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Rendemen, gel strength and viscosity of red algae *Kappaphycus alvarizii* (Doty) in Minahasa Peninsula

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Abstract

Seaweed *Kappaphycus alvarezii* is member of red algae (Rhodophyta) largely cultivated that is a carrageenan source highly needed in various industries worldwide. This study was aimed at knowing the rendemen, the gel strength and the gel viscosity of the carrageen production from *K. alvarezii* farming sites in Minahasa penninsula. Samples were collected from 4 regencies and extracted for carrageenan analysis and its physical properties. Results showed that the highest rendemen, 55.31%, was found in the product of Sondaken, South Minahasa, followed by Buku Tengah, Southeast Minahasa, 47.04%, Tumpaan, Minahasa, 39.62%, and the lowest in Jayakarsa, North Minahasa, 30.94%. The highest gel strength was recorded in the product of Buku Tengah, 1,391 g, followed by Tumpaan, 1,113 g, Jayakarsa, 626 g, and the lowest in Sondaken, 557 g. The highest viscocity was found in the product of Buku Tengah, 60 cps, then Sondaken 50 cps, Likupang, 40 cps, and Tumpaan, 30 cps, respectively. The highest rendemen occurred in the water with temperature range of 28.4 – 29.9^{o}C and pH of 8.14 – 8.15. The highest gel strength was found in thw water with nitrate concentration range of 0.1-0.2 gr/cm^2 , the highest viscosity in salinity range of 29.1 – 29.7 ppm. Carrageenan quality was highly dependent upon the interactions among water quality parameters and the supporting seaweed *K. alvarezii* farming site for optimal nutrient absorption around its habitat.

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Introduction

In the past, *Kappaphycus alvarezii* and *Eucheuma denticulatum* were harvested from nature in Indonesia and Philippine, then widespread to other countries, such as Tanzania, that are successful to cultivate (McHugh, 2003). *Kappaphycus alvarezii,* a red alga also known as *Eucheuma cotonii,* is a carrageenan-producing tropical seaweed occurring in Philippine and Indonesia. Total production of k-carragenan is 95 % from Southeast Asia, 55% from Philippine, 38% from Indonesia and 2.5% from Malaysia (Duduku *et al.*, 2008 and Campo *et al.*, 2009). North Sulawesi is part of Indonesia having potential for seaweed culture development due to occurring in the tropics, whose geographic position is adjacent to the Pacific Ocean with rich marine resources. Their distribution and habitats are coastal waters with reef flat, such as North Sulawesi and Mallucas waters. Carrageenan-producing seaweeds, particularly genus *Eucheuma,* have been cultivated in several locations of North Sulawesi Province. Soegiarto *et al.* (1978) claimed that algin, agar and carrageenan of marine algae were dependent upon species, region and climate.

Carrageenan is produced through extraction process of the red algae. Seaweeds, *K. Alvarezii* contain carrageenan of *kappa*-carrageenan group of relatively high concentration, about 50 % of the dry weight (Winarno, 1996). Kappa-carrageenan is a processed seaweed product with high economic value, 10 to 20 folds of the seaweed price. *K. alvarezii* is a very important k-carrageenan source due to high world demand for polysacharide of the cell wall of the seaweed (Muñoz *et al.*, 2004). Kappa carrageenan is used as raw materials of pharmaceutical, cosmetic, food and other industries Mubarak *et al.*, (1990) and mostly applied in food product (Prasetyowati *et al.*, 2008).

Carrgeenan has ability to synthesize stable gel at room temperature, and it is stable against enzymatic degradation to maintain the storing stability (Voight, 1994).

The quality of carrageenan determined based on its purity, gel strength and viscosity. The culture of *K. alvarezii* in Minahasa Peninsula has been long developed buts its carrageenan quality has not been identified. Bunga *et al.* (2013) analyzed the physical-chemical characteristic of carrageenan based on different harvest time in Noprth Minahasa, Harun *et al.* (2007) studied *K. alvarezii* in Gorontalo Province, while Mudeng *et al.* (2007) observed the growth rate in different depth in North Minahasa. However, the quality of carrageenan extracted from *K. alvarezii* cultivated in Minahasa Peninsula has not been studied. Determination of carrageenan is important due to its wide utilization in various industries. On the other hand, the carrageenan quality must meet the standard quality of FAO. This study was aimed to know the rendemen, viscosity and gel strength of *K. alvarezii* obtained from seaweed farming in Minahasa penninsula, North Sulawesi. Analyzing the carrageenan quality will be helpful for establishment of *K. alvarezii* development area.

Materials and methods

This study was carried out through *in situ* water quality measurements and sample collection of seaweed *K. alvarezii* cultured in Minahasa penninsular, North Sulawesi. Water quality parameter measurements were done in the farming site of 4 study sites in North Sulawesi, Jayakarsa, North Minahasa, Sondaken, South Minahasa, Tumpaan, Minahasa and Buku Tengah, Southeast Minahasa. The samples were extracted and analyzed under laboratory procedure to know the rendemen, the viscosity and the gel strength.

Extraction process started from sample cleasing and then dipped in water for 24 hours. Five grams of the sample were boiled at 70°C-90 °C using 100 ml of aqueous NaOH at pH 5 - 9 for 3 hours. The extract was filtered in hot condition and precipitated with ethanol, dried in an oven at 60°C for 24 hours, weighed and calculated the carrageenan (Winarno, 1990).

Rendemen

To determine the carrageenan content, the following formula was used (Winarno, 1990)

$$\% \text{ Rendemen } = \frac{carrageenan\ weight}{sample\ weight} \text{ X } 100\%$$

Gel Strength Test

Dry carrageenan was dissolved in aquadest at a concentration of 15% through heating. To obtain the gel strength, 10 ml of 1.5% carrageenan solution was poured into 3 cm-diametered glass with solution height ranging from 1.2 to 1.4 cm. After left for one night at room temperature, the glass was put on a balance. A stainless cylindrical rod (cross-sectional area of 0.786 cm^2) was laid on the sample, then pressed with hand until the gel was broken, and the weight was recorded. Three replications were used for the same sample. Gel strength is the difference between the gel weight before broken and after broken divided by the cross-sectional area of the stainless cylinder.

Viscosity Test

Extract solution of 1.5% concentration was heated in boiling water while regularly stirred until the temperature reached 75^{o}C. The viscosity was measured with *Viscometer Brookfield.* Spindel was previously heated at 75^{o}C then set on the measurer. Spindel position was set in the hot solution. The viscometer was activated and the solution temperature measured. At the solution temperature of 75^{o}C, the viscosity value appeared by reading the scale of the viscosimeter. Reading was done after 1 min. of 2 full rotations of spindel no 1.

Results and discussion

Water and Carrageenan Quality

Water quality is major factor affecting the seaweed growth. According to Gerung & Ohno (1997), water temperature, salinity, light, and nutrients are the mort important environmental factors beside other ecological factors. Water quality parameters are major environmental factors for seaweed growth. Different locations of *K. alvarezii* farming in Minahasa penninsular had dissimilar water and carrageenan quality (Table 1).

Table 1. water quality and carrageenan in 4 farmnig locations of *K. alvarezii* in Minahasa penninsular.

No	Parameter	North Minahasa	Minahasa	Southeast Minahasa	South Minahasa
A	Water quality				
1	Temperature (°C)	30.1– 30.4	28.1 – 30.2	28.6	28.4 – 29.9
2	Visibility (m)	5 – 6	2 – 8	8	5 – 7
3	Current(cm/sec)	20 – 40	20 – 40	8.15	20 – 40
4	Tide (m)	210	2	2	2
5	Depth (m)	7.1 – 8	1.20 - 6	6 - 8	4.8 – 10
6	pH	7.93– 8.07	8.10 – 8.20	8.14	8.02 – 8.15
7	Salinity (ppm)	30.3– 31.3	30.8 – 31.8	29.7	29.1 – 31.2
8	Nitrate (mg/l)	0.3 – 0.48	0.1 – 0.2	0.1	0.2 - 0.3
9	Phosphate (mg/l)	0.1 – 0.6	0.6	0.9	0.4 – 0.9
B	Carrageenan				
1	Rendemen (%)	30.94	39.62	47.04	55.31
2	Gel strength (g/cm2)	626	1,113	1.391	557
3	Viscosity (cps)	40	30	60	50
4	Ash (%)	35.47	35.31	41.30	36.81

Number of carrageenan in 4 study sites ranged from 30.94 to 55.31%, and these values are higher than the minimum standard value requisited by the Trade Department of Indonesia in 1989, 25% (Harun *et al.*, 2013), the FAO standard is 40%, and 30% carraheenan is categorized as low quality seaweed. Several factors known affecting the quantity and the quality of the carrageenan are seaweed species, seasonal fluctuation (Dawes *et al.*, 1977; Yakovleva *et al.*, 2001), rearing depth (Akmal *et al.*, 2013), rearing time, variety and extract condition (Hayashi *et al.*, 2013), alkaline solution (lye)

(Romenda *et al*., 2013), and plant age (Bunga *et al*,. 2013). Munoz *et al.* (2004) stated also that marine physico-chemical factors affected both seaweed growth and carrageenan rendemen.

According to Hayashi *et al.* (2007), difference in carrageenan content could also result from dissimilar extraction method and species. Carrageenan content varies with strain, species and depth, and in general, plant of higher growth rate has higher carrageenan content (Naguit *et al*., 2009). However, according to Syahlun *et al.* (2013), carrageenan content of the seaweed *K. alvarezii* is not influenced by culture method (line length).

Quality of Carrageenan

Rendemen

Carrageenan analysis of the seaweed *Kappaphycus. alvarezii* in Minahasa pennisular, it was found that the best rendemen level, 55,31%, was recorded in Sondaken (south Minahasa). Doty (1985) found that CAY of marine algae had a standard of 40%. In relation with water quality parameters, water temperature and pH might affect the rendemen level. It is supported by Yunkue *et al* (2010) that growth triggering agents in the culture media will work better if they are supported by suitable pH and temperature conditions. Water temperature is an important physical factor, and each temperature change tends to affect numerous chemical processes simultaneously occurring in plant and animal tissues that could entiraly influence the biotat, 2010). According to Lundsor (2002), good water temperature range for the seaweed *K. alvarezii* growth is 27 -30°C. Similar findings were also reported by Prajapati (2007) and Parenrengi *et al.* (2007), in which at this temperature range the carrageenan level could rise, but the present study found that the best carrageenan level occurred at the temperature range of 28.4 °C -29.9 °C and pH of 8.02-8.15 in south Minahasa waters and the lowest carrageenan level was found in north Minahasa with a temperature range of 30.1 °C-30.4 °C. The relationship between water temperature and pH and rendemen is presented in Fig. 01.

Fig. 1. Rendemen of *K. alvarezii.*

In tropical waters, seawater temperature variations are very little along the year. Sea surface temperature of Indonesian waters ranges from 27 °C – 32 °C. Small temperature change can cause mortality or physiological disturbance of marine biota (Romimohtarto, 2008). Bulboa and Paula (2006) examined growth temperature of. *K. alvarezi* at laboratoy scale and found that *K. alvarezi* could grow well at 21 °C, 24 °C, 27 °C and 30 °C, but died at 15 °C and 18 °C. Low water temperature could destruct lipid and protein membrane (Munoz *et al.,* 2004), but high water temperature could also cause protein denaturation and destruct enzymes and cell membranes labile to high temperature (Susanto *et al.,* 2001). Hayasi *et al* (2010) stated that in Brazil, the best growth occurred in summer with water temperature of 17.1 °C – 28.5 °C.

Water pH is highly needed in biochemical processes in the water. Water pH measurements in the farming site of *K. alvarezii* in south Minahasa ranged from 8.02 to 8.15 which gave the highest carrageenan. It is in line with Tri (1999) that good growth occurred at pH of 8.0-8.4, while it was slow at pH of 7.1-7.4, and the better the growth is the higher the carrageenan contant is (Munoz *et al.* (2004). This finding is different from Arisandi *et al.* (2012) that pH of tissue culture media affected the number of cells and the carrageenan rendemen, in which pH 8.5 gave the highest number of cells, but according to Barasanti and Gualtieri (2014), pH range for cultured algae was between 7 and 9, with optimum of 8.2-8.7. The lowest rendemen was recorded in north Minahasa with water pH range of 7.93-8.07. Phosphate is also a factor influencing the carrageenan content. According to Neis (2003), *Eucheuma* will produce the highest carrageenan at phosphate concentration of 0.62 mg/l – 0.66 mg/l. Nevertheless, present study demonstrated that the highest rendemen was found at phosphate concentrations of 0.4-0.9 mg/l, but lower rendemen was recorded at phosphate concentration of 0.6 mg/l.

Gel Strength

Gel strength is major paramter of the carrageenan. Nitrate is one of determining factors of *K. alvarezii* carrageenan gel strtength. Gel strength analysis on seaweed production in Minahasa penninsula ranged between 557g – 1,391g with the highest gel strength recorded in Buku Tengah, Southeast Minahasa. The relationship of nitrate concentration and gel strength is presented in Fig. 02.

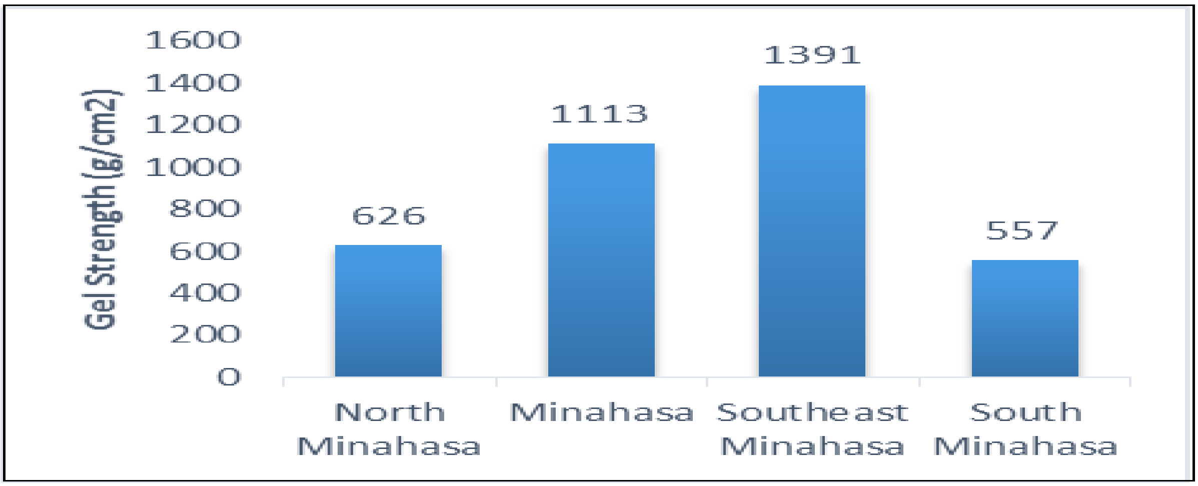

Fig. 2. Gel strength of *K. Alvarezii.*

The highest gel strength was recorded in Buku Tengah, southeast Minahasa. It occurred at nitrate concentrations of 0.1 – 0.2 mg/l with a gel strength of 1,391 g/cm², followed by Tumpaan, Minahasa at nitrate concentration of 0.1 mg/l with gel strength of 1,113 g/cm². This gel strengths is much higher than those in Jayakarsa waters, north Minahasa, 626 g/cm², at nitrate concentrations of 0.3 – 0.48 mg/l and Sondaken waters, south Minahasa, 557 g/cm², at nitrate concentrations of 0.2 – 0.3 mg/l. High seaweed rendemen in south Minahasa is not followed with high gel strength. Suitable nitrate concentration for seaweed fertility ranged from 0.1-3.5 ppm (Doty, 1988). The present study shows that nitrate concentration of the best gel strength were recorded at 0.1-0.2 mg/l, and higher nitrate concentration gave lower gel strength.

Harvest period gives influence on the carrageenan gel strength. Longer harvest time makes the gel strength tend to increase, then decrease after reaching the growth peak. It could result from increased sulphate concentration, and this is in agreement with Finlander *et al.* (1984) *in* Suryaningrum (1988) that increase in gel strength in proportional to sulphuric concentration. Zabik & Aldrich (1967) *in* Suryaningrum (1988) also stated that gel formation of the carrageenan is precipitation involving ionic binding between cation of certain metal and sulphate. If number of sulphates is excessive, it will bind with water. Therefore, if sulphate concentration of the carrageenan is high, three-dimensional structure formed will absorb much water. Such as carrageenan gel, if suppressed, will hardly maintain its structure due to its low gel strength. Nitrate concentrations, in form of ammonium >25 mg/l, are often toxic to phytoplankton, so that ammonium concentration should remain low (Barasanti and Gualtieri, 2014).

Viscosity

Viscosity test was carried out to know the viscosity level of the carrageenan. Varied salinity range in Minahasa penninsular results in different carrageenan characteristics. In this study, there may be relationship between salinity and viscosity (Fig. 03).

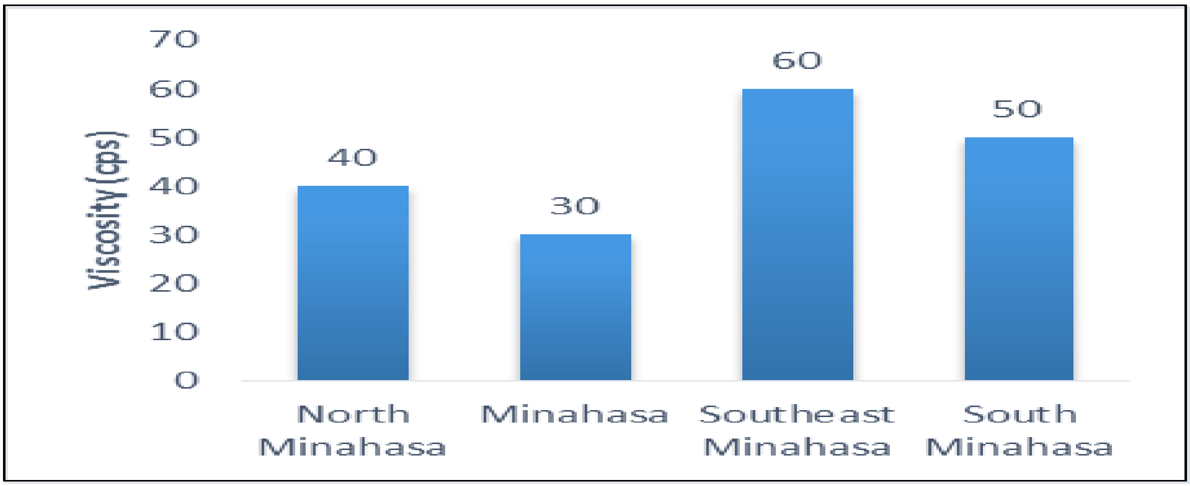

Fig. 3. Effect of salinity on *K. alvarezii* viscosity.

Viscosity of the samples ranged from 30 cps to 60 cps. Dense carrageenan solution varied from hard, breakable, solft and elastic. The viscosity is affected by concentration, temperature, type of carrageenan, molecular weight, dissolved metal ions (Towle, 1973). Low sulphate content of the carrageenan could decrease the viscosity.

Increased harvest period could reduce the carrageenan viscosity because of decreased sulphate content (Suryaningrum, 1988; Syamsuar, 2006). Harvest period of 40 days gave the highest sulphate content, and it would decline with time. Low sulphate content makes the viscosity declline (Wenno, *et al.*, 2012; and Syamsuar, 2006). Initial thallus weight causes intra-thallus competition, either spacde, sunlight, or nutrietns needed for photosynthesis.

Figure 03 indicates that water salinity of 29.7 ppm in Buku Tengah waters, southeast Minahasa gives the highest viscosity, 60 cps. Carrageenan viscosity and gel strength are main features needed for food and pharmaceutical industry applications. Sulphate content is parameter used for various polysacharides in red algae (Winarno, 1996). Solubility in the water is highly affected by the content of sulphate group (hydrophylic) and cation in the carrageenan. Number of sulphate fractions and cation balance in the water determine the viscosity or gel strength of the carrageenan (Campo *et al.*, 2009). Moirano (1977) further claimed that viscosity of carrageenan occurred due to rejectability between negativecharged sulphate groups along the polymer chain causing the polymer chain be stiff and strongly strecthed, and therefore, water molecules will bind with the carrageenan molecules making the viscosity rise.

Harvest period affects sulphate level of the carrageenan. Wenno *et al.* (2012) stated that the highest sulphate level was recorded in 40 days and the lowest in 50 days. Increased harvest time tends to cause sulphate content of the carrageenan flour decline. Low sulphate content of the carrageenan could make the viscosity decrease as well. Suryaningrum (1988) and Syamsuar (2006) further added that increased harvest time reduced the carrageenan viscosity because of declined sulphate content, since sulphate content is a parameter used for various polysacharides in red algae.

Ash content

Ash content analysis was done to know the mineral content in the carrageenan. Ash content of food material demonstrates the extent of the mineral content in the food material (Apriyantono *et al.,* 1989). The ash content found in all seaweed *K. alvarezii* samples ranged from 35.31% to 41,63%, while the FAO requirements are 14-40%. Thus, minerals contained in *K. alvarezii* along Minahasa penninsular meet food industry requirements. Ash content from all locations was presented in Fig. 04.

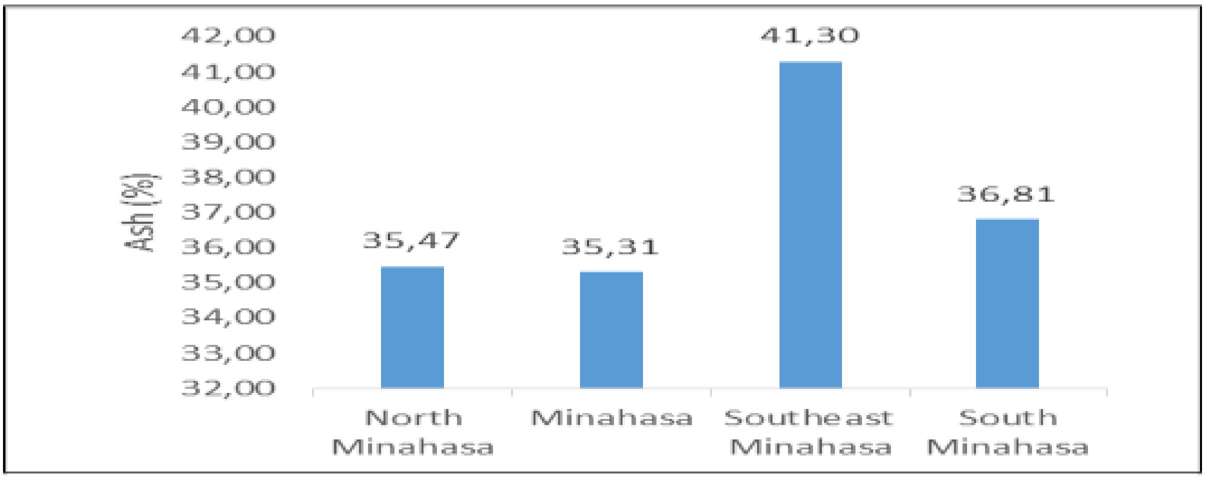

Fig. 4. Ash content in each sample.

Fig. 04 shows that salinity range of 29.1-29.7 ppm and phosphate concentration of 0.9 mg/l give higher ash content. Water salinity plays important role in osmotic process, in which high salinity will inhibit water and mineral absorption process in the water. Seaweed is categorized as food material containing sufficiently high minerals due to its ability of mineral absorption from the environment. Macrominerals, such as Na, K, Ca, Mg, Cl, S, P, , and I as a trace element (Sukri, 2006), Mn, Fe, and Cu are largely found in the seaweed (Ensminger *et al.,* 1995; Santoso *et al.,* 2006). Ash content reflects the extent of mineral content in the seaweed unburnt at the combustion (Bidwel, 1974 *in* Romenda *et al.*, 2013). Ash content is affected by other mineral salts, such as calcium and sodium attatched on the seaweed (Winarno, 1990) and farming time, in which the longer the farming time, the higher the ash content will be (Wenno *et al.*, 2012).

Conclusion

The best carrageenan quality of *Kappaphycus alvarezii* was found in southeast Minahasa with rendemen of 47.04 %, gel strength of 1,391 g/cm² and viscosity of 60 cps. Carrageenan quality was affected by water quality. rendemen was affected by temperature and pH, viscosity was influenced by salinity, gel strengh was affected by nitrate concentration, and ash content was influenced by phosphate concentration.

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