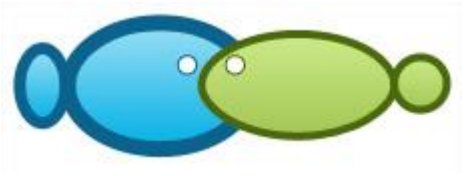

Development of snakehead fish (*Channa striata*) albumin extract capsules

Yohanes Kristianto, Nur Rahman, I Dewa N. Supariasa

Department of Nutrition, Polytechnic of Health Ministry of Health, Malang, Indonesia.
Corresponding author: Y. Kristianto, ykristianto@poltekkes-malang.ac.id

Abstract. Hypoalbuminemia is an important indicator of serious diseases and is associated with mortality risk of hospitalized patients. Oral fish albumin is commonly administered to relieve health problems. Unfortunately, available liquid albumin preparations inherit an unacceptably strong fishy odor. This experimental study was aimed to develop albumin capsules using snakehead extracts to address these problems. A factorial completely randomized design was used to run the experiments. The albumin as raw material was extracted from the local fresh snakehead (*Channa striata*) using water steam technique at 50°C for 60 minutes. The extracts were freeze-dried to form powder with the aid of dextrose, glucose, and starch at concentrations of 10, 20, and 30% as the excipients, respectively. The agglomerates obtained were crushed and transferred into gelatin hard capsule shells. The fishy odor was remarkably undetected in the final products. The excipient type and its concentration were significantly correlated to albumin content of the powder ($p<0.05$). Freeze drying of the fish albumin extracts increased albumin content by approximately five to seven times. The highest albumin content of the powder (9.77 g dL^{-1}) was obtained by inclusion of 30% glucose as excipient. Storage of the fish albumin capsules for one month increased the number of microorganisms, however, it did not exceed microbial quality standard for the capsules.
Key Words: channa, excipient, hypoalbuminemia, traditional medicine.

Introduction. Approximately 50% of the plasma protein consists of albumin that has prominent roles in the human metabolism. The primary role of albumin is to maintain oncotic pressure in the vascular compartment, which represents approximately 80% of the colloidal osmotic pressure. Albumin also serves as a carrier of key compounds for the metabolism of fatty acids, hormones and calcium. Additionally, it also contributes to acid-base balance (Gounden et al 2022). Hospitalized patients and critically ill patients are very likely to suffer from hypoalbuminemia. Hypoalbuminemia has a strong association with high risk of death for hospitalized patients. Inpatients who experience hypoalbuminemia at the time of the hospital admission have a higher risk of death as compared to those who have normal albumin levels (Jellinge et al 2014; Akirov et al 2017; Gounden et al 2022).

Increasing serum albumin level is crucial to reduce the risk of death (Akirov et al 2017). A common approach to treat hypoalbuminemia is albumin administration orally. Oral albumin formulas are conventionally prepared in the form of either fish albumin extract, fish meal, or formulated food products supplemented using fish as the active ingredient. Unfortunately, fish extract has a very strong undesirable fishy odor, very short shelf life, and requires frozen storage for distribution and long-term use. These decrease the acceptability of the products on the market. Previous research has been focused to improve the quality parameters of the product, including the use of specific ingredients such as honey and curcumin to mask the unpalatable odor (Suwita et al 2012), and the development of fish albumin extract powder (Wulansari et al 2022; Yuniarti et al 2013).

Albumin is sensitive to heat, therefore, albumin retention during processing or development of the oral preparation should be of main concern. The promising technology with respect to albumin retention is probably lyophilization or freeze-dry processing. This technology is a common option to develop products which contain sensitive materials such as protein compounds (Assegehegn et al 2020; Harel & Tang

2023). The advantages gained by the technology include reduced risk of denaturation and biological activity loss, protection of active components from oxidation, maintaining original structure, microbiological growth inhibition, and good rehydration of the final products (Hua et al 2010). The benefit offered by this process should be followed by further proper product handling, storage, and distribution to increase convenience and administration. All these requirements would be satisfied by developing fish extract high in albumin as an oral capsule.

Capsules have been the most widely used solid oral dose forms, making the oral route of medicine delivery the most practical for patients (Shah et al 2021). During oral capsule formulation, excipient selection is necessary to ensure that the product is capable to carry albumin. At the same time, the active ingredient can be released when the product is administered. The excipients are required to reduce the undesirable odor of the active ingredient, to help in getting desired properties during product manufacture, such as flow characteristics and the dosage determination (Narang et al 2017). Additionally, a suitable excipient ensures that the active ingredient is protected. There has been scarce work focused on eliminating the undesirable odor, preventing albumin loss during the processing, and developing convenience end products. This study was therefore aimed to develop snakehead fish albumin extract capsules using the freeze drying technique. Widely used excipients like glucose, dextrose, and starch, are examined for their effect on the quality of the capsules. The capsules are expected to eliminate the fishy odor, be easier to handle and to distribute, and have a reasonable shelf life.

Material and Method

Sample preparation and capsule making. The snakehead fish (*Channa striata*) samples were procured from a traditional market in Malang regency, Indonesia, based on the following criteria: alive, fresh, medium in size and relatively uniform. The samples were transported to the Food and Technology laboratory of the Polytechnic of Health, Malang, where they were killed and cut into cubes of approximately 0.5x0.5x0.5 cm, following fins, head, and offal removal. For a batch of processing, 100 g of snakehead fish were placed in filter cloth and extracted by water steaming at 50^{o}C for 60 minutes under a pH of above 4.7 (Pramesti 2006). Glucose, dextrose, and starch were added to the obtained extract according to the research design, and the mix was dried using a BioTron® Vacuum Freeze Dryer at -78^{o}C and -76 cm Hg^{-1}. The dried agglomerates obtained were crushed before being transferred into a gelatin capsule shell number 3, by the hand filling method aseptically and stored in a closed HDPE (high density polyethylene) plastic bag. Gelatin capsule shells were preferred as they are non-toxic, affordable, and have a very good safety history (Hoag 2017). The capsule shells and excipient were procured from local pharmacies in Malang. The study was conducted using a completely randomized design with two factors, namely the type of the excipient (glucose, dextrose, starch) and concentration of the excipient (10%, 20% and 30% w/w). Each treatment was repeated twice to obtain 18 experimental units.

Quality of capsule assessment. The capsule qualities were determined based on albumin content (Bromcresol Green Spectrophotometric method), solubility, water content (thermogravimetric method), and shelf life test in closed HDPE plastic container at ambient temperature. The solubility tests were conducted by dissolving 1 g of powder in 1 mL of distilled water in a test tube. The solvent required for dissolving the powder was used to determine the degree of the solubility (MoH RI 2014).

Statistical analysis. The mean of the obtained data was calculated. The treatment effects were determined using two-way analysis of variance. The Tukey Honest Significant Differences multiple pairwise-comparisons test was run to determine statistical mean differences between specific pairs of groups. All statistic tests were done using the statistical software R for Linux (R Core Team 2021). Statistical significances were obtained at confidence level of 95% ($p<0.05$).

Results and Discussion

Albumin contents. The albumin content of snakehead fish filtrate according to the type of excipient and concentration ranged from 1.10-1.38 g dL^{-1} (Figure 1). The type of excipient and concentration significantly affected the albumin filtrate level ($p<0.05$). However, there was no interaction detected between the two factors ($p>0.05$). The albumin levels of snakehead fish filtrate were slightly lower than the average value reported by previous researchers (Mustafa et al 2012; Khasani & Astuti 2019). This is most likely due to the addition of excipient used in the current study. A higher albumin level in the capsule could be achieved by using different fish as raw materials. The milkfish (*Chanos chanos*) originating from brackish water and Indian mackerel (*Rastrelliger kanagurta*) from seawater have been reported to contain higher albumin levels than snakehead (Fatma et al 2020).

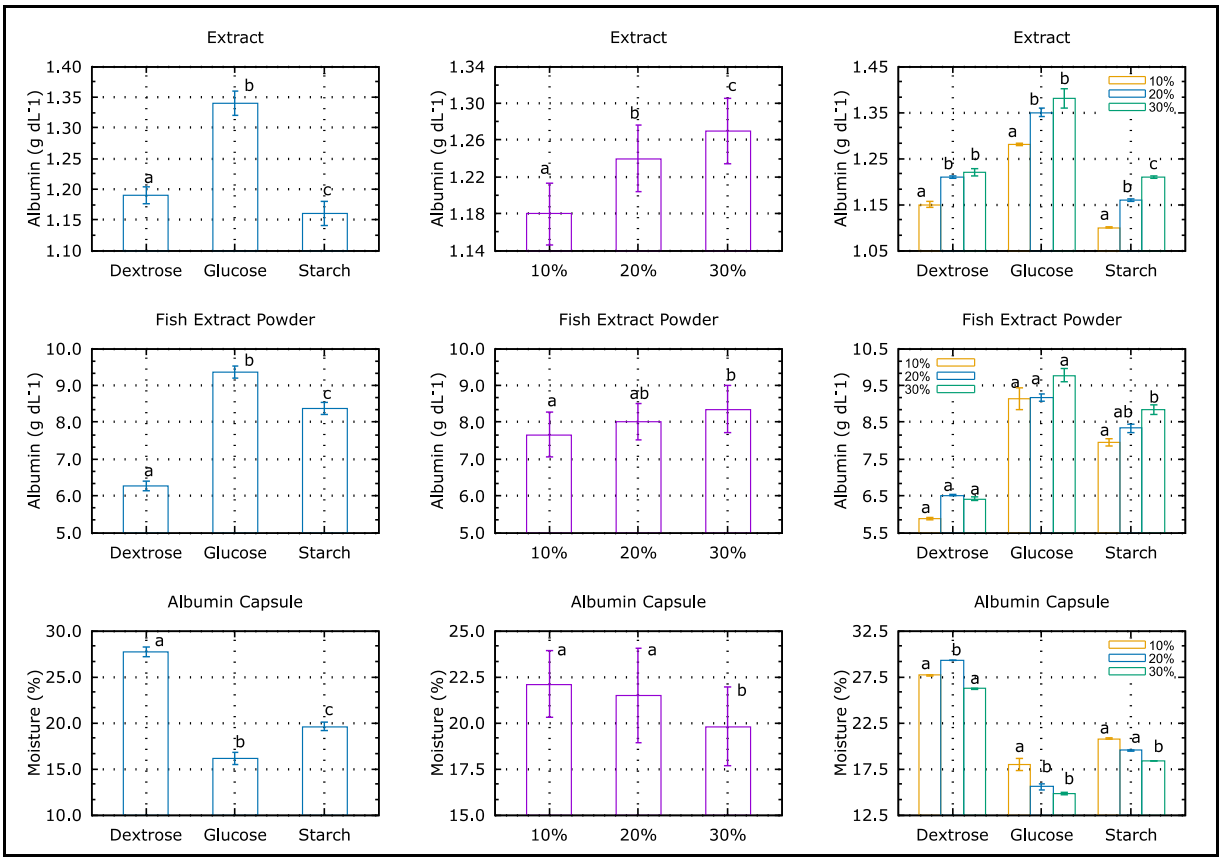

Figure 1. Albumin and moisture contents of snakehead (*Channa striata*) products. Different letter notations denote statistical significanct differences at $p<0.05$. Percentages on the X-axes indicate concentration of excipients.

Based on the type of excipients used, the agglomerates obtained from the filtrate through the freeze drying process have slightly different characteristics. The agglomerates resulting from the addition of glucose were more brittle than the others. This probably relates to the characteristic of glucose, which imparts a disintegrating tendency and is a diluent in the formulation (Haywood & Glass 2011; Kar et al 2019). After the agglomerates were crushed, the powder made with dextrose had a softer consistency than the other powders. Meanwhile, the agglomerate color for all groups of excipients was brownish white.

The type of excipient and its concentration had an effect on the albumin content of the snakehead filtrate powder ($p<0.05$). As depicted in Figure 1, the graphs in the second row show that both excipient type and its concentration significantly affect the albumin contents. The highest albumin content in the powder was obtained by the use of

30% glucose, although the albumin content differences with the same excipient at 10% and 20% were not statistically different ($p>0.05$). The use of starch and dextrose at 10, 20 and 30% resulted at lower albumin contents as compared to that of glucose at all concentrations. There was no interaction between excipient type and concentration ($p>0.05$). This suggests that the effect of excipient type on the albumin content of the powder is independent from its concentration. In this study, freeze drying increased the albumin content of snakehead extract from 5.06 to 7.40 times. The dehydration effect was achieved by initially freezing of the water before converting it to vapor by sublimation. The technique causes minimal solute movement and offers high retention of nutrient and sensory qualities. Changes to proteins are negligible (Walters et al 2014; Waghmare et al 2021; Fellows 2022). Freeze dying of protein can be safely conducted at a temperature higher than the thermal transition point to avoid denaturation (Tang & Pikal 2004). The highest albumin content of snakehead filtrate powder was produced from the use of 30% glucose, with 9.77 g dL^{-1} (Figure 1). Compared to other excipients, the incorporation of glucose in the formula resulted in higher albumin in the capsules. For the treatments with 10% and 20% glucose the yield of albumin was 9.14 and 9.17 g dL^{-1}, respectively. These results are higher when compared to the results of similar studies on the development of snakehead extract powder at low temperature with 25% lactose excipient, which produces 0.02 g dL^{-1} albumin (Wulansari et al 2022).

Moisture contents. The water content of snakehead filtrate powder ranged from 14.78 to 29.31% (Figure 1). Both type and concentration of excipients had a significant effect ($p<0.05$) on the moisture content of snakehead fish filtrate powder. Overall, glucose produced albumin capsules with lower moisture content compared to that obtained from dextrose and starch at 10, 20, and 30%. The lowest moisture content of the capsule was attained from 30% glucose. This content was not statistically different ($p>0.05$) from the value yielded from 20% glucose. The differences of the moisture content of albumin capsules as a result of different excipients are presented in the third row in Figure 1. The water content of the capsule shell usually ranges from 12 to 16% (Hoag 2017), and under normal storage conditions gelatin capsules have water contents between 13–16% (Chong et al 2016). It is required for the water content of the excipient material contained in the capsule shell to not have wide discrepancies from the shell water contents to avoid problems during storage. Excipient materials with very low water content may dry out the capsule shells. Furthermore, low moisture of freeze-dried products do not always result in a best storage stability of high protein content products (Tang & Pikal 2004).

Solubility. Solubility is one of the important properties of pharmaceutical preparations. The bioavailability of oral preparations is highly dependent on the solubility of oral preparations in the gastrointestinal tract and their permeability in cell membranes (Mantri et al 2017). Freeze drying technology was chosen in this study because the products resulted from the method exhibit good rehydration characteristics (Hua et al 2010). This study shows that the excipient used greatly affects the level of solubility of snakehead powder filtrate. Powders made with starch excipients are difficult to dissolve. Starch is a compound that is relatively insoluble in water, so it precipitates more. Table 1 shows that the solubility of powders made with dextrose and glucose are similar.

Starch is a complex carbohydrate, whereas dextrose and glucose are of simpler molecules. This could be the reason for the faster dissolving rate of dextrose and glucose. Starch is well known as a multifunctional excipient in pharmaceutical formulations. This affordable excipient has capabilities as a disintegrant, binder, matrix-former, and encapsulant (Mateescu et al 2015; Kunle 2019). In capsule formulations, starch improves the flow characteristics of the material required to facilitate the filling process into the shell (Hoag 2017). Starch and its derivatives are non-toxic and safe (Mateescu et al 2015). When starch is a choice, the solubility of capsules for future development might be improved by adding surfactants.

Table 1

The solubility of snakehead (*Channa striata*) extract powder capsules

Excipient	*Concentration (%)*	*Solvent (mL)*	*Solubility*
Dextrose	10	45	Fairly soluble
	20	45	Fairly soluble
	30	45	Fairly soluble
Glucose	10	49	Fairly soluble
	20	48	Fairly soluble
	30	48	Fairly soluble
Starch	10	>10000	Practically insoluble
	20	>10000	
	30	>10000	

Microbiological quality. The number of viable microorganisms in snakehead filtrate powder in all treatments (Table 2) was well below the limit for traditional medicine, that is $1x10^5$ CFU g^{-1} (NADFC 2019). It is likely that the low concentration of 10% of excipient could help inhibit microbiological growth. The inhibition effect of glucose according to the concentration seems to be more consistent than other excipients. When glucose is used as a binder in pharmaceutical products it is usually has a concentration of 50% (w/v) or 25% (w/w) (Kar et al 2019). Therefore, if required, the glucose concentration in the formula development may be increased. The capsules made by starch inclusion had higher microbiological count than other treatments. This probably relates to the starch protective effect on microbial growth during freeze drying by lowering water-binding capacity (Morgan & Vesey 2009).

Table 2

Microbiological load of snakehead fish extract powder capsules

Excipient	*Concentration (%)*	*Viable microorganisms (CFU g^{-1})*	
		Initial	*1 month storage*
Dextrose	10	$4.3x10^4$	$2x10^4$
	20	$1.6x10^3$	$6.7x10^5$
	30	$1.8x10^3$	$4.1x10^3$
Glucose	10	$5.5x10^3$	$1.3x10^4$
	20	$3x10^3$	$1.1x10^4$
	30	$1.1x10^3$	$2.2x10^4$
Starch	10	$9x10^4$	$1.6x10^4$
	20	$4.7x10^3$	$1.9x10^4$
	30	$9.3x10^4$	$4.4x10^3$

The number of microorganisms in the snakehead filtrate powder tends to increase with its water contents. The water content is an important factor that determines the degree of microbial contamination of pharmaceutical products. Other factors, including product characteristics, packaging materials, manufacturing process, and environment may also be significant (Dao et al 2018). Apart from albumin, snakehead filtrate powder contains other nutrients and is alkaline in nature. Therefore, it is very likely to get contaminated by microorganisms. The number of microorganisms in the snakehead filtrate capsule after storage for one month showed a slight increase. However, the increases did not exceed the standard for the quality of traditional medicines set by national regulation, which is 10^5 CFU g^{-1} (NADFC 2019), except for the 20% dextrose capsules treatment ($6.7x10^5$ CFU g^{-1}). This shows that the capsules had relatively limited microbiological growth during one month of storage. The growth of microorganisms, especially in capsules with 20% dextrose excipients, may be related to the higher water content (Figure 1).

The presence of certain microorganisms in the product may affect not only the active ingredients but also the product stability. The microorganisms may cause off odors and produce undesirable metabolites, which pose a risk to patient health. The

objectionable microorganisms used as indicators in pharmaceutical products include gram-negative bacteria with tolerance to bile salt, *E. coli*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa*, *Clostridia*, and *Candida albicans* (Sandle 2016). The freeze- drying technology can be aimed to target the gram-negative bacteria, as the survival ability of these microorganisms under lyophilization is weaker than that of gram-positive cells (Morgan & Vesey 2009).

Conclusions. This study has demonstrated the development of odorless high albumin capsules. The capsules could be produced by incorporating 30% glucose into snakehead albumin extracts followed by a freeze-drying treatment. The obtained capsules contained 9.77 g dL^{-1} albumin, 14.8% moisture, and $1.1x10^3$ CFU g^{-1} viable microorganisms. The capsules are fairly soluble in water and microbiologically stable for one month of storage in a conventional HDPE container. Further research might be conducted to assess biological activity of the capsules to treat hypoalbuminemia and related health problems.

Acknowledgements. We wish to acknowledge the financial assistance provided by the Polytechnic of Health Ministry of Health Malang, Indonesia. We thank Prof. Koentoro, dr. MPH, Dr. PH of Airlangga University, Indonesia, for guidance during the research proposal composition. Special thanks to the staff members of Food Technology Laboratory of the Department of Nutrition, Polytechnic of Health Ministry of Health Malang, Indonesia, for providing facilities to conduct this work.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

Akirov A., Masri-Iraqi H., Atamna A., Shimon I., 2017 Low albumin levels are associated with mortality risk in hospitalized patients. The American Journal of Medicine 130(12):1465.e11-1465.e19.

Assegehegn G., Fuente E. B., Franco J. M., Gallegos C., 2020 Freeze-drying: A relevant unit operation in the manufacture of foods, nutritional products, and pharmaceuticals, In: Advances in food and nutrition research. Toldrá F. (ed), Academic Press, pp. 1-58.

Chong R. H. E., Jones B. E., Díez F., Birchall J. C., Coulman S. A., 2016 Evaluating the sensitivity, reproducibility and flexibility of a method to test hard shell capsules intended for use in dry powder inhalers. International Journal of Pharmaceutics 500(1-2):316-325.

Dao H., Lakhani P., Police A., Kallakunta V., Ajjarapu S. S., Wu K. W., Ponkshe P., Repka M. A., Murthy S. N., 2018 Microbial stability of pharmaceutical and cosmetic products. AAPS PharmSciTech 19(1):60-78.

Fatma N., Metusalach, Taslim N. A., Nurilmala M., 2020 The protein and albumin contents in some species of marine and brackishwater fish of South Sulawesi, Indonesia. AACL Bioflux 13(4):1976-1985.

Fellows P. J., 2022 Freeze drying and freeze concentration. In: Food processing technology (5th Edition). Woodhead Publishing pp. 619-630.

Harel M., Tang Q., 2023 Protection and delivery of probiotics for use in foods. In: Microencapsulation in the food industry (2nd Edition). Sobel R. (ed), Academic Press, pp. 463-480.

Haywood A., Glass B. D., 2011 Pharmaceutical excipients – where do we begin? Australian Prescriber 34:112-114.

Hoag S. W., 2017 Capsules dosage form: Formulation and manufacturing considerations. In: Developing solid oral dosage forms (2nd Edition). Qiu Y., Chen Y., Zhang G. G. Z., Yu L., Mantri R. V. (eds), Academic Press, pp. 723-747.

Hua T. C., Liu B. L., Zhang H., 2010 Freeze drying of pharmaceuticals. In: Freeze-drying of pharmaceutical and food products. Hua T. C., Liu B. L., Zhang H. (eds), Woodhead Publishing, pp. 187-215.

Jellinge M. E., Henriksen D. P., Hallas P., Brabrand M., 2014 Hypoalbuminemia is a strong predictor of 30-day all-cause mortality in acutely admitted medical patients: a prospective, observational, cohort study. PLoS ONE 9(8):e105983.
Kar M., Chourasiya Y., Maheshwari R., Tekade R. K., 2019 Current developments in excipient science: Implication of quantitative selection of each excipient in product development. In: Basic fundamentals of drug delivery, advances in pharmaceutical product development and research. Tekade R. K. (ed), Academic Press, pp. 29-83.
Khasani I., Astuti D. N., 2019 Albumin level, growth and survival rate of snakehead fish (*Channa striata*) from three islands of Indonesia. AACL Bioflux 12(5):1688-1697.
Kunle O. O., 2019 Starch source and its impact on pharmaceutical applications, In: Chemical properties of starch. Intech Open, pp. 35-48.
Mantri R. V., Sanghvi R., Zhu H., 2017 Solubility of pharmaceutical solids. In: Developing solid oral dosage forms. 2nd Edition. Qiu Y., Chen Y., Zhang G. G. Z., Yu L., Mantri R. V., (eds), Academic Press, pp. 3-22.
Mateescu M. A., Ispas-Szabo P., Assaad E. (eds), 2015 Starch and derivatives as pharmaceutical excipients: From nature to pharmacy. In: Controlled drug delivery, woodhead publishing series in biomedicine. Woodhead Publishing, pp. 21-84.
Morgan C., Vesey G., 2009 Freeze-drying of microorganisms. In: Encyclopedia of microbiology. 3rd Edition. Schaechter M. (ed), Academic Press, pp. 162-173.
Mustafa A., Widodo M. A., Kristianto Y., 2012 Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. IEESE International Journal of Science and Technology 1(2):1-8.
Narang A. S., Mantri R. V., Raghavan K. S., 2017 Excipient compatibility and functionality. In: Developing solid oral dosage forms. 2nd Edition. Qiu Y., Chen Y., Zhang G. G. Z., Yu L., Mantri R. V. (eds), Academic Press, pp. 151-179.
Pramesti R. W., 2006 [The effect of excipient type and concentration on albumin, moisture, and solubility of eel (*Monopterus albus* Zuieuw) albumin extract capsules]. Thesis, Polytechnic of Health Ministry of Health Malang, Indonesia, 79 p. [In Indonesian].
Ryzkha C., 2009 [The effect of oven drying temperature on the quality of snakehead fish albumin (*Ophiocephalus striatus*)]. Thesis, Universitas Brawijaya, Malang, Indonesia, 119 p. [in Indonesian].
Sandle T., 2016 Specified and objectionable microorganisms. In: Pharmaceutical microbiology. Sandle T. (ed), Woodhead Publishing, pp. 93-101.
Shah H., Jain A., Laghate G., Prabhudesai D., 2021 Pharmaceutical excipients. In: Remington. 23rd Edition. Adejare A. (ed), Academic Press, pp. 633-643.
Suwita I. K., Kristianto Y., Purwaningsih F. Y., 2012 [Estimation of the shelf life of temulawak syrup (*Curcuma xanthorriza* Roxb) honey and snakehead fish extract (*Ophiocepalus striatus*) with the Arrhenius model and Q10 model]. AGROMIX 3(2):18-35. [In Indonesian].
Tang X. C., Pikal M. J., 2004 Design of freeze-drying processes for pharmaceuticals: practical advice. Pharmaceutical Research 21(2):191-200.
Waghmare R. B., Perumal A. B., Moses J. A., Anandharamakrishnan C., 2021 Recent developments in freeze drying of foods. In: Innovative food processing technologies. Knoerzer K., Muthukumarappan K. (eds), Elsevier, pp. 82-99.
Walters R. H., Bhatnagar B., Tchessalov S., Izutsu K. I., Tsumoto K., Ohtake S., 2014 Next generation drying technologies for pharmaceutical applications. Journal of Pharmaceutical Sciences 103(9):2673-2695.
Wulansari D., Prasetyati S. B., Ratnaningtyas S., Anasri A., Holis N. R., 2022 [Physical and hedonic quality characteristics of snakehead fish extract powder (*Channa striata*)]. PELAGICUS 3:29-37. [In Indonesian].
Yuniarti D. W., Sulistiyati T. D., Suprayitno H. E., 2013 [Effect of vacuum drying temperature on the quality of snakehead fish (*Ophiocephalus striatus*) albumin powder]. THPI Student Journal 1:1-9. [In Indonesian].
*** Gounden V., Vashisht R., Jialal I., 2022 Hypoalbuminemia. StatPearls, Treasure Island. Available at: https://europepmc.org/article/nbk/nbk526080.

*** MoH RI, 2014 [Indonesian harmacopoeia. 5th Edition]. Ministry of Health of the Republic of Indonesia, Jakarta, pp. 601-603. [In Indonesian].
*** NADFC, 2019 [Regulation No. 32/2019: Requirements for the safety and quality of traditional medicines]. National Agency of Drug and Food Control, Republic of Indonesia. [In Indonesian].
*** R Core Team, 2021 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Received: 19 December 2022. Accepted: 14 March 2023. Published online: 15 November 2023.
Authors:
Yohanes Kristianto, Department of Nutrition, Polytechnic of Health Ministry of Health, Jln. Besar Ijen 77c, 65112 Malang, Indonesia, e-mail: ykristianto@poltekkes-malang.ac.id
Nur Rahman, Department of Nutrition, Polytechnic of Health Ministry of Health, Jln. Besar Ijen 77c, 65112 Malang, Indonesia, e-mail: rahmancahaya@yahoo.com
I Dewa Nyoman Supariasa, Department of Nutrition, Polytechnic of Health Ministry of Health, Jln. Besar Ijen 77c, 65112 Malang, Indonesia, e-mail: dewa_gizimalang@yahoo.com

How to cite this article:
Kristianto Y., Rahman N., Supariasa I. D. N., 2023 Development of snakehead fish (*Channa striata*) albumin extract capsules. AACL Bioflux 16(6):2923-2930.