JOURNAL OF NATURAL SCIENCES AND MATHEMATICS RESEARCH

Available online at http://journal.walisongo.ac.id/index.php/jnsmr

The Influence of *Alginate* and $CaCl_2$ concentration Towards *Betacyanin* content and Antioxidant Activity of Red Dragon Fruit (*Hylocereus Polyrhizus*) restructuring product

Wira Wanti Bangalino[1], Lydia Ninan Lestario[2] dan Cucun Alep Riyanto[2]
[1]Mahasiswa Program Studi Kimia Fakultas Sains dan Matematika Universitas Kristen Satya Wacana, Salatiga
[2]Dosen Program Studi Kimia Fakultas Sains dan Matematika Universitas Kristen Satya Wacana, Salatiga

Corresponding author: 652012026@student.uksw.edu
Recived: 10 November 2016, Revised : 10 December 2016 Accepted: 28 December 2016.

Abstract

The purpose of this study was to determine the effect of alginate and $CaCl_2$ concentration of antioxidant activity and betacyanin content of restructuring product of red dragon fruit, and to determine the best formulation of the product restructuring that produces antioxidant activity and the content of the highest betacyanin on product restructuring. Data were analyzed by 4x2 Factorial Design and Randomized Completely Block Design (RCBD) with 4 replications. As the first factor is the alginate concentration which are 2 %, 3 %, 4 % and 5 %. The second factors are calcium cloride ($CaCl_2$) 0.5 % and 0.75 %, while as the block is the time analysis. To test the differences between treatments means, the Honestly Significant of Differences (HSD) were used at 5% level of significant.The results showed that the higher concentrations of alginate and $CaCl_2$ causes declining content of antioxidant activity and betacyanin, the best results are obtained is at a concentration of 2% alginate and CaCl2 0.5% with betacyanin levels 0.537 ± 0.062 mg/g dry weight basis and 0.132 ± 0.011 mg / g wet weight basis. The antioxidant activity of H. polyrhyzus restructuring amounted to 77.452% ± 0.624.

Key words: Antioxidant; Betacyanin; Dragon fruit; Restructured.

1. Introduction

Red dragon fruit is one of food material that have good enough profit for health because red dragon fruit contains A, C and E vitamin, protein, fiber, and mineral source such as calcium, phosphor, and magnesium [1], beside that red dragon fruit potentially help reduce blood sugar content and prevent the risk of heart disease of diabetes medical patient [2]. Beside that, red dragon fruit (*Hylocereus polyrhizus*) contains antioxidant compound such as ascorbat acid and betacyanin. Betacyanin that contained in red dragon fruit between 0.32 – 0.41 mg/g where

is the betacyanin functioned as antioxidant [3].

Antioxidant is a compound that can obstruct oxidation with reaction way with reactive free radical to made non reactive free radical that more stable. Compound of fenolic antioxidant is a compound that have a role by multi function because it have a role as reducing agent, free radical captor, metal astringent, or device at the form of singlet oxygen [4]. Nature antioxidant can protect the body from the damage that caused by reactive oxygen species, it can hamper the degenerative disease and can hamper the lipid peroxide in food [5].

Form all the overbalance of antioxidant in red dragon fruit, it inclined have soft texture and have high water content so that its very easy to broken and the smell is not strong, it made some people does not like this fruit. Because of that, it need continue processing, one of restructuring, the purpose of restructuring technique is to return made of food material with binding agent help so it become durable and can consume without relieve the benefit of red dragon fruit. Restructuring is a way of processing with gel system as binding agent that made by chemical, without through heating process (non-thermal) with using alginate combination and calcium ion [6]. Some overbalance of this product are fruit percentage on the product reach 99%, it still protrude the real taste food, it can be saved more over 5-7 days, and the nutrition value is not decrease.

Based on the background above, the purpose of the research is to know the influence of alginate concentration and $CaCl_2$ toward antioxidant activity and betacyanin content of restructuring product of red dragon fruit, and also determined the best formulation toward result product of restructuring that produce antioxidant activity and high betacyanin content in restructuring product.

2. Experiments Procedure

Sample used is red dragon fruit that gain from Salatiga market. Chemical material used in this research is ethanol, chloride acid 0.5% (PA), chloride calcium (Merk, Jerman), 1,1-diphenyl-2-pycrylhydrazil (Sigma, USA), and algynate (technique). Instrument used such as spektrofotometry UV-VIS (Optizen UV 2120), analytic balance of 2 decimal (OHAUS, TAJ602), analytic balance of 4 decimal (OHAUS Pioneer, PA214), *mixer* (Philips, HR1538), pH meter (HANNA Instrument 9812), moisture analyzer (OHAUS MB25), blender (Vitara).

Restructuring of fruit porridge [13]

Restructuring of red dragon fruit is is done used algynate variation with concentration of 2%, 3%, 4% and 5% of each algynate added $CaCl_2$ with concentration 0.5% and 0.75%. Algynate is added to the porridge of red dragon fruit (100 gram) and then homogeneity with mixer with rotation of 484 rpm during 5 minutes. Still in rotation of 484 rpm, it is added kitchen salt and sugar with certain amount according to the appetite. The mixing is done during 1 minute. Beside that, $CaCl_2$ (0.5%; 0.75%) in the form of solution is got in to the blend that in mixed condition in rotation of 484 rpm during 15 seconds. The blend which got in to plastic container diameter 5 cm, covered and saved in temperature of 9 °C during 18-20 hours and then doing analysis the betacyanin content, antioxidant activity and water content.

Water content measurement

Water content is measure used moisture analyzer (Ohaus, MB25) which is done to the pulp of red dragon fruit restructuring result.

Measurement of Betacyanin content [7]

The weight of red dragon fruit amounted 0.5 gram is macerated with aquades amounted 50 ml by high rise. Filtrate that gain is fulfilling in measure squash 50 ml that layered aluminum foil. Filtrate is soluble in buffer phosphate (0,05 M, pH 6.5) is fulfilling until 5 ml so absorption is gain in the shift 0,4

until 0.5±0.02 on the wavelength 538 nm. Absorption value is measured in wavelength 538 nm, 476 nm and 600 nm. This method is also used to test the restructure result. Calculation of betalain content used equation as follows:

$$X = 1{,}095\ (A_{528} - A_{600})$$

$$Z = A_{538} - x$$

$$Y = A_{476} - z - \frac{x}{3{,}1}$$

where, X = Absorption of betanin reduce the polluter

Y = Absorption of vulgaxanthin – I

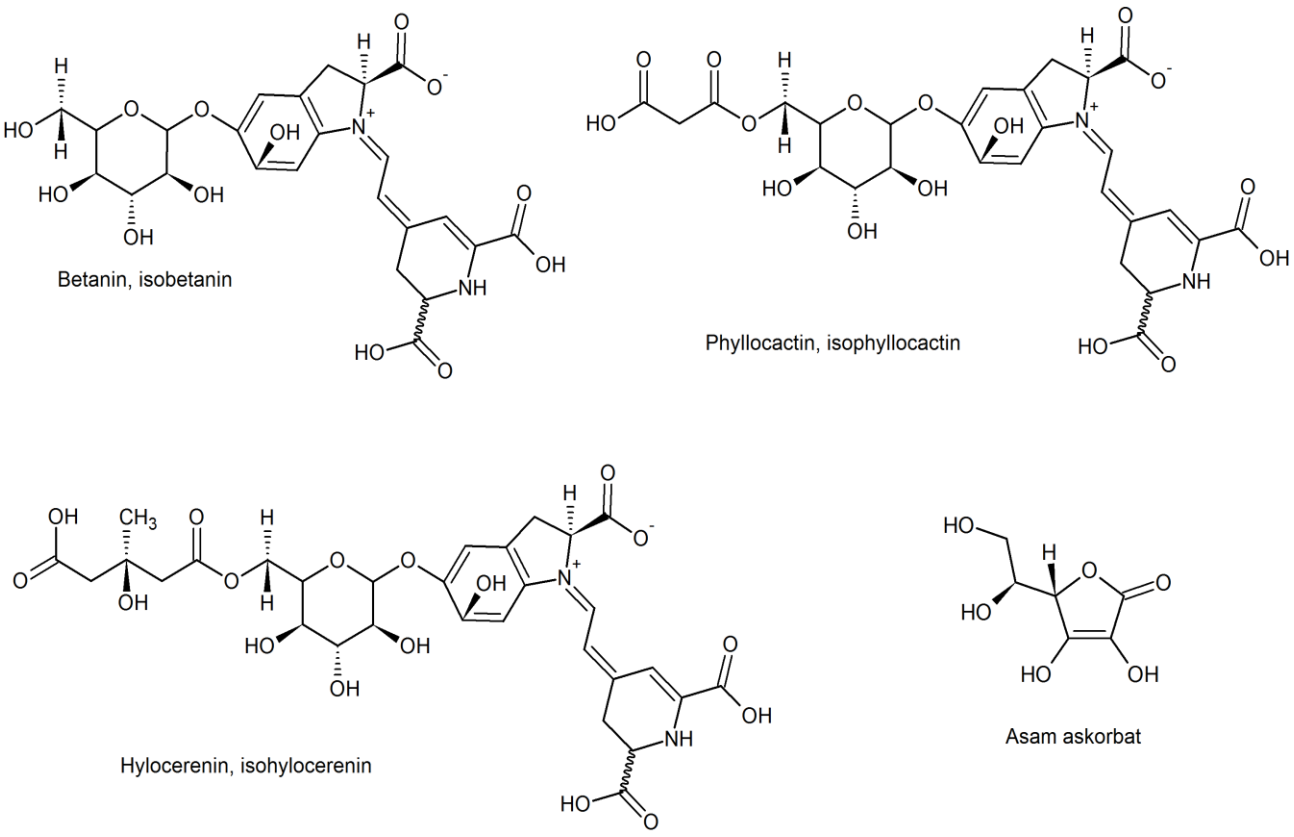
Z = Absorption of polluter

Concetration of *betanin* and ulgaxanthin – I is determined with the equation as follows:

$C_{betanin}$ = (x/1120) x *dilution factor*

$C_{vulgaxanthin\ -\ I}$ =*(y/750) x dilution factor*

Content of betalain pigment is amounted of $C_{betanin} + C_{vulgaxanthin}$ with unit g/100ml.



Pigure 1. Betacyanin compound of red dragon fruit

Preparation of fruit extract to antioxidant activity test [8]

Weight of 1 g red dragon fruit that have been smoothed or red dragon fruit of restructure result is macerate in 10 ml ethanol - HCl 0.5% (v/v), in the temperature 9°C more over one night, then it is done filtration with filter paper. The waste that still have purple color is extracted with 1x10 mL ethanol - HCl 0,5% (v/v) more over 30 minutes, filtrate is joined and volume is appropriated to 25 mL.

Test of antioxidant activity with method of free radical catching [9]

Antioxidant activity is measured with method of free radical catching DPPH. A number of 2 mL DPPH 0.1 mM in ethanol is added with 0,1 mL extract from 1 g red dragon fruit in 25 mL ethanol - HCl 0.5% (v/v), the mixture is liquid with ethanol until 3 mL then it kept during 30 minutes, it is also made blank with the same way but its not used fruit

extract. Furthermore, it is done measurement with spektrofotometry UV-VIS λ=517 nm. The same method used to measure antioxidant activity of fresh red dragon fruit. Antioxidant activity is counted by comparing the sample absorbed with blanko, this method also used to restructure result, with formulation:

$$\text{Antioxidant activity } (\%) = \{1 - (\frac{A_{sampel}}{A_{blanko}})\} \times 100\%$$

Data Analysis

Research data is analyzed with statistically with Factorial treatment plan 4 x 2 with based plan of Group random plan, 4 repetition. As first factor is alginate concentrate consist of 4 limit that is 1%, 2%, 3% and 4%, while the second factor is chloride calcium that is 0,5% and 0,75%. As group is analysis time. Mean test between treatment is done with honest real different test with meaning level 5% [10].

3. Result and Discussion

Betacyanin content based on dry weight of restructure red dragon fruit between 0.286 ± 0.031 mg/g until 0.537 ± 0.062 mg/g (Table 1). Calculation result of betacyanin content based on wet weight of restructure result of red dragon fruit in various concentrate of algynate and $CaCl_2$ is presented at Table 2.

Research result show that based on treatment between Algynate concentrate at $CaCl_2$ 0,5%, betacyanin content on fruit of restructure result with 2% Algynate concentrate have higher betacyanin content if it compared with fruit of restructure result with 3%, 4% and 5% Algynate concentrate. If it compared among algynate concentrate at $CaCl_2$ 0,75%, the highest betacyanin content is in 2% Algynate concentrate, while betacyanin content faced declined in 3%, 4% and 5% Algynate concentrate. It is shown that the higher of algynate and $CaCl_2$ concentrate, caused decline towards betacyanin content, it is assumed that there is a bond between algynate calcium and betacyanin, so that the betacyanin is less extracted. If the betacyanin is tied by alginate, actually that betacyanin is inside the product and still consumed, its only not extracted in the betacyanin content measurement. So, the benefit of betacyanin still can gain when its consumed.

If its compared between $CaCl_2$, concentrate of betacyanin content in fruit of restructure result that given $CaCl_2$ 0.5% is different if compared if it given $CaCl_2$ 0.75%. it is shown that addition $CaCl_2$ is influenced betacyanin content in red dragon fruit of restructure result, it is accordance with research result of Herawati (2015) who doing restructure research at duwet fruit with alginate comparison of 1%, 2%, 3% and 4% also $CaCl_2$ 0.75% and 1% [11]. Based on that research is gain that best result whether from antocyanin content or antioxidant activity on algynate concentrate 1% and $CaCl_2$ 0.75%.

Betacyanin content of red dragon fruit restructure result is range from 0.286 – 0.537 mg/g (BK) and betacyanin content of red dragon fruit of restructure result based wet weigh is range from 0.102±0.020 – 0.132±0.011 mg/g, if it compared with betacyanin content of fresh red dragon fruit is 1.2671 mg/g (BK) and 0.1776 mg/g (BB) apparently the betacyanin content of fruit restructure result experience descent after doing processing treatment of fresh fruit become restructure product. Furthermore, the antioxidant activity of red dragon fruit restructuring at various concentrations of alginate and $CaCl_2$ is shown in Table 3. More over, restructure antioxidant activity of red dragon fruit at various alginate and $CaCl_2$ concentrate show in Table 3.

Based on Table 3 seen that more and more rise of algynate concentrate caused in descent towards antioxidant activity, it is accordance with increasing of $CaCl_2$ that more rise and also caused descent towards antioxidant activity. It is gain highest antioxidant activity in 2% concentrate of algynate and 0.5% concentrate $CaCl_2$ even though the descent on antioxidant activity is less than descent on betacyanin content. It is because of other compound in red dragon fruit which can rolled as antioxidant that is *poly-phenol* and fitoalbumin that contain in pulp red dragon fruit [12].

Table 1. Betacyanin content (mg/g) based on dry weight of restructure red dragon fruit in various concentrate of Alginate and $CaCl_2$

$CaCl_2$ content	Algynate content				control (0%)
	2%	3%	4%	5%	
	O.537±0.062	0.420±0.018	0.376±0.018	0.286±0.059	
0.5%	(b)	(b)	(b)	(a)	
	(d)	(c)	(b)	(a)	1.267
	0,410±0,064	0.366±0.063	0.304±0.016	0.286±0.031	
0.75%	(a)	(a)	(a)	(a)	
	(c)	(bc)	(ab)	(a)	
W = 0.055		W = 0.159			

Information: numbers that followed by same letter in the under side is show that among treatment is no different meaning in horizontal direction, while the number that followed by the same letter in right side show that among treatment is no different meaning in vertical direction.

Table 2. Betacyanin content (mg/g) based on wet weight of restructure result of red dragon fruit in various concentrate of algynate and $CaCl_2$

Content $CaCl_2$	Algynate content				control (0%)
	2%	3%	4%	5%	
	0.132±0.011	1.122±0.025	0.122±0.005	0.102±0.020	
0.5%	(a)	(a)	(a)	(a)	
	(b)	(ab)	(a)	(a)	0. 177
	0.131±0.015	0.128±0.017	0.114±0.009	0.124±0.015	
0.75%	(a)	(a)	(a)	(a)	
	(a)	(a)	(a)	(a)	
W = 0.021		W = 0.016			

Numbers that followed by same letter in the under side is show that among treatment is no different meaning in horizontal direction, while the number that followed by the same letter in right side show that among treatment is no different meaning in vertical direction.

Table 3. Antioxidant activity (%) restructure of red dragon fruit at various *alginate* and $CaCl_2$ concentrate

Content $CaCl_2$	Alginate content				Control 0%
	2%	3%	4%	5%	
	77.452±0.624	75.517±0.581	74.875±0.515	73. ±0,931	
0.5%	(b)	(b)	(b)	(b)	
	(d)	(c)	(b)	(a)	78.90
	73.35±0.414	74.507±0.900	71.407±0.631	71.047±0.512	
0.75%	(a)	(a)	(a)	(a)	
	(d)	(c)	(b)	(a)	
W = 0.452		W = 0.337			

Numbers that followed by same letter in the under side is show that among treatment is no different meaning in horizontal direction, while the number that followed by the same letter in right side show that among treatment is no different meaning in vertical direction.

Table 4. Water content (%) restructure of red dragon fruit in various *algynate* concentrtae and $CaCl_2$

Content $CaCl_2$	*Algynate* content 2%	3%	4%	5%	Control 0%
0.5%	75.292±1.479 (b) (b)	73.642±1.714 (b) (b)	67.452±1.113 (b) (a)	64.645±1.275 (b) (a)	85.88
0.75%	67.547±1.698 (a) (c)	65.075±2.455 (a) (bc)	62.300±2.455 (a) (b)	57.070±5.224 (a) (a)	
W = 3.409		W = 2.543			

Numbers that followed by same letter in the under side is show that among treatment is no different meaning in horizontal direction, while the number that followed by the same letter in right side show that among treatment is no different meaning in vertical direction.

Antioxidant activity of red dragon fruit restructure result is range from 71.047±0,512 – 77.452±0.624. Restructure result of red dragon fruit have antioxidant activity that is no different from fresh red dragon fruit without processing i.e. amounted 78.90%. it is shown that processing with restructure way is not damage antioxidant activity from red dragon fruit. Restructure water content of red dragon fruit is range from 57.070 ± 5.224 until 75.792 ± 1.479 (Table 4). If it compared between algynate concentrate at $CaCl_2$ 0.5% higher water content get in 2% algynate concentrate, it is possibility because the quantity of algynate that add in small quantity so the ability in tie the water is not good as in add 5% algynate ,while in algynate concentrate of $CaCl_2$ 0.75% higher water content also gain in adding 2% algynate and the lowest in in adding 5% algynate. If it compared between 0.5% and 0.75% of $CaCl_2$ concentrate show that the real influence toward water content accordance with research [6] whho doing restructure research used comparison of calcium kind that is calcium of lactate and $CaCl_2$, and it gain in strong gel form for $CaCl_2$ added [13]. The different texture from each treatment is because of algynate interaction with chloride calcium in forming gel. Algynae can make a gel with calcium ion where is take place a bond between algynate and calcium ion. The form is added look like an egg in a box (egg box, see Figure 1) [14] the function of $CaCl_2$ is to push the algynate chain in order to unite through ionic interaction where is they will make hydrogen union between chain [13]. From the result can be seen that more adding the algynate and $CaCl_2$ will influence the water content of restructure result fruit (see Figure 2). Because of that, according to the observation result, if it more and more adding the alginate, its more firm gel structure that gain and more water content that can trapped in those gel structure (see Figure 3).

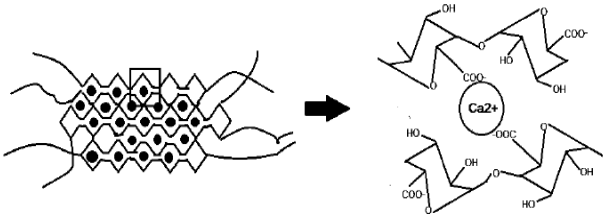


Figure 1. Egg box from G-G coordination of algynate and ion Ca^{2+} [16]

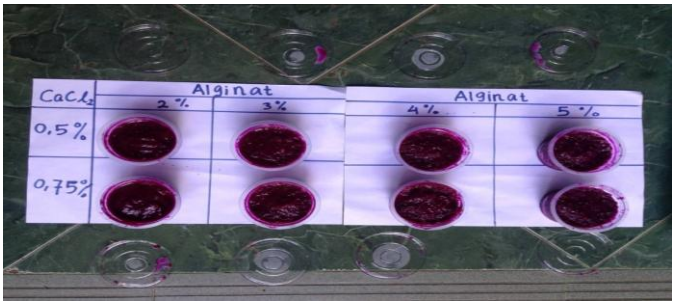
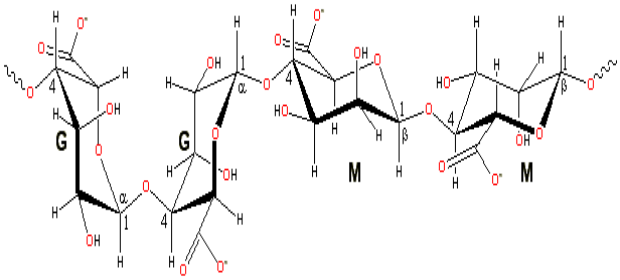


Figure 2. restructure result of red dragon fruit (*H. polyrhyzus*)



a. -Guluronat-Guluronat- (b) -Guluronat-Manuronat- (c) -Marnuronat-Manuronat

Figure 3. Algynate structure [15]

4. Conclusion

From this research, it can be drawn a conclusion that more and more algynate concentrate and $CaCl_2$ concentrate caused antioxidant activity and betacyanin content is descent, and best result that gain is 2% of algynate concentrate and 0.5% of $CaCl_2$ with beacyanin content of 0.537±0.062 mg/g based dry weight and 0.132±0.011 mg/g based on wet weight and also antioxidant activity of restructure H. polyrhyzus amounted 77.452%±0.624.

Acknowledgment

The authors would like to thank Ministry of Research Technology and Higher Education. who has contributed this research through a competing grant program in 2015.

References

[1] M. R. Ramadhan, N. Harun, dan F. Hamzah, Kajian Pemanfaatan Buah Naga Merah (*Hylocereus polyrhizus*) dan Mangga (*Mangifera indicalinn*) dalam Pembuatan Fruit Leather. *SAGU,* Vol.14 No. 1, pp. 23-31, 2015.

[2] R. Wahyuni, Pemanfaatan Buah Naga Super Merah *(Hylocereus costaricensis)* dalam Pembuatan Jenang dengan Perlakuan Penambahan Daging Buah yang Berbeda*. Jurnal. Teknologi Pangan* 4 (1), pp.71-92, 2012.

[3] R.E.M. Pakhpahan, Aktivitas Antioksidan dan Kadar Fenolik Total pada Buah Naga Putih *(Hylocereus Undatus)* dan Buah Naga Merah *(Hylocereus Polyhizus)*. *Skripsi*. Salatiga: Universitas Kristen Satya Wacana. 2006.

[4] L. N. Lestario, , A.E. Christian, , dan M. Yohanes, Aktivitas Antioksidan Daun Ginseng Jawa (*Talinum paniculatum* Gaertn). AGRITECH 29 (2), 71-78, 2009.

[5] E.P. Oktaviani, L.M.E. Purwijantiningsih, F.S. Pranata, Kualitas dan Aktivitas Antioksidan Minuman Probiotik dengan Variasi Ekstrak Buah Naga Merah (*Hyloreceus polyrhizus*). *Skripsi*. Yogyakarta: Universitas Atma Jaya, 2012.

[6] S. Raharjo, dan Z. Utama, Sifat - Sifat Fisik dan Sensoris Buah Hasil Restrukturisasi Non-termal Selama Penyimpanan Dingin. *Jurnal Teknologi dan Industri Pangan* 13 (1), pp.11-19, 2002.

[7] R.E. Wrosltad, and M. Giusti, M. Antthocyanins Characterization and measure with UV-Visible Spectroscopy. In: Wrostald, R. E,editor. *Current protocols in food analytical chemistry*. New york: John Wiley & spons.p.F1.2.1 - 1.2.13, 2001.

[8] M.A.W. Setiawan, E.K. Nugroho, dan Lestario, L. N. Ekstraksi Betasianin dari Kulit Umbi Bit *(Beta vulgaris)* sebagai Pewarna Alami. *Agritech 27*(1) dan (2), pp.38 – 43, 2015.

[9] L.N. Lestario, P. Hastuti, S. Raharjo, dan Tranggono. Sifat Antioksidatif Ekstrak Buah Duwet (*Syzygium cumini*). *Agritech* 25, pp.24-31, 2005.

[10] R.G.D. Steel, dan J.H.Torrie, Prinsip dan Prosedur Statistika Suatu Pendekatan Biometrika. Edisi Cetakan ke 2, Jakarta: PT. Gramedia Pustaka Utama, pp.168-208, 1989.

[11] D. Herawati, N.L. Lydia. A. Silvia. Pengaruh Alginat dan $CaCl_2$ Terhadap Kadar Antosianin, Aktivitas antioksidan Dan Karakteristik Sensorik Buah Duwet (*Syzygium cumini Linn*) Hasil

Restrukturisasi. *Agritech* 36(3), pp. 24-3, 2015.

[12] V. Lianiwati, Pemberian Ekstrak Buah Naga Merah (*Hylocereus Polyrhizus*) Menurunkan Kadar F_2 Isoprostan pada Tikus Putih Jantan (*Albino rat*) yang Diberi Aktivitas Berlebih. *Skripsi*. Bali: Universitas Udayana. 2011.

[13] S. Raharjo, dan Z. Utama, Pengaruh Ukuran Partikel dan Proporsi Guluronat/ Manuronat dalam Alginat Terhadap Sifat Fisik dan Sensoris Produk Hasil Restrukturisasi dari Buah Sirsak. *Jurnal. Teknol. dan Industri Pangan,* Vol. XIV (3), pp. 206-213, 2003.

[14] S. Sellimi, Y. Islem, B.A. Hanen, M. Hana, M. Veronique, R. Marguerite, D. Mostefa, M. Tahar, H. Mohamed, N. Moncef, Structural, Physicochemical and Antioxidant Properties of Sodium Alginate Isolated from a Tunisian Brown Seaweed. *International Journal of Biological Macromolecules* 72, pp. 1358–1367, 2014.

[15] M. Chaplin, *Alginate*. http://www1.lsbu.ac.uk., 10 April 2015.

[16] R. Yamdech, P. Aramwit, dan Kanokpanont, S. Stability of Anthocyanin in Mulberry Fruits Extract Adsorbed on Calcium Alginate Beads. *International Conference Chulalongkorn University, Bangkok Thailand,* 2012.