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Effect of grape seed extract as potent antioxidant on swelling properties and antioxidant activity of biocompatible carrageenan-based hydrogels

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Abstract - Grape seed extract has attracted a lot of interest as an effective natural antioxidant. It is extensively studied as it is rich in flavonoids, phenolic compounds, unsaturated fatty acids and vitamins showing particular promise in anti-inflammatory, anti-microbial, and anti-oxidative properties. We aim to highlight the effect of grape seed extract on the physicochemical properties and biological functions as antioxidant of an edible and biocompatible carrageenan-based hydrogels. The carrageenan-based hydrogels containing grape seed extract were prepared at various concentrations of grape seed extract (0-2% w/v) and two concentrations of glycerol (6% and 12% v/v). The *k*-carrageenan polysaccharide can be extracted from red edible seaweeds which has an ability to form gel with potential utilization in wound-healing products. The physical properties of carrageenan-based hydrogels were analyzed i.e. color, moisture content, swelling properties. In addition, the antioxidant properties of carrageenan-based hydrogels were studied as total phenolic content and % scavenging activity. Among 6 formulae, the results reported their percentages of opacity, moisture contents and swelling properties were 99-100%, 9-12%, and 14-29%, respectively. For antioxidant activities, the highest total phenolic content at 21.8 mg gallic acid equivalents per mL and scavenging activity (%) at 87.8%

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with 2% grape seed extract. The findings suggest that grape seed extract holds promise as an active ingredient in carrageenan hydrogel formulation, offering potential benefits for pharmaceutical purposes.

Keywords: Hydrogels, carrageenan, grape seed extract, antioxidant activity

1. Introduction

Grape seed extract is an industrial derivative of whole grape seeds which contains proanthocyanidins (Ma et al., 2017). 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 (Freitas et al., 1999). 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 benefits i.e. 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 (Kwatra, 2020; Rajakumari, et al. 2020; Ribeiro et al., 2015).

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) (Byrne et al., 2002). 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 (Byrne et al., 2008). For most medical applications, the novel engineering of hydrogels for drug delivery dividing them to biodegradable hydrogels which are favored over non-degradable hydrogels since they degrade in clinically relevant time scale, smart hydrogel or stimuli-sensitive hydrogel that respond to environmental changes, such as temperature, pH, light, and specific molecules such as glucose and finally biomimetic hydrogels which are relatively inert polymer chains can be tailored with the selected biological moieties to yield bioactive hydrogels (Kakkar & Narula, 2022; Bustamante-Torres et al., 2021; Kamath & Park, 1993; Li & Su, 2018).

The carrageenan is polymer used for preparing hydrogels. Carrageenan corresponds 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 β-D-galactose-4-sulfated units (Mano et al., 2007). The three main types of carrageenans include the kappa (k), with one sulfate group, iota (i) and lambda (l) with two and three sulfate groups, respectively (Campo et al., 2009). 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 (Jayakody et al., 2023; Shafie et al., 2022). 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 (Rode et al., 2018).

Carrageenan hydrogels can be function as a bioactive material for wound healing to help heal wounds by attracting new skin cells to the wound site to promote healing and provide a platform for new tissue growth (Jangdey et al., 2024). Carrageenan hydrogels for wound dressings can help heal chronic wounds which has no respond to other treatments and wounds that expel bodily fluids such as urine, granulating wounds, necrotic or rotting wounds, partial and full-thickness wounds, second-degree burns, and sites of skin donation and skin grafts. In tissue regeneration, hydrogel-based membranes have been used in periodontal and implant therapy to promote the growth of specific types of cell. In oral surgery, hydrogel barriers can prevent fast-growing cells around the gum from migrating to a wound in a tooth (Samiei et al., 2021). Carrageenan-based hydrogels have been addressed for sustained drug release and various applications in bone and cartilage tissue engineering, however, they have been rarely studied the presence of active pharmaceutical ingredients in hydrogel formulation which may improve physiochemical properties and biological performance of the hydrogels.

Grape-seed extract has high contents of flavonoids with oligomeric proanthocyanidins, monomeric phenolic compounds such as (−)-epicatechin, (+)-catechins, and (−)-epicatechin-3-o-gallate and dimeric, trimeric, and tetrameric proanthocyanidins (condensed tannins) which was previously demonstrated antioxidant, anti-inflammatory, anti-neurodegenerative, anti-microbial, anti-cancer, and cardioprotective activities (Ajit et al., 2021). Furthermore, antioxidant and anti-inflammatory properties of grape seed extract have been reported for its use in tissue regeneration and wound healing (Locilento et al., 2019). Due to impairment of the healing by bacterial biofilm (BBF), the effective combinations of traditional antibiotics or specific anti-BBF agents and grape seed extract exhibited antibiotic-resistant isolates in these biofilms to maintain both potential cytotoxicity and anti-bacterial efficacy or prevent the formation of BBF (Wei et al. 2019). The grape seed extract itself has been reported for cell regeneration property in prevention of Alzheimer's and cancer (Faki et al., 2019; Gupta et al., 2020; Kwatra, 2020) and reduction of the bacterial lipopolysaccharide-induced intracellular reactive oxygen species (ROS) production and mitochondrial superoxide production (Nallathambi et al., 2020). For melanoma therapy and wound healing, grape seed-inspired smart hydrogel was investigated as a photothermal agent and the hydrogel scaffolds demonstrated excellent and controlled photothermal ability (Ma et al., 2019). Recently, Garcia and co-workers reported a potential use of scaffold of anionic collagen from porcine serosa and bovine tendon and grape seed extract in tissue engineering. The increase in absorption capacity and decrease in the collagen degradation of scaffold was due to the addition of grape seed extract (Garcia et al. 2019). In another

study, hydrogel films consisting of sodium carboxymethylcellulose (CMC)-grapefruit seed extract (GSE) was synthesized for potential wound healing applications. The hydrogel films have demonstrated excellent antimicrobial activity for increasing GSE concentration varying from 0.25% to 1.5% (v/v) (Koneru et al., 2020). In this study, we aim to highlight the effect of grape seed extract on the physicochemical properties and biological functions as antioxidant of an edible and biocompatible carrageenan-based hydrogels. The developed carrageenan-based hydrogels that contain grape seed extract had good compressive characteristics and may serve in the wound healing promoted angiogenesis and skin regeneration.

2. Materials and methods

2.1 Materials

k-Carrageenan was purchased from Chemipan (Thailand), sorbitol from Sigma Aldrich (US), and glycerin from Lab Valley (Thailand). Natural substances i.e. grape seed extract, vitamin C, collagen, hyaluronic acid from Chemipan were used as cosmetic grade. The AR laboratory grade reagents used in the preparation of phosphate buffer saline (PBS) buffer solution were as follows: Sodium Chloride (NaCl), Potassium Chloride (KCl), di-Sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and Monopotassium phosphate (KH_2PO_4).

2.2 Methods

2.2.1 Preparation of carrageenan-based hydrogels

To prepare for carrageenan-based hydrogels (CH), 2% w/v *k*-Carrageenan and 5% w/v sorbitol were mixed with 6% and 12% w/v glycerol for CH1-6, respectively. Subsequently the mixture was heated to 60-70˚C for 30 minutes. According to each of formulae, the various ratio of grape seed extract (GSE) was gradually added into the warm mixture (Table 1). Afterwards, small amount of ingredients are as follows: vitamin C, collagen and hyaluronic acid were added, and then poured in the silicone molds with 2.8 cm diameter and 1.7 cm height. Finally, the solutions were cooled at 4˚C overnight and stored for further analysis.

Table 1. The formulation of carrageenan-based hydrogels

	Carrageenan (%w/v)	Sorbitol (%w/v)	Glycerol (%v/v)	Vit C (%w/v)	GSE (%w/v)	Collagen (%w/v)	Hyaluronic acid (%w/v)	Water (mL)
CH1	2.0	5.0	6.0	0.1	-	0.2	0.2	86.5
CH2	2.0	5.0	6.0	0.1	1.0	0.2	0.2	85.5
CH3	2.0	5.0	6.0	0.1	2.0	0.2	0.2	84.5
CH4	2.0	5.0	12.0	0.1	-	0.2	0.2	80.5
CH5	2.0	5.0	12.0	0.1	1.0	0.2	0.2	79.5
CH6	2.0	5.0	12.0	0.1	2.0	0.2	0.2	78.5

2.2.2 Color measurement

Commission International de l'Eclairage (CIE) whiteness, brightness, L*a*b* color and opacity of the gels were measured with Hunter Lab CIE L*a*b* system colorimeter adapted from De Carvalho et al. (2006). The color and opacity of hydrogel samples were measured as follow: covering with white ceramic plate and the black cover. Opacity (%) is a measure for the transparency of hydrogels can be calculated by using the following equation:

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

where Rwb refers to reflectance of a sample sheet over perfect black, and Rw refers to reflectivity of the same sample over perfect white. The test samples were completely done in triplicate for each.

Moisture content measurement

A method was developed from Jakubczyk et al. (2022). Carrageenan 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 80˚C for 2 hours. The weight of each of samples were weighed after taking out from the oven. The moisture content (% M) was calculated according to the following formula:

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

where Wi represents the initial weight of sample, and Wd corresponds to the weight of sample after drying. The test samples were done in triplicate for each.

2.2.3 Analysis of swelling properties

Swelling properties and resistance to degradation of hydrogels were adapted from Kozlowska et al. (2018). In this study, this experimental part and analysis of swelling properties were performed by placing hydrogel samples with a diameter of 2.8 cm and a height of 1.7 cm in each condition. 5 g of the hydrogel sample was immersed in 40 mL of PBS buffer pH 7.4 and 5.5, respectively, under different conditions. After 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 can be calculated by using the below equation:

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

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

As a function of swelling temperature and pH, the swelling capacity of carrageenan hydrogels were studied at room temperature (RT) ~25˚C and physiological temperature at 37˚C.

2.2.4 Determination of total phenolic compounds

Folin-Ciocalteu (FC) method was modified from Lawag et al. (2023) and used to determine the total phenolic compounds of carrageenan-based hydrogel (Lawag et al., 2023). Briefly, 0.1 mL of carrageenan-based hydrogel solution obtained from degradation in buffer pH 5.5 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 RT 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.

2.2.5 Determination of antioxidant activity

The DPPH free radical scavenging activity was determined using the method modified by Watcharin et al. (2023). 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 3 mL of methanol and 0.5 mL carrageenan-based hydrogel solution degraded in buffer pH 5.5. 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 (UV 2600, Shimadzu). Methanol was used as blank. And 3 mL of methanol mixed with 0.3 mL of DPPH was used as control. The test were completely done in triplicate for each.

$$\text{Inhibition (\%)} = \frac{\text{ODcontrol-ODsampl}}{\text{ODcontrol}} \times 100$$

Where ODcontrol is absorbance of methanol and DPPH solution (10:1)

ODsample is absorbance of sample in methanol and DPPH solution (10:1)

2.2.6 Data analysis

Statistical analysis was accomplished using SAS software version 9.4 for Total phenolic compounds and antioxidant activity, and by SPSS (IBM SPSS Statistics Processor version 26) for color analysis. A Completely Randomized Design (CRD) was used for the experimental design and One-way ANOVA was used to assess the data statistically. The least significant difference (LSD) at 0.05 level of probability was utilized to analyze comparisons among treatments. All experiments were conducted in triplicate.

3. Results and discussion

Preparation and physical observation of carrageenan-based hydrogels

The appearance of carrageenan-based hydrogels with variations of GSE concentrations (0-2% w/v) is shown in Figure 1A. The carrageenan-based hydrogels (CH) were formulated into semi-solid gel with variations of GSE concentrations, vitamin C, collagen, and hyaluronic acid. A three-dimensional gel network structure formation of carrageenan was contributed to a change in temperature during the sol-gel and gel-sol transitions (Bhattacharyya et al., 2024). The CH pass through a unique critical gel state resulting in associative, interactive network formation in the mixture. The GSE can easily incorporated with CH *via* hydrogen bonding, and thereby strengthening the crosslinking hydrogel matrix (Figure 1B). In general, abundant hydrophilic groups present in

flavonoids and phenolic compounds in GSE can easily be complexed with hydrophilic moieties of CH *via* hydrogen bonding interactions (Ribeiro et al., 2015). The qualitative composition of CH and function in the formulation are given in Table 2. (Merck Index, 2006).

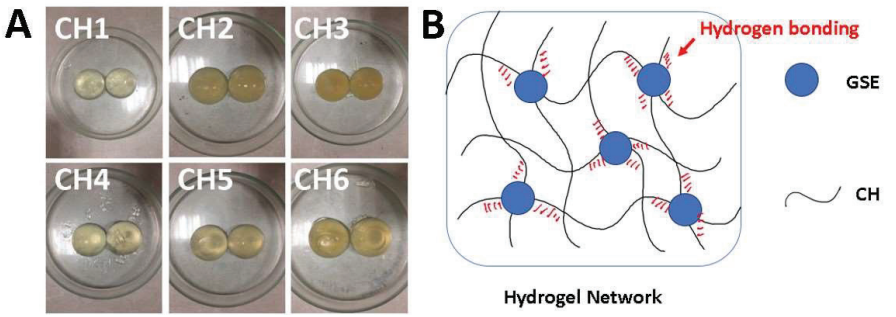

Figure 1. Carrageenan-based hydrogels with variations of grape seed extract concentrations (A) and an illustration for the formation of crosslinking hydrogel network with hydrogen bond distributed in hydrogel (B).

Table 2. The qualitative composition and function in the formulation of carrageenan-based hydrogels

Component	Function in the formulation
Sorbitol	Humectant, thickener - improving texture
Glycerol	Humectant, viscosity-decreasing agent
Vitamin C	Antioxidant, anti-inflammatory, anti-aging
Grape Seed Extract	Antioxidant, anti-aging, skin-brightening and hydration properties
Collagen	Moisturizing, film-forming properties
Hyaluronic acid	Anti-aging, moisturizing, wound healing

The color analysis of the CH was tested by Hunter Lab CIE L*a*b* system colorimeter. The results showed as L*, a*, b*, and opacity (%). According to Table 3, hydrogel samples exhibited lightness values (L*) between 30.58 and 34.52; reddish (a*) and yellowish (b*) values were in the ranges of -1.05 to 1.47 and 6.62 to 13.66, respectively. The positive b* values of all hydrogels indicated that the hydrogel started to appear light yellow. As the amount of GSE increased from 0-2%, the L* and b* significantly increased demonstrating that the concentration of GSE had an impact on the color of the hydrogels. However, the b* of CH2 was significantly higher than that of CH5, as well as the b* of CH3 was also significantly greater than that of CH6. It can be seen that the yellowness of the hydrogel decreased with higher glycerol content, while maintaining the same concentration of GSE. This indicated that an increase of the glycerol content from 6% to 12% caused significant increase in yellowness but did not significantly affect the lightness of the hydrogel. The presence of pale-yellow color of the hydrogels with increasing concentration of the GSE might result from a denser polymer network inside the hydrogel at higher

GSE concentration due to a stronger interaction between molecular chains of hydrogels. According to the literature, the polymer-polymer interactions (i.e. ionic, hydrogen, other electrostatic interactions and covalent bonds) are the main factor for hydrogel to crosslink which increase the crosslink density and thus form a hydrogel network (3D structure) (Mahinroosta et al., 2018). The concentration of the polymer, the mass fractions between the polymers or other components in the hydrogel blends have contributed to the hydrogen interactions to the hydrogel network (Kopač et al., 2022).

For the opacity, it can be seen that the hydrogels showed high opacity and percent opacity of all prepared hydrogels were observed in a range from 98 to 100% (Table 3). There was no significant difference in the opacity among these hydrogels. Based on the amount of the light materials transmit, the prepared CH almost completely blocked the transmission of light resulting in almost 100% opacity. As reported by De Oliveira et al, hydrogels produced from rice and oat cellulose by using epichlorohydrin as a crosslinker exhibited %opacity around 96-99%. The loss of light transparency resulted from an increase of crystalline regions within the three-dimensional network (De Oliveira et al., 2017). Moreover, the increased opacity can be attributed to polymer hydrogel matrix of rice and oat cellulose fiber, which had a high content of cellulose, lignin and ash. In another study, Dalei et al. (2024) prepared cucumber peel extract-imbued dragon fruit peel pectin hydrogel films and reported that the addition of peel extract improved the tensile strength and antioxidant properties, as well as enhanced the density and opacity of hydrogel films (Dalei et al., 2024). Garcia and co-workers developed κ-Carrageenan hydrogels by ionic crosslinking, using KCl and NaCl as crosslinkers and showed that the transparency of κ-Carrageenan hydrogels decreased from a transparent to an opaque gel as carrageenan concentration increased due to the change in the structure, pore size and degree of homogeneity of the polymer network (Garcia et al., 2024)

The optical transmittance and transparency appearance did not observe for all CH. However, CH1 without GSE was visually the most translucent which could allow partial transmission of light through them and the incident light may get reflected or scattered as it passes through an interior polymer network of the material exhibiting color density appearance of these hydrogels. Moreover, percent opacity increased when b* value increased as well, this can be explained that positive b* interpreted the yellow color result which means when grape seed extract increased, it affected to b* values resulting in the higher percent opacity. As these results, CH1 and CH2 formulae which contain 0-1% GSE demonstrated the highest percent opacity, however, the CH2 and CH3 exhibited the strong color intensity due to the presence of GSE and lower amount of glycerol. Therefore, the color of carrageenan-based hydrogels were obviously affected by GSE concentration and glycerol content. Moreover, the polymer molecular weight and concentration, hydrogel crosslinking density, and high water content can offer great prospect to hydrogel transparency (Ding et al., 2022).

Table 3. Color analysis as L*, a*, b*, and opacity (%) of carrageenan-based hydrogels

	L*	a*	b*	% Opacity
CH1	30.58 ± 0.48 [b]	1.25 ± 0.13 [a]	5.27 ± 0.28 [e]	99.97 ± 0.37 [ns]
CH2	33.38 ± 0.24 [a]	-1.05 ± 0.05 [d]	11.15 ± 0.58 [b]	99.95 ± 0.47 [ns]
CH3	33.38 ± 0.66 [a]	0.17 ± 0.08 [b]	13.66 ± 0.14 [a]	99.45 ± 0.20 [ns]
CH4	30.58 ± 0.82 [b]	1.47± 0.13 [a]	6.62 ± 0.66 [d]	99.08 ± 0.53 [ns]
CH5	34.52 ± 1.18 [a]	-0.49 ± 0.11[c]	8.34 ± 0.25 [c]	98.71 ± 0.32 [ns]
CH6	33.40 ± 1.61 [a]	0.23 ± 0.24 [b]	10.29 ± 1.01 [b]	99.52 ± 0.48 [ns]

[a-e] letters in the same column are designated the statistically significant difference ($p < 0.05$) and [ns] designated no significant difference.

3.1 Moisture content of carrageenan-based hydrogels

For moisture content properties, moisture content of CH was determined at hot air oven 80°C for 2 h, As shown in Figure 2, CH4, CH5, and CH6 hydrogels with 12 % glycerol showed the slightly higher moisture content than that of CH1, CH2, and CH3 hydrogels with 6% glycerol. However, there was no statistically significant difference in moisture content among these carrageenan hydrogels. The hydrogel composition influences its compression analysis. 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 (Gong et al., 2003). As moisturizing agent, glycerol has high ability to absorb water from the surroundings that could affect its evaporating from the deeper layers of the hydrogels. These effect is known as transepidermal water loss (TEWL) (Coupland et al., 2000). Therefore, the greater amount glycerol evaporated and released from hydrogels was correspond to the higher moisture content. Glycerol is a trihydroxyalcohol with localized osmotic diuretic and laxative effects (Pleuvry, 2008). Glycerol 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. In addition, glycerol is used as a solvent, humectant and vehicle in various pharmaceutical preparations (Fluhr et al., 2008). Glycerol 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. Several researches reported the function of glycerol including its 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 (Gesslein, 1999).

For wound healing applications, Mokhtari et al. (2021) reported hybrid carrageenan-based platform modified

with starch/cellulose nanofiber to have a considerable swelling ability with optimum mechanical strength and stability to apply at wound site and stop bleeding (Mokhtari et al., 2021). It has been demonstrated that the mechanical performance including stiffness, viscoelastic behavior, and initial state recovery for effective wound healing of carrageenan-based hydrogels, can be improved by chemical modification, crosslinking, copolymerization and incorporation of nanoparticles or biomolecules. As they mimic natural microenvironments, CH can be enable to improve cell-cell and tissue interactions, and support cell function including proliferation, migration and re-epithilization by providing suitable matrix with optimum moisture content, evaporation and degradation rate.

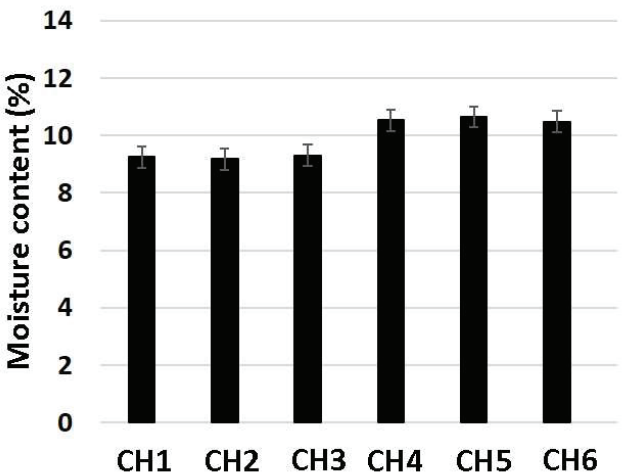

Figure 2. Moisture content (%) of carrageenan-based hydrogels *with 0-2% w/v GSE*

3.2 Study of swelling properties

Hydrogels have strong water adsorption, swelling, and water retention abilities due to hydrophilic chain segments of hydrogel network (Jin et al., 2023). Based on highly hydrophilic moieties, CH with a three-dimensional network structure can swell without dissolving in water. The swelling properties were studied at both physiological and acidic pH. To examine the swelling properties, carrageenan-based hydrogels were placed in two different pH, i.e., neutral (pH = 7.4) and stimulated acidic (pH = 5.5), separately. The swelling properties of hydrogels were also observed under two different conditions at body temperature (37° C) and RT (20-25° C) after 24 h (Figure 3). It was found that the swelling behavior of hydrogels in PBS buffer pH 7.4 was significantly increased in comparison with pH 5.5 at both 37˚C and RT. At pH 7.4, CH1- CH6 demonstrated the highest degree of swelling capacity in comparison to another condition. At constant pH, the %swelling of CH1-CH6 was not statistically significant different. Interestingly, the presence of GSE and glycerol in carrageenan-based hydrogels in each formulation varying from 0-2% and 6-12%, respectively, did not show the statistically significant difference in the swelling behavior of hydrogels.

To investigate the swelling behavior of CH at 37˚C and RT, the degree of swelling slightly rose with the increase of temperature. This could be explained that the rate of water penetration inside the polymeric network at the temperature at 37˚C was greater than the rate of penetration at RT. In addition, hydrogel is sensitive to basic pH conditions that it could absorb greater amount of liquids when pH is higher (Anseth et al., 1996). At higher pH, water molecules possibly undergo hydrogen-bonding forming to generate more space for the molecules to penetrate inside indicating higher swelling (Chen et al., 2003). 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 (Beltran et al., 1991).

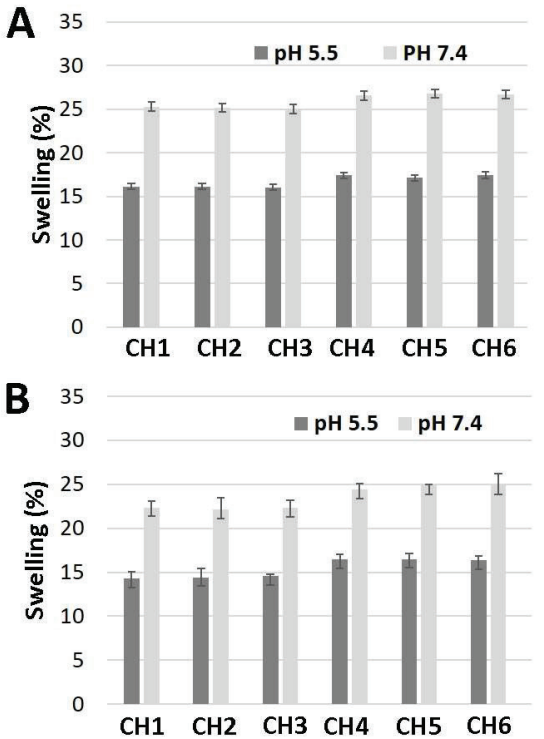

Figure 3. *Swelling capacity of carrageenan-based hydrogels containing 0-2% w/v* GSE *in distilled water (pH 5.5) and PBS buffer (pH 7.4) at 37˚C (A) and at RT (B)*

3.3 Total phenolic compounds and antioxidant activity

The total phenolic compounds of hydrogels were 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 equivalent (mg GAE/mL). As shown in Figure 4, the total phenolics of the prepared hydrogels determined by the Folin−Ciocalteu method ranged from 14.6 to 21.8 mg GAE /g dry material. The carrageenan-based hydrogels containing

2% GSE (CH3) exhibited highest total phenolic compounds and there was significant difference in total phenolic compounds of carrageenan-based hydrogels containing 2% GSE with 6% and 12% glycerol (CH3 and CH6) in comparison with the prepared hydrogels containing 0% and 1% GSE. The GSE acts as powerful antioxidant agent, thus the high total phenolic content was observed in the samples with higher amount of grape seed extract.

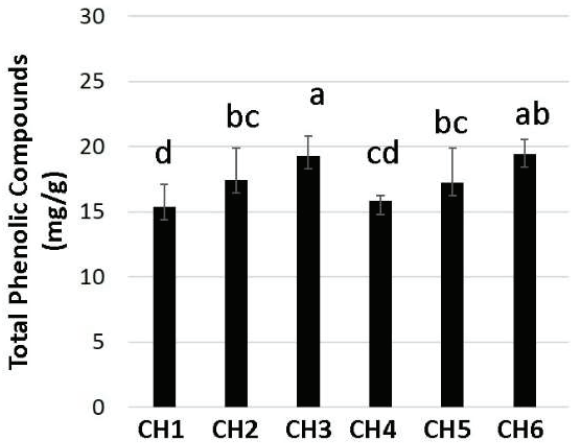

Figure 4. Total phenolic compounds of carrageenan-based hydrogels

For antioxidant properties, scavenging activity of carrageenan-based hydrogels containing GSE was determined by 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 (Adjimani & Asare, 2015). It can be seen that the scavenging activity of carrageenan-based hydrogels ranged from 51.8 to 87.8 % (Figure 5). The carrageenan-based hydrogels containing 1-2% GSE has statistically significant difference in the scavenging activity in comparison with the hydrogels without GSE. Additionally, an increase of GSE from 1% to 2% at the same concentration of glycerol demonstrated the significantly higher amount of antioxidant agent as shown in CH2 and CH3, as well as CH5 and CH6, respectively. From these results, the ratio of GSE could impact the antioxidant properties including total phenolic content and scavenging activity. Based on the data, the highest DPPH free radical scavenging activity was found in the concentration of GSE at 2% (CH3) with statistically significant difference among other formulae CH. The greater the percentage of free radical scavenging was corresponding to more GSE content to stabilize free radicals. Interestingly, it can be seen that increasing glycerol from 6% to 12%, the antioxidant value significantly exhibited a downward trend for hydrogels containing both 1% and 2% GSE found in CH2-CH5 and also CH3-CH6, respectively. Figure 5 reveals that the radical scavenging activity (%) of CH2 was significantly greater than CH5, and CH3 also exhibited significantly greater antioxidant activity than CH6. According to our results, while maintaining

GSE concentration, an increase in glycerol content from 6% to 12% did not increase the antioxidant activity of the hydrogel. However, it was previously reported that the presence of glycerol in sodium alginate/poly(vinyl alcohol) (SA/PVA) hydrogel altered physical properties such as gel fraction, swelling ability, degradation in simulated body fluids, and thermal resistance (Bialik-Wąs et al., 2021). In our findings, the total phenolic compounds and antioxidant activity of hydrogels was significantly improved by incorporating with higher concentration of GSE in the hydrogels.

For hydrogels without GSE (CH1 and CH4), the percentage of free radical scavenging resulted from the antioxidant properties of carrageenan and other compositions presence in CH such as vitamin C. The antioxidant activity of commercial *k*-Carrageenan with various molecular weight and the degraded *k*-carrageenan was found in the range of 32-40% measured by DPPH assay (Diah et al., 2022). Furthermore, the antioxidant activity can be influenced by pH and also depends upon the oxidation rate of antioxidant compounds, and this oxidation rate was influenced by the surrounding environments and pH (Aksoy et al., 2013).

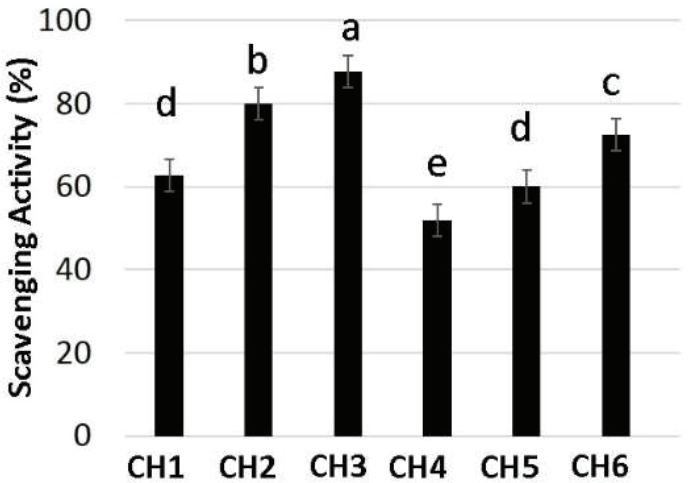

Figure 5. Radical scavenging activity (%) of carrageenan-based hydrogels

It is well established that GSE acts as powerful antioxidant and antimicrobial agent due to rich phenolic compounds and other antioxidant compositions (Kwatra, 2020; Memar et al., 2019). The prevention of cellular oxidative damage, and control of anti-oxidant enzyme expression *via* inhibition of the Nuclear factor kappa B (NF-κB) pathway could lead to anti-atherosclerotic effects accelerated by the existence of antioxidants (Ajit et al., 2021). Qureshi et al. (2024). reported that hydrogels based on carrageenan have good gastro-retentive and antioxidant properties (Qureshi et al., 2024). They can be utilized for controlled drug delivery and tissue engineering i.e. curcumin releases for delivery in cancerous lung cells, quercetin for anti-inflammatory and anti-cancerous, cephradine for injectable hydrogel system, ketoprofen and mupirocin for wound healing, octenyl succinic anhydride/β-cyclodextrin

for controlled release of food functional ingredients, storax balsam for wound dressing (Qureshi et al., 2024; Deng et al., 2024; Nakipoglu et al., 2024). Additionally, the encapsulation of probiotic in carrageenan-alginate hydrogels has been investigated for the development of carrageenan hydrogel bead system (Premjit et al., 2024). The swelling behavior of hydrogels significantly affected by pH in both gastric and intestinal phases, and the carrageenan content in the optimized hydrogels.

According to a previous study and these results, the GSE and carrageenan had an impact on total phenolic compounds, DPPH radical scavenging activity and other biological properties of hydrogel. The study could successfully establish an appropriate encapsulation of GSE to CH. It was found that the incorporation of grape seed extract in carrageenan-based hydrogels helped to enhance their antioxidant activities which is essential in the reduction in ROS in the food and cosmetics applications. GSE exhibited high antioxidant activities in carrageenan-based hydrogels which could prevent oxidative damage of the cells. However, the mechanical and other biological applications of carrageenan-based hydrogels need to be further studied.

4. Conclusion

The formulated carrageenan-based hydrogels containing various ratio of grape seed extract were evaluated in terms of their swelling capacity and antioxidant properties. The swelling behavior experiments showed that the grape seed extract did not affect swelling behavior of hydrogels and swelling capacity of hydrogels was depend on pH. The carrageenan-based hydrogels contained 2% w/v grape seed extract and 6% v/v glycerol was contributed to a highest total phenolic compounds and DPPH radical scavenging activity. It is worth evaluating the behavior of this hydrogel for the potential to be used in drug delivery, tissue engineering and wound healing applications.

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Conflicts of interests

The authors declare no conflict of interest.

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