Carrageenan-Based Hydrogels Containing Grape Seed Extract: Effect of their Antioxidant Properties

MS. YATINUN NA SONGKHLA

ID: 5712500

A SPECIAL PROJECT SUBMITTED TO SCHOOL OF BIOTECHNOLOGY, ASSUMPTION UNIVERSITY, IN PART FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

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LEVEL OF STUDY : Bachelor of Science

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Assumption University

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Thank you,

Ms. Yatinun Na Songkhla

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Abstract

The kappa carrageenan polysaccharide can be extracted from red edible seaweeds which has an ability to form gel. Carrageenan-based hydrogels containing grape seed extract were prepared with 9 formulations: 0.5%, 1% and 2% grape seed extract with various concentrations of glycerol (0%, 12.5%, and 25%). The physical properties of carrageenan-based hydrogels were analyzed i.e. color measurement, moisture content, swelling properties, degradation, firmness. In addition, the antioxidant properties of carrageenan-based hydrogels were studied as total phenolic content and % scavenging activity. Among 9 formulations, the results reported their percentages of opacity were 99-100%, moisture contents were 9-12%, swelling properties were 14-29%, degradations were 20-72%, and firmness were 10-55%, respectively. The high firmness and high resistance to degradation were found in 3 formulations containing 25% glycerol and 0.5%, 1% and 2% grape seed extract. For antioxidant activities, the results showed the highest total phenolic content at 23.3±1.0 mg GAE/mL and scavenging activity (%) at 87.8±1.6% in formula containing 25% glycerol and 2% grape seed extract. The results suggested that grape seed extract was useful in the prevention of diseases in which free radicals implicated.

Key words: hydrogels, carrageenan, grape seed extract, antioxidant activity

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Introduction

Hydrogel is a three-dimensional (3D) network of hydrophilic polymers which can highly swell in water and absorb large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains. Hydrogels were first reported by Wichterle and Lím (1960) [1]. By definition, water must constitute at least 10% of the total weight (or volume) for a material to be a hydrogel. Hydrogels also possess a degree of flexibility very similar to natural tissue due to their significant water content. The hydrophilicity of the network is due to the presence of hydrophilic groups such as $-NH_2$, -COOH, -OH, $-CONH_2$, - CONH -, and $-SO_3H$ [2]. For most medical application , the novel engineering of hydrogels for drug delivery require dividing them to biodegradable hydrogels which are favoured over non-degradable hydrogels since they degrade in clinically relevant time scale [3], smart hydrogel or stimuli-sensitive hydrogel that respond to environmental changes, such as temperature, pH, light, and specific molecules such as glucose [4] and finally biomimetic hydrogels which are relatively inert polymer chains can be tailored with the selected biological moieties to yield bioactive hydrogels [5].

In this project, carrageenan is polymer used for preparing hydrogels. Carrageenans correspond to a family of hydrophilic polysaccharides consisting of linear and highly sulfated galactans found in several marine red algae species (class of Rhodophyceae) composed by repeating 3,6-anhydro-D-galactose and b-D-galactose-4-sulfated units [6]. The three main types of carrageenans include the kappa (j-), with one sulfate group, iota (i-) and lambda (k-) with two and three sulfate groups, respectively [7]. These natural polymers are widely used in several industrial, environmental, and commercial applications as gelling, thickening, emulsifying, and stabilizing agents due to their ability to form thermo-reversible gels and viscous solutions [8]. More recently, carrageenan hydrogels started to be explored in regenerative medicine as scaffolds and controlled-release systems for ophthalmic/oral drug preparations, growth factors, enzyme immobilization, and cell encapsulation [9]. Several bioactive agents are used in this study (i.e. grape seed extract, hyaluronic acid) as well as other pharmaceutical and cosmetic ingredients such as allantoin, glycerol, sorbitol and collagen.

Grape seed extract is an industrial derivative of whole grape seeds. The extract contains proanthocyanidins [10]. Grape seed extract quality is measured by the content of procyanidins which are formed from proanthocyanidins. Generally, grape seed extract quality contains 95% procyanidins, but potency varies among products [11]. Grape seed extract is an industrial derivative of grape seeds. It is rich in antioxidants and oligomeric proanthocyanidin complexes and has been linked to a wide range of possible health benefit. treating tooth decay, protecting against pathogens, improving night vision, Alzheimer's disease, treating diabetic retinopathy and improving blood sugar control, relieving symptoms of chronic venous insufficiency, anti-aging properties (protecting collagen and elastin), reducing edema, relieving symptoms of chronic venous insufficiency, reducing iron levels in people with hemochromatosis, and reducing inflammation [12].

Sorbitol is a sugar alcohol found in fruits and plants with diuretic, laxative and cathartic property. Unabsorbed sorbitol retains water in the large intestine through osmotic pressure thereby stimulating peristalsis of the intestine and exerting its diuretic, laxative and cathartic effect. In addition, sorbitol has one-third fewer calories and 60 % the sweetening activity of sucrose and is used as a sugar replacement in diabetes [13].

Glycerin is a trihydroxyalcohol with localized osmotic diuretic and laxative effects. Glycerin elevates the blood plasma osmolality thereby extracting water from tissues into interstitial fluid and plasma. This agent also prevents water reabsorption in the proximal tubule in the kidney leading to an increase in water and sodium excretion and a reduction in blood volume. Administered rectally, glycerin exerts a hyperosmotic laxative effect by attracting water into the rectum, thereby relieving constipation. In addition, glycerin is used as a solvent, humectant and vehicle in various pharmaceutical preparations [14]. Glycerin is used safely in numerous cosmetics and personal care products such as soaps, toothpaste, shaving cream, and skin/hair care products to provide smoothness and lubrication. It is also a well-known humectant that prevents the loss of moisture from products so they don't dry out as quickly. Other reported functions for glycerin include use as a fragrance ingredient, denaturant, hair conditioning agent, oral care agent, skin conditioning agent— humectant, skin protectant, oral health care drug, and viscosity decreasing agent [15].

Collagen is a hard, insoluble, and fibrous protein that makes up one-third of the protein in the human body. In most collagens, the molecules are packed together to form long, thin fibrils. These act as supporting structures and anchor cells to each other. They give the skin strength and elasticity. There are at least 16 different types of collagen, but 80 to 90 percent of them belong to types 1, 2, and 3. These different types have different structures and functions. The collagens in the human body are strong and flexible. Collagen is resorbable. This means it can be broken down, converted, and absorbed back into the body. It can also be formed into compacted solids or lattice-like gels [16]. Its diverse range of functions and the fact that it is naturally occurring make it clinically versatile and suitable for various medical purposes. Collagen for medical use can originate from humans, cows, pigs, or sheep. Skin fillers, collagen injections can improve the contours of the skin and fill out depressions. Fillers that contain collagen can be used cosmetically to remove lines and wrinkles from the face. It can also improve scars, as long as these do not have a sharp edge. These fillers are sourced from humans and cows. Skin tests should be done before using collagen from cows, to avoid aggravating any allergies. Collagen can fill relatively superficial volumes. More extensive gaps are usually filled with substances such as fat, silicone, or implants. Wound dressing, collagen can help heal wounds by attracting new skin cells to the wound site. It promotes healing and provides a platform for new tissue growth. Collagen dressings can help heal: chronic wounds that do not respond to other treatment, wounds that expel bodily fluids such as urine or sweat, granulating wounds, on which different tissue grows, necrotic or rotting wounds, partial and full-thickness wounds, second-degree burns, and sites of skin donation and skin grafts. Guided tissue regeneration, collagen-based membranes have been used in periodontal and implant therapy to promote the growth of specific types of cell. In oral surgery, collagen barriers can prevent fast-growing cells around the gum from migrating to a wound in a tooth. This preserves a space where tooth cells have the chance to regenerate. Collagen-based membranes can aid healing in these cases and they are resorbable, so this barrier does not need to be surgically removed after the main operation [17].

Hyaluronic acid or HA is a non-sulphated GAG and is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic β $(1\rightarrow3)$ bond [18]. In aqueous solutions HA forms specific and stable tertiary structures. Despite the simplicity in its composition, without variations in its sugar composition or without branching points, HA has a variety of physicochemical properties. HA polymers occur in a vast number of configurations and shapes, depending on their size, salt concentration, pH, and associated cations [19]. Unlike other GAG, HA is not covalently attached to a protein core, but it may form aggregates with proteoglycans. HA encompasses a large volume of water giving solutions high viscosity, even at low concentrations [20].

Allantoin is a skin active ingredient with keratolytic, moisturizing, soothing, anti-irritant properties, promotes the renewal of epidermal cell and accelerates wounds healing. Allantoin is safe and non-irritant, highly compatible with the skin and with cosmetic raw materials. Allantoin enjoys a long history of use in cosmetics and topical pharmaceuticals with no findings of toxicity or adverse reactions. Comply with CTFA and JSCI requirements [21]. The beneficial effects on the skin of Allantoin were well documented. Allantoin is a mild keratolytic agent that dissolves the intercellular cement that holds the cornified cells together, helping the natural desquamation of stratum corneum and increasing skin smoothness. The moisturizing effect results from its ability to increase the water bounded to the intercellular matrix and keratin, thereby softening skin and making the skin look healthier. The soothing, anti-irritant and skin protectant effect is due to the ability of Allantoin to form complexes and neutralize many irritant and sensitizing agents. Allantoin enhances epidermal cell-proliferation, promotes the regeneration of damaged epithelium and accelerates wound healing [22]. Moreover, allantoin is suitable for any personal care application. Its use notably increases the performance of every cosmetic preparation: used at low levels on intact skin gives a smooth and healthy appearance; used on irritated, chapped and cracked skin provide relief from pain and promote healing. Allantoin is also useful as only active ingredient. The many cosmetic applications include: Body and face care, Hand-care: gels, lotions, creams, Shaving-care: shaving soaps, aftershaves, gels, lotions, creams, Baby-care: diaper rash, bath products, gels, lotions, creams, powders, wipes, Lips-care: sticks, creams, Sun-care: sunscreens, aftersuns, suntans, gels, lotions, creams, Hair products: shampoos, tonics, Bath products: shower gels, bubble baths, intimate, powders, wipes, Oral preparations: toothpastes, mouthwashes [23].

Objectives

1. To develop the formulation of carrageenan-based hydrogel containing grape seed extract.
2. To investigate the physical properties of the prepared carrageenan-based hydrogel.
3. To determine radical scavenging properties of the prepared carrageenan-based hydrogel.

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Materials and Methods

1. Materials

Carrageenan was purchased from Chemipan (Thailand), sorbitol from Sigma Aldrich, and glycerin from Lab Valley (Thailand). Substances used were vitamin c, collagen, grape seed extract, hyaluronic acid and allantoin (Chemipan, Thailand). The laboratory grade reagents used in the preparation of PBS buffer solution were as follows: NaCl, KCl, $Na_2HPO_4 \cdot 12H_2O$ and KH_2PO_4.

Equipments

1. Hot Air Oven
2. Color measurement, Hunter Lab CIEL*a*b*
3. Texture analysis, TA.XTpulsC Texture analyser
4. Shaking incubator
5. Spectrophotometer

2. Methods

2.1 Preparation of hydrogels

In the first step, carrageenan solutions (2% w/v), sorbitol solutions (5% w/v) were prepared, then mixed with glycerol and water following each formulae, in order to obtain the final volume of 100 mL solution. According to each of formulae, grape seed extract was continually added. Afterwards, small amount of ingredients are as follows: vitamin c, collagen, hyaluronic acid (HA) and allantoin (AL) were added to each of formulae. After mixing all ingredients, heated to 60-70°C for 30 minutes, then poured in the semi-circle silicone molds with 2.8 cm diameter and 1.7 cm height. In the final step, the solutions were cooled at 4°C for a day. The composition of each of formulae to prepare hydrogels is shown in Table 1.

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Table1. The composition of mixtures to prepare hydrogels

Sample no.	2% w/v Carrageenan (mL)	5% w/v Sorbitol (mL)	Glycerol (mL)	Water (mL)	Vitamin C (g)	Grape Seed Extract (mL)	Collagen (g)	HA (g)	AL (g)
F81	50	25	-	25	0.1	0.5	0.2	0.2	0.5
F82	50	25	-	25	0.1	1	0.2	0.2	0.5
F83	50	25	-	25	0.1	2	0.2	0.2	0.5
F111	50	25	12.5	12.5	0.1	0.5	0.2	0.2	0.5
F112	50	25	12.5	12.5	0.1	1	0.2	0.2	0.5
F113	50	25	12.5	12.5	0.1	2	0.2	0.2	0.5
F121	50	25	25	-	0.1	0.5	0.2	0.2	0.5
F122	50	25	25	-	0.1	1	0.2	0.2	0.5
F123	50	25	25	-	0.1	2	0.2	0.2	0.5

2.2. Moisture content properties

Hydrogel samples with a diameter of 2.8 cm and a height of 1.7 cm were weighed and taken in the hot air-drying oven at 60°C for 2 hours. The weight of each samples was weighed after taking out from the oven. The moisture content (% M) was calculated according to the following formula:

$$\text{Moisture Content (\%)} = \frac{Wi - Wd}{Wi} \times 100$$

Where W_i represents the initial weight of sample, and W_d corresponds to the weight of sample after drying. The test samples were completely done in triplicate for each.

2.3. Swelling properties

As a function of swelling time, temperature and pH, swelling capacity of hydrogel in PBS buffer (pH 7.4) at 37°C, distilled water (pH 5.5) at 37°C, PBS buffer (pH 7.4) at 25°C and distilled water (pH 5.5) at 25°C were determined. 5g of the sample was immersed in 40 ml each of condition.

This experimental part was performed by placing hydrogel samples with a diameter of 2.8 cm and a height of 1.7 cm in each condition. At each time intervals; 0, 2, 4, 6, and 24 h, swollen gels were taken out from the swelling medium, and dried to remove the excess from the surface by using filter paper. The degree of swelling (% S) can be calculated by using the below equation:

$$\text{Degree of swelling (\%)} = \frac{Ws - Wd}{Wd} \times 100$$

Where W_s is the weight of swollen sample at t time, and W_d is the weight of initial sample at 0 time. The test samples were completely done in triplicate for each.

2.4. Mechanical strength properties

Semi-circle samples with a diameter of 2.8 cm and a height of 1.7 cm were used for mechanical test using texture analyzer. Compression platen probe with 100 mm diameter was used to analyze the firmness, test speed is 20 mm/sec and target distance is 5 mm. The result was obtained from the raw data and force (N) was plotted.

2.5. Color Measurement

CIE whiteness, brightness, L*a*b* color and opacity of the gels were measured with Hunter Lab CIE L*a*b* system colorimeter. The followings are the ways that were used to measure samples in this measurement: covering with white ceramic plate, and covering with white ceramic plate and the black cover. Opacity (% O) is a measure for the transparency of hydrogels can be calculated by using the following equation:

$$\text{Opacity (\%)} = \frac{Rwb}{Rw} \times 100$$

Where R_{wb} refers to reflectance of a sample sheet over perfect black, and R_w refers to reflectivity of the same sample over perfect white. The test samples were completely done in triplicate for each.

2.6. In vitro degradation properties

The enzymatic degradation behavior of the carrageenan-based hydrogels was performed by immersing 5 g of hydrogel into PBS buffer (pH 7.4) and distilled water (pH 5.5) and incubated them in a shaking incubator with 50 rpm for 24 hours. After 4 hours passed, the gels were taken out and weighed (w_t). The weight loss (% W) was calculated by using the below equation:

$$\text{Weight loss (\%)} = \frac{W0-Wt}{W0} \times 100$$

Where W_0 is the initial weight of each sample, and W_t is the weight of sample at each time intervals. The test samples were completely done in triplicate for each.

2.7. Total phenolic compounds

Folin-Ciocalteu (FC) method was used to determine the total phenolic compounds of carrageenan-based hydrogel. 0.1 ml of carrageenan-based hydrogel released solution which is obtained from degradation test, was mixed with 1.25 mL of 0.1 N FC reagent solution, and vortexed immediately. After 4 minutes, 1 ml of 0.7 M Na_2CO_3 was added to the mixture. Afterwards, the mixture was totally shaken and left to incubate at room temperature for 1 hour. In the final step, UV-Vis spectroscopy at 760 nm was used to measure the absorbance of the solution. Gallic acid was used as the standard curve for determining the phenol content. Results were reported in the gallic acid equivalents and the total phenols were expressed as milligram gallic acid equivalents per mL (mgGAE/mL). The test samples were completely done in triplicate for each.

2.8. DPPH free radical scavenging activity

The DPPH free radical scavenging activity was determined using the method proposed by Brand-Williams. The test samples were compared to a known antioxidant, ascorbic acid (1000 ppm). 0.3 ml of DPPH solution (0.1 mM, in methanol) was mixed with 3ml of methanol and 0.5 ml carrageenan-based hydrogel release solutions at different pH solutions; pH 5.5 and pH 7.4 which obtained from degradation test. After mixing, the samples were kept for 1 hour at room temperature in the dark. The DPPH radical was determined by measuring the absorbance at 517 nm via UV-Vis spectroscopy. Methanol was used as blank, and 3ml of methanol mixed with 0.3 ml of DPPH was used as control. The test samples were completely done in triplicate for each.

$$\text{Inhibition (\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where $Abs_{control}$ is absorbance of methanol and DPPH solution (10:1)

Abs_{sample} is absorbance of sample in methanol and DPPH solution (10:1)

2.9 Shape retention and microbial growth studies

Hydrogel samples with a diameter of 2.8 cm and a height of 1.7 cm were placed into petri dishes and left at room temperature to observe the shape retention and microbial growth at 1, 5 and 7 days, respectively.

Results and discussions

1.Moisture content properties

Moisture Content was determined at hot air oven 60°C for 2 hours, As shown in figure1, F121, F122, and F123 formulae showed the higher moisture content (%) than the others. These formulae contain the highest 25% glycerol, this can be explained that glycerol acts as moisturizing agent which this moisturizing agent has high ability to absorb water from the surroundings that could effect on its evaporating from the deeper layers of the hydrogels, an effect known as trans-epidermal water loss (TEWL) [24]. Thus, when glycerol evaporated and released from the hydrogels increased, it means that moisture content (%) increased as well. While F81, F82 and F83 formulae contain no glycerol showed the lower moisture content (%) among the rest, this can be illustrated that there is no glycerol which acts as moisturizing agent to be evaporated and released from the deeper layers of the hydrogels.

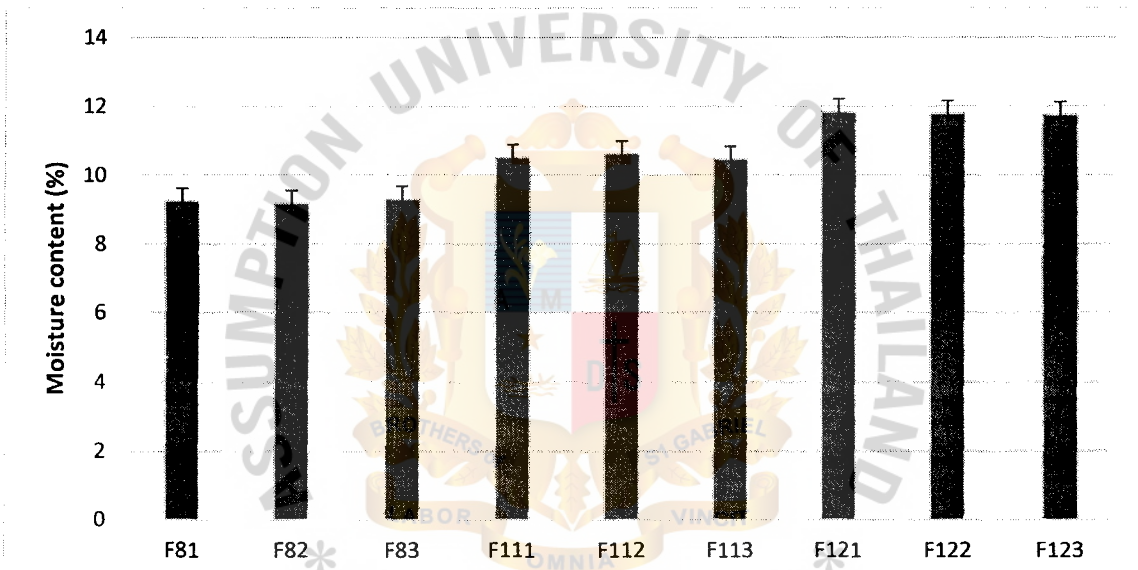

Figure1. Moisture content (%) of carrageenan-based hydrogels

2. Swelling Properties

2.1Effect of temperature on the swelling capacity

Effect of temperature on percent swelling was determined at two different temperature (RT and 37°C). It was found that both pH conditions, the degree of swelling rose with the increase of temperature, this could be explained that at the temperature at 37°C the rate of water penetration inside the polymeric network was greater than the rate of penetration at RT. Furthermore, water molecules form hydrogen bonds with hydrophilic groups of the candidate polymer leading to the stable shell of hydration around these hydrophilic groups and resulting in the greater absorption of water and making the increase of percent swelling [25].

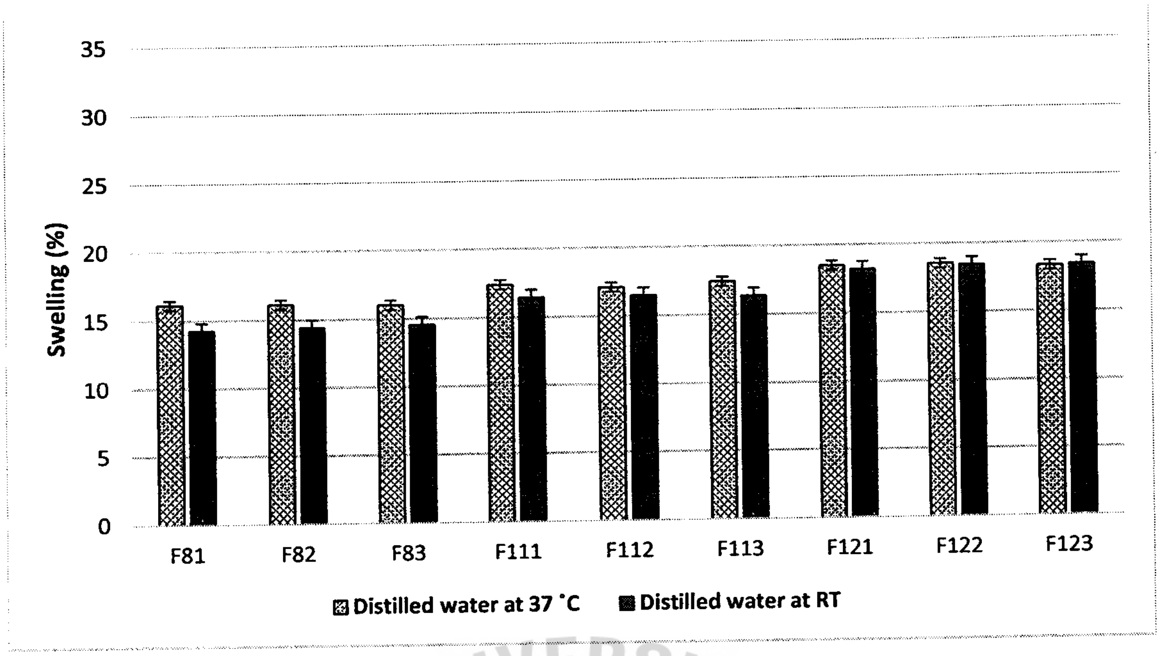

Figure2. Effect of temperature at 37˚C and RT on the swelling capacity of carrageenan-based hydrogels in distilled water (pH 5.5)

2.2 Effect of pH at 37˚C on the swelling capacity

Effect of temperature on percent swelling was studied at two different pH intervals (pH 5.5 and pH 7.4). After 24 hours, the swelling behavior was clearly observed. Hydrogel samples in pH 7.4 were very differently from those in pH 5.5 as shown in the Figure3. In pH 7.4, water molecules highly forms hydrogen-bonding so they generate more space for the molecules to penetrate inside [26], thus swells more, and hydrogel is sensitive to basic pH conditions that makes absorbing more basic liquids [27]. Both pH conditions, F121, F122 and F123 (25% glycerol) showed the maximum degree of swelling among the rest, this can be explained that their main composition consisted of the same amount of carrageenan and sorbitol, however it contained higher amount of glycerol (25% glycerol or glycerol:water as 2:0). Glycerol is a hydroscopic so it has a higher ability to absorb water than sorbitol, thus swells more than the other formulae [28].

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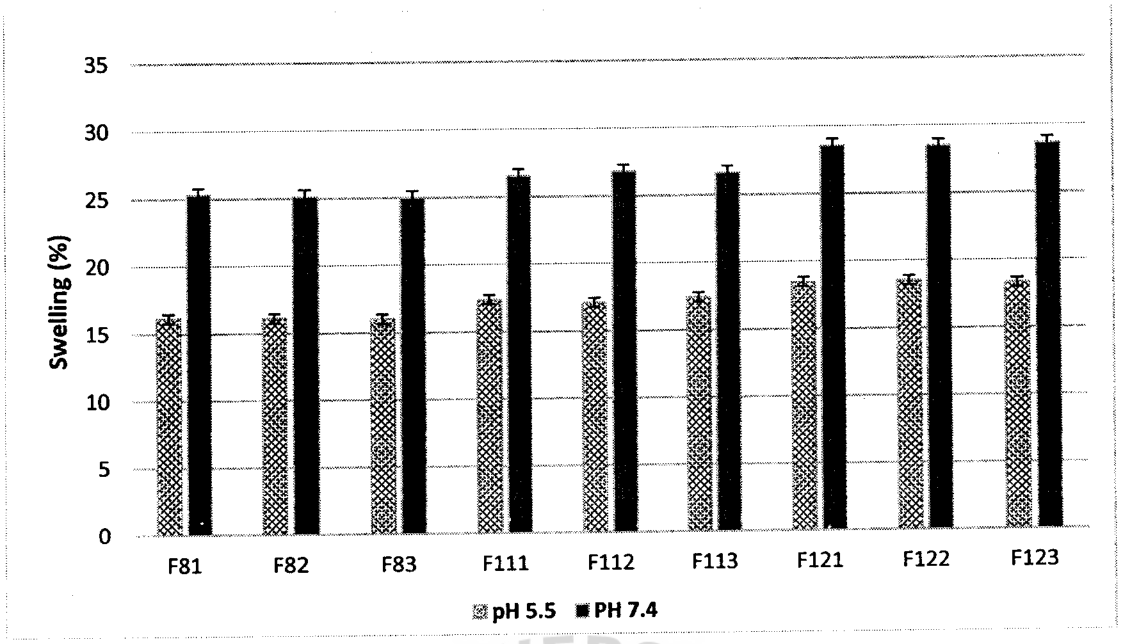

Figure3. Effect of pH at 37˚C on the swelling capacity of carrageenan-based hydrogels in distilled water (pH 5.5) and PBS buffer (pH 7.4)

2.3 Effect of pH at room temperature on the swelling capacity

Effect of pH at room temperature on percent swelling was investigated in two different pH (pH 5.5 and pH 7.4). After 24 hours, the swelling capacity could be obviously seen that hydrogels sample in pH 7.4 were significantly different from samples in pH 5.5 as shown in Figure 4. This can be explained that in pH 7.4, water molecules undergo hydrogen-bonding forms so generates more space which results in swelling more. Also, hydrogel is sensitive to basic pH that makes absorbing more basic liquids [29]. Both pH conditions, F121, F122 and F123 reached the highest degree of swelling capacity among the others, due to glycerol which is a hydroscopic so it has a higher ability to absorb water than sorbitol, thus swells more than the other formulae [30].

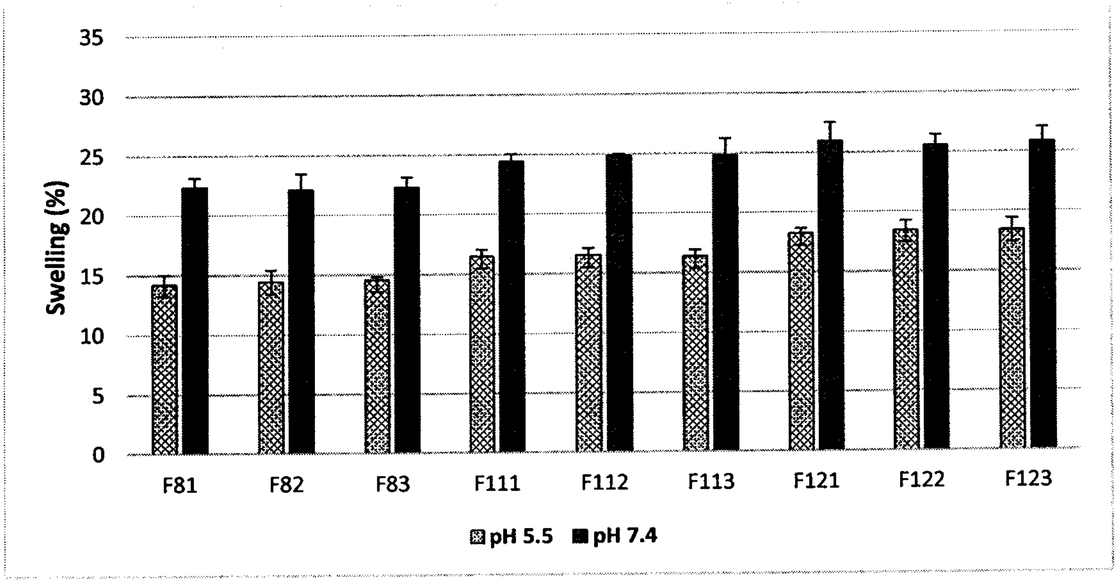

Figure4. Effect of pH at RT on the swelling capacity of carrageenan-based hydrogels in distilled water (pH 5.5) and PBS buffer (pH 7.4)

3. Texture Compression Analysis

Hydrogel firmness is used as the quality evaluation for texture parameter. Texture analyses were performed using a probe that compressed on the prepared samples, with a defined force, the result of force was plotted. The hydrogel composition influences its compression analysis. Figure 5 represents the obtained result of firmness from textural analysis. According to the obtained result, it can be seen that F81, F82, F83 exhibited the lowest compression force, and F121, F122, and F123 exhibited the highest compression force comparing to the others. Carrageenan-based hydrogels with higher water content firmed at a slower rate than hydrogels with the lower water content. Besides, it was found that the application of glycerol caused changes in the mechanical properties of carrageenan-based hydrogels as well. By increasing the amount of glycerol, the firmness values of all of the samples increased due to the tightly cross-linking of glycerol polymers [31].

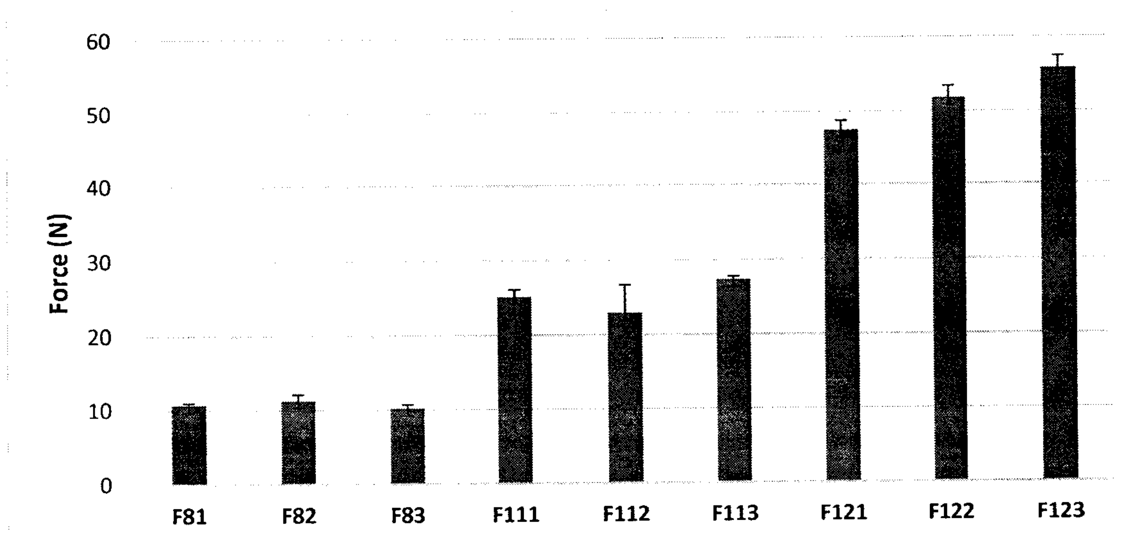

Figure5. Firmness force of carrageenan-based hydrogels

4. Color measurement

For color measurement, the results showed as L*, a*, b*, and opacity (%). According to the table2, it can be seen that percent opacity of all prepared hydrogels were quite similar from 98-100 percent. Moreover, when b* value increased, making percent opacity increased as well, this can be explained that positive b* interpreted the yellow color which means when grape seed extract increased, it affected to b* values. Therefore, F83, F113, F123 formulae which contain 2% grape seed extract showed the highest percent opacity thus, the color of carrageenan-based hydrogels were obviously affected by grape seed extract concentration.

Sample no.	L*	a*	b*	% Opacity
F81	33.38 ± 0.24	-1.05 ± 0.05	5.27 ± 0.28	99.95 ± 0.47
F82	33.38 ± 0.66	0.17 ± 0.08	8.34 ± 0.25	99.45±0.20
F83	30.58 ± 0.48	1.25 ± 0.13	11.15±0.58	99.97 ± 0.37
F111	34.52 ± 1.18	-0.49 ± 0.11	6.62±0.66	98.71 ± 0.32
F112	33.40 ± 1.61	0.23±0.24	10.29±1.01	99.52 ± 0.48
F113	30.58 ± 0.82	1.47±0.13	13.66±0.14	99.08 ± 0.53
F121	36.47 ± 0.58	-0.69±0.12	-0.69±0.37	99.78 ± 0.56
F122	31.33 ± 1.03	0.40±0.04	9.99±0.65	100.04 ± 0.81
F123	27.55±0.68	1.90±0.10	11.61±0.08	99.94±0.15

Table2. Color measurement as L*, a*, b*, and opacity (%) of carrageenan-based hydrogels

5. Antioxidant activities

5.1 Total phenolic compounds and DPPH assay

The antioxidant properties of the prepared hydrogels were determined from the degraded hydrogel solution after degrading 24 hours in PBS buffer (pH 7.4) and distilled water (pH 5.5) using total phenolic compound test and DPPH assay. Herein, total phenolic content of degraded solution was determined by the Folin-Ciocalteu colorimetric method and gallic acid was used as a standard phenolic compound. The content of total phenols is expressed as gallic acid equivalents (mg GAE/mL). As the figure6 shown, all carrageenan-based hydrogels degraded in pH 7.4 exhibited higher total phenolic compounds than that of pH 5.5 medium. At pH 5.5, the prepared hydrogel released grape seed extract molecules more effective than pH 7.4 resulting from the further hydrolysis of carrageenan hydrogels in acidic medium. At the same pH condition, the carrageenan-based hydrogels containing grape seed extract 2% has higher total phenolic content in comparison with the prepared hydrogels containing grape seed extract 0.5% and 1%. There was significant difference between the total phenolic content of prepared hydrogels containing grape seed extract 0.5%, 1% and 2% at each storage condition. The grape seed extract could act as antioxidant agent, and it was indicated that high total phenolic content was observed in the samples which contain phenolic compounds in high amounts [32].

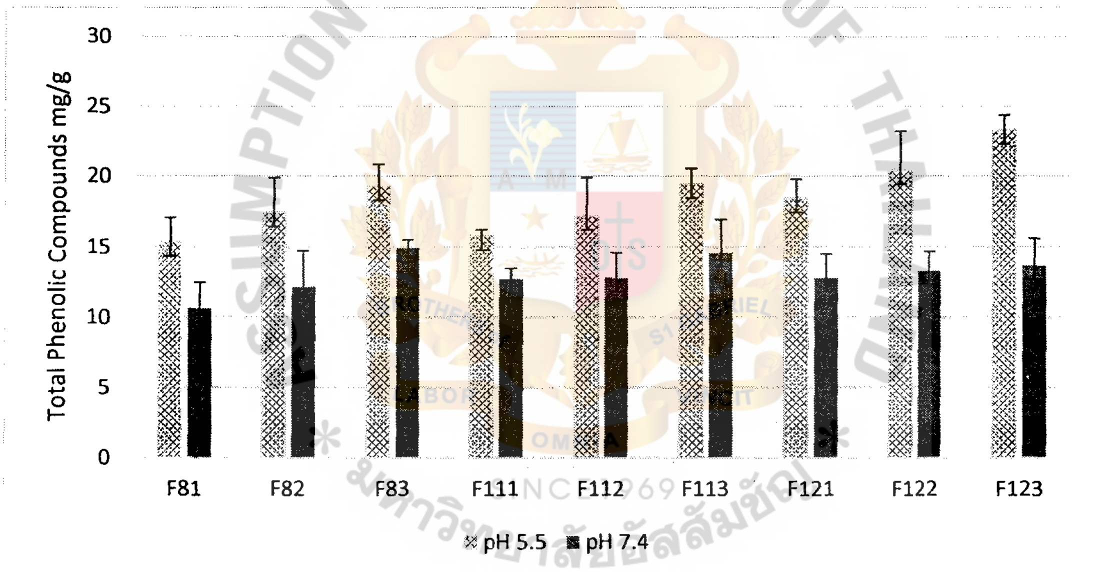

Figure6. Total phenolic content of carrageenan-based hydrogels in distilled water (pH5.5) and PBS buffer (pH 7.4)

For antioxidant properties, scavenging activity of carrageenan-based hydrogel containing grape seed extract was observed using DPPH assay. DPPH free radicals have ability to receive electron or bind with hydrogen from antioxidant molecules becoming a stable molecule. The decrease of DPPH radical quantity resulted in discoloration originally from purple to yellow. Degree of discoloration depended on radical scavenging activity of antioxidant compound in samples. Because the antioxidant activity was influenced by pH, and antioxidant activity could depend upon the oxidation rate of antioxidant compounds, and this oxidation rate was influenced by the surrounding pH [33].

As figure 7 shown, the prepared hydrogels had greater antioxidant properties at pH 5.5 medium than pH 7.4 medium. In acidic media (pH 5.5), the prepared hydrogel released degrading molecules more effective than pH 7.4 as observed in the previous results. At the same pH condition, the carrageenan-based hydrogels containing grape seed extract 2% had considerably higher scavenging activity in comparison with the prepared hydrogels containing grape seed extract 0.5% and 1% as it contained the higher amount of antioxidant agent. There was significant difference between scavenging activity of prepared hydrogels containing grape seed extract 0.5%, 1% and 2% at each storage condition. From these results, it can be seen that both the ratio of grape seed extract and the different pH conditions could impact the antioxidant properties including total phenolic content and scavenging activity.

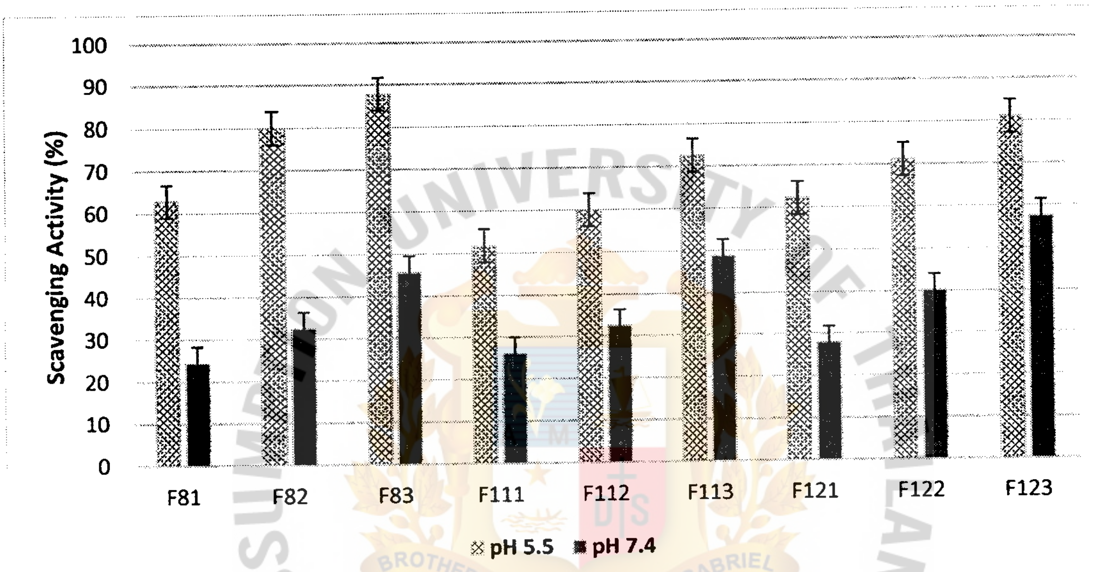

Figure7. Radical scavenging activity (%) of carrageenan-based hydrogels in distilled water (pH5.5) and PBS buffer (pH 7.4)

6. Shape retention and microbial growth

According to the figure8, F121, F122, and F123 demonstrated no microbial growth on the surface of hydrogels after keeping at room temperature for 7 days. This can be explained that these formulae contained the highest percentages of glycerol (25% from the total volume). As glycerol property, it could bind to adjacent water molecules with hydrogen bonds. When water molecules were bound, water molecules were not free and not released as the free water content to microbes. Thus, this was resulted from low water activity as the above supporting reasons.

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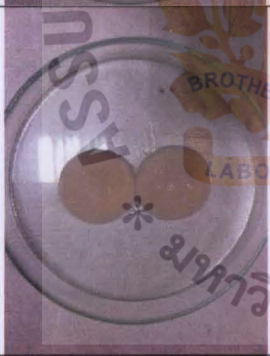
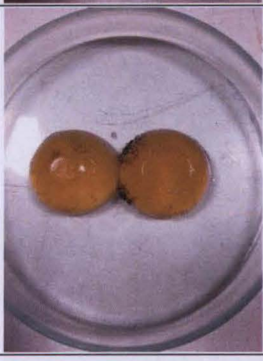
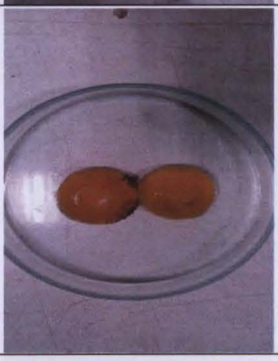
Sample no.	Day 1	Day 5	Day 7
F81			
F82			
F83			
F111			

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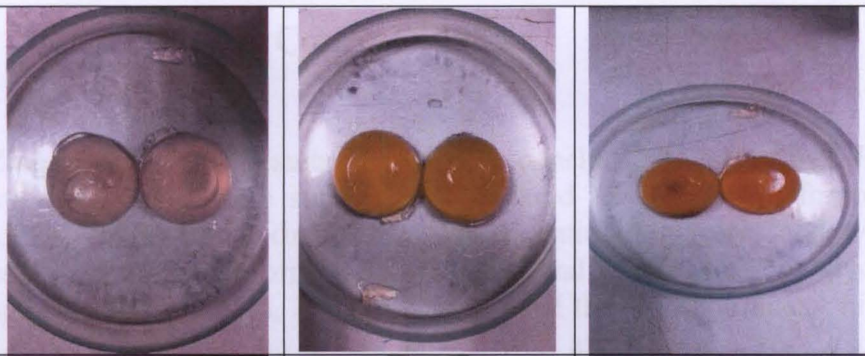
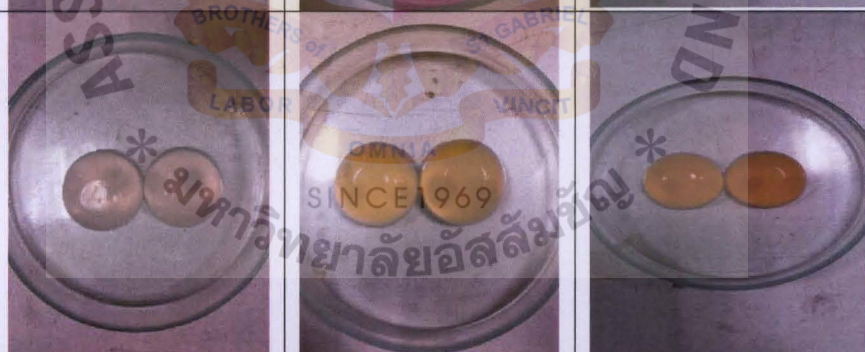
F112	
F113	
F121	
F122	
F123	

Figure8. Physical appearance of carrageenan-based hydrogels

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Conclusions

According to the overall results, they can be concluded as followings: First, carrageenan-based hydrogels were successfully prepared for 9 different formulae; 0.5%, 1% and 2% grape seed extract with various ratio of glycerol and water. Second, the results obtained that formulae F121, F122 and F123 (with 25% glycerol containing grape seed extract 0.5%, 1% and 2%) showed the higher firmness, higher resistance to degradation, and no microbial growth after storage for 7 days, comparable to all formulae. Finally, comparing formulae F121, F122 and F123, F123 showed the highest total phenolic content and % scavenging activity. Thus, the most optimum formula of carrageenan-based hydrogels was F123 containing 25% glycerol and 2% grape seed extract. Grape seed extract exhibited high antioxidant activities in carrageenan-based hydrogels which could enhance the repair and healing of cells and tissues in chronic wounds, and prevent oxidative damage.

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Appendix

Appendix A: Moisture content properties after 2 hours

Sample no.		Wt. plate	Wt. after oven	Wt. sample	percent
F81	1	40.7953	47.7604	6.9651	12.05726
	2	45.3865	51.7289	6.3424	12.98089
	3	46.5618	53.249	6.6872	12.37439
mean					12.47084
SD					0.469311
F82	1	43.4859	49.2003	5.7144	12.605
	2	44.0085	50.1011	6.0926	13.03056
	3	49.9683	55.8102	5.8419	12.15529
mean					12.59695
SD					0.43769
F83	1	44.6835	51.1325	6.449	12.90122
	2	44.5898	50.8807	6.2909	12.39886
	3	42.7737	48.4251	5.6514	12.82337
mean					12.70782
SD					0.270382
F111	1	47.1521	54.123	6.9709	13.63095
	2	42.4645	49.5287	7.0642	14.56924
	3	45.7392	52.9422	7.203	14.15244
mean					14.11754
SD					0.470115
F112	1	44.2315	51.7687	7.5372	14.93791
	2	40.0519	47.3085	7.2566	13.25414
	3	47.884	54.8639	6.9799	14.25379
mean					14.14861
SD					0.846796
F113	1	43.633	51.0552	7.4222	13.06082
	2	46.7901	53.7573	6.9672	14.51516
	3	47.4851	53.9835	6.4984	14.62976
mean					14.06858
SD					0.874624
F121	1	44.9174	52.457	7.5396	15.77537

	2	46.0869	53.395	7.3081	15.33641
	3	45.4122	53.7388	8.3266	15.06377
mean					15.39185
SD					0.359026
F122	1	44.8807	52.8259	7.9452	15.84101
	2	45.0308	53.83	8.7992	14.75248
	3	41.3023	50.2012	8.8989	16.05816
mean					15.55055
SD					0.699628
F123	1	47.8965	55.3519	7.4554	16.27545
	2	43.0745	50.7472	7.6727	15.69721
	3	44.4749	52.6105	8.1356	14.63322
mean					15.53529
SD					0.833005

Appendix B: Swelling properties

Appendix B-1 Swelling (%) in distilled water buffer at RT, after 2 hours

Sample no.		Wt. plate	0 h	Wt. sampl e	2 h	Wt. Swollen sample	percent
F81	1	40.795	46.921	6.125	47.468	6.672	8.932
	2	45.387	50.906	5.519	51.463	6.077	10.099
	3	46.562	52.422	5.860	53.098	6.537	11.550
% Swelling							10.194
SD							1.312
F82	1	43.486	48.480	4.994	49.034	5.548	11.089
	2	44.009	49.307	5.299	49.816	5.808	9.602
	3	49.968	55.100	5.132	55.595	5.627	9.652
% Swelling							10.114
SD							0.845
F83	1	44.684	50.3005	5.617	50.8872	6.2037	10.445 1
	2	44.590	50.1007	5.5109	50.6107	6.0209	9.2544
	3	42.773 7	47.7004	4.9267	48.2482	5.4745	11.119 0
% Swelling							10.272 8
SD							0.9442

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F111	1	47.1521	53.1728	6.0207	53.8324	6.6803	10.9555
	2	42.4645	48.4995	6.035	49.1654	6.7009	11.0340
	3	45.7392	51.9228	6.1836	52.749	7.0098	13.3611
% Swelling							11.7836
SD							1.3668
F112	1	44.2315	50.6428	6.4113	51.3738	7.1423	11.4017
	2	40.0519	46.3467	6.2948	47.1206	7.0687	12.2943
	3	47.884	53.869	5.985	54.5902	6.7062	12.0501
% Swelling							11.9154
SD							0.4613
F113	1	43.633	50.0858	6.4528	50.7362	7.1032	10.0793
	2	46.7901	52.746	5.9559	53.449	6.6589	11.8034
	3	47.4851	53.0328	5.5477	53.7712	6.2861	13.3100
% Swelling							11.7309
SD							1.6166
F121	1	44.9174	51.2676	6.3502	52.0398	7.1224	12.1602
	2	46.0869	52.2742	6.1873	53.0601	6.9732	12.7018
	3	45.4122	52.4845	7.0723	53.58	8.1678	15.4900
% Swelling							13.4507
SD							1.7867
F122	1	44.8807	51.5673	6.6866	52.3731	7.4924	12.0510
	2	45.0308	52.5319	7.5011	53.5203	8.4895	13.1767
	3	41.3023	48.7722	7.4699	49.887	8.5847	14.9239

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% Swelling							13.383 9
SD							1.4476
F123	1	47.896 5	54.1385	6.242	55.0736	7.1771	14.980 8
	2	43.074 5	49.5428	6.4683	50.4184	7.3439	13.536 8
	3	44.474 9	51.42	6.9451	52.221	7.7461	11.533 3
% Swelling							13.350 3
SD							1.7313

Appendix B-2 Swelling (%) in distilled water buffer at RT, after 4 hours

Sample no.		Wt. plate	0 h	Wt. sampl e	4 h	Wt. Swollen sample	percent
F81	1	40.7953	46.920 6	6.125 3	47.566 4	6.7711	10.543 2
	2	45.3865	50.905 6	5.519 1	51.589 6	6.2031	12.393 3
	3	46.5618	52.421 5	5.859 7	53.105 9	6.5441	11.679 8
% Swelling							11.538 8
SD							0.9331
F82	1	43.4859	48.48	4.994 1	49.113 4	5.6275	12.683 0
	2	44.0085	49.307 2	5.298 7	49.897 3	5.8888	11.136 7
	3	49.9683	55.100 1	5.131 8	55.630 6	5.6623	10.337 5
% Swelling							11.385 7
SD							1.1924
F83	1	44.6835	50.300 5	5.617	50.909 2	6.2257	10.836 7
	2	44.5898	50.100 7	5.510 9	50.752 4	6.1626	11.825 7
	3	42.7737	47.700 4	4.926 7	48.297 2	5.5235	12.113 6
% Swelling							11.592 0

SD							0.6697
F111	1	47.1521	53.1728	6.0207	53.8909	6.7388	11.9272
	2	42.4645	48.4995	6.035	49.2788	6.8143	12.9130
	3	45.7392	51.9228	6.1836	52.6974	6.9582	12.5267
% Swelling							12.4556
SD							0.4967
F112	1	44.2315	50.6428	6.4113	51.3957	7.1642	11.7433
	2	40.0519	46.3467	6.2948	47.1367	7.0848	12.5500
	3	47.884	53.869	5.985	54.6233	6.7393	12.6032
% Swelling							12.2988
SD							0.4818
F113	1	43.633	50.0858	6.4528	50.8368	7.2038	11.6384
	2	46.7901	52.746	5.9559	53.4695	6.6794	12.1476
	3	47.4851	53.0328	5.5477	53.7801	6.295	13.4704
% Swelling							12.4188
SD							0.9457
F121	1	44.9174	51.2676	6.3502	52.1093	7.1919	13.2547
	2	46.0869	52.2742	6.1873	53.1551	7.0682	14.2372
	3	45.4122	52.4845	7.0723	53.6055	8.1933	15.8506
% Swelling							14.4475
SD							1.3106
F122	1	44.8807	51.5673	6.6866	52.4118	7.5311	12.6297
	2	45.0308	52.5319	7.5011	53.6122	8.5814	14.4019

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	3	41.3023	48.7722	7.4699	49.9351	8.6328	15.5678
% Swelling							14.1998
SD							1.4794
F123	1	47.8965	54.1385	6.242	55.1068	7.2103	15.5127
	2	43.0745	49.5428	6.4683	50.4985	7.424	14.7751
	3	44.4749	51.42	6.9451	52.2988	7.8239	12.6535
% Swelling							14.3138
SD							1.4844

Appendix B-3 Swelling (%) in distilled water buffer at RT, after 6 hours

Sample no.		Wt. plate	0 h	Wt. sample	6 h	Wt. Swollen sample	percent
F81	1	40.7953	46.9206	6.1253	47.6102	6.8149	11.2582
	2	45.3865	50.9056	5.5191	51.6119	6.2254	12.7974
	3	46.5618	52.4215	5.8597	53.1247	6.5629	12.0006
% Swelling							12.0187
SD							0.7697
F82	1	43.4859	48.48	4.9941	49.1427	5.6568	13.2697
	2	44.0085	49.3072	5.2987	49.9128	5.9043	11.4292
	3	49.9683	55.1001	5.1318	55.7016	5.7333	11.7210
% Swelling							12.1400
SD							0.9892
F83	1	44.6835	50.3005	5.617	51.0074	6.3239	12.5850
	2	44.5898	50.1007	5.5109	50.7695	6.1797	12.1359
	3	42.7737	47.7004	4.9267	48.3013	5.5276	12.1968

% Swelling							12.3059
SD							0.2436
F111	1	47.1521	53.1728	6.0207	54.0092	6.8571	13.8921
	2	42.4645	48.4995	6.035	49.4075	6.943	15.0456
	3	45.7392	51.9228	6.1836	52.8089	7.0697	14.3298
% Swelling							14.4225
SD							0.5823
F112	1	44.2315	50.6428	6.4113	51.5851	7.3536	14.6975
	2	40.0519	46.3467	6.2948	47.2898	7.2379	14.9822
	3	47.884	53.869	5.985	54.6954	6.8114	13.8079
% Swelling							14.4958
SD							0.6126
F113	1	43.633	50.0858	6.4528	50.9721	7.3391	13.7351
	2	46.7901	52.746	5.9559	53.6095	6.8194	14.4982
	3	47.4851	53.0328	5.5477	53.847	6.3619	14.6764
% Swelling							14.3032
SD							0.5000
F121	1	44.9174	51.2676	6.3502	52.2895	7.3721	16.0924
	2	46.0869	52.2742	6.1873	53.2719	7.185	16.1250
	3	45.4122	52.4845	7.0723	53.6782	8.266	16.8785
% Swelling							16.3653
SD							0.4448
F122	1	44.8807	51.5673	6.6866	52.707	7.8263	17.0445

	2	45.0308	52.531 9	7.501 1	53.691 2	8.6604	15.455 1
	3	41.3023	48.772 2	7.469 9	50.006 8	8.7045	16.527 7
% Swelling							16.342 4
SD							0.8108
F123	1	47.8965	54.138 5	6.242	55.184 6	7.2881	16.759 1
	2	43.0745	49.542 8	6.468 3	50.632 8	7.5583	16.851 4
	3	44.4749	51.42	6.945 1	52.47	7.9951	15.118 6
% Swelling							16.243 0
SD							0.9749

Appendix B-4 Swelling (%) in distilled water buffer at RT, after 24 hours

Sample no.		Wt. plate	0 h	Wt. sampl e	24 h	Wt. Swollen sample	percent
F81	1	40.7953	46.920 6	6.125 3	47.760 4	6.9651	13.710 3
	2	45.3865	50.905 6	5.519 1	51.728 9	6.3424	14.917 3
	3	46.5618	52.421 5	5.859 7	53.249	6.6872	14.121 9
% Swelling							14.249 8
SD							0.6136
F82	1	43.4859	48.48	4.994 1	49.200 3	5.7144	14.423 0
	2	44.0085	49.307 2	5.298 7	50.101 1	6.0926	14.982 9
	3	49.9683	55.100 1	5.131 8	55.810 2	5.8419	13.837 3
% Swelling							14.414 4
SD							0.5729
F83	1	44.6835	50.300 5	5.617	51.132 5	6.449	14.812 2
	2	44.5898	50.100	5.510	50.880	6.2909	14.153

			7	9	7		8
	3	42.7737	47.7004	4.9267	48.4251	5.6514	14.7096
% Swelling							14.5585
SD							0.3543
F111	1	47.1521	53.1728	6.0207	54.123	6.9709	15.7822
	2	42.4645	48.4995	6.035	49.5287	7.0642	17.0539
	3	45.7392	51.9228	6.1836	52.9422	7.203	16.4855
% Swelling							16.4405
SD							0.6370
F112	1	44.2315	50.6428	6.4113	51.7687	7.5372	17.5612
	2	40.0519	46.3467	6.2948	47.3085	7.2566	15.2793
	3	47.884	53.869	5.985	54.8639	6.9799	16.6232
% Swelling							16.4879
SD							1.1470
F113	1	43.633	50.0858	6.4528	51.0552	7.4222	15.0229
	2	46.7901	52.746	5.9559	53.7573	6.9672	16.9798
	3	47.4851	53.0328	5.5477	53.9835	6.4984	17.1368
% Swelling							16.3799
SD							1.1777
F121	1	44.9174	51.2676	6.3502	52.457	7.5396	18.7301
	2	46.0869	52.2742	6.1873	53.395	7.3081	18.1145
	3	45.4122	52.4845	7.0723	53.7388	8.3266	17.7354
% Swelling							18.1933
SD							0.5020

F122	1	44.8807	51.5673	6.6866	52.8259	7.9452	18.8227
	2	45.0308	52.5319	7.5011	53.83	8.7992	17.3055
	3	41.3023	48.7722	7.4699	50.2012	8.8989	19.1301
% Swelling							18.4194
SD							0.9769
F123	1	47.8965	54.1385	6.242	55.3519	7.4554	19.4393
	2	43.0745	49.5428	6.4683	50.7472	7.6727	18.6200
	3	44.4749	51.42	6.9451	52.6105	8.1356	17.1416
% Swelling							18.4003
SD							1.1645

Appendix B-5 Swelling (%) in PBS buffer at RT, after 2 hours

Sample no.		Wt. plate	0 h	Wt. sample	2 h	Wt. swollen sample	percent
F81	1	43.9957	49.69	5.6943	50.4548	6.4591	13.4310
	2	46.9715	52.665	5.6935	53.369	6.3975	12.3650
	3	42.8712	48.8643	5.9931	49.6208	6.7496	12.6228
% Swelling							12.8063
SD							0.5562
F82	1	46.3421	52.2102	5.8681	52.9457	6.6036	12.5339
	2	42.1481	47.5851	5.437	48.2608	6.1127	12.4278
	3	44.0648	49.3277	5.2629	49.9592	5.8944	11.9991
% Swelling							12.3203
SD							0.2831

F83	1	45.9985	50.749	4.7505	51.3415	5.343	12.4724
	2	44.1828	49.0801	4.8973	49.6981	5.5153	12.6192
	3	42.0913	46.9464	4.8551	47.4973	5.406	11.3468
% Swelling							12.1461
SD							0.6961
F111	1	42.6867	48.9261	6.2394	49.8113	7.1246	14.1873
	2	43.5803	49.5124	5.9321	50.4025	6.8222	15.0048
	3	40.4521	46.7752	6.3231	47.6968	7.2447	14.5751
% Swelling							14.5891
SD							0.4089
F112	1	41.9873	48.016	6.0287	48.923	6.9357	15.0447
	2	46.9364	53.2796	6.3432	54.1874	7.251	14.3114
	3	50.5126	57.2386	6.726	58.1827	7.6701	14.0366
% Swelling							14.4642
SD							0.5212
F113	1	47.5966	53.607	6.0104	54.5094	6.9128	15.0140
	2	48.0842	53.9375	5.8533	54.8157	6.7315	15.0035
	3	49.213	55.2742	6.0612	56.1263	6.9133	14.0583
% Swelling							14.6919
SD							0.5488
F121	1	51.7424	58.2126	6.4702	59.2127	7.4703	15.4570
	2	43.1434	49.7486	6.6052	50.7569	7.6135	15.2652
	3	43.6577	49.7251	6.0674	50.773	7.1153	17.2710
% Swelling							15.997

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							8
SD							1.1068
F122	1	45.0422	51.9215	6.8793	53.0661	8.0239	16.6383
	2	41.0285	47.4	6.3715	48.487	7.4585	17.0603
	3	48.336	55.6345	7.2985	56.6744	8.3384	14.2481
% Swelling							15.9823
SD							1.5166
F123	1	49.1081	55.6176	6.5095	56.6744	7.5663	16.2347
	2	45.133	51.4712	6.3382	52.5534	7.4204	17.0742
	3	43.6215	50.5628	6.9413	51.5545	7.933	14.2869
% Swelling							15.8653
SD							1.4299

Appendix B-6 Swelling (%) in PBS buffer at RT, after 4 hours

Sample no.		Wt. plate	0 h	Wt. sample	4 h	Wt. swollen sample	percent
F81	1	43.9957	49.69	5.6943	50.5018	6.5061	14.2564
	2	46.9715	52.665	5.6935	53.566	6.5945	15.8251
	3	42.8712	48.8643	5.9931	49.7804	6.9092	15.2859
% Swelling							15.1224
SD							0.7970
F82	1	46.3421	52.2102	5.8681	53.1455	6.8034	15.9387
	2	42.1481	47.5851	5.437	48.373	6.2249	14.4914
	3	44.0648	49.3277	5.2629	50.1281	6.0633	15.2083
% Swelling							15.2128

SD							0.7236
F83	1	45.9985	50.749	4.750 5	51.498 1	5.4996	15.768 9
	2	44.1828	49.080 1	4.897 3	49.854 3	5.6715	15.808 7
	3	42.0913	46.946 4	4.855 1	47.629 8	5.5385	14.075 9
% Swelling							15.217 8
SD							0.9891
F111	1	42.6867	48.926 1	6.239 4	50.042 5	7.3558	17.892 7
	2	43.5803	49.512 4	5.932 1	50.504 2	6.9239	16.719 2
	3	40.4521	46.775 2	6.323 1	47.885 5	7.4334	17.559 4
% Swelling							17.390 5
SD							0.6047
F112	1	41.9873	48.016	6.028 7	48.934 5	6.9472	15.235 5
	2	46.9364	53.279 6	6.343 2	54.476 7	7.5403	18.872 2
	3	50.5126	57.238 6	6.726	58.424 3	7.9117	17.628 6
% Swelling							17.245 4
SD							1.8484
F113	1	47.5966	53.607	6.010 4	54.661 9	7.0653	17.551 2
	2	48.0842	53.937 5	5.853 3	54.869 3	6.7851	15.919 2
	3	49.213	55.274 2	6.061 2	56.389 2	7.1762	18.395 7
% Swelling							17.288 7
SD							1.2589
F121	1	51.7424	58.212 6	6.470 2	59.494 1	7.7517	19.806 2
	2	43.1434	49.748 6	6.605 2	50.970 3	7.8269	18.496 0

	3	43.6577	49.7251	6.0674	50.8121	7.1544	17.9154
% Swelling							18.7392
SD							0.9686
F122	1	45.0422	51.9215	6.8793	53.1608	8.1186	18.0149
	2	41.0285	47.4	6.3715	48.6753	7.6468	20.0157
	3	48.336	55.6345	7.2985	56.9471	8.6111	17.9845
% Swelling							18.6717
SD							1.1640
F123	1	49.1081	55.6176	6.5095	56.8875	7.7794	19.5084
	2	45.133	51.4712	6.3382	52.7641	7.6311	20.3985
	3	43.6215	50.5628	6.9413	51.7309	8.1094	16.8283
% Swelling							18.9117
SD							1.8584

Appendix B-7 Swelling (%) in PBS buffer at RT, after 6 hours

Sample no.		Wt. plate	0 h	Wt. sample	6 h	Wt. swollen sample	percent
F81	1	43.9957	49.69	5.6943	50.6965	6.7008	17.6756
	2	46.9715	52.665	5.6935	53.6247	6.6532	16.8561
	3	42.8712	48.8643	5.9931	49.9356	7.0644	17.8756
% Swelling							17.4691
SD							0.5402
F82	1	46.3421	52.2102	5.8681	53.1631	6.821	16.2386
	2	42.1481	47.5851	5.437	48.5591	6.411	17.9143
	3	44.0648	49.3277	5.2629	50.2815	6.2167	18.1231

% Swelling							17.425 3
SD							1.0330
F83	1	45.9985	50.749	4.750 5	51.543 8	5.5453	16.730 9
	2	44.1828	49.080 1	4.897 3	49.912 6	5.7298	16.999 2
	3	42.0913	46.946 4	4.855 1	47.843 5	5.7522	18.477 5
% Swelling							17.402 5
SD							0.9406
F111	1	42.6867	48.926 1	6.239 4	50.088 2	7.4015	18.625 2
	2	43.5803	49.512 4	5.932 1	50.670 3	7.09	19.519 2
	3	40.4521	46.775 2	6.323 1	48.087 3	7.6352	20.750 9
% Swelling							19.631 8
SD							1.0673
F112	1	41.9873	48.016	6.028 7	49.135 9	7.1486	18.576 1
	2	46.9364	53.279 6	6.343 2	54.518 3	7.5819	19.528 0
	3	50.5126	57.238 6	6.726	58.661 5	8.1489	21.155 2
% Swelling							19.753 1
SD							1.3042
F113	1	47.5966	53.607	6.010 4	54.750 4	7.1538	19.023 7
	2	48.0842	53.937 5	5.853 3	55.039 6	6.9554	18.828 7
	3	49.213	55.274 2	6.061 2	56.544 6	7.3316	20.959 5
% Swelling							19.604 0
SD							1.1780
F121	1	51.7424	58.212 6	6.470 2	59.612 8	7.8704	21.640 8

	2	43.1434	49.7486	6.6052	51.0803	7.9369	20.1614
	3	43.6577	49.7251	6.0674	50.974	7.3163	20.5838
% Swelling							20.7953
SD							0.7620
F122	1	45.0422	51.9215	6.8793	53.3301	8.2879	20.4759
	2	41.0285	47.4	6.3715	48.7781	7.7496	21.6291
	3	48.336	55.6345	7.2985	57.0875	8.7515	19.9082
% Swelling							20.6711
SD							0.8769
F123	1	49.1081	55.6176	6.5095	56.9983	7.8902	21.2105
	2	45.133	51.4712	6.3382	52.8841	7.7511	22.2918
	3	43.6215	50.5628	6.9413	51.8905	8.269	19.1275
% Swelling							20.8766
SD							1.6083

Appendix B-8 Swelling (%) in PBS buffer at RT, after 24 hours

Sample no.		Wt. plate	0 h	Wt. sample	24 h	Wt. swollen sample	percent
F81	1	43.9957	49.69	5.6943	51.012	7.0163	23.2162
	2	46.9715	52.665	5.6935	53.9028	6.9313	21.7406
	3	42.8712	48.8643	5.9931	50.1875	7.3163	22.0787
% Swelling							22.3452
SD							0.7730
F82	1	46.3421	52.2102	5.8681	53.4634	7.1213	21.3561
	2	42.1481	47.5851	5.437	48.7486	6.6005	21.3997

	3	44.0648	49.3277	5.2629	50.5722	6.5074	23.6467
% Swelling							22.1342
SD							1.3100
F83	1	45.9985	50.749	4.7505	51.7801	5.7816	21.7051
	2	44.1828	49.0801	4.8973	50.2202	6.0374	23.2802
	3	42.0913	46.9464	4.8551	48.0098	5.9185	21.9027
% Swelling							22.2960
SD							0.8580
F111	1	42.6867	48.9261	6.2394	50.4074	7.7207	23.7411
	2	43.5803	49.5124	5.9321	50.9651	7.3848	24.4888
	3	40.4521	46.7752	6.3231	48.3569	7.9048	25.0146
% Swelling							24.4148
SD							0.6400
F112	1	41.9873	48.016	6.0287	49.5238	7.5365	25.0104
	2	46.9364	53.2796	6.3432	54.8511	7.9147	24.7746
	3	50.5126	57.2386	6.726	58.9042	8.3916	24.7636
% Swelling							24.8495
SD							0.1394
F113	1	47.5966	53.607	6.0104	55.03	7.4334	23.6756
	2	48.0842	53.9375	5.8533	55.368	7.2838	24.4392
	3	49.213	55.2742	6.0612	56.8735	7.6605	26.3859
% Swelling							24.8336
SD							1.3975

F121	1	51.7424	58.2126	6.4702	59.9985	8.2561	27.6019
	2	43.1434	49.7486	6.6052	51.4461	8.3027	25.6994
	3	43.6577	49.7251	6.0674	51.21	7.5523	24.4734
% Swelling							25.9249
SD							1.5764
F122	1	45.0422	51.9215	6.8793	53.6227	8.5805	24.7293
	2	41.0285	47.4	6.3715	49.0853	8.0568	26.4506
	3	48.336	55.6345	7.2985	57.4944	9.1584	25.4833
% Swelling							25.5544
SD							0.8629
F123	1	49.1081	55.6176	6.5095	57.3782	8.2701	27.0466
	2	45.133	51.4712	6.3382	53.1037	7.9707	25.7565
	3	43.6215	50.5628	6.9413	52.2755	8.654	24.6741
% Swelling							25.8257
SD							1.1878

Appendix B-9 Swelling (%) in distilled water at 37°C, after 2 hours

Sample no.		Wt.plate	0 h	Wt. sample	2 h	Wt. Swollen Sample	Percent
F81	1	44.2637	49.7536	5.4899	50.3568	6.0931	10.9874
	2	42.6779	48.1796	5.5017	48.8703	6.1924	12.5543
	3	48.4955	54.1548	5.6593	54.8131	6.3176	11.6322
% Swelling							11.7246
SD							0.7875
F82	1	48.4662	53.3136	4.8474	53.848	5.3818	11.0245

	2	39.9001	45.6447	5.7446	46.2411	6.341	10.3819
	3	43.3798	47.641	4.2612	48.2422	4.8624	14.1087
% Swelling							11.8384
SD							1.9922
F83	1	45.5504	50.596	5.0456	51.1452	5.5948	10.8847
	2	48.3849	53.0104	4.6255	53.5591	5.1742	11.8625
	3	47.5267	52.1539	4.6272	52.7534	5.2267	12.9560
% Swelling							11.9011
SD							1.0362
F111	1	48.1159	54.9693	6.8534	55.8385	7.7226	12.6828
	2	45.0931	52.1263	7.0332	53.0925	7.9994	13.7377
	3	40.3431	47.3598	7.0167	48.2278	7.8847	12.3705
% Swelling							12.9303
SD							0.7164
F112	1	46.5516	53.3303	6.7787	54.252	7.7004	13.5970
	2	49.865	56.378	6.513	57.2261	7.3611	13.0216
	3	43.898	50.572	6.674	51.3446	7.4466	11.5763
% Swelling							12.7316
SD							1.0411
F113	1	42.6517	49.4668	6.8151	50.3725	7.7208	13.2896
	2	43.9811	50.9565	6.9754	51.8724	7.8913	13.1304
	3	47.3722	53.9782	6.606	54.8003	7.4281	12.4447
% Swelling							12.9549
SD							0.4489

F121	1	43.1568	49.4124	6.2556	50.3009	7.1441	14.2033
	2	45.7585	51.9824	6.2239	52.8429	7.0844	13.8257
	3	40.6043	46.7031	6.0988	47.6305	7.0262	15.2063
% Swelling							14.4118
SD							0.7135
F122	1	42.7165	49.2816	6.5651	50.159	7.4425	13.3646
	2	44.7745	51.1847	6.4102	52.0962	7.3217	14.2195
	3	45.2933	51.4752	6.1819	52.4034	7.1101	15.0148
% Swelling							14.1996
SD							0.8253
F123	1	43.0029	49.1324	6.1295	49.9877	6.9848	13.9538
	2	39.7667	46.3918	6.6251	47.3593	7.5926	14.6036
	3	43.5533	50.0824	6.5291	51.0169	7.4636	14.3128
% Swelling							14.2901
SD							0.3255

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Appendix B-10 Swelling (%) in distilled water at 37°C, after 4 hours

Sample no.		Wt.plate	0 h	Wt. sample	4 h	Wt. Swollen Sample	Percent
F81	1	44.2637	49.7536	5.4899	50.474	6.2103	13.1223
	2	42.6779	48.1796	5.5017	48.8664	6.1885	12.4834
	3	48.4955	54.1548	5.6593	54.9042	6.4087	13.2419
% Swelling							12.9492
SD							0.4078

F82	1	48.4662	53.3136	4.8474	53.918	5.4518	12.4685
	2	39.9001	45.6447	5.7446	46.3911	6.491	12.9931
	3	43.3798	47.641	4.2612	48.1722	4.7924	12.4660
% Swelling							12.6425
SD							0.3036
F83	1	45.5504	50.596	5.0456	51.2452	5.6948	12.8667
	2	48.3849	53.0104	4.6255	53.5591	5.1742	11.8625
	3	47.5267	52.1539	4.6272	52.7734	5.2467	13.3882
% Swelling							12.7058
SD							0.7755
F111	1	48.1159	54.9693	6.8534	55.9043	7.7884	13.6429
	2	45.0931	52.1263	7.0332	53.1872	8.0941	15.0842
	3	40.3431	47.3598	7.0167	48.2341	7.891	12.4603
% Swelling							13.7291
SD							1.3141
F112	1	46.5516	53.3303	6.7787	54.301	7.7494	14.3199
	2	49.865	56.378	6.513	57.3127	7.4477	14.3513
	3	43.898	50.572	6.674	51.4268	7.5288	12.8079
% Swelling							13.8264
SD							0.8821
F113	1	42.6517	49.4668	6.8151	50.4252	7.7735	14.0629
	2	43.9811	50.9565	6.9754	51.9033	7.9222	13.5734
	3	47.3722	53.9782	6.606	54.9085	7.5363	14.0827
% Swelling							13.906

							3
SD							0.2885
F121	1	43.1568	49.4124	6.2556	50.4027	7.2459	15.8306
	2	45.7585	51.9824	6.2239	52.8601	7.1016	14.1021
	3	40.6043	46.7031	6.0988	47.7551	7.1508	17.2493
% Swelling							15.7273
SD							1.5761
F122	1	42.7165	49.2816	6.5651	50.2908	7.5743	15.3722
	2	44.7745	51.1847	6.4102	52.1857	7.4112	15.6157
	3	45.2933	51.4752	6.1819	52.5	7.2067	16.5774
% Swelling							15.8551
SD							0.6373
F123	1	43.0029	49.1324	6.1295	50.1852	7.1823	17.1760
	2	39.7667	46.3918	6.6251	47.3682	7.6015	14.7379
	3	43.5533	50.0824	6.5291	51.0687	7.5154	15.1062
% Swelling							15.6734
SD							1.3143

Appendix B-11 Swelling (%) in distilled water at 37°C, after 6 hours

Sample no.		Wt.plate	0 h	Wt. sample	6 h	Wt. Swollen sample	Percent
F81	1	44.2637	49.7536	5.4899	50.5002	6.2365	13.5995
	2	42.6779	48.1796	5.5017	48.9035	6.2256	13.1578
	3	48.4955	54.1548	5.6593	54.9887	6.4932	14.7350
% Swelling							13.8308

SD							0.8137
F82	1	48.4662	53.3136	4.8474	54.0041	5.5379	14.2447
	2	39.9001	45.6447	5.7446	46.4038	6.5037	13.2141
	3	43.3798	47.641	4.2612	48.2577	4.8779	14.4724
% Swelling							13.9771
SD							0.6705
F83	1	45.5504	50.596	5.0456	51.2568	5.7064	13.0966
	2	48.3849	53.0104	4.6255	53.6392	5.2543	13.5942
	3	47.5267	52.1539	4.6272	52.8414	5.3147	14.8578
% Swelling							13.8495
SD							0.9080
F111	1	48.1159	54.9693	6.8534	55.9824	7.8665	14.7824
	2	45.0931	52.1263	7.0332	53.2064	8.1133	15.3572
	3	40.3431	47.3598	7.0167	48.4006	8.0575	14.8332
% Swelling							14.9909
SD							0.3182
F112	1	46.5516	53.3303	6.7787	54.3992	7.8476	15.7685
	2	49.865	56.378	6.513	57.3501	7.4851	14.9255
	3	43.898	50.572	6.674	51.5034	7.6054	13.9556
% Swelling							14.8832
SD							0.9072
F113	1	42.6517	49.4668	6.8151	50.5509	7.8992	15.9073
	2	43.9811	50.9565	6.9754	51.95	7.9689	14.2429

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	3	47.3722	53.9782	6.606	54.9212	7.549	14.2749
% Swelling							14.8084
SD							0.9518
F121	1	43.1568	49.4124	6.2556	50.5035	7.3467	17.4420
	2	45.7585	51.9824	6.2239	52.9241	7.1656	15.1304
	3	40.6043	46.7031	6.0988	47.7822	7.1779	17.6936
% Swelling							16.7553
SD							1.4129
F122	1	42.7165	49.2816	6.5651	50.3709	7.6544	16.5923
	2	44.7745	51.1847	6.4102	52.2778	7.5033	17.0525
	3	45.2933	51.4752	6.1819	52.5412	7.2479	17.2439
% Swelling							16.9629
SD							0.3349
F123	1	43.0029	49.1324	6.1295	50.2055	7.2026	17.5071
	2	39.7667	46.3918	6.6251	47.464	7.6973	16.1839
	3	43.5533	50.0824	6.5291	51.1997	7.6464	17.1126
% Swelling							16.9346
SD							0.6793

Appendix B-12 Swelling (%) in distilled water at 37°C, after 24hours

Sample no.		Wt.plate	0 h	Wt. sample	24 h	Wt. Swollen sample	Percent
F81	1	44.2637	49.7536	5.4899	50.6721	6.4084	16.7307
	2	42.6779	48.1796	5.5017	48.9963	6.3184	14.8445
	3	48.4955	54.1548	5.6593	55.1057	6.6102	16.8024

% Swelling							16.1259
SD							1.1103
F82	1	48.4662	53.3136	4.8474	54.1369	5.6707	16.9844
	2	39.9001	45.6447	5.7446	46.53	6.6299	15.4110
	3	43.3798	47.641	4.2612	48.3215	4.9417	15.9697
% Swelling							16.1217
SD							0.7976
F83	1	45.5504	50.596	5.0456	51.3974	5.847	15.8831
	2	48.3849	53.0104	4.6255	53.7228	5.3379	15.4016
	3	47.5267	52.1539	4.6272	52.9301	5.4034	16.7747
% Swelling							16.0198
SD							0.6967
F111	1	48.1159	54.9693	6.8534	56.1213	8.0054	16.8092
	2	45.0931	52.1263	7.0332	53.4107	8.3176	18.2620
	3	40.3431	47.3598	7.0167	48.56	8.2169	17.1049
% Swelling							17.3920
SD							0.7678
F112	1	46.5516	53.3303	6.7787	54.5218	7.9702	17.5771
	2	49.865	56.378	6.513	57.4925	7.6275	17.1119
	3	43.898	50.572	6.674	51.6823	7.7843	16.6362
% Swelling							17.1084
SD							0.4705
F113	1	42.6517	49.4668	6.8151	50.7105	8.0588	18.2492

	2	43.9811	50.9565	6.9754	52.0958	8.1147	16.3331
	3	47.3722	53.9782	6.606	55.148	7.7758	17.7081
% Swelling							17.4301
SD							0.9878
F121	1	43.1568	49.4124	6.2556	50.6447	7.4879	19.6991
	2	45.7585	51.9824	6.2239	53.022	7.2635	16.7034
	3	40.6043	46.7031	6.0988	47.864	7.2597	19.0349
% Swelling							18.4791
SD							1.5733
F122	1	42.7165	49.2816	6.5651	50.4575	7.741	17.9114
	2	44.7745	51.1847	6.4102	52.4131	7.6386	19.1632
	3	45.2933	51.4752	6.1819	52.6201	7.3268	18.5202
% Swelling							18.5316
SD							0.6260
F123	1	43.0029	49.1324	6.1295	50.3372	7.3343	19.6558
	2	39.7667	46.3918	6.6251	47.5914	7.8247	18.1069
	3	43.5533	50.0824	6.5291	51.2039	7.6506	17.1769
% Swelling							18.3132
SD							1.2522

Appendix B-13 Swelling (%) in PBS buffer at 37°C, after 2 hours

Sample no.		Wt. plate	0 h	Wt. Sample	2 h	Wt. Swollen Sample	Percent
F81	1	46.8921	52.1105	5.2184	52.8774	5.9853	14.6961
	2	45.0906	48.564	3.4734	49.0526	3.962	14.0669

	3	41.767	46.9464	5.1794	47.7026	5.9356	14.6001
%Swelling							14.4544
SD							0.3390
F82	1	42.3082	47.7775	5.4693	48.5769	6.2687	14.6161
	2	49.1529	54.2041	5.0512	54.9239	5.771	14.2501
	3	34.2891	39.7491	5.46	40.5378	6.2487	14.4451
% Swelling							14.4371
SD							0.1832
F83	1	48.0556	52.2054	4.1498	52.8196	4.764	14.8007
	2	42.354	49.8632	7.5092	50.9185	8.5645	14.0534
	3	45.956	51.2572	5.3012	52.0272	6.0712	14.5250
% Swelling							14.4597
SD							0.3779
F111	1	44.8677	50.5115	5.6438	51.3872	6.5195	15.5161
	2	50.7629	57.1107	6.3478	58.0835	7.3206	15.3250
	3	45.0226	51.4146	6.392	52.4761	7.4535	16.6067
% Swelling							15.8159
SD							0.6914
F112	1	46.92454	53.1179	6.19336	54.0804	7.15586	15.5408
	2	44.9967	50.4587	5.462	51.3169	6.3202	15.7122
	3	45.6549	51.2569	5.602	52.1876	6.5327	16.6137
% Swelling							15.9556
SD							0.5764

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F113	1	43.2178	48.8204	5.6026	49.6806	6.4628	15.3536
	2	45.912	51.5405	5.6285	52.4473	6.5353	16.1109
	3	46.8727	52.8394	5.9667	53.7934	6.9207	15.9887
% Swelling							15.8177
SD							0.4066
F121	1	43.7936	49.3315	5.5379	50.2979	6.5043	17.4507
	2	43.0321	48.6685	5.6364	49.6763	6.6442	17.8802
	3	42.8203	48.4604	5.6401	49.4819	6.6616	18.1114
% Swelling							17.8141
SD							0.3353
F122	1	48.6362	54.595	5.9588	55.6532	7.017	17.7586
	2	44.5476	50.7495	6.2019	51.864	7.3164	17.9703
	3	44.7346	52.0181	7.2835	53.2838	8.5492	17.3776
% Swelling							17.7022
SD							0.3003
F123	1	42.3951	48.3445	5.9494	49.3876	6.9925	17.5329
	2	42.6253	48.3689	5.7436	49.3715	6.7462	17.4560
	3	42.3595	48.4661	6.1066	49.5381	7.1786	17.5548
% Swelling							17.5145
SD							0.0519

Appendix B-14 Swelling (%) in PBS buffer at 37°C, after 4 hours

Sample no.		Wt. plate	0 h	Wt. Sample	4 h	Wt. Swollen Sample	Percent
F81	1	46.8921	52.1105	5.2184	52.9984	6.1063	17.0148
	2	45.0906	48.564	3.4734	49.1577	4.0671	17.0928
	3	41.767	46.9464	5.1794	47.8781	6.1111	17.9886
%Swelling							17.3654
SD							0.5411
F82	1	42.3082	47.7775	5.4693	48.7172	6.409	17.1814
	2	49.1529	54.2041	5.0512	55.1147	5.9618	18.0274
	3	34.2891	39.7491	5.46	40.6852	6.3961	17.1447
% Swelling							17.4511
SD							0.4994
F83	1	48.0556	52.2054	4.1498	52.9038	4.8482	16.8297
	2	42.354	49.8632	7.5092	51.1898	8.8358	17.6663
	3	45.956	51.2572	5.3012	52.1804	6.2244	17.4149
% Swelling							17.3037
SD							0.4293
F111	1	44.8677	50.5115	5.6438	51.5706	6.7029	18.7657
	2	50.7629	57.1107	6.3478	58.2761	7.5132	18.3591
	3	45.0226	51.4146	6.392	52.6563	7.6337	19.4258
% Swelling							18.8502
SD							0.5384
F112	1	46.92454	53.1179	6.19336	54.2632	7.33866	18.4924

	2	44.9967	50.4587	5.462	51.4915	6.4948	18.9088
	3	45.6549	51.2569	5.602	52.3198	6.6649	18.9736
% Swelling							18.7916
SD							0.2611
F113	1	43.2178	48.8204	5.6026	49.8354	6.6176	18.1166
	2	45.912	51.5405	5.6285	52.6011	6.6891	18.8434
	3	46.8727	52.8394	5.9667	53.9897	7.117	19.2787
% Swelling							18.7462
SD							0.5871
F121	1	43.7936	49.3315	5.5379	50.4827	6.6891	20.7877
	2	43.0321	48.6685	5.6364	49.8248	6.7927	20.5149
	3	42.8203	48.4604	5.6401	49.6396	6.8193	20.9074
% Swelling							20.7367
SD							0.2012
F122	1	48.6362	54.595	5.9588	55.8289	7.1927	20.7072
	2	44.5476	50.7495	6.2019	52.0458	7.4982	20.9017
	3	44.7346	52.0181	7.2835	53.5053	8.7707	20.4188
% Swelling							20.6759
SD							0.2430
F123	1	42.3951	48.3445	5.9494	49.5828	7.1877	20.8139
	2	42.6253	48.3689	5.7436	49.5371	6.9118	20.3392
	3	42.3595	48.4661	6.1066	49.7327	7.3732	20.7415
% Swelling							20.6315
SD							0.2558

Appendix B-15 Swelling (%) in PBS buffer at 37°C, after 6 hours

Sample no.		Wt. plate	0 h	Wt. Sample	6 h	Wt. Swollen Samples	Percent
F81	1	46.8921	52.1105	5.2184	53.0905	6.1984	18.7797
	2	45.0906	48.564	3.4734	49.2834	4.1928	20.7117
	3	41.767	46.9464	5.1794	47.9681	6.2011	19.7262
%Swelling							19.7392
SD							0.9661
F82	1	42.3082	47.7775	5.4693	48.8276	6.5194	19.1999
	2	49.1529	54.2041	5.0512	55.2453	6.0924	20.6129
	3	34.2891	39.7491	5.46	40.7748	6.4857	18.7857
% Swelling							19.5328
SD							0.9580
F83	1	48.0556	52.2054	4.1498	53.0467	4.9911	20.2733
	2	42.354	49.8632	7.5092	51.2782	8.9242	18.8436
	3	45.956	51.2572	5.3012	52.2869	6.3309	19.4239
% Swelling							19.5136
SD							0.7191
F111	1	44.8677	50.5115	5.6438	51.6909	6.8232	20.8973
	2	50.7629	57.1107	6.3478	58.4614	7.6985	21.2782
	3	45.0226	51.4146	6.392	52.7238	7.7012	20.4819
% Swelling							20.8858
SD							0.3983

F112	1	46.92454	53.1179	6.19336	54.3681	7.44356	20.1861
	2	44.9967	50.4587	5.462	51.5838	6.5871	20.5987
	3	45.6549	51.2569	5.602	52.4819	6.827	21.8672
% Swelling							20.8840
SD							0.8761
F113	1	43.2178	48.8204	5.6026	49.9711	6.7533	20.5387
	2	45.912	51.5405	5.6285	52.7603	6.8483	21.6718
	3	46.8727	52.8394	5.9667	54.0165	7.1438	19.7278
% Swelling							20.6461
SD							0.9765
F121	1	43.7936	49.3315	5.5379	50.5761	6.7825	22.4742
	2	43.0321	48.6685	5.6364	50.0016	6.9695	23.6516
	3	42.8203	48.4604	5.6401	49.7022	6.8819	22.0173
% Swelling							22.7144
SD							0.8432
F122	1	48.6362	54.595	5.9588	55.865	7.2288	21.3130
	2	44.5476	50.7495	6.2019	52.1903	7.6427	23.2316
	3	44.7346	52.0181	7.2835	53.6925	8.9579	22.9889
% Swelling							22.5112
SD							1.0447
F123	1	42.3951	48.3445	5.9494	49.6814	7.2863	22.4712
	2	42.6253	48.3689	5.7436	49.6187	6.9934	21.7599
	3	42.3595	48.4661	6.1066	49.9533	7.5938	24.3540
%							22.861

Swelling							7
SD							1.3404

Appendix B-16 Swelling (%) in PBS buffer at 37°C, after 24 hours

Sample no.		Wt. plate	0 h	Wt. Sample	24 h	Wt. Swollen Samples	Percent
F81	1	46.8921	52.1105	5.2184	53.4587	6.5666	25.8355
	2	45.0906	48.564	3.4734	49.46	4.3694	25.7960
	3	41.767	46.9464	5.1794	48.2021	6.4351	24.2441
%Swelling							25.2919
SD							0.9076
F82	1	42.3082	47.7775	5.4693	49.2104	6.9022	26.1990
	2	49.1529	54.2041	5.0512	55.4741	6.3212	25.1425
	3	34.2891	39.7491	5.46	41.063	6.7739	24.0641
% Swelling							25.1352
SD							1.0675
F83	1	48.0556	52.2054	4.1498	53.288	5.2324	26.0880
	2	42.354	49.8632	7.5092	51.6902	9.3362	24.3302
	3	45.956	51.2572	5.3012	52.5607	6.6047	24.5888
% Swelling							25.0023
SD							0.9491
F111	1	44.8677	50.5115	5.6438	52.0502	7.1825	27.2635
	2	50.7629	57.1107	6.3478	58.7846	8.0217	26.3698
	3	45.0226	51.414	6.392	53.078	8.0557	26.027

			6		3		8
% Swelling							26.553 7
SD							0.6381
F112	1	46.92454	53.117 9	6.1933 6	54.806	7.88146	27.256 6
	2	44.9967	50.458 7	5.462	51.872 8	6.8761	25.889 8
	3	45.6549	51.256 9	5.602	52.785 4	7.1305	27.284 9
% Swelling							26.810 4
SD							0.7974
F113	1	43.2178	48.820 4	5.6026	50.345 2	7.1274	27.215 9
	2	45.912	51.540 5	5.6285	53.057 8	7.1458	26.957 4
	3	46.8727	52.839 4	5.9667	54.380 6	7.5079	25.830 0
% Swelling							26.667 8
SD							0.7370
F121	1	43.7936	49.331 5	5.5379	50.931 1	7.1375	28.884 6
	2	43.0321	48.668 5	5.6364	50.278 2	7.2461	28.559 0
	3	42.8203	48.460 4	5.6401	50.053	7.2327	28.237 1
% Swelling							28.560 2
SD							0.3238
F122	1	48.6362	54.595	5.9588	56.294 7	7.6585	28.524 2
	2	44.5476	50.749 5	6.2019	52.549 2	8.0016	29.018 5
	3	44.7346	52.018 1	7.2835	54.051 8	9.3172	27.922 0
% Swelling							28.488 2
SD							0.5491
F123	1	42.3951	48.344	5.9494	50.084	7.6891	29.241

			5		2		6
	2	42.6253	48.3689	5.7436	50.0257	7.4004	28.8460
	3	42.3595	48.4661	6.1066	50.1644	7.8049	27.8109
% Swelling							28.6328
SD							0.7388

Appendix C: Texture compression analysis

Test ID	Batch		Firmness N Force 1
Start Batch F81	F81		
F811	F81		2.48
F812	F81		2.48
F813	F81		2.91
F814	F81		2.99
End Batch F81	F81		
Average:	F81 (F)	AVERAGE("BATCH")	2.71
S.D.	F81 (F)	STDEVP("BATCH")	0.24
C.V.	F81 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	8.73
Start Batch F82	F82		
F825	F82		10.27
F826	F82		12.12
F827	F82		10.73
F828	F82		11.91
End Batch F82	F82		
Average:	F82 (F)	AVERAGE("BATCH")	11.26
S.D.	F82 (F)	STDEVP("BATCH")	0.78
C.V.	F82 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	6.91
Start Batch F83	F83		
F839	F83		9.85
F8310	F83		10.44
F8311	F83		10.9

F8312	F83		9.58
End Batch F83	F83		
Average:	F83 (F)	AVERAGE("BATCH")	10.19
S.D.	F83 (F)	STDEVP("BATCH")	0.51
C.V.	F83 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	5.04
Start Batch F111	F111		
F11113	F111		24.45
F11114	F111		25.55
F11115	F111		24.04
F11116	F111		26.49
End Batch F111	F111		
Average:	F111 (F)	AVERAGE("BATCH")	25.13
S.D.	F111 (F)	STDEVP("BATCH")	0.96
C.V.	F111 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	3.82
Start Batch F112	F112		
F11217	F112		23.62
F11218	F112		21.29
F11219	F112		18.49
F11220	F112		28.48
End Batch F112	F112		
Average:	F112 (F)	AVERAGE("BATCH")	22.97
S.D.	F112 (F)	STDEVP("BATCH")	3.66
C.V.	F112 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	15.94
Start Batch F113	F113		
F11321	F113		7.68
F11322	F113		7.5
F11323	F113		6.68
F11324	F113		7.49
End Batch	F113		

F113			
Average:	F113 (F)	AVERAGE("BATCH")	7.34
S.D.	F113 (F)	STDEVP("BATCH")	0.39
C.V.	F113 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	5.27
Start Batch F121	F121		
F12125	F121		8.11
F12126	F121		9.23
F12127	F121		6.48
F12128	F121		6.05
End Batch F121	F121		
Average:	F121 (F)	AVERAGE("BATCH")	7.47
S.D.	F121 (F)	STDEVP("BATCH")	1.27
C.V.	F121 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	17.06
Start Batch F122	F122		
F12229	F122		19.39
F12230	F122		21.71
F12231	F122		21.84
F12232	F122		23.87
End Batch F122	F122		
Average:	F122 (F)	AVERAGE("BATCH")	21.7
S.D.	F122 (F)	STDEVP("BATCH")	1.59
C.V.	F122 (F)	STDEVP("BATCH")/AVERAGE("BATCH") *100	7.32
Start Batch F123	F123		
F12333	F123		54.15
F12334	F123		57.45
F12335	F123		54.07
F12336	F123		57.19
End Batch F123	F123		

Average	F123 (F)	AVERAGE("BATCH")	55.72
S.D.	F123 (F)	STDEVP("BATCH")	1.61
C.V.	F123 (F)	STDEVP("BATCH")/AVERAGE("BATCH")*100	2.89

Appendix D: Color measurement

ID	L*	a*	b*
F811	18.69	-1.37	-0.46
F811 w	37.39	-1	5.58
F811 wb	37.18	-1	5.49
F811 b	14.48	-0.75	-1.52
F812	13.57	-0.65	-2.37
F812 w	37.44	-1.05	5.03
F812 wb	37.58	-1.04	5
F812 b	15.45	-0.82	-1.93
F813	16.37	-0.62	-1.12
F813 w	37.83	-1.09	5.2
F813 wb	37.84	-1.05	5.13
F813 b	14.81	-0.9	-1.35
ID	L*	a*	b*
F821	19.68	-1.99	-3.84
F821 w	34.13	0.09	8.39
F821 wb	34.01	0.05	8.27
F821 b	19.35	-1.9	-3.78
F822	18.95	-1.95	-3.77
F822 w	33.11	0.24	8.56
F822 wb	32.93	0.09	8.53
F822 b	18.44	-1.96	-3.65
F823	19.46	-1.64	-3.71
F823 w	32.89	0.18	8.07
F823 wb	32.64	0.08	7.85
F823 b	19.41	-1.85	-3.38
ID	L*	a*	b*
F831	19.2	-1.99	-0.97
F831 w	30.68	1.4	11.4
F831 wb	30.8	1.42	11.37
F831 b	18.84	-1.8	-1.02
F832	17.72	-2.04	-1.84

F832 w	31	1.2	11.56
F832 wb	30.93	1.25	11.41
F832 b	18.4	-1.98	-1.15
F833	19.07	-2.04	-0.97
F833 w	30.05	1.15	10.48
F833 wb	29.97	1.13	10.43
F832 b	18.45	-2.16	-1.43
ID	L*	a*	b*
F111 (1)	13.11	-1.07	-3.73
F111 (1) w	34.26	-0.52	6.59
F111 (1) wb	33.74	-0.63	6.17
F111 (1) b	13.35	-1.32	-3.66
F111 (2)	14.07	-0.64	-2.98
F111 (2) w	35.81	-0.36	7.3
F111 (2) wb	35.3	-0.43	6.97
F111 (2) b	14.08	-1.09	-3.01
F111 (3)	14.46	-2.26	-3.87
F111 (3) w	33.5	-0.58	5.98
F111 (3) wb	33.19	-0.58	5.71
F111 (3) b	14.04	-1.17	-4.08
ID	L*	a*	b*
F112 (1)	14.49	-1.61	-2.79
F112 (1) w	31.55	0	9.14
F112 (1) wb	31.4	-0.03	8.94
F112 (1) b	14.27	-1.71	-2.9
F112 (2)	14.48	-1.84	-3.45
F112 (2) w	34.16	0.47	11.05
F112 (2) wb	34.16	0.5	11.22
F112 (2)	14.27	-1.69	-3.16

b			
F112 (3)	14.27	-1.36	-2.34
F112 (3) w	34.5	0.23	10.67
F112 (3) wb	34.17	0.21	10.49
F112 (3) b	14.14	-1.37	-2.59
ID	L*	a*	b*
F113 (1)	12.72	-1.19	0.92
F113 (1) w	30.84	1.6	13.59
F113 (1) wb	30.44	1.42	13.05
F113 (1) b	13.28	-0.17	1.44
F113 (2)	10.64	-1.46	-0.74
F113 (2) w	31.24	1.34	13.82
F113 (2) wb	31.14	1.33	13.65
F113 (2) b	10.48	-1.29	-0.75
F113 (3)	11.64	-0.6	0.94
F113 (3) w	29.67	1.48	13.58
F113 (3) wb	29.33	1.34	13.36
F113 (3) b	12.37	-0.2	1.57
ID	L*	a*	b*
F121 (1)	12.12	-0.7	-0.34
F121 (1) w	35.8	-0.57	7.13
F121 (1) wb	35.89	-0.53	7
F121 (1) b	10.3	-0.84	-1.14
F121 (2)	14.48	-0.47	1.16
F121 (2) w	36.79	-0.69	7.71
F121 (2) wb	36.48	-0.65	7.51
F121 (2) b	7.78	-0.71	-1.17

F121 (3)	7.3	-0.91	-1.78
F121 (3) w	36.83	-0.81	7.02
F121 (3) wb	36.81	-0.71	6.98
F121 (3) b	7.19	-1.03	-1.86
ID	L*	a*	b*
F122 (1)	12.13	-1.35	-1.28
F122 (1) w	32.48	0.39	10.54
F122 (1) wb	32.64	0.45	10.4
F122 (1) b	11.35	-1.32	-1.65
F122 (2)	11.65	-0.93	-1.62
F122 (2) w	31.02	0.45	10.17
F122 (2) wb	30.74	0.48	10.04
F122 (2) b	11.42	-1.03	-1.94
F122 (3)	14.09	-1.04	-1.47
F122 (3) w	30.49	0.37	9.28
F122 (3) wb	30.65	0.4	9.45
F122 (3) b	13.22	-1.11	-1.55
ID	L*	a*	b*
F123 (1)	16.89	-0.65	0.9
F123 (1) w	27.21	1.84	11.17
F123 (1) wb	27.24	1.8	10.92
F123 (1) b	15.92	-1.25	-0.59
F123 (2)	13.81	-1.12	-1.29
F123 (2) w	28.33	2.01	12.53
F123 (2) wb	28.3	1.95	12.42
F123 (2) b	14.25	-1.3	-1.22
F123 (3)	14.91	-1.38	-1.07
F123 (3)	27.1	1.84	11.12

w			
F123 (3) wb	27.05	1.85	11.05
F123 (3) b	15.09	-1.33	-0.98

	L*	mean	SD	a*	mean	SD	b*	mean	SD
F81 (1)	37.39			-1			5.58		
F81 (2)	37.44			-1.05			5.03		
F81 (3)	37.83	37.55	0.24	-1.09	-1.05	0.05	5.2	5.27	0.28
F82 (1)	34.13			0.09			8.39		
F82 (2)	33.11			0.24			8.56		
F82 (3)	32.89	33.38	0.66	0.18	0.17	0.08	8.07	8.34	0.25
F83 (1)	30.68			1.4			11.4		
F83 (2)	31			1.2			11.56		
F83 (3)	30.05	30.58	0.48	1.15	1.25	0.13	10.48	11.15	0.58
F111 (1)	34.26			-0.52			6.59		
F111 (2)	35.81			-0.36			7.3		
F111 (3)	33.5	34.52	1.18	-0.58	-0.49	0.11	5.98	6.62	0.66
F112 (1)	31.55			0			9.14		
F112 (2)	34.16			0.47			11.05		
F112 (3)	34.5	33.40	1.61	0.23	0.23	0.24	10.67	10.29	1.01

F113 (1)	30.84			1.6			13.59		
F113 (2)	31.24			1.34			13.82		
F113 (3)	29.67	30.58	0.82	1.48	1.47	0.13	13.58	13.66	0.14
F121 (1)	35.8			-0.57			7.13		
F121 (2)	36.79			-0.69			7.71		
F121 (3)	36.83	36.47	0.58	-0.81	-0.69	0.12	7.02	7.29	0.37
F122 (1)	32.48			0.39			10.54		
F122 (2)	31.02			0.45			10.17		
F122 (3)	30.49	31.33	1.03	0.37	0.40	0.04	9.28	10.00	0.65
F123 (1)	27.21			1.84			11.17		
F123 (2)	28.33			2.01			12.53		
F123 (3)	27.1	27.55	0.68	1.84	1.90	0.10	11.12	11.61	0.80

Appendix E: Degradation studies

Appendix E-1 Degradation test in pH 7.4 medium

pH 7.4		0 h	4 h	% degradation
F81	1	4.625	1.2264	73.4832
	2	4.1946	1.186	71.7256
mean				72.6044
SD				1.2429
F82	1	3.7163	1.0377	72.0771
	2	3.3906	1.008	70.2707
mean				71.1739
SD				1.2773
F83	1	3.9004	1.0374	73.4027

	2	4.3592	1.1461	73.7085
mean				73.5556
SD				0.2162
F111	1	4.6507	1.994	57.1247
	2	3.943	1.75	55.6176
mean				56.3711
SD				1.0657
F112	1	4.4727	1.9849	55.6219
	2	4.1463	1.7708	57.2920
mean				56.4570
SD				1.1810
F113	1	4.3472	1.8553	57.3220
	2	3.7707	1.587	57.9123
mean				57.6171
SD				0.4175
F121	1	5.3246	3.0757	42.2360
	2	5.1583	3.0094	41.6591
mean				41.9476
SD				0.4080
F122	1	6.2301	3.6812	40.9127
	2	4.9698	2.9209	41.2270
mean				41.0698
SD				0.2223
F123	1	4.7216	2.7727	41.2763
	2	4.9828	2.9339	41.1195
mean				41.1979
SD				0.1109

Appendix E-2 Degradation test in pH 5.5 medium

pH 5.5		0 h	4 h	% degradation
F81	1	4.3525	2.0525	52.8432
	2	3.97	1.7901	54.9093
mean				53.8763
SD				1.4610
F82	1	4.5217	2.2254	50.7840
	2	4.0499	1.9499	51.8531
mean				51.3186
SD				0.7560
F83	1	4.9008	2.4206	50.6081

	2	4.631	2.3023	50.2850
mean				50.4465
SD				0.2284
F111	1	4.7844	3.142	34.3282
	2	3.5078	2.2154	36.8436
mean				35.5859
SD				1.7786
F112	1	4.8928	3.2067	34.4608
	2	4.366	2.8208	35.3917
mean				34.9263
SD				0.6582
F113	1	4.6445	3.0116	35.1577
	2	3.7672	2.4092	36.0480
mean				35.6029
SD				0.6295
F121	1	5.1128	4.036	21.0609
	2	5.1945	4.1552	20.0077
mean				20.5343
SD				0.7447
F122	1	5.8466	4.5251	22.6029
	2	5.6977	4.5395	20.3275
mean				21.4652
SD				1.6089
F123	1	4.7436	3.6716	22.5989
	2	4.6941	3.5436	24.5095
mean				23.5542
SD				1.3510

Appendix F: Total phenolic content

Samples		pH 5.5	x values	pH 7.4	x values
F81	1	0.291	14.126214	0.189	9.1747573
	2	0.301	14.61165	0.207	10.048544
	3	0.357	17.330097	0.262	12.718447
mean			15.355987		10.647249
SD			1.726773		1.84615
F82	1	0.397	19.271845	0.258	12.524272
	2	0.302	14.660194	0.3	14.563107
	3	0.378	18.349515	0.194	9.4174757
mean			17.427184		12.168285
SD			2.4402561		2.5912207

F83	1	0.366	17.76699	0.321	15.582524
	2	0.429	20.825243	0.304	14.757282
	3	0.397	19.271845	0.298	14.466019
mean			19.288026		14.935275
SD			1.5291904		0.5791434
F111	1	0.334	16.213592	0.262	12.718447
	2	0.316	15.339806	0.278	13.495146
	3	0.327	15.873786	0.247	11.990291
mean			15.809061		12.734628
SD			0.4404744		0.7525577
F112	1	0.294	14.271845	0.243	11.796117
	2	0.401	19.466019	0.243	11.796117
	3	0.369	17.912621	0.307	14.902913
mean			17.216828		12.831715
SD			2.6660755		1.7937096
F113	1	0.401	19.466019	0.247	11.990291
	2	0.377	18.300971	0.339	16.456311
	3	0.423	20.533981	0.317	15.38835
mean			19.433657		14.61165
SD			1.1168566		2.3321189
F121	1	0.411	19.951456	0.266	12.912621
	2	0.367	17.815534	0.298	14.466019
	3	0.361	17.524272	0.227	11.019417
mean			18.430421		12.799353
SD			1.3252812		1.7260905
F122	1	0.463	22.475728	0.26	12.621359
	2	0.445	21.601942	0.307	14.902913
	3	0.355	17.23301	0.257	12.475728
mean			20.436893		13.333333
SD			2.8088303		1.3612445
F123	1	0.494	23.980583	0.307	14.902913
	2	0.456	22.135922	0.302	14.660194
	3	0.492	23.883495	0.237	11.504854
mean			23.333333		13.68932
SD			1.0381239		1.8956917

Appendix G: Radical scavenging activity (DPPH assay)

samples		pH 5.5	% AA	pH 7.4	% AA
F81	1	0.069	63.874346	0.14	26.701571
	2	0.081	57.591623	0.15	21.465969
	3	0.063	67.015707	0.143	25.13089
mean			62.827225		24.43281
SD			4.7985086		2.6867023
F82	1	0.037	80.628272	0.123	35.602094
	2	0.045	76.439791	0.128	32.984293
	3	0.033	82.722513	0.136	28.795812
mean			79.930192		32.460733
SD			3.1990057		3.4332139
F83	1	0.026	86.387435	0.105	45.026178
	2	0.02	89.528796	0.107	43.979058
	3	0.024	87.434555	0.099	48.167539
mean			87.783595		45.724258
SD			1.5995029		2.179755
F111	1	0.087	54.450262	0.143	25.13089
	2	0.091	52.356021	0.145	24.08377
	3	0.098	48.691099	0.135	29.319372
mean			51.832461		26.17801
SD			2.9150599		2.7704202
F112	1	0.076	60.209424	0.132	30.890052
	2	0.08	58.115183	0.127	33.507853
	3	0.073	61.780105	0.128	32.984293
mean			60.034904		32.460733
SD			1.838683		1.3852101
F113	1	0.058	69.633508	0.098	48.691099
	2	0.052	72.774869	0.1	47.643979
	3	0.047	75.39267	0.095	50.26178
mean			72.600349		48.86562
SD			2.8835448		1.3175976
F121	1	0.074	61.256545	0.14	26.701571
	2	0.073	61.780105	0.132	30.890052

	3	0.069	63.874346	0.141	26.17801
mean			62.303665		27.923211
SD			1.3852101		2.5826612
F122	1	0.055	71.204188	0.113	40.837696
	2	0.057	70.157068	0.119	37.696335
	3	0.053	72.251309	0.11	42.408377
mean			71.204188		40.314136
SD			1.0471204		2.3992543
F123	1	0.04	79.057592	0.087	54.450262
	2	0.039	79.581152	0.08	58.115183
	3	0.03	84.293194	0.076	60.209424
mean			80.977312		57.591623
SD			2.8835448		2.9150599

Appendix H: The ANOVA Procedure

Appendix H-1 Dependent Variable: total phenolic content in pH 5.5

Df Sum Sq Mean Sq F value Pr(>F)

a 8 5519 690 114.817 < 2e-16 ***

b 1 14361 14361 2390.294 < 2e-16 ***

rep 2 42 21 3.531 0.0404 *

a:b 8 881 110 18.326 2.97e-10 ***

Residuals 34 204 6

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 9 23.33

ab 8 20.44

b 6 19.43

bc 3 19.29

bcd 7 18.43

bcd 2 17.43

bcd	5	17.22
cd	4	15.81
d	1	15.36

Appendix H-2 Dependent Variable: radical scavenging activity in pH 5.5

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
a	8	5519	690	114.817	< 2e-16	***
b	1	14361	14361	2390.294	< 2e-16	***
rep	2	42	21	3.531	0.0404	*
a:b	8	881	110	18.326	2.97e-10	***
Residuals	34	204	6			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a	3	87.78
b	9	80.98
b	2	79.93
c	6	72.6
c	8	71.2
d	1	62.83
d	7	62.3
d	5	60.03
e	4	51.83

Appendix I : PBS buffer preparation (1 liter)

Step no.	Added chemicals
1.	Start with 800 mL of distilled water.
2.	Add 8 grams of NaCl
3.	Add 0.2 gram of KCl
4.	Add 1.44 grams of Na_2HPO_4
5.	Add 0.24 gram of KH_2PO_4
6.	Adjust pH to 7.4 with HCl
7.	Add distilled water to a total volume of 1 liter.

Appendix J: DPPH solution 0.1 mM, 100 mL

From DPPH M.W. = 394.33 g/mol, % Assay = 85 %

Solution 1000 mL have DPPH 0.1 mmol

Solution 100 mL have DPPH $\frac{100\ mL \times 0.1\ mmol}{1000\ mL}$

= 0.00001 mol

M.W. = 394.33 g/mol

DPPH 1 mol mass 394.33 g

DPPH 0.00001 mol mass $\frac{(0.00001\ \text{mol}) \times 394.33\ g}{1\ mol}$

= 0.0039433 g

% Assay = 85

DPPH 85 g from the chemical 100 g

DPPH 0.0039433 g from the chemical $\frac{0.0039433\ g \times 100\ g}{85\ g}$

= 0.004639 g

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