Review Article

49

INDONESIAN SCHOLAR SOCIETY

Indones. J. Chem. Stud.
2022, 1(2), 49–53
Available online at journal.solusiriset.com
e-ISSN: 2830-7658; p-ISSN: 2830-778X

Indonesian Journal of Chemical Studies

Evaluation of The Biochemical Contents in Guillard f/2 and Walne Growth Medium to Fulfill the Animal-Free Aspects of Microalgal Bioprocessing

Elva Stiawan*

Department of Chemistry, Faculty of Military Mathematics and Natural Sciences, Republic of Indonesia Defense University, Kawasan IPSC, Sentul, Bogor 16810, Indonesia

Received: 03 Nov 2022; Revised: 07 Dec 2022; Accepted: 08 Dec 2022;
Published online: 13 Dec 2022; Published regularly: 31 Dec 2022

Abstract—Marine microalgae with their diverse biomolecule contents could be used as potential sources of food, cosmetics, and pharmaceutical ingredients. In accordance with regulations in some countries and to competitively engage huge numbers of consumers, microalgae-based products should be properly manufactured using non-animal-derived materials. As a cultivable microorganism using a scalable bioreactor technique, consideration of the origin of the material used in the upstream process of marine microalgae was inevitable. Currently, the material origin of chemical contents within common artificial microalgal seawater medium had not been evaluated. This article evaluated Guillard f/2 and Walne medium as common artificial microalgal nutrients used in marine microalgal bioprocess-related activities. The risk assessment results showed that the largest portion of Guillard f/2 and Walne media were inorganic salts considered as low, while the remaining biochemical contents of vitamins were categorized as high risk due to their relatively complex chemical synthesis and enzymatic stages during the manufacturing process. As a suggestion, several plant-based bioproducts were proposed as alternative sources to substitute related biochemical actions to fulfill non-animal-origin aspects in the initial stages of the bioprocessing of marine microalgal-based products.

Keywords— Algal bioprocessing; Animal-free product; Guillard f/2; Marine microalga; Walne.

1. INTRODUCTION

Bioproduct manufacturing is an industrial activity generated using biological systems to produce commercial biomolecule-based products, from the simplest biomolecule, such as glucose for dietary purposes, to very complex biomacromolecules, such as Biosimilar Active Pharmaceutical Ingredients (APIs) of some therapeutic hormones and monoclonal antibodies (mAb) for medical uses. To steadily fulfill consumers' needs and enhance the existing market share, many industrial researchers and practitioners focused on the bioprocessing of foods, cosmetics, and pharmaceuticals must keep their ears open to the dynamic changes in market preferences.

Currently, consumers' preferences toward commercial bio-based products are technically exaggerated. Some consumers are used to consuming products directly obtained or bio-processed from natural resources rather than products that are synthetically processed using diverse complex inorganic chemicals. Yet, large numbers of consumers are not only concerned about the "natural product" labelling but also about the origin and detailed processes of each ingredient of the products. This condition broadly occurs in some countries or regions with a great population of particular religious or social backgrounds who are stringently concerned about the origin of the typical food or additives they consume. For example, Moslem and Jewish consumers follow their halal and kosher as a part of their religious rules, respectively, which strictly prohibits consuming porcine-origin materials as a food ingredient, and in some occasions, including pharmaceuticals and cosmetics.

Halal and kosher product assurance as a guarantee system that has been legally established and properly implemented by the government in some countries to accommodate a particular section of consumers, i.e., Moslem consumers contribute a significant impact on the technical aspects of bioprocessing in the consumables industry. In this law, every stage of the manufacturing process is strictly handled to be free from inappropriate substances, importantly free from porcine origin material. Proper fulfillment at the cultivation stage (upstream process) is considered one

***Corresponding author**.
Email address: elvastiawan@gmail.com
DOI: 10.55749/ijcs.v1i2.16

of the main challenges in the implementation of product assurance in bioprocessing, even occurs as a controversy or a bottleneck in some biotech product manufacturers, such as for some vaccine manufacturers that routinely use complex ingredients of growth medium. In some cases, the existing growth medium for the bioprocessing of bioproducts is still unreplaceable by alternative sources due to the complexity of nutrients for microbes or cells. A long-term research scheme must provide fully suitable ingredients for diverse components of the growth medium.

Declaring free animal-origin raw material or product is a common approach that on some occasions, can briefly convince the market that there are no porcine-origin components in the final product. In actual, it is still partially difficult to obtain all vegetable-based materials in the upstream process of bioprocessing activities for a biotech product because most cells cultivated in the bioreactor and microbes cultured in the fermenter still require essential nutrients, such as amino acids, serum, blood extract, etc., that are still widely obtained from animal extracts, or even solely from the porcine origin, and are considered critical for cell growth so that they are inevitable to be removed from the process.

Another approach that can be selected to establish the proper bioprocessing activities is the usage of marine resources. This approach is principally proper because it has been clearly stated that all organisms originating from seawater are categorized as halal to consume as written in the holy Al-Qur'an as the Moslems' primary guidance, especially in Al Maidah verse 96 below:

"*It is lawful for you to hunt and eat seafood, as a provision for you and the travellers. ...*"

Meanwhile, regarding the allowance of seawater resource consumption, all fish with visual scales are considered acceptable under kosher rules [1]. There are probably no particular prohibition concerns regarding the use of marine unicellular resources. Although further confirmation is still required, the manufacturers of bioprocesses marine microalgal-based products should consider the proper aspects of their consumers. To fulfill the kosher rules for cell-based products, product processing must properly consider the cell origin (and its animal origin), the contents of the growth medium, and the detailed material used in each process stage [2].

With a great number of Moslem consumers as a potential target market for halal biotech products, Indonesia as a maritime country should be at the forefront of a country that proactively initiates and consistently develops marine resources for bioprocess activities, especially for industrial purposes. Many biotech manufacturing features can be gradually substituted by marine resources. For example, converting porcine-origin-gelatin to fish-based-gelatin during particular chromatography resins as a bed supporting material in the bioproduct purification step, employing fish serum albumin as a part of the enriched cell growth medium, using silica poriferans as adsorbents, and so forth.

Diverse fish species provide various functional forms of gelatin [3] with warm water fish skins as the fittest substitute for mammalian-origin gelatin [4]. Fish gelatin can also be further modified to produce a particular three-dimensional gel structure for different purposes [5]. While, proper and standardized manufacturing activities should be developed to provide high-quality fish gelatin that furtherly can be physically and chemically processed as competitive material rather than mammalian gelatin [6].

From the perspective of cell development and upstream stages, the use of marine microorganisms as host cells to produce high-value bioproducts can also be an alternative innovation in bioprocessing. Several studies show a bright prospect of high saline water usage in bioprocessing activities, such as the use of marine yeast for bioethanol [7], and emulsifiers [8], marine bacteria and fungi for functional enzymes production [9], green microalga for fatty acid production [10], and so forth.

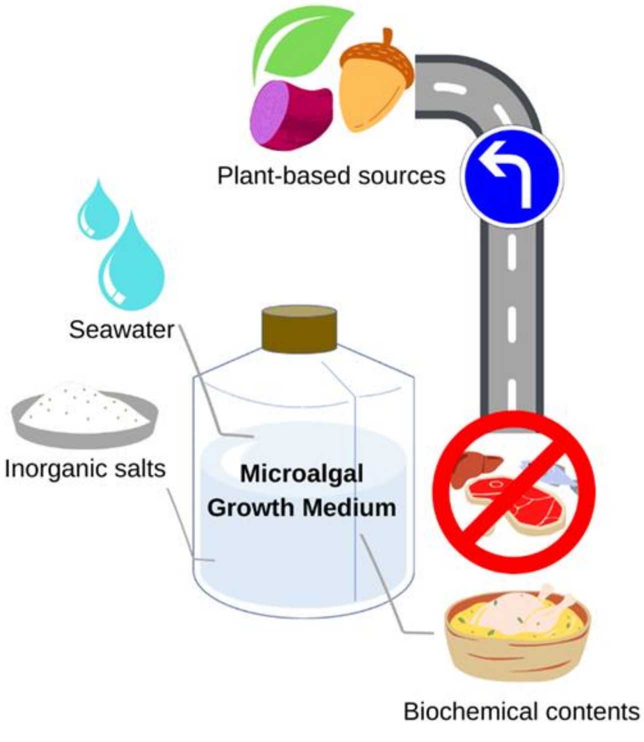

Fig. 1. Schematic diagram of animal-free growth medium to support the marine microalgae bioprocess industry.

Marine diatoms as a part of seawater phytoplankton are prospective cultivable resources that can be developed as working cell bank that are regularly cultivated to yield targeted bioproducts. Currently, studies on the bioprocessing of marine microalgae are still popular for their use as biodiesel sources due to their high lipid content. Some species of marine microalgae have specific biomolecule contents that show potential activity to be used as the main ingredients or active substances in several foods or pharmaceuticals. According to the global market trends for both types of products that should meet non-animal-origin requirements, the aspects should also be properly fulfilled from working microalgal cell development as the initial stage of marine microalgae bioprocessing. Two main concerns are often evaluated for non-animal-origin status in the cell development stage, i.e., the origin of species and growth medium. Note that further features must be considered if any genetic engineering, molecular modification, or related molecular modification is applied to the marine microalgae candidates before their use as a routine host cell.

Native marine microalgae living in high-salinity water bodies as a part of seawater creatures are considered halal organisms from an Islamic perspective. Yet, after being primarily isolated and properly cultivated to maintain the stock culture, other non-animal-origin aspects of the microalgae growth medium for refreshment and subsequent microalgae culture activities should also be assessed (**Fig. 1**). f/2 Guillard and Walne nutrient solutions are common use artificial growth mediums for microalgal, including diatoms (with addition of Sodium metasilicate). This microalgae growth medium is suitable to be used in the initial step of primary cell cultivation of fresh captured marine diatoms, such as *Chaetoceros debilis* [11].

2. DISCUSSION

The f/2 Guillard and Walne nutrient solution as enriched seawater growth medium consists of sterilized seawater, inorganic salts, and some minor biochemical components. To provide non-animal-origin information on the components, an assessment is conducted according to each component source and processing method with an additional consideration based on guidance released by BPJPH (Halal Product Assurance Agency of the Republic of Indonesia), especially for chemicals listed in the related halal positive list are categorized as a very low risk of a halal discrepancy, while the low-risk category is defined by the availability of halal supporting documents of related chemical products that have been successfully obtained by chemical manufacturers (data not shown). Three microalgal species have been already been written in the halal positive list of BPJPH, i.e., *Chlorella ellipsoids*, *Spirulina* spp, and *Scenedesmus* spp [12]. Furthermore, medium risks are applied to chemicals that are not listed in the halal positive list and are not attributed to halal supporting documents, such as legal halal certificates, pork-free statements, non-animal origin statements, etc. Meanwhile, chemicals or components that cannot fulfill all the above criteria and are commonly processed by relatively complex production steps, such as involving many organic solvents and reagents or through enzymatic reaction using a fermentor, are categorized as high risk.

2.1. Evaluation of Microalgal Medium Component

Table 1 and **Table 2** display the assessment results for f/2 Guillard and Walne nutrient solutions, respectively, with each risk level related to animal-origin ingredients.

Table 1. f/2 Guillard nutrient solution components and each risk level for animal origin.

No	Components	Risk Level	Remarks
1.	$NaNO_3$	Very low	Natural deposits [13, 14]
2.	$NaH_2PO_4 \cdot H_2O$	Low	Hydrated salt
3.	$FeCl_3 \cdot 6\,H_2O$	Medium	Hydrated salt
4.	$Na_2EDTA \cdot 2\,H_2O$	Low	Hydrated salt
5.	$MnCl_2 \cdot 4\,H_2O$	Medium	Hydrated salt
6.	$ZnSO_4 \cdot 7\,H_2O$	Very low	Hydrated salt
7.	$CoCl_2 \cdot 6\,H_2O$	Medium	Hydrated salt
8.	$CuSO_4 \cdot 5\,H_2O$	Very low	Hydrated salt
9.	$Na_2MoO_4 \cdot 2\,H_2O$	Very low	Hydrated salt
10.	Thiamine.HCl (vitamin B_1)	High	Sequential chemical processes [15,16].
11.	Biotin (vitamin H)	High	Sequential chemical processes [17,18].
12.	Cobalamin (vitamin B_{12})	High	Can only be produced by fermentation [19].
13.	Seawater	Very low	Mixture of inorganic salts
14.	$Na_2SiO_3 \cdot 9H_2O$	Very low	Hydrated salt

Table 2. Walne nutrient solution components and each risk level for animal origin

No	Components	Risk Level	Remarks
1.	$NaNO_3$	Very low	Natural deposits [13,14]
2.	$NaH_2PO_4 \cdot H_2O$	Low	Hydrated salt
3.	$Na_2SiO_3 \cdot 9H_2O$	Very low	Hydrated salt
4.	Na_2EDTA	Very low	Inorganic salt
5.	$ZnCl_2$	Very low	Inorganic salt
6.	$CoCl_2 \cdot 6\,H_2O$	Low	Hydrated salt
7.	$CuSO_4 \cdot 5\,H_2O$	Very low	Hydrated salt
8.	$(NH_4)_2Mo_7O_{24} \cdot 4\,H_2O$	Low	Hydrated salt
9.	Tiamin. HCl (vitamin B_1)	High	Sequential chemical processes [15,16]
10.	Sianokobalamin (vitamin B_{12})	High	Can only be produced by fermentation [19]
11.	Seawater	Very low	Mixture of inorganic salts

The results show that the largest portion of f/2 Guillard and Walne microalgal growth medium (10 inorganic salts and 8 inorganic salts in seawater, respectively) is probably safe from animal parts. Meanwhile, the remaining components of vitamins that function as supplements for microalgal growth should be attributed to non-animal origin information because they can be derived from biomolecular precursors of animal origin sources and are commonly manufactured using various organic reagents or through several enzymatic reactions in the biological fermentor that the enzymes themselves must also be assessed.

Some studies have shown several alternative sources of biochemical nutrients, i.e., Thiamine, Cyanocobalamin, and Biotin, that can be selected for proper animal-free marine microalgae bioprocessing.

2.2. Alternative Sources of Thiamine

As in plants, thiamine (Vitamin B_1) may be involved in carbon metabolism, including between C and N assimilation [19]. Based on a particular halal certificate released by The Islamic Food and Nutrition Council of America IFANCA, a particular Thiamine-HCl product manufactured has been certified as halal. Yet, it should be further confirmed to the related manufacturer whether its halal status based on particularly non-porcine-origin or completely non-animal-origin materials. Several vegetables show relatively high thiamine content. Beans and oats contain the prospective amounts of thiamine, i.e., 7.59 µg/mL and 5.06 µg/mL, respectively [21], while rice milk that is mainly formed from brown rice shows 11.95 µg/mL thiamine content is considered very potential to be developed as thiamine source for microalgal growth medium [22]. Note that the level of Thiamine from its food sources could significantly decrease after boiling [23] so that it is required appropriate extraction method with optimal condition instead of high temperature.

2.3. Alternative Sources of Cyanocobalamin

In humans, Cyanocobalamin (Vitamin B_{12}) is categorized as a vital nutrient that is commonly un-existed in unfortified plant-based foods [24]. However, dried purple laver, called nori by the Japanese, is considered the fittest source of Cyanocobalamin for vegetarians [25] so it can also be extracted into the Vitamin B_{12} nutrient to support microalgal growth. Thus, beans, oats, and rice milk can be prospective plant-based sources for thiamine as microalgal biochemical content. Moreover, some plant-based foods are also displayed to be rich in Vitamin B_{12}, for example, fermented meals of natto and tempeh [26]. Although both fermented foods may have high cyanocobalamin levels, these typical foods should be thoroughly assessed regarding the aim of providing vegetable-origin biochemical nutrients since it can be an extra effort that should be performed to ensure the natto and tempeh processing stages are completely free of animal parts, especially in the stages of microorganism introduction for providing starters, i.e., *Bacillus subtilis* natto and *Saccharomyces cerevisiae*, respectively.

2.4. Alternative Sources of Biotin

Some vegetables have a relatively high biotin (Vitamin H), for example nuts that are categorized as biotin-rich food [27]. Peanuts and red peppers show biotin content of 50 µg/100g per food, while soybeans and their derivatives exhibit 19.3 µg biotin [28]. Although sweet potato leaves show relatively low biotin levels of 3–8 µg/100 g [29], they can be proposed as an alternative source of biotin from crop waste. Meanwhile, other plant-based sources are confirmed with small amounts of biotin, such as fruit, cereals, and bread contained (0.01–0.1 µg/100g wet weight) [30]. Hence, peanuts and red peppers can be positioned as the main option of biotin sources for plant-based biochemical content of microalgal medium with sweet potato leaves extraction into biotin, which is a promising approach with another purpose of agricultural-waste reduction.

3. CONCLUSION

To establish non-animal-origin bioprocessing of marine microalgal-based products, the artificial f/2 Guillard and Walne microalgal growth medium could be used in the upstream process after being labeled as non-animal-origin-grade growth medium using only proper inorganic salts and fully biochemical components from common vegetable sources with the prospective level of related biomolecule contents. Furthermore, consideration of using appropriate reagents, solvents, and separation components that were also free of animal parts should be precisely performed during the extraction and purification of these alternative plant resources.

As a suggestion, the substitution of animal-origin vitamins as biochemical contents into alternative vegetable-origin sources could be performed as an approach from the material origin scope. Moreover, a comprehensive discussion should be also properly generated between the food, drugs, and cosmetics regulatory institutions and academics as well as practitioners with strong biochemical or bioprocess backgrounds and experiences, especially regarding the technical aspects that should be concerned (or if applicable, modified) related to enzymatic chemical transformation during the vitamin manufacturing process using fermentor or bioreactor technology.

ACKNOWLEDGEMENT

The authors acknowledge the Chemistry Department of the Republic of Indonesia Defense University, and the LP2M Unhan RI for the supporting facilities in writing this article.

CONFLICT OF INTEREST

We have no conflicts of interest to disclose regarding this article.

REFERENCES

[1] Regenstein, J.M., Chaudry, M.M., & Regenstein, C.E. 2003. The kosher and halal food laws. *Compr. Rev. Food Sci. Food Saf.,* 2(3), 111-127. doi: 10.1111/j.1541-4337.2003.tb00018.x

[2] Kenigsberg, J.A., & Zivotofsky, A.Z. 2019. A Jewish religious perspective on cellular agriculture. *Front. Sustain. Food Syst.* 3 59. doi: 10.3389/fsufs.2019.00128

[3] Muhammad, K.M.L., Ariffin, F., Abd Razak, H.K.B., & Sulaiman, P.D.S. 2016. Review of fish gelatin extraction, properties and packaging applications. *Food Sci. Nutr.* 56. 47-59.

[4] Nitsuwat, S., Zhang, P., Ng, K., & Fang, Z. 2021. Fish gelatin as an alternative to mammalian gelatin for food industry: A meta-analysis. *LWT.* 141. 110899. doi: 10.1016/j.lwt.2021.110899

[5] Derkach, S.R., Voron'ko, N. G., Kuchina, Y. A., & Kolotova, D.S. 2020. Modified fish gelatin as an alternative to mammalian gelatin in modern food technologies. *Polymers*. 12(12). 3051. doi: 10.3390/polym12123051

[6] Karim, A. A., & Bhat, R. 2009. Extraction and characterization of gelatin from Atlantic salmon (Salmo salar) skin. *Food Hydrocoll.* 23(3). 563-576. doi: 10.1016/j.foodhyd.2008.07.002

[7] Zaky, A.S., Greetham, D., Tucker, G.A. and Du, C. 2018. The establishment of a marine focused biorefinery for bioethanol production using seawater and a novel marine yeast strain. *Sci. Rep.* 8(1). 1-14. doi: 10.1038/s41598-018-30660-x

[8] Souza, F.A.S.D., Salgueiro, A.A. and Albuquerque, C.D.C. 2012. Production of bioemulsifiers by Yarrowia lipolytica in sea water using diesel oil as the carbon source. *Braz. J. Chem. Eng.* 29(1). 61-67. doi: 10.1590/S0104-66322012000100007

[9] Sarkar, S., Pramanik, A., Mitra, A. and Mukherjee, J. 2010. Bioprocessing data for the production of marine enzymes. *Mar. Drugs*. 8(4). 1323-1372. doi: 10.3390/md8041323

[10] Kim, Z. H., Park, H., & Lee, C. G. 2016. Seasonal assessment of biomass and fatty acid productivity by Tetraselmis sp. in the ocean using semi-permeable membrane photobioreactors. *J. Microbiol. Biotechnol.* 26(6). 1098–1102. doi: 10.4014/jmb.1601.01031

[11] Stiawan, E., Rachmawati, Y., Nurachman, Z., Sutomo, S., & Sapulete, S. 2022. Identification of Tropical Marine Diatom Chaetoceros dayaensis CBO from Bokor Island through Morphology and Genetic Marker Analysis. *Indones. J. Chem. Stud.* 1(1). 1–7. doi: 10.55749/ijcs.v1i1.2

[12] Keputusan Menteri Agama Republik Indonesia No. 1360 Tahun 2021 Tentang Bahan yang dikecualikan dari kewajiban bersertifikat Halal.

[13] Kirk, R.E., Othmer, D.F., & Eckroth,D. 2004. *Encyclopedia of Chemical Technology Vol. 5*. John Wiley & Sons, Inc.

[14] Wisniak, J. and Garcés, I., 2001. The rise and fall of the salitre (sodium nitrate) industry. *Indian J. Chem. Technol.* 8(9). 427-438.

[15] Jiang, M., Liu, M., Huang, H., & Chen, F. 2021. Fully Continuous Flow Synthesis of 5-(Aminomethyl)-2-methylpyrimidin-4-amine: A Key Intermediate of Vitamin B_1. *Org. Process. Res. Dev.* 25(10). 2331–2337. doi: 10.1021/acs.oprd.1c00253

[16] Chaudry, M.M., 2022. *Halal Product Certificate* (Report No. 4739.15720.II220009, pp. 1-99). The Islamic Food and Nutrition Council of America (IFANCA). https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/396/950/halal_certificate.pdf

[17] Seki, M. 2006. Biological significance and development of practical synthesis of biotin. *Med. Res. Rev.* 26(4). 434–482). doi: 10.1002/med.20058

[18] Seki, M. 2008. (+)-Biotin: A Challenge for industrially viable total synthesis of natural products. *Stud. Nat. Prod. Chem.* 34(C). 265–307. doi:10.1016/S1572-5995(08)80029-4

[19] Calvillo, Á., Pellicer, T., Carnicer, M., & Planas, A. 2022. Bioprocess Strategies for Vitamin B_{12} Production by Microbial Fermentation and Its Market Applications. *Bioengineering*. 9(8). 365. doi: 10.3390/bioengineering9080365

[20] Fitzpatrick, T.B., & Chapman, L.M. 2020. The importance of thiamine (vitamin B1) in plant health: From crop yield to biofortification. *J. Biol. Chem.* 295(34). 12002–12013. doi: 10.1074/jbc.REV120.010918

[21] Kerns, J.C., & Gutierrez, J.L. 2017. Thiamin. In Advances in Nutrition. *ASN.* 8(2). 395–397). doi:10.3945/an.116.013979

[22] Lalić, J., Denić, M., Sunarić, S., Kocić, G., Trutić, N., Mitić, S., & Jovanović, T. 2014. Assessment of thiamine content in some dairy products and rice milk. *CYTA-J. Food.* 12(3). 203–209. doi: 10.1080/19476337.2013.814713

[23] Hrubša, M., Siatka, T., Nejmanová, I., Vopršalová, M., Krčmová, L. K., Matoušová, K., Javorská, L., Macáková, K., Mercolini, L., Remião, F., Máťuš, M., & Mladěnka, P. 2022. Biological Properties of Vitamins of the B-Complex, Part 1: Vitamins B_1, B_2, B_3, and B_5. *Nutrients*. 14(3). doi: 10.3390/nu14030484

[24] Niklewicz, A., Smith, A.D., Smith, A., Holzer, A., Klein, A., McCaddon, A., Molloy, A.M., Wolffenbuttel, B.H.R., Nexo, E., McNulty, H., Refsum, H., Gueant, J.L., Dib, M.J., Ward, M., Murphy, M., Green, R., Ahmadi, K.R., Hannibal, L., Warren, M.J., & Owen, P.J. 2022. The importance of vitamin B_{12} for individuals choosing plant-based diets. *Eur. J. Nutr.* doi: 10.1007/s00394-022-03025-4

[25] Watanabe, F., Yabuta, Y., Bito, T., & Teng, F. 2014. Vitamin B_{12}-containing plant food sources for vegetarians. *Nutrients*. 6(5). 1861–1873. doi: 10.3390/nu6051861

[26] Chandra-Hioe, M.V., Lee, C. and Arcot, J., 2019. What is the cobalamin status among vegetarians and vegans in Australia?. *Int. J. Food Sci. Nutr.* 70(7). 875-886. doi: 10.1080/09637486.2019.1580681

[27] Jungert, A., Ellinger, S., Watzl, B., & Richter, M. 2022. Revised D-A-CH reference values for the intake of biotin. Eur. J. Nutr. 61(4). 1779–1787. doi: 10.1007/s00394-021-02756-0

[28] Watanabe, T., Kioka, M., Fukushima, A., Morimoto, M. and Sawamura, H. 2014. Biotin content table of select foods and biotin intake in Japanese. *Int. J. Anal. Bio-Sci.* 2(4). 109-125.

[29] Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Tadokoro, T. and Maekawa, A. 2000. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* poir). *Food Chem.* 68(3). 359-367. doi: 10.1016/S0308-8146(99)00206-X

[30] Agostoni, C., Canani, R.B., Fairweather-Tait, S., Heinonen, M., Korhonen, H., La Vieille, S., Marchelli, R., Martin, A., Naska, A., Neuhaeuser-Berthold, M. and Nowicka, G. 2014. Scientific Opinion on Dietary Reference Values for zinc. *Efsa Journal*, 12(10). 3844. doi: 10.2903/j.efsa.2014.3844