0	9.04 ± 3.812	8.87 ± 1.05	5.87 ± 3.06	1.00 ± 1.05	75.22
Green spinach	5	10.48 ± 1.555	8.61 ± 1.29	11.2 ± 3.30	2.00 ± 3.30	67.71
	10	7.21 ± 0.854	9.08 ± 1.47	12 ± 3.33	2.67 ± 1.15	69.04
	15	3.35 ± 0.832	14.01 ± 8.07	13.33 ± 8.57	3.00 ± 1.67	66.31
Red spinach	5	9.76 ± 1.114	7.87 ± 1.86	11.07 ± 2.37	2.00 ± 1.67	69.30
	10	8.40 ± 3.175	9.48 ± 1.17	11.27 ± 0.61	2.78 ± 0.45	68.07
	15	7.24 ± 0.367	9.79 ± 0.86	13.93 ± 1.10	3.00 ± 3.34	66.04

Table C.6: Mean score of the overall acceptance for each of the formulations

Descriptives

Overall acceptance of spinach cracker

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
G5	40	5.13	1.181	.187	4.75	5.50	2	7
G10	40	5.10	1.057	.167	4.76	5.44	2	7
G15	40	4.70	1.454	.230	4.24	5.16	1	7
R5	40	5.33	1.228	.194	4.93	5.72	2	7
R10	40	3.85	1.312	.207	3.43	4.27	1	7
R15	40	4.55	1.552	.245	4.05	5.05	1	7
C	40	4.13	1.202	.190	3.74	4.51	1	7
Total	280	4.68	1.377	.082	4.52	4.84	1	7

Table C.7: ANOVA results for overall acceptance of spinach cracker

ANOVA

Overall acceptance of spinach cracker

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72.186	6	12.031	7.194	.000
Within Groups	456.525	273	1.672		
Total	528.711	279			

Table C.8: Post hoc test results for overall acceptance of spinach cracker

Multiple Comparisons

Dependent Variable: Overall acceptance of spinach cracker

Tukey HSD

(I) Formulation of spinach cracker	(J) Formulation of spinach cracker	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
G5	G10	.025	.289	1.000	-.83	.88
	G15	.425	.289	.763	-.43	1.28
	R5	-.200	.289	.993	-1.06	.66
	R10	1.275*	.289	.000	.42	2.13
	R15	.575	.289	.424	-.28	1.43
	C	1.000*	.289	.011	.14	1.86
G10	G5	-.025	.289	1.000	-.88	.83
	G15	.400	.289	.810	-.46	1.26
	R5	-.225	.289	.987	-1.08	.63
	R10	1.250*	.289	.000	.39	2.11

	R15	.550	.289	.481	-.31	1.41
	C	.975*	.289	.015	.12	1.83
G15	G5	-.425	.289	.763	-1.28	.43
	G10	-.400	.289	.810	-1.26	.46
	R5	-.625	.289	.320	-1.48	.23
	R10	.850	.289	.054	-.01	1.71
	R15	.150	.289	.999	-.71	1.01
	C	.575	.289	.424	-.28	1.43
R5	G5	.200	.289	.993	-.66	1.06
	G10	.225	.289	.987	-.63	1.08
	G15	.625	.289	.320	-.23	1.48
	R10	1.475*	.289	.000	.62	2.33
	R15	.775	.289	.107	-.08	1.63
	C	1.200*	.289	.001	.34	2.06
R10	G5	-1.275*	.289	.000	-2.13	-.42
	G10	-1.250*	.289	.000	-2.11	-.39
	G15	-.850	.289	.054	-1.71	.01
	R5	-1.475*	.289	.000	-2.33	-.62
	R15	-.700	.289	.194	-1.56	.16
	C	-.275	.289	.964	-1.13	.58
R15	G5	-.575	.289	.424	-1.43	.28
	G10	-.550	.289	.481	-1.41	.31
	G15	-.150	.289	.999	-1.01	.71
	R5	-.775	.289	.107	-1.63	.08
	R10	.700	.289	.194	-.16	1.56
	C	.425	.289	.763	-.43	1.28
C	G5	-1.000*	.289	.011	-1.86	-.14
	G10	-.975*	.289	.015	-1.83	-.12
	G15	-.575	.289	.424	-1.43	.28
	R5	-1.200*	.289	.001	-2.06	-.34
	R10	.275	.289	.964	-.58	1.13
	R15	-.425	.289	.763	-1.28	.43

*. The mean difference is significant at the 0.05 level.

APPENDIX D

Figure D.1: Dried red spinach

Figure D.2: Grinding process of spinach leaves using grinder

Figure D.3: Green spinach powder

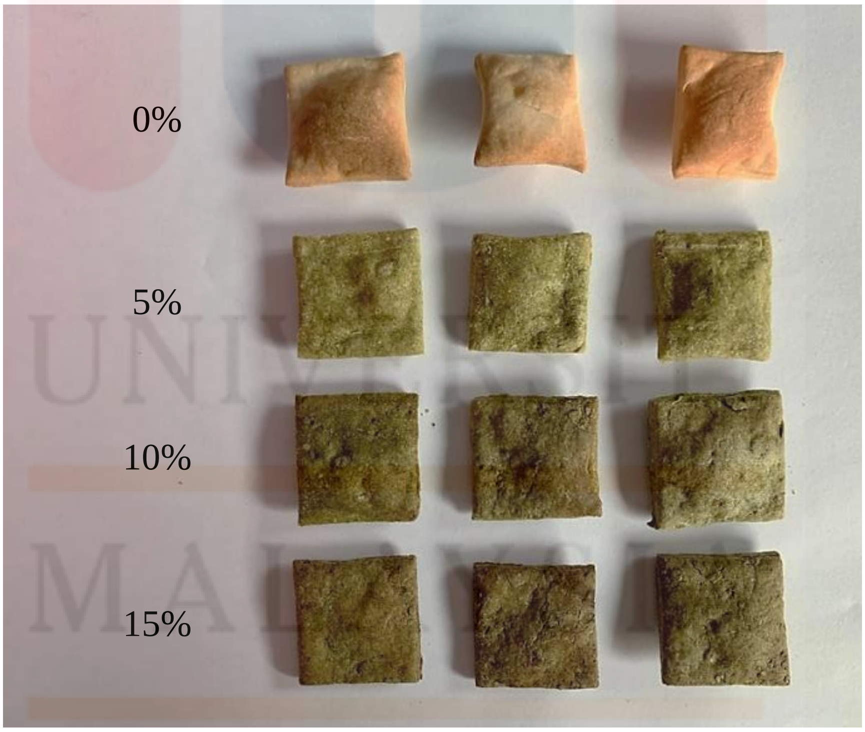

Figure D.4: Green spinach cracker with different percentages of flour substitution

Figure D.5: Red spinach powder

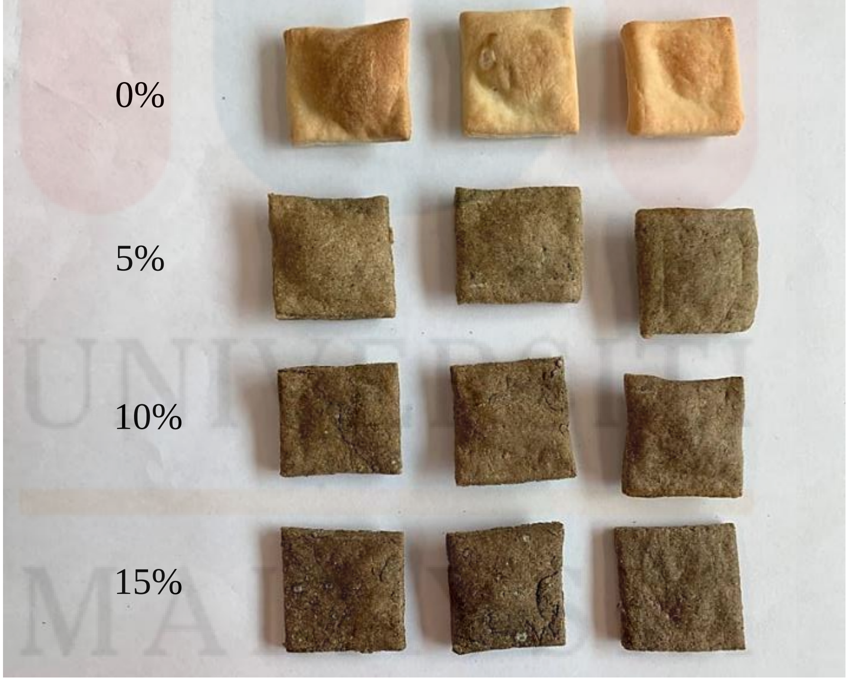

Figure D.6: Red spinach cracker with different percentages of flour substitution

Figure D.7: Spinach cracker in sealed plastic bag for shelf life study

Figure D.8: Baking process of spinach cracker

Figure D.9: Ash content analysis using muffle furnace

Figure D.10: Fat content analysis using Buchner funnel and separating funnel

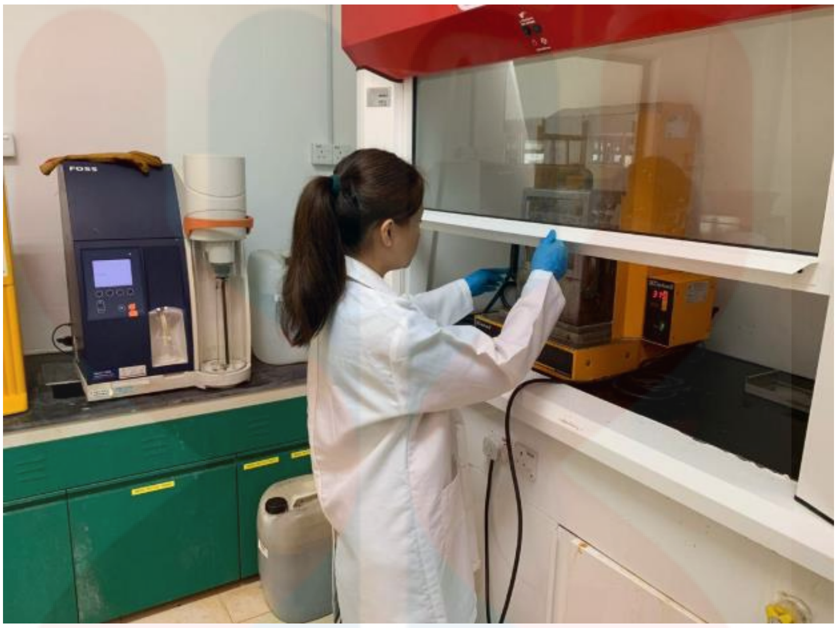

Figure D.11: Protein analysis using Kjedahl auto distillation analyzer (Kjeltec 8200)

Figure D.12: Sensory evaluation

Figure D.13: Table layout during sensory evaluation

APPENDIX E

Table E.1: Cost of production for red spinach cracker with 5% flour substitution per 100 g of crackers

Item	Amount Required/100 g of crackers	Price (RM)
Fresh red spinach	1	3.00/kg
Wheat flour	1	2.35
Sugar	1	2.39
Salt	1	0.37
Butter	1	11.70
Sealed pouch bag	50	8.00
Cracker mould	1	7.90
Cost Price per 100 g of Crackers	**56**	**35.71**

APPENDIX F

Figure F.1: Packaging for red spinach cracker (front)

Figure F.2: Packaging for red spinach cracker (back)