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Bio-Hydrogels Derived from Agarose and Cellulose: Characterization and Swelling Properties

Bio-Hydrogels Derived from Agarose and Cellulose: Characterization and Swelling Properties

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Abstract. There are a lot of postharvest techniques to extend the shelf-life of foods. One of them was the use of materials such as hydrogel. Hydrogel which is made from bio-based materials usually known as bio-hydrogel. The functional properties of bio-hydrogel were in the ability to absorb water which is usually the main problem of food spoilage. In this study, bio-hydrogel was made from agarose and cellulose to form robust material which can be incorporated into packaging or stand alone as an addition material into food system in order to get more sustainable option packaging for the environment. Bio-hydrogel was formulated using Agarose, Natrium carboxymethylcellulose (CMCNa), Hydroxyethyl cellulose (HEC), and 1-Ethyl-3-(3-dimethylaminopropyhyl). The resulted hydrogel showed the firm hydrogel which has hardness value of 261 g-1116 g. P1,5%C0% (polymer concentration-P; crosslinker concentration-C) had maximum swelling value of 46,86 g/g when in the form of beads, whereas in the form of particle material P2%C2,5% had maximum swelling value of 34,83 g/g. The materials derived from this study have firm shapes with their specific characteristic and swelling value in both forms (bead and particle). They can be utilize for many applications includes food.

1. Introduction

Food spoilage usually came from the chemical, physical, and biological damage, coming from inside or from the outside barrier of the packaging. Water activity usually the main problem, the cause damage of foods, so that recently the usage of functional material such as hydrogel with many combination forms as a packaging layer (incorporated inside the packaging sheet or addition substances into packaging systems can be a solution. Lots of works had been done regarding the situation to extend shelf life of food for example the making of hydrogel as one of food material support, including Roy et al[1] who made biodegradable hydrogel film for food packaging. Another works had been done by Al-Mekawy et al[2] produce highly stretchable, smooth, and biodegradable hydrogel films made from chitosan as a food packaging. The

development of antimicrobial hydrogel to control foodborne pathogens was also done by Oh et al[3]. Another hydrogel for food research to make various material in food technology for example the ability as an indicator of fish freshness was also examined by Ding et al[4] CEE incorporated PVA-polymeric hydrogel was also studied by Kannan et al[5] which resulted that it had potential use as a food packaging with antibacterial also antifouling effects on MRSA and other MDR S.aureus strains. For the purpose of biosensors also intelligent food packaging system it was also examined by Jun Yang et al[6] who found that chitosan-based hydrogels can convert environment signal through its swelling behaviour and have great application potentials in intelligent food packaging system because of its favourable antibacterial, antioxidant activity and good biodegradability option. Glycerol as a plasticizer to produce bioplastic was identified by Suryani et al[7] which capture that the addition of it can softened the PLA texture as the increasing level of glycerol. Peelman et al[8] studied about heat resistance hydrogel made from starch, cellulose, PLA, and PHA. They found that the use of nucleating agents (with plasticizers usage combination), also a high mould temperature, inducing slower cooling rate could be the best solution for higher crystallinity to produce a higher heat-resistant product. Francis et al[9] studied about hybrid hydrogel for low fat and heat resistant chocolate. It resulted that 50% (v/v) hydrogel dispersed chocolate gave a glossy appearance, less surface roughness and highest melting resistance (80°C), also non-Newtonian behaviour. Hydrogel beads for designing future foods had been studied by Saqib et al[10] which is potential to flourish in the future food system. Particle-filled food protein gels also researched by Gravelle et al[11], whereas nanogels as a stable delivery vehicles for curcumin also had been studied by Hu et al[12]. Biodegradable active films for food packaging played important role on preventing food spoilage, extending shelf life of food, and also preserving freshness and quality of food[13]. Qin et al[14] also made a functional hydrogel in the purpose of thermal management using boron nitride network and PVA hydrogel resulted two times higher conductivity up to 1.12 W m-1 K-1. Hydrogel for cooling ability was also studied by Zamengo et al[15] made from polyvinyl alcohol for thermal management.

Hydrogels in many kinds of researches were modified by alternatives materials whether it is synthetic polymer, renewable materials, or combination of the two. Examination of synthetic SAH efficiency in water absorption had been done by Yu et al[16]. Swelling capacity increased by raised NaOH concentration (maximum of 3.0 N). Hydrolized kC-g-PAAm had a better swelling capacity than un-hydrolized ones. Synthesized copolymer hydrogel of Kappa carrageenan and acrylamide using potassium persulfate as initiator were evaluated its absorbent and adhesive properties by Meena et al[17]. They obtained that hydrogel with %N 10,56 had superior swelling property with maximum swelling up to 22 g/g in the alkaline pH. Former cellulose-based hydrogel researchers Esposito et al[18], were crosslinked sodium salt of carboxymethylcellulose and hydroxyethyl cellulose with divynilsulvone. They found that the highest equilibrium water uptake up to 400 g H2O/g polymer was in the ratio of 3:1 CMCNa/HEC which dried by extraction with acetone rather than under vacuum or atmospheric condition. The cellulose based hydrogel was also being studied by Astrini et al[19] using divinylsulfone as a crosslinker. A ratio of 5:1 CMCNa/HEC with a reaction temperature of 60°C gave the best absorption capacity in distilled water with a swelling ratio of ±40 g/g.

In this research, we were focusing on bio hydrogel formulation as one of functional material options to prevent food spoilage with mixing technique to discover the effects of crosslinker and polymer concentration. The concentrations become a valuable input to design hydrogel material using Agarose and Cellulose. With the physical result options it gives us choices which formula is needed for a certain purpose of biobased hydrogel. The mixing options used in this research also

possible to get the economist way to produce bio-based material for many applications including on foods. The bio-hydrogel can be added to a food system as an absorbent and it can expanded to other uses also. The evaluation were in the area of physical and swelling properties of resulted bio-hydrogel.

2. Materials and Methods

2.1. Materials

In this study, bio-based hydrogel (bio-hydrogel) was made from Agarose, Natrium carboxymethylcellulose (CMCNa), Hydroxyethyl cellulose (HEC), and 1-Ethyl-3-(3-dimethylaminopropyhyl). Agarose was in brand Vivantis (USA), whereas CMCNa, HEC and 1-Ethyl-3-(3-dimethylaminopropyhyl) (EDC) were obtained from Sigma-Aldrich (USA). It was also used citric acid which purchased from Merck (Germany), ethanol 96% and distilled water from chemical store. They were directly used without any further purification.

2.2. Bio-hydrogel preparation

The preparation of bio-hydrogel solution was the modification from Esposito et al, Anah et al, and Astrini et al[18,20,21] and those modification techniques had been registered in Indonesian patent number P00201911821. Preparation used was through synthesis process with 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride as crosslinker. The formulation composition was CMC:HEC:Agarose with the ratio of 5:1:10. The polymer and concentration variations were displayed in Table 1. There were also several treatments after crosslinking step which include stabilizing, drying, and moulding process. The hydrogels were made in two forms, beads or ball shape and semi-powdered or particle shape. For particles shape instead of ball-shaped bio-hydrogel it was done by size reduction process from its gel shape.

Table 1. Research Design of Agar-based Bio-Hydrogel Formulation

	1,5% Polymer Concentration	2% Polymer Concentration	2,5% Polymer Concentration	3% Polymer Concentration
0% Crosslinker Concentration	P1,5C0	P2C0	P2,5C0	P3C0
1% Crosslinker Concentration	P1,5C1	P2C1	P2,5C1	P3C1
2,5% Crosslinker Concentration	P1,5C2,5	P2C2,5	P2,5C2,5	P3C2,5

2.3. Characterization

2.3.1. FTIR Analysis

The functional groups of the hydrogels were evaluated by using the Fourier transmission infrared (FTIR, Vertex 80, Bruker). The technique of attenuated total reflection (ATR) was employed to analyse FTIR spectra at 400–4000 cm−1.

2.3.2. Rheological Properties

Rheology profiles were examined using Rheometer MCR 302 Anton Paar with two tests which were viscosity curve using 0,01-1000 1/s shear rate, at constant temperature of

46°C and viscosity value using 100 1/s shear rate, at the temperature range of 36-68°C. The test was conducted with measuring system PP-50 following Fernandez et al[22].

2.3.3. Texture analysis

Bio-hydrogel texture was tested using TexturePro CT V1.4 Build 17-Texture Analyzer (Brookfield Engineering-Germany). The test was done with the trigger load of 10 g, target of 5.0 mm, and test speed of 0,5 mm/s. The data collected were hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness.

2.3.4. TGA analysis

TG-DTA curves of bio-hydrogel were conducted using Perkin Elmer STA 6000 thermal analyzer under nitrogen atmosphere with dynamic heating rate of 10 ◦C min−1. All experiments were conducted from room temperature to 900 ◦C according to Phang et al[23].

2.3.5. Morphological Properties

The surface morphology of bio-hydrogel was recorded employing scanning electron microscopy (SEM). The samples were sputtered with 10-20 nm thick layer gold and scanned using scanning electron microscope (SEM) Hitachi SU 3500 series (Hitachi, Japan).

2.3.6. Swelling Index

The swelling test was done using 2 methods which were specifically for beads shape and particle-sized shape. For beads shape it was done by weighing the swelled hydrogel, un-swelled hydrogel, and calculate the swelling ratio divided by un-swelled form hydrogel. For particle shape it was done using methods from Astrini et al[24] with modification by using tea bag method. The formula variations of hydrogel were the concentration of crosslinker agent and total polymer used in the mixture.

2.4. Statistical Analysis

The data were evaluated using CoStat software with triplicate data generation using Two Way Annova, One Way Annova, and LSD for Post-Hoc Test.

3. Results and Discussions

3.1. FTIR Analysis

The FTIR analysis was done to examine the spectral analysis of the bead's hydrogels, shown in Figure 1.

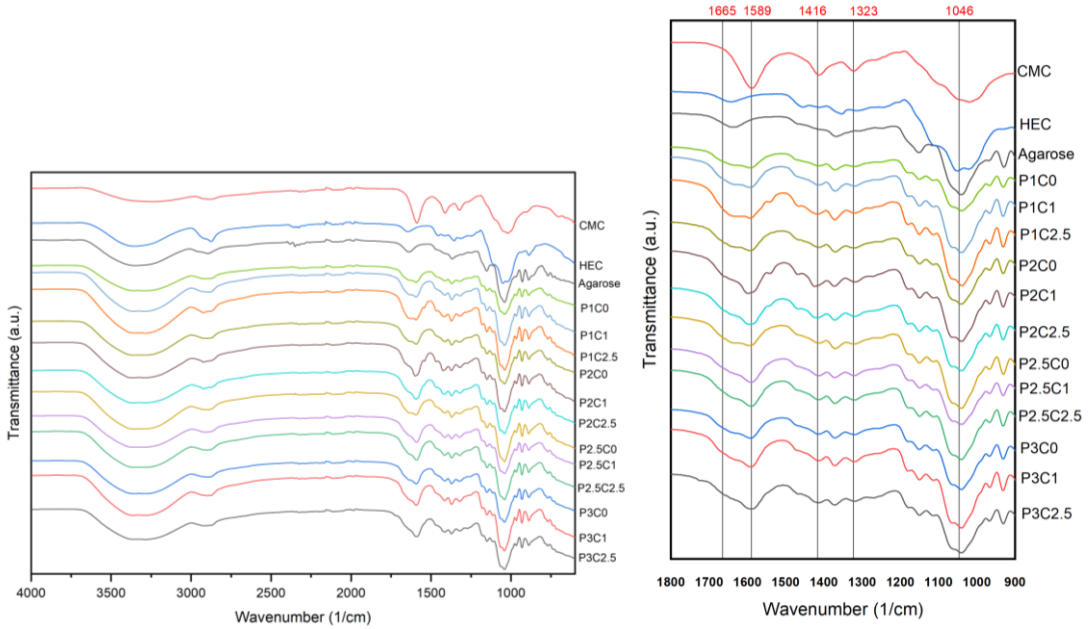

Figure 1. FTIR spectra of hydrogels and its single compositions

Figure 1 showed that native carboxyl methyl cellulose exhibited the appearance of a broad hydroxy group (O-H) at a wavenumber of 3340 cm^{-1}, C-H bond at 2902 cm^{-1} and C-O-C at 1034 cm^{-1}. Due to two double bonds in CMC were identical; there was no difference between single and double bonds because the carboxyl groups were present as sodium salt, which led to the formation of the carboxylate negative ion. As a result, asymmetric and symmetric stretching vibration peaks were seen at 1589 and 1416 cm^{-1}, respectively. Meanwhile, hydrogels are composed of CMC:HEC:Aga with ratio 5:1:10, showed peaks of C-O and O-H bond at 1589 cm^{-1} and 3300 cm^{-1}, respectively. Crosslinking by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in between the polymer matrix revealed new shoulder peak of CO at 1665 cm^{-1}. Furthermore, the more addition of EDC/DMAP increased the ratio of shoulder peak at 1665 to 1589 cm^{-1} and improved hardness value due to crosslinking effect.

3.2. Rheological properties

The rheological properties was examined using method by Fernandez et al[22] which were divided into several tests which showed in Figure 2A-Figure 2D.

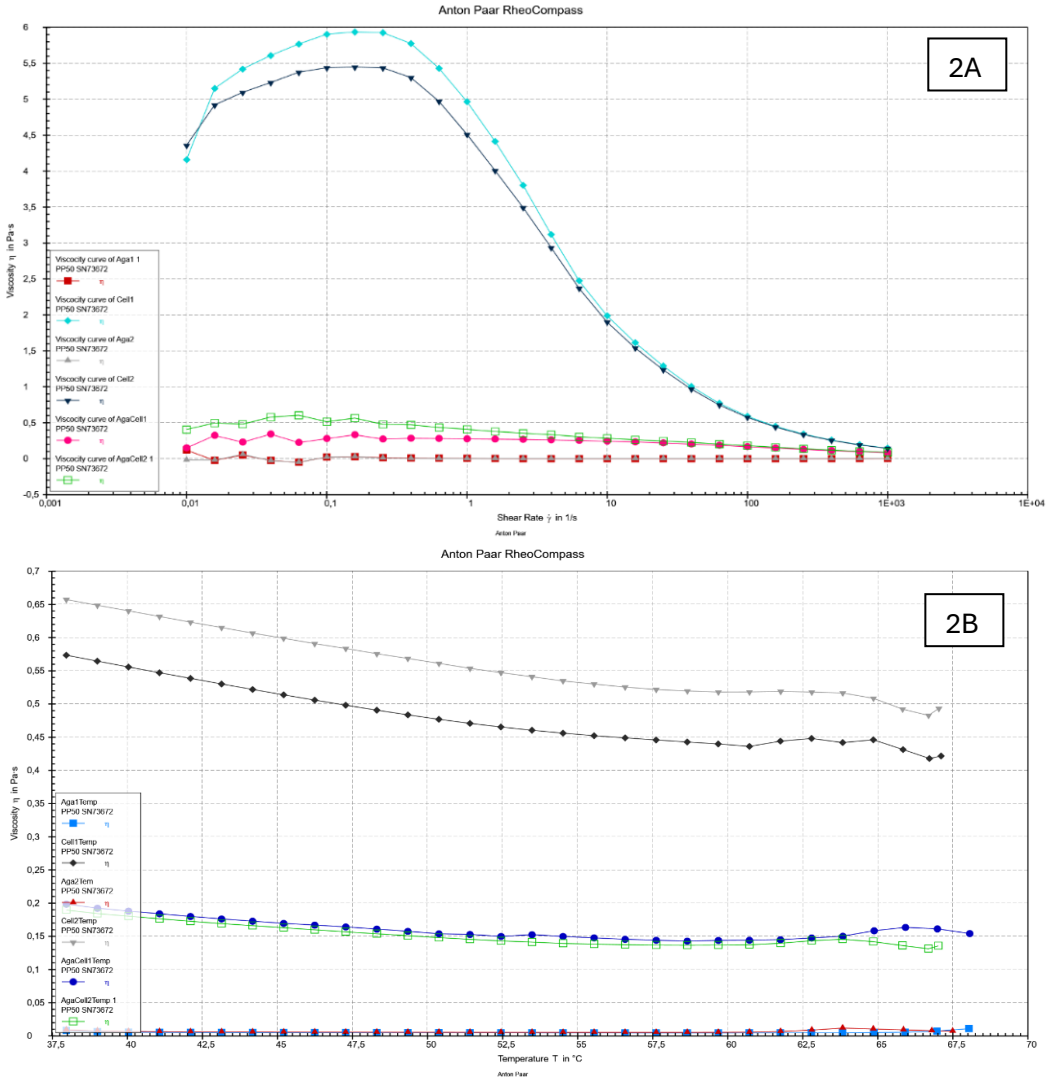

Figure 2. Viscosity value vs temperature - **2A (**Aga2%:Aga1; Aga2%C1%:Aga2; Cell2%:Cell1; Cell2%C1%:Cell2; AgaCell2%: AgaCell1; AgaCell2%C1%: AgaCell2); **2B (**Aga2%:Aga1; Aga2%C1%:Aga2; Cell2%:Cell1; Cell2%C1%:Cell2; AgaCell2%: AgaCell1; AgaCell2%C1%: AgaCell2)

Figure 2A represented the viscosity value in various shear rate from 0.01 – 1000 1/s. The lowest viscosity value was Aga1 (Aga2%) and Aga2 (Aga2%C1%). Cellulose formulations Cell1 (Cell2%) and Cell2 (Cell2%C1%) had the highest viscosity. While the viscosity value of mixture formulations AgaCell1 and AgaCell2 was in between Agarose and Cellulose, due to the property's combination of Agarose and Cellulose. From the above graph we could also see that crosslinker incorporation contributed to lowering the viscosity value of single backbone (Cell1>Cell2 and Aga1>Aga2). Whereas in the mixture formulation its opposite, AgaCell1 (without crosslinker) had the lower viscosity value compared to AgaCell2 (with crosslinker). Maybe it was caused by the mixture bond properties which made the backbone more reactant with the crosslinker.

Figure 2B showed the viscosity value in various Temperature from 67,5°C to 37,5°C. Overall, alike with viscosity value vs shear rate graph above, the highest viscosity value was Cellulose formulations, and the lowest value was Agarose formulations. While the mixture of Agarose and Cellulose was in between. Opposite with the effect of different shear rate to the viscosity value in Figure 1A, the effect of different temperatures ranged from 67,5°C to 37,5°C resulted different which indicated that in single backbone formulations crosslinker addition contributed to rise the viscosity value in each temperature, represented by the viscosity value of Cell2 was higher than Cell1 (Cell2>Cell1) and also in Agarose formulations (Aga2>Aga1) in each temperature., AgaCell1>AgaCell2. Temperature and shear rate effects played different role in viscosity value.

3.3. Texture

The texture analysis result was presented in Table 2, for 5 tests which were Hardness, Springiness, Gumminess, Adhesiveness, and Cohesiveness.

Table 2. Texture properties of Bio-hydrogel (1-Way Completely Randomized-1WCR)

Hydrogels	Hardness (g)	Springiness (mm)	Gumminess (g)	Adhesiveness (mJ)	Cohesiveness
P1,5C0	261±33,65^{i}	3,54±0,28^{cd}	69±34,77^{g}	0,03±0.06^{a}	0,27±0,13^{de}
P1,5C1	579,33±44,52^{fg}	3,89±0,17^{abc}	225,33±62,08^{ef}	0.03±0.06^{a}	0,39±0,08^{cd}
P1,5C2,5	715±70,53^{de}	4,09±0,31^{ab}	274,33±70,12^{e}	0.3±0.36^{a}	0,38±0,08^{cd}
P2C0	699±58,51^{def}	4,19±0,20^{ab}	213±127,17^{ef}	0±0^{a}	0,30±0,19^{de}
P2C1	1116,33±65,32^{a}	4,32±0,19^{a}	875,67±95,55^{a}	0.33±0.15^{a}	0,78±0,06^{a}
P2C2,5	872,67±24,09^{bc}	4,02±0,11^{abc}	638,67±24,83^{bc}	0.07±0.06^{a}	0,73±0,04^{a}
P2,5C0	448,67±26,01^{h}	3,54±0,66^{bcd}	229,67±53,59^{e}	0,1±0,26^{a}	0,51±0,10^{bc}
P2,5C1	629,67±104,71^{efg}	3,81±0,36^{abc}	428±64,21^{d}	0,1±0,17^{a}	0,68±a0,02^{ab}
P2,5C2,5	502,67±156,52^{gh}	1,85±0,56^{d}	90,33±45,54^{fg}	0,1±0,10^{a}	0,2±0,14^{ef}
P3C0	529,33±52,29^{gh}	3,09±1,36^{bcd}	48,67±29,96^{g}	0,13±0,15^{a}	0,09±0,07^{f}
P3C1	948,33±20,50^{b}	4,03±0,07^{abc}	745,33±96,76^{ab}	0,1±0,10^{a}	0,79±0,11^{a}
P3C2,5	787,33±116,90^{cd}	3,85±0,49^{abc}	578±158,52^{c}	0,23±0,25 ^{a}	0,73±0,11^{a}

*Different letter shows statistically significantly different (Post Hoc Test with LSD, P<0.05)

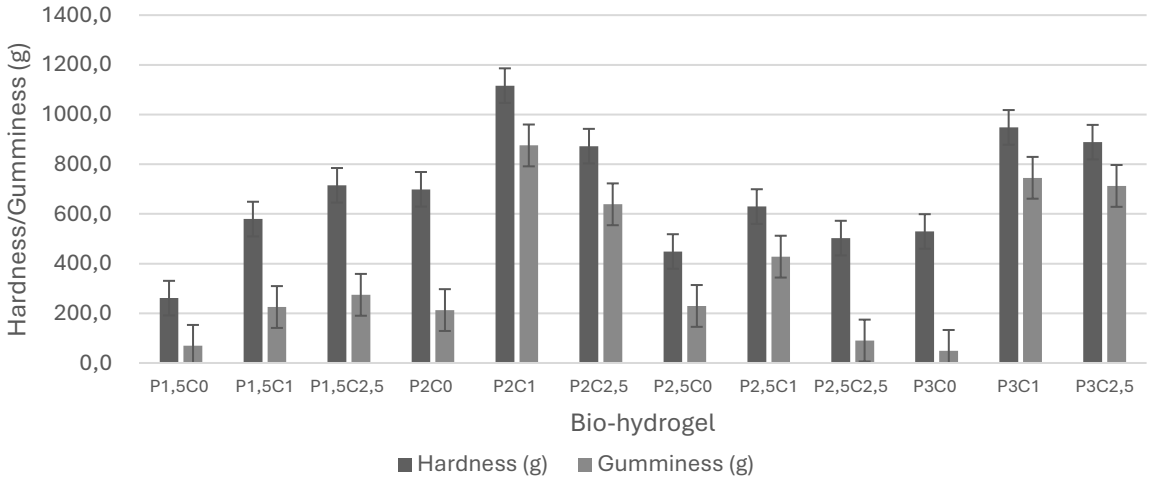

Figure 3. Hardness and Gumminess value of Beads Bio-hydrogels

Figure 3 showed the hardness and gumminess test for beads hydrogels. The 2WCR (two-ways anova test) showed that the hardness value was significantly different for polymer and crosslinker concentration, however, interaction between polymer and crosslinker was not significant. From the result of Least Significance Different (LSD) test, the use of 2% polymer had a greater value than 3%, continue with 2,5% polymer, and the last was 1,5%. For the crosslinker factor, they had a different subset for each, with the order among others of 1%, 2,5%, and 0%, respectively. It seems that without crosslinker the beads hydrogels had the lowest value of hardness if it compared with the use of crosslinker. To examined them as a single individual viewpoint, the test was continued with 1-WCR (one-way anova test) and the result showed that the variations were statistically different. The post hoc test results using LSD revealed the same order of treatments P2C1, P3C1, P3C2,5, P2C2,5, P2,5C1, P2C0, P1,5C2,5, P1,5C0, P2,5C0, P3C0, P2,5C2,5, P1,5C0, respectively from the highest to the lowest. Anova test for gumminess indicated that the value was significantly different for polymer and crosslinker. Interaction between them was also significant according to 2-WCR test. From the result of Least Significance Different (LSD) test, the use of 2% polymer had greater value than 3%, continue with 2,5% polymer, and the last was 1,5%. For the crosslinker factor, they had a different subset for each, with the order among others of 1%, 2,5%, and 0%, respectively. Just the same as hardness value, it seems that without crosslinker the beads hydrogels had the lowest value of gumminess if it compared with the use of crosslinker. 1-WCR (one-way anova test) of gumminess showed that the variations were statistically different. For LSD test in 1-WCR it resulted different order which was P2C1, P3C1, P2C2,5, P3C2,5, P2,5C1, P1,5C2,5, P2,5C0, P1,5C1, P2C0, P2,5C2,5, P1,5C0, and P3C0 respectively from the highest value to the lowest.

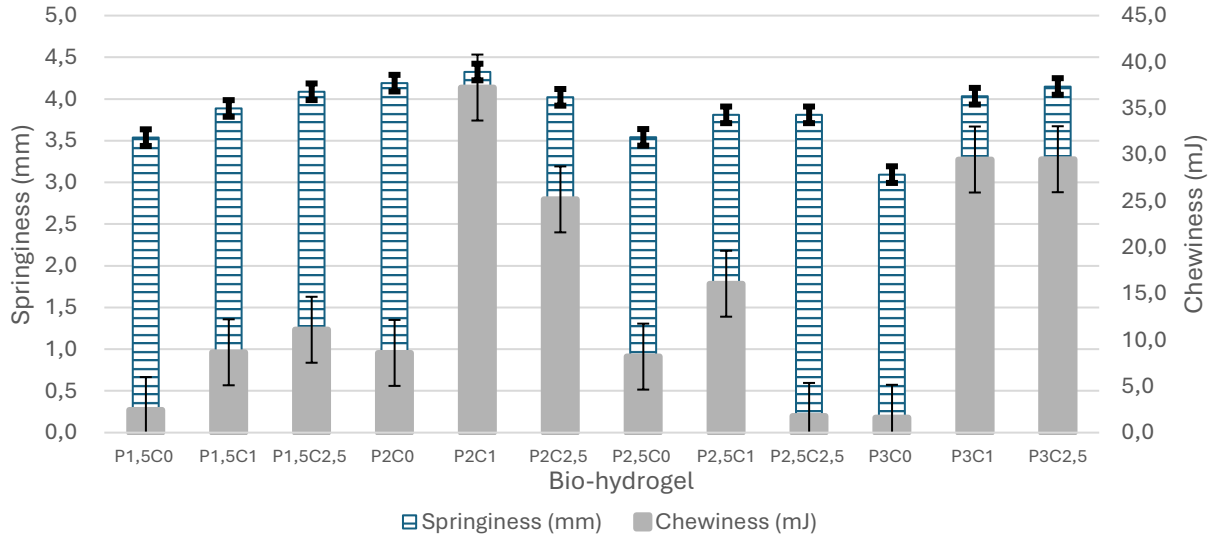

Figure 4. Springiness and Chewiness of Bio-hydrogels

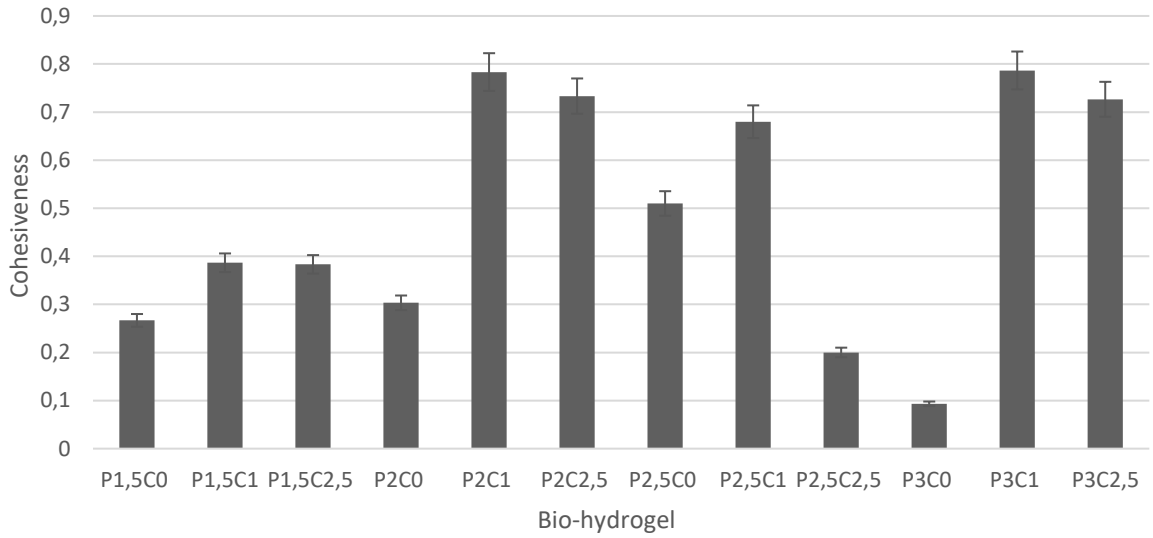

Figure 5. Cohesiveness of Bio-hydrogels

Figure 4 and Figure 5 showed the springiness, chewiness, and cohesiveness test for beads hydrogels. The anova test showed that the springiness value was not significantly different for crosslinker concentration and interaction, however, it was significant for polymer concentration. From the result of Least Significance Different (LSD) test, the use of 2% polymer had a greater swelling than the others, followed by 1,5%, 3%, and 2,5% polymer, respectively. For the crosslinker factor, there is only one subset with the order among others of 1%, 2,5%, and 0% respectively. To examined them as a single individual viewpoint, the test was continued with one way Completely Randomized. The post hoc test results using LSD revealed the order of treatments P2C1; P2C0; P1,5C2,5; P2C2,5; P3C1; P3C2,5; P2,5C1; P1,5C1; P2,5C0; P3C0; P1,5C0; P2,5C2,5, respectively from the highest to the lowest, with the graph interpretation above.

Anova test for chewiness indicated that the value was significantly different for polymer, crosslinker, and interaction between them according to 2-WCR test. From the result of Least Significance Different (LSD) test, the use of 2% polymer had a greater value than 3%, followed by 2,5% polymer and 1,5%. For the crosslinker factor, they also had a different subset for each, with the order among others of 1%, 2,5%, and 0% respectively. Those results of 2WCR order were same with hardness, gumminess, and cohesiveness. LSD test in 1-WCR of chewiness had different order which was P2C1, P3C1, P2C2,5, P3C2,5, P2,5C1, P1,5C2,5, P1,5C1, P2C0, P2,5C0, P1,5C0, P2,5C2,5, and P3C0, respectively from the highest value to the lowest. Whereas, the cohesiveness value can be seen at Figure 5. It showed similar trends behaviour as the other texture parameters examined.

3.4. TGA

The thermal gravimetry analysis was used to measure the degradation of material against heat struck. The results can be seen at Figure 6. Agarose as a single material (Aga) resulted 3 inflection points (299.65°C, 428.92°C, and 546.62°C). Cellulose mixture from CMC and HEC showed 2 inflection points (315.59°C and 345.29°C). Whereas in the bio-hydrogel it showed more – 6 inflection points (225.07°C, 246.86°C, 474.08°C, 525.52°C, 601.24°C, and 858.94°C). The combined components made new material which more complex material which might be applied in many applications due to more stable form for further usage including food.

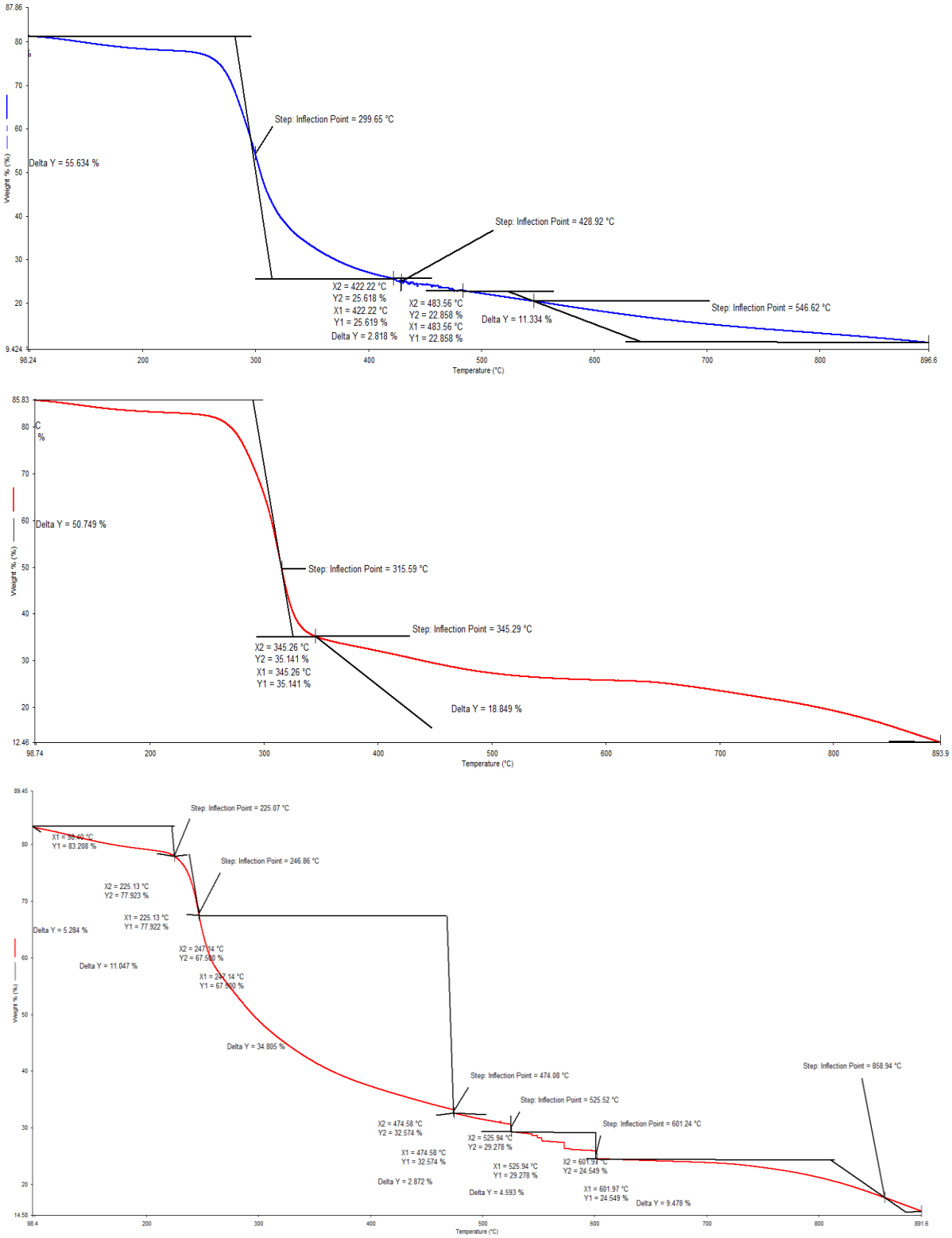

Figure 6. TGA profile of component and bio-hydrogel (a. Aga, b. Cell, c. Aga-Cell)

3.5. Morphological properties

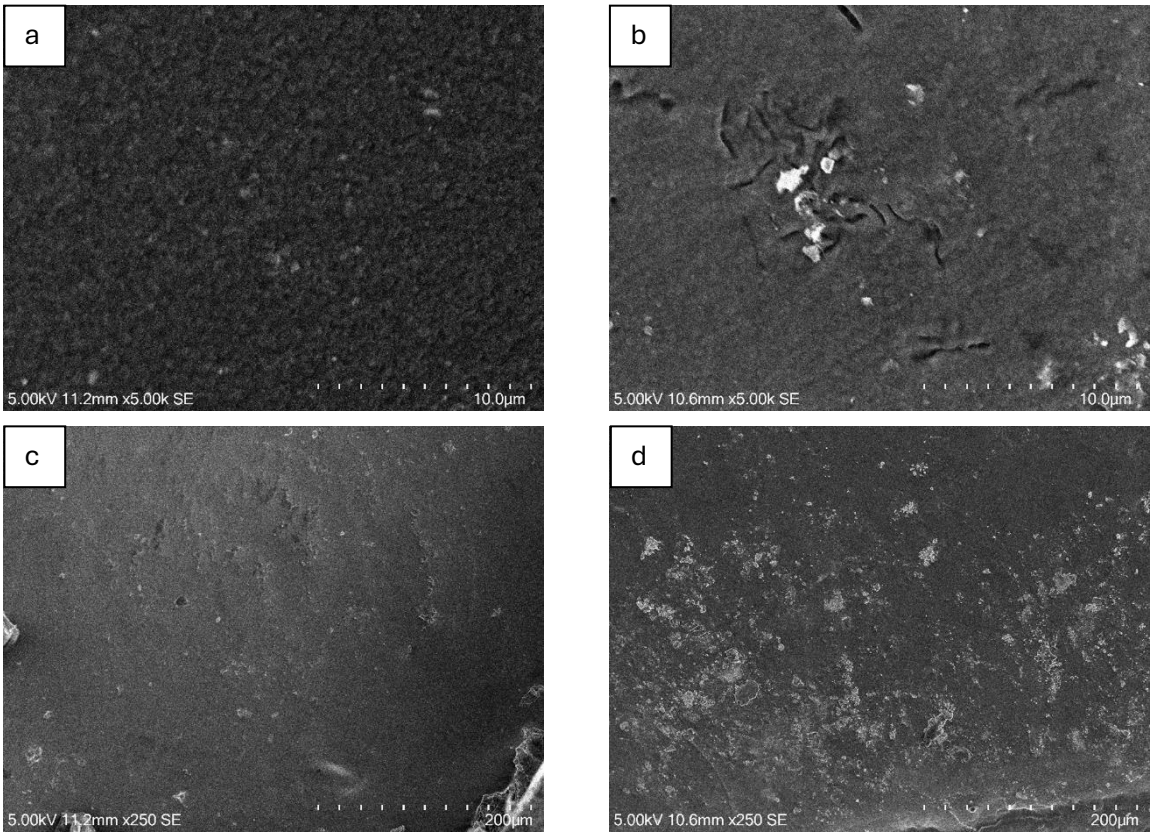

Figure 7. SEM Morphology of beads hydrogel
(a) before swelling, 5.00k SE; (b) after swelling-dried in room temperature, 5.00k SE; before swelling, 250 SE; (d) after swelling-dried in room temperature, 250 SE

The morphology of the hydrogels is seen in Figure 7. The hydrogel's surfaces were corrugated, while the interior structure included numerous sockets and voids, as shown in SEM pictures. The absorption of water molecules is aided by the corrugation, sockets, and cavities. Figure A shows a hydrogel surface before swelling and Figure B after swelling. The surface of the A and B hydrogels appeared corrugated, with visible sockets. Water absorption is favored by these holes and corrugation 25. After the process of water absorption, the surface of the hydrogel slightly changed with the appearance of a gap on the surface of the hydrogel as shown in the picture. This could be due to space left by the absorbed water.

3.6. Swelling studies

Swelling properties were measured in two variants of hydrogels shape, beads/round and in particle sized. The results are represented in Table 3.

Table 3. Swelling value of formulated Agar-based Bio-hydrogel

Hydrogels	Swelling beads (g/g)	Swelling particle (g/g)
P1,5%C0%	46,86±3,04^{a}	12,18±1,65^{d}
P1,5%C1%	18,46±1,03^{cd}	15,68±6,39^{d}
P1,5%C2,5%	23,92±0,36^{b}	18,78±7,51^{cd}
P2%C0%	16,06±0,34^{e}	15,62±1,92^{d}
P2%C1%	17,93±0,44^{d}	20,55±2,48^{bcd}

P2%C2,5%	19,20±0,05^{c}	34,83±8,78^{a}
P2,5%C0%	19,31±0,42^{c}	13,35±1,72^{d}
P2,5%C1%	17,84±0,45^{d}	27,62±2,32^{ab}
P2,5%C2,5%	8,18±0,18^{f}	30,36±7,06^{a}
P3%C0%	23,92±0,36^{b}	15,99±3,23^{d}
P3%C1%	16,54±0,78^{e}	26,61±0,76^{abc}
P3%C2,5%	15,96±0,64^{e}	33,58±6,58^{a}

* Different letter shows statistically significantly different-LSD Post Hoc Test (P<0,05)

Figure 8 shows the swelling ratio of bio-hydrogel beads in 12 variations of formula (P1,5C0; P1,5C1; P1,5C2,5; P2C0; P2C1; P2C2,5; P2,5C0; P2,5C1; P2,5C2,5; P3C0; P3C1; and P3C2,5). The highest swelling value was P1,5C0 with the constant value (in 10-24 hours swelling time) approximately at 46,8606 g/g. Followed by P3C0 (23,9169 g/g); P1,5C2,5 (22,6407 g/g); P2,5C0 (19,3102 g/g); P2C2,5 (19,2022 g/g); P1,5C1 (18,0488); P2C1 (17,9211 g/g); P2,5C1 (17,8378); P3C1 (16,5491); P2C0 (16,0662); P3C2,5 (15,9548); and P2,5C2,5 (8,1779 g/g). We also could see that from approximately at 10-12 hours the swelling value start begin in constant value. Swelling value between 24 to 48 hours were not that different. The values obtained were similar when it is examined with statistic. The ANOVA test using CoStat with two-way Completely Randomized (two-WCR) anova for swelling test of particle hydrogels showed that the results are significantly different. The interaction between two factors (polymer and crosslinker) was also significant. From the result of Least Significance Different (LSD) test, the use of 1,5%, 3%, 2%, and 2,5% polymer respectively from the highest to the lowest swelling value had the different subset for each. For the crosslinker factor, they also had a different subset for each, with the order among others of 0%, 1%, and 2,5%, respectively. It seems that without crosslinker the beads hydrogels have better performance from that result. To examined them as a single individual viewpoint, the test was continued with one way Completely Randomized. The post hoc test results using LSD revealed the same order of treatments P1,5C0; P3C0; P1,5C2,5; P2,5C0; P2C2,5; P1,5C1; P2C1; P2,5C1; P3C1; P2C0; P3C2,5; P2,5C2,5, respectively from the highest to the lowest, with the graph interpretation below.

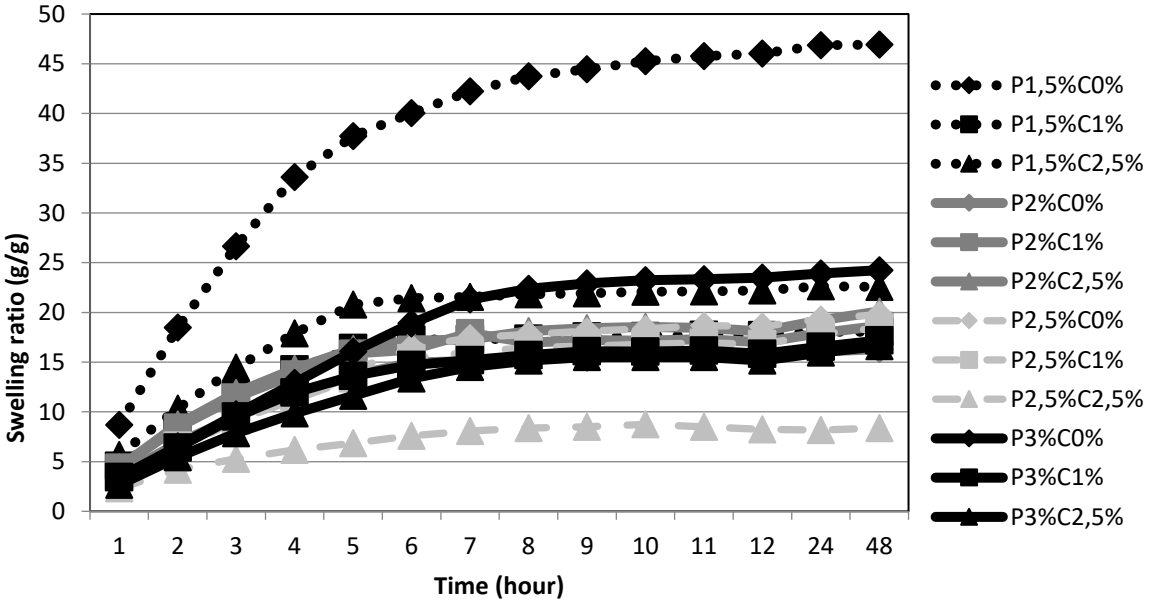

Figure 8. Swelling ratio bead-shape bio-hydrogel

From the above graph we could see the different swelling ratio values in many samples tested for bead-shape bio-hydrogel. In the use of 1% crosslinker, the greater the polymer concentration the lower the swelling ratio value will be. Crosslinking density was higher, so the competition reactions inside the macromolecules were more difficult. For the lowest polymer concentration in this research (1,5%), the use of 0%, 1%, and 2,5% (all variant of crosslinker concentration used) gave the highest swelling ratio value compared with higher polymer concentrations (2%, 2,5%, and 3%). In the lower polymer concentration, it was easier for crosslinker to bond because the low crosslinking density. If it compared between 2%;2,5%;3% polymer, we could see that in 3% polymer the swelling value was higher if it reacted with the lowest crosslinker concentration (0%) because there was enough space between water and molecules, whilst the use of 2,5% polymer resulted higher swelling ratio than 2% polymer for the same crosslinker concentration (in 0%) because there were more ionic group. In 2% polymer, the swelling value was higher if it reacted with the higher crosslinker concentration because the network elastic response to swelling increased, whilst the use of 3% polymer resulted higher swelling ratio than 2% polymer for the same crosslinker concentration (2,5%) because there were more ionic group.

Figure 9 below shows us the swelling ratio of particle-sized bio-hydrogels. There were also 12 formulas which was the same formula as the beads, but with different shape by mashed the gel into small parts. Different phenomena were appeared in which formula better in swelling ratio than the others. The highest swelling value was P2C2,5, with the maximum value of ±34,8305 g/g. Followed by P3C2,5 (33,5786 g/g); P2,5C2,5 (30,3567 g/g); P2,5C1 (27,6219 g/g); P3C1 (26,6086 g/g); P2C1 (20,5521 g/g); P1,5C2,5 (18,7874 g/g); P3C0 (15,9917 g/g); P1,5C1 (15,6829 g/g); P2C0 (15,6205 g/g); P2,5C0 (13,3537 g/g); and P1,5C0 (12,1830 g/g). The values obtained were similar when it is examined with statistic. The ANOVA test using CoStat with two-WCR anova for swelling test of particle hydrogels showed that the results are significantly different, but the interaction of the two factors was not significant. From the result of LSD test, the use of 3%, 2,5%, and 2% polymer respectively from the highest to the lowest swelling value had the same subset, but different subset with 1,5% polymer which has the lowest swelling value

amongst. Whereas in the crosslinker factor, the use of 2,5% crosslinker had the greater value of swelling rather than 1%. The use of 0% crosslinker (without crosslinker) had the lowest swelling value between them, and they were in different subset for each. To examined them as a single individual viewpoint, the test was continued with one way Completely Randomized. The post hoc test results using LSD revealed the same order of treatments P2C2,5; P3C2,5; P1,5C2,5; P2,5C1; P3C1; P2C1; P1,5C2,5; P3C0; P1,5C1; P2C0; P2,5C0; P1,5C0 respectively from the highest to the lowest, with the graph interpretation above.

From the Figure 9, we also could see that the swelling ratio begin constant start in the 70-90 minutes with slightly twist between P3C0 – P1,5C1 – P2C0 and P2,5C0 - P1,5C0. In the early 30 minutes swelling capacity was in greater increase and then began slightly down before reach the equilibrium in constant value. This phenomenon was rather different than beads shape above. It might be caused by the different surface area which was greater than beads shape so it might be adsorbs water faster than beads shape.

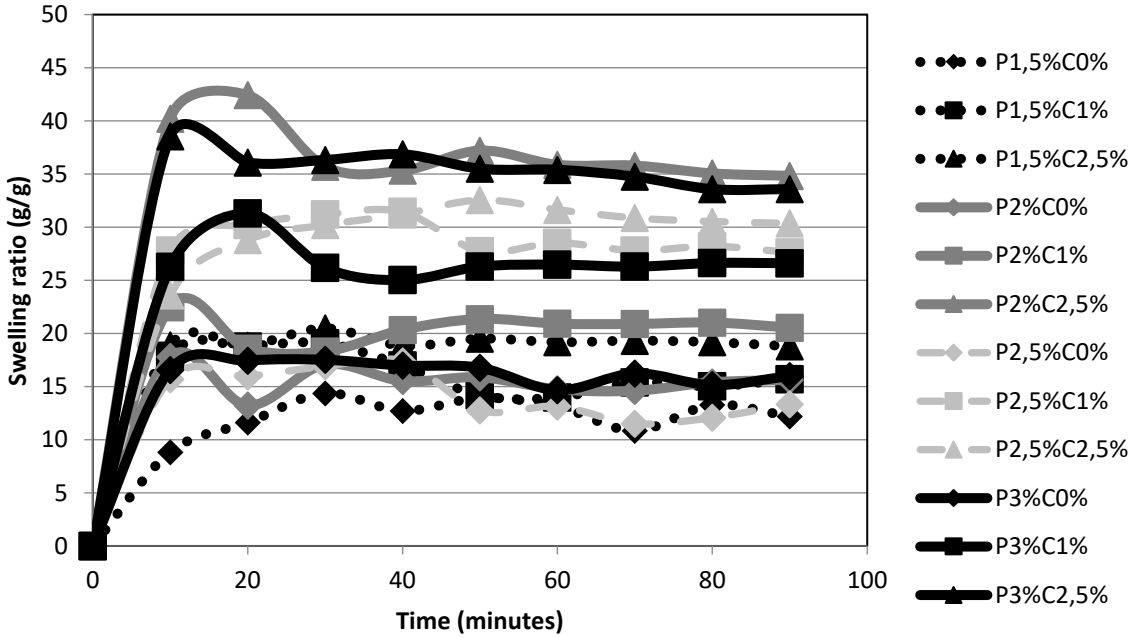

Figure 9. Swelling ratio for particle-shaped hydrogel

Figure 9 above shows us the different swelling ratio values in many samples tested for particle-shape bio-hydrogel. We could see that for the use of 3% polymer, the lowest swelling ratio was if it reacted with 0% crosslinker (without crosslinker). For 2,5% polymer, the highest swelling ratio was if it reacted with 1% crosslinker because the network elastic responds to swelling more increased than 0% crosslinker and crosslinking density was more decrease than 3% polymer. For 2% polymer, the highest swelling ratio was if it reacted with 2,5% crosslinker because network elastic response to swelling more increase than 1% crosslinking. In the use of 1,5% polymer (the lowest polymer concentration), the use of 0%, 1%, and 2,5% crosslinker (all variant crosslinker concentration in this research) resulted the lowest swelling ratio compared with higher polymer concentrations (2%, 2,5%, 3%) because the CMCNa/HEC ratio in that polymer concentration was the lowest so the ionic group was the lowest amongst. If it compared between 2%, 2,5%, and 3% polymer, it might be needs to balancing to determine the appropriate polymer and crosslinker concentration to get optimum swelling ratio. Without crosslinker (0% crosslinker), it resulted the

higher swelling ratio for 2% polymer concentration than 2,5%, might be because there was optimum space between water and molecules for the lower polymer concentration. The addition of 1% crosslinker resulted higher swelling ratio for 3% polymer than 2% because the higher ionic group. The addition of 2,5% crosslinker resulted higher swelling ratio for 3% polymer than 2,5% polymer due to the higher ionic group. The use of 2,5% (the highest crosslinker concentration in this research) resulted the highest swelling ratio. In opposite, without crosslinker (0% crosslinker as the lowest crosslinker concentration in this research) resulted the lowest swelling ratio. The more crosslinker concentration used, the higher swelling ratio attained. That phenomena was also found by 18 that it caused by the increase of network elastic response to swelling. In the higher crosslinker concentration, the average molecule weight between two crosslinking sites decreases. Swelling value between 24 to 48 hours were not that different.
The swelling values in this research was still lower than those done by 18 who examined the water sorption in cellulose-based hydrogels reached up to 400 g/g. The greater swelling value was the combination of soaking in water-soaking in methanol-desiccation as a sequential protocol. The backbone in this research was not only cellulose but it combined with agarose so it resulted lower swelling value due to the mixed properties of cellulose and agarose.

4. Conclusions

New material of bio-hydrogel has been made successfully using agarose and cellulose derivatives of Carboxymethyl Cellulose and Hydroxyethyl Cellulose, using EDAC. The material has a good physical appearance and characteristic make it possible for broader used including to maintain food quality. The resulted hydrogel showed the firm hydrogel which has hardness value of 261 g-1116 g. P1,5%C0% had maximum swelling value of 46,86 g/g when in the form of beads, whereas in the form of particle material P2%C2,5% had maximum swelling value of 34,83 g/g. Bio-hydrogel made from agarose and cellulose derivatives might become promising solution to food applications with environmental friendly option.

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