Retention of albumin in Indonesian shortfin eel meat (Anguilla bicolor) by freeze-drying encapsulation using maltodextrin and gum Arabic as coating materials

R D S Rawendra*, H Kosasih and D Lo

Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia

*reynetha.rawendra@binus.edu, reyrawendra@gmail.com

Abstract. Indonesian shortfin eel (Anguilla bicolor) or locally known as sidat eel is one of Indonesia’s important export commodity owing to its nutritional value such as albumin. In this study, encapsulation of freeze-dried sidat eel meat (EM) by biopolymers such as maltodextrin (MD) and gum Arabic (GA) was examined to find the most effective method to retain its albumin content. Emulsion of biopolymers with a constant weight ratio percentage of 8.00% (w/w, coating material/ sidat eel meat) and four different proportions of MD and GA were prepared using a homogenizer, followed by freeze-drying. The 4 different proportions were named T1 (EM 92% + GA 5.33% + MD 2.67%), T2 (EM 92% + GA 2.91% + MD 5.09%), T3 (EM 92% + GA 2.00% + MD 6.00%) and T4 (EM 92% + GA 1.52% + MD 6.46%), respectively. Powders were characterized for its physico-chemical characteristics including colour measurements, moisture content, water solubility, and retention of albumin content. It was observed that T4 consisting of EM with addition of GA (1.52%) and MD (6.46%) as coating materials retained the highest amount of albumin. The encapsulation efficiency were positively influenced by moisture content, solubility and colour measurement differences. To conclude, a mixture of gum Arabic and maltodextrin as coating materials was efficient to retain albumin in EM freeze-drying encapsulation. This necessary information may be valuable to researchers working on food supplements or functional food development.

1. Introduction

Indonesian shortfin eel (Anguilla bicolor) or locally known as sidat eel has 19 species that spreads throughout the world, in which seven of them are available in Indonesian waters [1]. Sidat eel meat (EM) is consumed widely in countries like Japan, Hong Kong, Germany and Italy mainly because of its economical aspects following its nutritional contents [2]. It has a unique taste, rich in vitamin A, B1, B2, B6, C, D, protein albumin, DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid) or better known as omega-3, and several other minerals [3]. Albumin has many benefits for the body, one of which is regulating osmotic pressure in the blood and act as means of transportation that carries less water-soluble ingredients such as free fatty acids, calcium, iron and certain types of drug, through blood plasma and cell fluids. Albumin is also useful in forming new body tissues. According to Astuti (2006), albumin content in such fish can be packaged in capsules form to treat patients suffering tuberculosis, diabetic ulcers, malnutrition and cancer [4]. However, being widely found in blood plasma, albumin is easily denatured. Therefore, an addition
step is required in its encapsulation process to preserve its bioactivity including adding coating materials such as GA and MD. They are expected to protect albumin protein binding from the encapsulation processes. Previous study from Krishnan et al. (2006), shows that the combination of GA and MD was more effective in protecting active ingredients compared to other coating materials [5]. Many studies have been conducted in regards to using GA as flavor encapsulation. Kanakdande, et al. (2007), used GA, MD and commercialized modified starch (Hi-cap) as Cumin Oleoresin coating material [6]. Each coating material has advantages and disadvantages, so it is required to include multiple coating material to get optimal results. The coating for EM encapsulation is expected to be an albumin protective material to achieve its potential as food supplement. This study formulates a composition between GA and MD to determine its effects on the characteristic of EM encapsulation.

2. Materials and Methods

2.1 Materials

*Anguilla bicolor* was obtained from Pelabuhan Ratu and cultivated in freshwater waters of West Java. The study began with sample preparations, where the EM was freeze-dried. The next step is to formulate a composition between EM with GA and MD to generate encapsulation. The GA and MD total concentration is 8% (w/w), because it produces the best and fastest dissolving stability from preliminary study.

2.2 Preparation of coating material composition

Compositions of MD and GA mixtures at different design points are listed as follows: T1 (EM 92% + GA 5.33% + MD 2.67%), T2 (EM 92% + GA 2.91% + MD 5.09%), T3 (EM 92% + GA 2.00% + MD 6.00%) and T4 (EM 92% + GA 1.52% + MD 6.46%). Each formulation was prepared by blending and rehydrating the carriers in distilled water (40°C) by magnetic stirrer (IKA® C-MAG HS 7) at 3600 rpm for 2 hours. After dissolution, the mixture was placed overnight in the refrigerator (15°C) to obtain full hydration. The next day, freeze dried EM was added to the feed at proportion of 1:12.5 (coating material: freeze dried EM) (w/w) and homogenized by a rotor-stator homogenizer (Ultra-Turrax IKA® T25) at 13600 rpm for 2 minutes.

2.3 Colorimetry

Color analysis is conducted using a portable colorimeter 3nh NH310. The first stage is a colorimeter calibration with a white standard and a black color calibration. Next, the sample is placed in the container, then measured. The obtained data were expressed in L *, a *, b * and L *, c, ° h which was displayed on the viewer screen.

2.4 Moisture content and solubility

Moisture content and solubility were conducted based on AOAC method 2005 [7].

2.5 Albumin content determination

To determine albumin levels in the sample, 500 µl of sample was taken and diluted in 5 ml of aquadest, and 4 ml of Biuret Reagent was added and stored at room temperature for 30 minutes to form perfect purple color. Next, the sample was measured using a UV-VIS spectrophotometer at maximum wavelength.

2.6 Experimental Design and Data Analysis

The experimental design was using a one-factor complete randomized design (RAL) method with 5 treatment levels (T0, T1, T2, T3, T4) and three replications. The collected data was analyzed using IBM SPSS Statistics version 19 software. Data processing was accomplished using analysis of variance and Duncan's Multiple Range Test (DMRT) test to determine the level of difference.
3. Results and Discussion

3.1 Effect of Wall Material Proportion on the Retention of Albumin during Encapsulation

UV-VIS spectrophotometry method was used to determine albumin level on formulation of EM encapsulation samples. This method uses machine that provide the advantages, which are easy to use, greater accuracy in terms of quantitative measurements, high sensitivity, and quick result [8]. The variance analysis showed the formulation treatment had a significant effect (P <0.05) on albumin levels encapsulation of EM. The results of the data were continued by the DMRT, which determined the level of treatment would have a difference. From the further testing, T0 and T4 were similar to each other, but significantly different from the treatments of T1, T2 and T3.

![Figure 1. Albumin levels in encapsulated sidat meat with different coating material proportions](image)

Regarding the albumin content, the result shows in T0 (control) was obtained at 13.0497 ± 1.6250 mg/g. Based on Figure 1, highest albumin content formulation between MD and GA coating material on EM, T4 treatment has 12.8369 ± 1.5969 mg/g and the lowest is T2 treatment which is 5.1733 ± 0.2457 mg/g. Therefore, the T4 formulation shows a higher concentration of MD formulations than GA, which can well protect albumin content in EM sample. The average range of AL levels obtained in this study has almost the same value based on Putri et al. (2016), values of albumin levels from EM extract of Anguilla bicolor species at 8.998 ± 0.242 mg/100 g and Anguilla Marmorata at 13.269 ± 0.508 mg/g [9]. The addition of MD was based on the fact that it can bind water-soluble proteins; therefore the dissolved protein will remain bounded even in small amounts. A previous study [10] suggests that inclusion of MD up to 5% can bind to protein even more. Furthermore, the higher the concentration of GA used in the formulation, the fewer levels of albumin that can be protected. This is because GA contains proteins that are easily denatured [11].

Based on the results, the best treatment of variations in formulations between GA coating material and MD is T4 (dried EM + GA 1.54% + MD 6.46%), which shown in the high value of albumin that has a result insignificantly vary from the control. The formulation of T4 treatment is has the optimum ability to protect albumin content of EM samples.

3.2 Effect of Coating Material Proportion on the Powder Color Value during Encapsulation

The color analysis results on the variations in samples are presented in Table 1. From the variance analysis, the formulation difference of MD and GA as EM coating shown significant effects (P <0.05) on the values of L *, a *, c and * h, except for b *. The color of object contains 3 elements, i.e. hue, chroma and lightness. The hue has units in the form of degrees (°) representing the dominant
wavelengths in Red, Yellow, Green, Blue and Purple. The chroma shows the color intensity. The lightness is based on mixing white elements that indicates color brightness.

Table 1. Results of Colorimetry on formulation treatment of EM Encapsulation

| Treatment | Parameter | L*          | a*         | b*          | c         | °h         |
|-----------|-----------|-------------|------------|-------------|-----------|------------|
| T0        |           | 56.5633 ± 0.4980 | 7.6167 ± 0.1804 | 12.6267 ± 0.3493 | 14.7467 ± 0.3786 | 58.9067 ± 0.4043 |
| T1        |           | 66.8500 ± 0.2946b | 3.7233 ± 0.2065c | 13.1467 ± 0.4881 | 13.6633 ± 0.5258b | 74.1967 ± 0.2902a |
| T2        |           | 67.8900 ± 0.1493a | 3.8133 ± 0.0404c | 12.9633 ± 0.0961 | 13.5100 ± 0.0985b | 73.6100 ± 0.0954b |
| T3        |           | 61.3800 ± 0.0265c | 4.5667 ± 0.2112b | 13.0467 ± 0.3614 | 13.8233 ± 0.4072b | 70.7100 ± 0.3464c |
| T4        |           | 57.1700 ± 0.1572d | 3.6700 ± 0.0400b | 12.7133 ± 0.1060 | 13.2333 ± 0.09018b | 73.9067 ± 0.2608ab |

Different superscripts in the same column show significant differences (P<0.05)

The L* represents brightness level ranging between 0 and 100. The value of a* is chromatic level of green to red, ranges from -100 to +100. The value of b* is also chromatic degree which states the level of blue to yellow, ranges from -100 to +100. Chroma (c) shows the level of sample color intensity or color sharpness level, which is obtained from the coordinates of the values a* and b*. The higher the chroma value, the stronger the intensity of the color produced. Based on Table 1, the highest L* value is the T2 formulation, because it is the brightest. This might be due to the addition of MD and GA as coating material. A low L* value can increase the chroma value of color sharpness; therefore, the control has a thick color because there is MD and GA additions to dried EM. The value of ° hue shows the real color of the measurement results, whereas in the range 54 - 90, it has mixed of red and yellow color, while between the interval 90 and 126 is yellow. The average of color measurements ranges from 58 to 74, which means the sample has red color with lower yellow intensity.

3.3 Effect of Coating Material Proportion on the Moisture Content during Encapsulation

In particular to dry powdered food supplements, moisture content is an important parameter to determine its quality, whereas low level moisture content prevents the growth of damaging microbes. The result of variance analysis, the formula treatment had a significant effect (P <0.05) on the moisture content of encapsulated EM. Furthermore, the results was tested with DRMT to determine the level of different treatment. Figure 2 shows the result of T0 (control) dried EM was significantly different that the ones with formulation treatments (T1, T2, T3 and T4), based on significant difference in moisture content levels.
Figure 2. Moisture content in encapsulated EM with different coating material proportions

(T0) as the control has the lowest moisture content. The treatment of coating material between MD and GA has an average moisture content of 4.6% - 6.5%. The highest was T2 at 6.8302 ± 0.0535% and the lowest was T4 at 4.6691 ± 0.0580%. By national standard, the maximum requirements for powdered milk moisture content (SNI 01-2970-1999) and instant coffee (SNI 01-2983-1992) are 4.0%, where mixed coffee products are 7.0% (SNI 01-4446-1998). Hence, the moisture content of EM encapsulation remains within the national standard of dried products.

The relationship between the coating material composition and the moisture content can be attributed to the viscosity of the emulsion of coating material. High viscosity causes high moisture content on encapsulated EM. As shown in Figure 2, T4 has lowest moisture content due to high concentration of MD and less GA. Addition of MD can reduce moisture content due to low viscosity [12] as well as minimum use of GA that can cause decreased viscosity or water trapped in the smaller structure. This way, the shelf life of this product can be lengthen by minimising the chances of fungal growth.

According to Gardjito et al. (2006), MD has lower molecular weight (less than 4000) and its simpler structure enables easy water removal through evaporation on drying process [13]. Whereas with GA, it has larger molecular weight of ± 500,000 and complex molecular structure. It can create strong bond with water molecule, therefore it requires more evaporation energy to remove when bonded with GA.

3.4 Effect of Wall Material Proportion on the Solubility during Encapsulation

Solubility in water is a parameter associated with the release of active ingredients in the application of food supplements. Food supplements (microcapsules) should have high solubility in commonly used solvents, water. Solubility analysis was carried out by dissolving the supplement formulated with coating material into distilled water, then filtered with Whatman No. filter paper. The more residue left on the filter paper, the lower the solubility of the material. The average solubility in the sample is 56 - 76%, where the T0 (control) solubility had the lowest value of 56.9524 ± 0.2315%. The treatment formulation with the highest solubility is T4 of 76.9687 ± 0.6956%, while the one with the lowest value is T2 at 68.9801 ± 8.9052%.

Figure 2. Moisture content in encapsulated EM with different coating material proportions
Figure 3. Average of solubility in encapsulated EM with different coating material proportions

The presence of GA and MD as coatings allows for high solubility in all treatment of formulations. In Figure 3, the T4 has more MD content than GA, which optimises solubility factor. In addition, according to Mahdavi et al., (2016), GA also highly soluble, hence the usage of combination of the two in a product delivers a high moisture content goods [14]. Low moisture content causes the sample to be more hygroscopic and easier to absorb water that works hand in hand with high solubility. This is due to large difference in water vapor pressure between solid and liquid. This study deduces that the best formulation when implementing a combination of GA and MD as coating for dried EM is T4. It has the lowest moisture content from other formulation variations and has the highest solubility value. From this study, it is concluded that GA and MD can be used as encapsulant materials. In addition, the best treatment of encapsulation of dried EM i.e. samples with the treatment of 1.54% AG coating and 6.46% MD coating was able to protect albumin levels equivalent compared to the control treatment, which significantly affected the color, moisture content, and solubility of samples in water (P <0.05).

References
[1] Budimawan 2007 Identifikasi Spesies Glass Eel dan Elver Ikan Sidat (Anguilla spp) Berdasarkan Jumlah Vertebrata (Makassar: Universitas Hasanuddin)
[2] Subekti S, Prawesti M, and Arief M 2011 Indo. J. Mar. Sci. Technol. 5(2) 28 - 34.
[3] Rovara O, Setiawan I E, and Amarullah M H 2010 Mengenal Sumberdaya Ikan Sidat (Jakarta: BPPT-HSF)
[4] Astuti N 2006 Potensi Albumin Ikan Gabus (Makassar: Universitas Hasanuddin)
[5] Khrisnan S, A C Kshirsagar and R S Singhal 2005 Carb. Polym. 62(7), 309 - 315
[6] Kanakdande D, Bhosale R, and Singh R S 2007 Carb. Polym. 67(6), 536 – 541
[7] AOAC 2005 Official Methods of Analysis (Washington: Benjamin Franklin Station)
[8] Day R A, and Underwood A L 2002 Qualitative Analysis (Jakarta: Erlangga)
[9] Putri A A, Yuliet, and Jamaluddin 2016 J. of Pharm. 2(2) 90 - 95.
[10] Balasubramani P, Palaniswamy P T, Visvanathan R, Thirupathi V, Subbayaran A, and Maran J P 2015. Int. J. Biol. Macromol. 72(1), 210 - 217.
[11] Mirhosse I H, Tan C, Hamid N, and Yusof S 2008 Food Chem 107: 1161 - 1172.
[12] Tirgar M, Jinap S, Zaidul I S M and Mirhosseini H 2015 J Food Sci Technol 52(7):4441–4449
[13] Tonon R V, Grosso C R F, and Hubinger M D 2011 Food Res Int 44(1):282–289.
[14] Mahdavi S A, Jafari S M, Assadpoor A 2016 Int. J. Biol. Macro. 85(4):379-386