
UNIVERSITI PUTRA MALAYSIA

# *DEVELOPMENT OF HALAL HYDROLYSATES-BASED NANOCOSMECEUTICAL FORMULATION FROM Actinopyga lecanora*

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# DEVELOPMENT OF HALAL HYDROLYSATES-BASED NANOCOSMECEUTICAL FORMULATION FROM *Actinopyga lecanora*

**By**

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*Actinopyga lecanora* is classified among the edible species of sea cucumber. Its hydrolysates, which obtained from enzymatic hydrolysis was reported to contain relatively high antioxidant activities, mostly applied in food industries. *Actinopyga lecanora* hydrolysates (ALH) is halal depending on its source (marine animal), extraction process and the safety measurement. Even though ALH have high potential of antioxidant activities, which is essential in skin care products, no work has been reported specifically on ALH as an actives ingredient. Likewise, no related antityrosinase activity of ALH has been recorded. Therefore, to diversify the usage of ALH in the cosmeceutical application, nanoemulsion containing ALH was developed, particularly to alleviate skin aging and to improve skin pigmentation.

This research consists of two parts; Part A is the optimization of hydrolysis conditions of ALH, while Part B covers the formulation of nanoemulsion containing ALH obtained from Part A. In Part A, ALH was hydrolysed from raw sample of *Actinopyga lecanora* using papain enzyme. The best conditions selected from the optimization were at pH 5.00, 70°C of reaction temperature, 9 h of hydrolysis time and 1.00% E/S ratio with the ALH having 55.00% of DH, 46.30% of DPPH radical scavenging activity and 108.20 $Fe^{2+}$µg/mL of FRAP activity.

The amount of hydrophobic amino acid (286.40 mg/g sample) was higher than hydrophilic amino acid (253.60 mg/g sample), indicated that the hydrophobic amino acid was responsible for the antioxidant and antityrosinase activities. SEM image of ALH pictured a smooth structures with pores as the protein was degraded into smaller peptides.

In Part B, nanoemulsion formulation containing ALH was optimized using D-optimal mixture design. The optimum compositions were at 10.00% of safflower oil: argan oil (ratio 9:1), 1.00% of ALH, 13.75% of Tween 80: Span 80 (ratio 8:2) surfactants, 0.87% of xanthan gum, 73.69 % of water and 0.70% of phenonip. The actual particle size obtained was 139.80 nm, with the acceptable polydispersity index (0.302) and zeta potential (-27.50 mV). High conductivity of the nanoemulsion was observed (1311 µS/cm) with pH 5.38 and displayed a pseudoplastic behaviour. Morphology analysis disclosed a spherical shape of the nanoemulsion particles, monodispersed without any aggregation. Moreover, the nanoemulsion showed good stability within 28 days when stored separately at different temperatures (4 and 25°C). However, for long term storage, the suggested storage condition was at 4°C due to the unchanged of physical appearances (colour and odour) after 28 days.

*In vitro* permeation study revealed that the permeability of ALH was improved to 66.48% of ALH released after 8 h of application. The kinetic released of ALH followed first-order model, in which the released was based on the concentration-gradient pattern. *In vitro* cytotoxicity test of all samples against normal 3T3 fibroblast cell recorded $IC_{50}$ values more than 500 µg/mL, proved that the optimized nanoemulsion is safe, halal and suitable for topical application.

Apart from that, an improved in antioxidant activity was shown after incorporating ALH to the nanoemulsion. The DPPH radical scavenging activity of nanoemulsion containing ALH had a significant difference ($p<0.05$) at concentration of more than 5000 µg/ml compared to the blank nanoemulsion. The FRAP activity of both samples also showed a significant difference ($p<0.05$) at every sample concentration. Similar pattern was found for antityrosinase activity whereby the optimized nanoemulsion containing ALH displayed a significant difference ($p<0.05$) in tyrosinase inhibition activities for both substrates, L-DOPA (43.20%) and L-tyrosine (46.60%). Overall, the nanoemulsion containing halal hydrolysates *from A. lecanora* was successfully formulated and have a full potential to be applied in cosmeceutical industries.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGUNAN FORMULASI NANOKOSMESEUTIKAL BERASASKAN HIDROLISAT HALAL DARIPADA *Actinopyga lecanora***

Oleh

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*Actinopyga lecanora* dikelaskan di antara spesies gamat yang boleh dimakan. Hidrolisat yang diperoleh daripada hidrolisis berenzim ini dilaporkan mengandungi aktiviti antioksida yang tinggi secara relatif dan kebanyakannya diaplikasikan di dalam industri makanan. Hidrolisat daripada *Actinopyga lecanora* (ALH) adalah halal bergantung kepada sumbernya (haiwan laut), proses pengekstrakan dan juga pengukuran keselamatannya. Walaupun ALH mempunyai potensi aktiviti antioksida yang tinggi, dimana ianya penting di dalam produk penjagaan kulit, tiada kajian khusus dilaporkan ke atas ALH sebagai kandungan aktif. Selain itu, tiada kajian berkaitan aktiviti perencatan tirosina oleh ALH direkodkan. Oleh itu, bagi meluaskan kegunaan ALH di dalam aplikasi kosmeseutikal, nanoemulsi yang mengandungi ALH telah direka bentuk khusus untuk mengurangkan penuaan kulit dan menambahbaikkan pempigmenan kulit.

Penyelidikan ini terdiri daripada dua bahagian; Bahagian A ialah pengoptimuman keadaan reaksi ALH, sementara Bahagian B merangkumi formulasi nanoemulsi yang mengandungi ALH yang telah dioptimumkan dan diperoleh daripada Bahagian A. Di Bahagian A, ALH telah dihidrolisis daripada sampel mentah *Actinopyga lecanora* menggunakan enzim papain. Keadaan tindakbalas terbaik yang dipilih dari pengoptimuman adalah tindakbalas yang dijalankan pada pH 5.00, suhu tindakbalas pada 70°C, 9 jam masa hidrolisis dan 1.00% nisbah E/S dengan hidrolisat menunjukkan 55.00% DH, 46.30% aktiviti DPPH radikal skaveng dan 108.20 $Fe^{2+}$ µg/mL aktiviti FRAP.

Jumlah asid amino hidrofobik (286.4 mg/g sampel) adalah lebih tinggi daripada asid amino hidrofilik (253.6 mg/g sampel), menunjukkan bahawa asid amino hidrofobik bertanggungjawab terhadap aktiviti-aktiviti antioksidan dan antitirosina.

Imej SEM bagi ALH memperlihatkan struktur yang licin dan berliang dimana ianya menunjukkan bahawa protein telah dipecahkan kepada peptida yang lebih kecil.

Di bahagian B, formulasi nanoemulsi yang mengandungi ALH telah dioptimumkan menggunakan reka bentuk campuran D-optimal. Tahap komposisi optimum adalah 10.00% minyak safflower: minyak argan (nisbah 9:1), 1.00% ALH, 13.75% surfaktan Tween 80: Span 80 (nisbah 8:2), 0.87% gam xanthan, 73.69% air dan 0.70% phenonip.

Ukuran saiz partikel sebenar yang diperoleh adalah 139.80 nm, dengan indeks kepoliserakan (0.302) dan potensi zeta (-27.50 mV). Kekonduksian yang tinggi oleh nanomeulsi telah diperhatikan (1311 µS/cm) dengan pH 5.38 dan menunjukkan tingkah laku pseudoplastic. Analisis morfologi mendedahkan bentuk sfera oleh partikel nanoemulsi, ekaserakan tanpa sebarang pengagregatan. Selain itu, nanoemulsi adalah stabil dalam masa 28 hari apabila disimpan berasingan pada suhu yang berbeza (4 dan 25°C). Walau bagaimanapun, untuk penyimpanan jangka panjang, keadaan penyimpanan yang dicadangkan adalah pada suhu 4°C berikutan penampilan fizikal yang tidak berubah (warna dan bau) selepas 28 hari.

Kajian penelapan *in vitro* menunjukkan kebolehtelapan ALH terlepas dipertingkatkan kepada 66.48% selepas 8 jam diaplikasikan. Kinetik pelepasan bagi ALH adalah mengikut model kinetik tertib pertama dimana pelepasan ALH berdasarkan corak kecerunan-kepekatan. Ujian sitotoksisiti *in vitro* bagi kesemua sampel terhadap sel normal fibroblas 3T3 merekodkan nilai $IC_{50}$ melebihi 500 µg/mL, membuktikan nanoemulsi mengandungi ALH adalah selamat, halal dan sesuai digunakan untuk aplikasi topikal.

Selain itu, peningkatan dalam aktiviti antioksidan telah ditunjukkan apabila ALH ditambah ke dalam nanoemulsi. Aktiviti pembersihan radikal DPPH bagi nanoemulsi mengandungi ALH memperlihatkan perbezaan keertian ($p<0.05$) pada kepekatan lebih daripada 5000 µg/ml berbanding nanoemulsi kosong. Aktiviti FRAP bagi kedua-dua sampel juga menunjukkan perbezaan keertian ($p<0.05$) pada setiap kepekatan sampel. Pola yang sama diperlihatkan dalam aktiviti antitirosina dimana nanoemulsi mengandungi ALH menunjukkan perbezaan keertian ($p<0.05$) ke atas kedua-dua substrat L-DOPA (43.20%) dan L-tirosina (46.60%). Secara keseluruhannya, nanoemulsi yang mengandungi hidrolisat halal daripada *A. lecanora* telah berjaya diformulasi dan berpotensi penuh untuk diaplikasikan di dalam industri kosmeseutikal.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AABA | α-Aminobutyric acid |
| AhR | Arylhydrocarbon receptor |
| ALH | *Actinopyga lecanora* hydrolysates |
| ANOVA | Analysis of variance |
| AO | Argan oil |
| AP-1 | Protein 1 |
| BNE | Blank nanoemulsion |
| CA | Citric acid |
| DH | Degree of hydrolysis |
| DLS | Dynamic light scattering |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| E/S | Enzyme/substrate |
| ECM | Extracellular matrix |
| FRAP | Ferric reducing antioxidant power |
| G’ | Storage modulus |
| G” | Loss modulus |
| GHK | Tripeptide glycyl-L-lysine |
| HLB | Hydrophilic-lipophilic balance |
| $IC_{50}$ | Concentration that cause 50% inhibition |
| HPLC | High performance liquid chromatography |
| L-DOPA | 3,4-Dihydroxyphenylalanine |
| LVR | Linear viscoelastic region |

| | |
|---|---|
| MAPK | Mitogen activated protein kinase |
| MARDI | Malaysian Agricultural Research and Development Institute |
| MCT | Medium chain triglyceride |
| MED | Mixture experimental design |
| MMPs | Matrix metalloproteinases |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide |
| NE | Nanoemulsion |
| NEB | Nonenzymatic browning |
| OD | Optical density |
| O/W | Oil-in-water |
| OPA | *O*-phthaldialdehyde |
| PBS | Phosphate buffer solution |
| PDI | Polydispersity index |
| PIC | Phase inversion composition |
| PIT | Phase inversion temperature |
| PITC | Phenyl-isocyanate |
| PKOE | Palm kernel oil ester |
| PUFAs | Polyunsaturated fatty acids |
| ROS | Reactive oxygen species |
| RSE | Residual standard error |
| $R^2$ | Coefficient of determination |
| S80 | Span 80 (sorbitan monooleate) |
| SEM | Scanning electron microscopy |
| SO | Safflower oil |
| TA | Tannic acid |
| TIMP-2 | Tissue inhibitor metalloproteinases 2 |

| | |
|---|---|
| T80 | Tween 80 (polyoxyethylene sorbitan monooleate) |
| TEM | Transmission electron microscopy |
| TPTZ | 2,4,6-tris(2-pyridyl)-s-triazine |
| UV | Ultraviolet |
| W/O | Water-in-oil |
| ZH | Zein hydrolysates |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Cosmeceutical products are those in between cosmetic products that cleanse and beautify; and pharmaceuticals that can cure and treat. In the late of 1970s, the word “cosmeceutical” was created by Raymond Reed (Founder of U.S. Society of Cosmetic Chemist) but then commercialized by Albert M. Kligman (dermatologist) in the medical fields (Wanjari *et al.,* 2015). Since cosmetics are no longer visualized as products that can only cover up imperfections in personal appearances, the combination of clinically proven active ingredients with a good delivery system is the current trend. Thus, the actives must hold therapeutic, disease-fighting, and healing properties that can be topically applied to the skin to improve the physiological effect on the cellular level (Mukul *et al.,* 2011).

Adding to that, halal cosmeceuticals are more demanding now because of the safety issues and the adverse effects arise from other harmful and doubtful products. Certain cosmeceutical products contained heavy metal, for example mercury, a common ingredient in skin whitening product. This harmful ingredient might root several adverse effects for example eczema, acne, eye inflammation and allergic reaction (Mohiuddin, 2019). Prolong used over time can cause mercury to accumulate in the body and bring damages to kidney and memory loss (Sahu *et al.,* 2014). Besides that, the growing number of Muslim population consumers worldwide also contributed to the increasing needs, as they become more religious through knowledge and information on concern towards halal cosmeceutical products (Che Mohd Hashim *et al.,* 2014).

In general, halal (lawful) refers to things or actions permitted by Islamic law (Shariah law) for Muslim consumption and practice. The halal requirements not only applied to the foods and drinks but also extend to cosmetic and personal care products. According to the MS 2200: Part 1: 2008 standard, the sources of ingredients of halal cosmetic products can include halal animals (land and aquatic), plants, microorganism, alcohol, chemicals, soil, and water as long as they are not hazardous and najs (Standards Malaysia, 2008; Hashim *et al.,* 2013). Not only that, the preparation, processing, manufacturing and storing of halal cosmeceutical products should be clearly distinguished from non-halal things. Besides, the toxicology and safety of the products must be checked and verified.

Today, cosmetic industries are likely to produce products that have many good traits to the skin such as antioxidant and antityrosinase properties.

Several common actives added to the anti-aging products are flavonoid compound, glycogen and protein. Protein based ingredients such as collagen, peptides, placenta and elastin usually derived from animal origins. The uncertainty of the ingredients and the manufacturing process can be doubtful and could lead to haram (unlawful), which is forbidden in Islam (Hashim *et al.,* 2013). For example, peptides derived from halal animals that is not slaughtered according to Syariah procedure was disqualified to be consumed by Muslim. Hence, the research and study on the halal cosmeceuticals is an important task so that the Muslim consumers have a wholesome and thoyyiban (good) choices for cosmeceuticals products.

Hydrolysates from *Actinopyga lecanora (A. lecanora),* an edible marine sea cucumber comes from halal source, depending on the extraction process and the safety of the hydrolysates to be used in the formulation. Its application is widely utilized in the functional food and therapeutic agents as a supplement for antihypertensive. The antioxidant and antibacterial properties of *A. lecanora* hydrolysates (ALH) has been reported for its excellent bioactivity (Auwal *et al.,* 2017a; Ghanbari *et al.,* 2012). Despite this, ALH has not been extensively study for cosmeceutical products development. To the author's knowledge, no work has been reported on its bioactivity as one of the halal cosmeceutical ingredient, especially in tackling skin aging or skin pigmentation.

Therefore, in this study ALH was introduced as a new bioactive compound in the formulation. Applying the new generation of nanotechnology in the cosmeceutical formulation, nanoemulsions (NE) are appraised in skin care products because they have good sensorial properties such as rapid penetration and good biophysical properties especially the hydrating power (Arora *et al.,* 2012). Moreover, NE also enhance the stability of cosmeceutical ingredients and improve the sustained release of the actives for long-lasting effect (Nagda *et al.,* 2010).

## 1.2 Problem Statement

In recent years, anti-aging and whitening products from biopeptides become more acceptable due to their beneficial ability to stimulate the physiological processes at the cellular level. However, in view of the fact that bioactivities of some single isolated biopeptides may reduce when tested alone, the use of hydrolysates as a whole could improves the activity due to their synergistic effects with other components present in the hydrolysates (Daliri *et al.,* 2017).

Most of the hydrolysates sources are from plants, human serum, animals collagen, protein and elastin (Zhang *et al.,* 2009). Either targeted for topical application or consumed as nutraceutical, Muslim should consider the source and the manufacturing process of the products. The doubtful and uncertainty of the ingredient's origin and production process may lead to haram, and it is prohibited to be use by Muslim consumers.

*A. lecanora* is a marine invertebrate of sea cucumber and it is halal to be consumed. Despite being commonly and abundantly found in Malaysia and other South Asian countries, this species is still underutilized especially in cosmeceutical application. The ALH produced from hydrolysis of *A. lecanora* has been claimed to have relatively high potential with antioxidant, antibacterial and wound healing properties that are essential in skin care products (Bordbar *et al.,* 2011; Ghanbari *et al.,* 2015). However, no work has been reported specifically on the use of ALH as an actives ingredient in the cosmeceutical products. Moreover, there is also no records on the antityrosinase activity of the ALH. Thus, in order to expand the possibility of ALH as an active ingredient in the cosmeceutical product, a nanoemulsion formulation containing halal hydrolysates from *A. lecanora* was developed, especially to alleviate skin aging and to improve skin pigmentation.

Skin aging is a complex biological process affected by the combination of endogenous and exogenous factors. In aged skin, fibroblast collapsed and produced low level of collagen with high level of collagen degrading enzymes demonstrated visible and measurable characteristics. Loss of elasticity, flattening, thinning, decreased barrier function, irregular keratinization leading to a yellow skin tone and decreased skin lipids were the visible signs for skin aging (Badenhorst *et al.,* 2014).

Skin pigmentation is one of the results of accumulation of over production of melanin. Melanin is biosynthesized in the melanosome of the melanocyte and tyrosinase is an oxidase containing copper enzyme that is responsible for the skin melanin biosynthesis. Not only that, over production of melanin also correlated with other skin problems such as post-inflammatory melanoderma, age spots and freckles (Karkouch *et al.,* 2017; Wu *et al.,* 2013).

Therefore, the quest for cosmeceuticals that have both tyrosinase inhibitors with anti-aging properties have led to numerous screening tests, research on new agents, particularly from natural sources (plants and animals) such as biopeptides and hydrolysates. Some single isolated biopeptides showed reduce bioactivities when tested alone. Hence, the use of hydrolysates as a whole will improve the activity due to their synergistic effects with other components in the hydrolysates (Daliri *et al.,* 2017).

The challenging part in delivering ALH to the skin is the strong barrier function of the skin itself. A suitable delivery system such as nanoemulsion was chosen to encapsulate ALH, since small particle sized of emulsion (100 to 200 nm) can provide an effective delivery of the actives by enhancing the skin absorption. The nanoemulsion containing ALH was formulated using statistical method approach of D-optimal mixture design. This approach was appropriate to be used in cosmeceutical formulation because the interaction between each components of the nanoemulsion can be fully analyzed and the well-designed model could benefit in achieving high desirable results.

## 1.3 Scope of Study

In this study, the ALH was used as a bioactive compound, which encapsulated in oil-in-water nanoemulsion for cosmeceutical purposes, specifically to combat or slow down skin aging and skin pigmentation. The dried and sieved *A. lecanora* sample was proceeded with papain enzymatic hydrolysis to obtain the hydrolysates. Optimization of hydrolysis conditions of ALH was conducted in order to check the best reaction conditions of hydrolysis such as pH, temperature, time and enzyme to substrate (E/S) ratio.

The nanoemulsion containing ALH was prepared by low and high-energy emulsification method, optimized using D-optimal mixture design. The physicochemical characterizations of the formulations were evaluated. The storage condition of nanoemulsion containing ALH was also determined and expected to be at lower temperature. Nanoemulsions containing ALH stored at higher temperature (45°C) had greater negative potentials than those stored at lower temperature (4 or 25°C), possibly due to increased ionization of adsorbed charge groups, as well as ions in water at elevated temperatures that accumulated at the droplet interfaces (Balcão *et al.,* 2013).

Other than that, *in vitro* efficacy assessments of the optimized nanoemulsion containing ALH were also investigated. The efficacy assessments comprised of the permeation through cellulose membrane acetate, the cytotoxicity effect against normal fibroblast 3T3 cell line, the antioxidant and the antityrosinase activities.

## 1.4 Objectives

The main objective of this study is to design and develop a halal hydrolysates-based nanocosmeceutical formulation from *A. lecanora*, a marine by-catch in fisheries industry. Therefore, the following specific objectives were as followed:

1. To optimize the hydrolysis conditions of *A. lecanora* hydrolysates.
2. To characterize the *A. lecanora* hydrolysates using amino acid profile and scanning electron microscopy.
3. To prepare and optimize the nanoemulsion formulation containing *A. lecanora* hydrolysates.
4. To determine the physicochemical properties and stability of the optimized nanoemulsion containing *A. lecanora* hydrolysates.
5. To investigate the safety and efficacy of the optimized nanoemulsion containing *A. lecanora* hydrolysates using *in vitro* permeation release, cytotoxicity, antioxidant and antityrosinase activities.

## REFERENCES


Ab Aziz, N. A., Salim, N., Zarei, M., Saari, N., & Md Yusoff, F. (2020). Extraction, anti-tyrosinase, and antioxidant activities of the collagen hydrolysate derived from *Rhopilema hispidum*. *Preparative Biochemistry and Biotechnology*, 1–10.

Abd Gani, S. S., Basri, M., Abdul Rahman, M. B., Kassim, A., Raja Abd Rahman, R. N. Z., Salleh, A. B., & Ismail, Z. (2011). Engkabang fat esters for cosmeceutical formulation. *Journal of Surfactants and Detergents*, *14*, 227–233.

Adjonu, R., Doran, G., Torley, P., & Agboola, S. (2014a). Formation of whey protein isolate hydrolysate stabilised nanoemulsion. *Food Hydrocolloids*, *41*, 169–177.

Adjonu, R., Doran, G., Torley, P., & Agboola, S. (2014b). Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities. *Journal of Food Engineering*, *122*, 15–27.

Al Azad, S., Muhammad Shaleh, S. R., & Siddiquee, S. (2017). Comparison of fatty acid and proximate composition between *Holothuria edulis* and *Holothuria scabra* collected from coastal water of Sabah , Malaysia. *Advances in Bioscience and Biotechnology*, *8*, 91–103.

Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2020). Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University - Science*, *32*(1), 7–16.

Alzorqi, I., Ketabchi, M. R., Sudheer, S., & Manickam, S. (2016). Optimization of ultrasound induced emulsification on the formulation of palm-olein based nanoemulsions for the incorporation of antioxidant β-D-glucan polysaccharides. *Ultrasonics Sonochemistry*, *31*, 71–84.

Amberg, N., & Fogarassy, C. (2019). Green consumer behavior in the cosmetics market. *Resources*, *8*(137), 1–19.

Amri, E., & Mamboya, F. (2012). Papain, a plant enzyme of biological importance: a review. *American Journal of Biochemistry and Biotechnology*, *8*(2), 99–104.

Anton, N., Benoit, J. P., & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates- a review. *Journal of Controlled Release*, *128*(3), 185–199.

Anton, N., & Vandamme, T. F. (2011). Nano-emulsions and micro-emulsions: clarifications of the critical differences. *Pharmaceutical Research*, *28*(5), 978–985.

Aqil, M., Kamran, M., Ahad, A., & Imam, S. S. (2016). Development of clove oil based nanoemulsion of olmesartan for transdermal delivery: Box-Behnken design optimization and pharmacokinetic evaluation. *Journal of Molecular Liquids*, *214*, 238–248.

Arancibia, C., Navarro-Lisboa, R., Zúñiga, R. N., & Matiacevich, S. (2016). Application of CMC as thickener on nanoemulsions based on olive oil: Physical properties and stability. *International Journal of Polymer Science*, *2016*, 1–10.

Arbain, N. H., Basri, M., Salim, N., Wui, W. T., & Abdul Rahman, M. B. (2018). Development and characterization of aerosol nanoemulsion system encapsulating low water soluble quercetin for lung cancer treatment. *Materials Today: Proceedings*, *5*, S137–S142.

Aswathanarayan, J. B., & Vittal, R. R. (2019). Nanoemulsions and their potential applications in food industry. *Frontiers in Sustainable Food Systems*, *3*, 1–21.

Aumeeruddy-Elalfi, Z., Gurib-Fakim, A., & Mahomoodally, M. F. (2016). Kinetic studies of tyrosinase inhibitory activity of 19 essential oils extracted from endemic and exotic medicinal plants. *South African Journal of Botany*, *103*, 89–94.

Auwal, S. M., Zarei, M., Abdul-Hamid, A., & Saari, N. (2017a). Optimization of bromelain-aided production of angiotensin I-converting enzyme inhibitory hydrolysates from stone fish using response surface methodology. *Marine Drugs*, *15*(104), 1–14.

Auwal, S. M., Zarei, M., Abdul-Hamid, A., & Saari, N. (2017b). Response surface optimisation for the production of antioxidant hydrolysates from stone fish protein using bromelain. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–10.

Auwal, S. M., Zarei, M., Tan, C. P., & Saari, N. (2018). Comparative physicochemical stability and efficacy study of lipoid S75-biopeptides nanoliposome composite produced by conventional and direct heating methods. *International Journal of Food Properties*, *21*(1), 1646–1660.

Aydin, M., Sevgili, H., Tufan, B., Emre, Y., & Köse, S. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science and Technology*, *46*(3), 500–508.

Azeem, A., Rizwan, M., Ahmad, F. J., Iqbal, Z., Khar, R. K., Aqil, M., & Talegaonkar, S. (2009). Nanoemulsion components screening and selection: a technical note. *American Association of Pharmaceutical Scientists*, *10*(1), 69–76.

Aziz, M., Husson, F., & Kermasha, S. (2015). Optimization of the hydrolysis of safflower oil for the production of linoleic acid, used as flavor precursor.

*International Journal of Food Science*, *2015*, 1–10.

Azmi, N. A. N., Elgharbawy, A. A. M., Motlagh, S. R., Samsudin, N., & Mohd Salleh, H. (2019). Nanoemulsions: factory for food, pharmaceutical and cosmetics. *Processes*, *7*(9).

Badenhorst, T., Darren, S., & Wu, Z. (2014). Pharmaceutical strategies for the topical dermal delivery of peptides/proteins for cosmetic and therapeutic applications. *Austin Journal of Pharmacology and Therapeutics*, *2*(6), 1–10.

Bakour, M., Soulo, N., Hammas, N., El Fatemi, H., Aboulghazi, A., Taroq, A., Abdellaoui, A., Al-Waili, N., & Lyoussi, B. (2018). The antioxidant content and protective effect of argan oil and *Syzygium aromaticum* essential oil in hydrogen peroxide-induced biochemical and histological changes. *International Journal of Molecular Sciences*, *19*(610), 1–14.

Balcão, V. M., Costa, C. I., Matos, C. M., Moutinho, C. G., Amorim, M., Pintado, M. E., Gomes, A. P., Vila, M. M., & Teixeira, J. A. (2013). Nanoencapsulation of bovine lactoferrin for food and biopharmaceutical applications. *Food Hydrocolloids*, 32(2), 425–431.

Barzkar, N., Attaran Fariman, G., & Taheri, A. (2017). Proximate composition and mineral contents in the body wall of two species of sea cucumber from Oman sea. *Environmental Science and Pollution Research*, *24*(23), 18907–18911.

Bechtel, P. J., Oliveira, A. C. M., Demir, N., & Smiley, S. (2012). Chemical composition of the giant red sea cucumber, *Parastichopus californicus* , commercially harvested in Alaska. *Food Science & Nutrition*, *1*(1), 63–73.

Bhat, Z. F., Kumar, S., & Bhat, H. F. (2015). Bioactive peptides of animal origin: a review. *Journal of Food Science and Technology*, *52*(9), 5377–5392.

Bialek, A., Bialek, M., Jelinska, M., & Tokarz, A. (2016). Fatty acid profile of new promising unconventional plant oils for cosmetic use. *International Journal of Cosmetic Science*, *38*(4), 382–388.

Bordbar, S., Anwar, F., & Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods: a review. *Marine Drugs*, *9*, 1761–1805.

Bordbar, S., Ebrahimpour, A., Abdul Hamid, A., Abdul Manap, M. Y., Anwar, F., & Saari, N. (2013). The improvement of the endogenous antioxidant property of stone fish *(Actinopyga lecanora)* tissue using enzymatic proteolysis. *BioMed Research International*, *2013*, 1–9.

Bordbar, S., Ebrahimpour, A., Zarei, M., Abdul Hamid, A., & Saari, N. (2018). Alcalase-generated proteolysates of stone fish *(Actinopyga lecanora)* flesh as a new source of antioxidant peptides. *International Journal of Food Properties*, *21*(1), 1541–1559.

Bouchemal, K., Briançon, S., Perrier, E., & Fessi, H. (2004). Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *International Journal of Pharmaceutics*, *280*(2), 241–251.

Bougatef, A., Nedjar-Arroume, N., Manni, L., Ravallec, R., Barkia, A., Guillochon, D., & Nasri, M. (2010). Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle *(Sardinella aurita)* by-products proteins.

Campos, F. F., Calpena Campmany, A. C., Delgado, G. R., Serrano, O. L., & Naveros, B. C. (2012). Development and characterization of a novel nystatin-loaded nanoemulsion for the buccal treatment of candidosis: ultrastructural effects and release studies. *Journal of Pharmaceutical Sciences*, *101*(7), 1–29.

Capek, I. (2004). Degradation of kinetically-stable o/w emulsions. *Advances in Colloid and Interface Science*, *107*(2–3), 125–155.

Cernasov, D. (2008). The design and development of anti-aging formulations. In N. Dayan (Ed.), *Skin Aging Handbook: An Integrated Approach to Biochemistry and Product Development* (1st ed., pp. 291–325). William Andrew Inc.

Chang, T. S. (2009). An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences*, *10*(6), 2440–2475.

Charrouf, Z., Harhar, H., Gharby, S., & Guillaume, D. (2008). Enhancing the value of argan oil is the best mean to sustain the argan grove economy and biodiversity, so far. *Oilseeds and Fats, Crops and Lipids*, *15*(4), 269–271.

Chaves-López, C., Serio, A., Paparella, A., Martuscelli, M., Corsetti, A., Tofalo, R., & Suzzi, G. (2014). Impact of microbial cultures on proteolysis and release of bioactive peptides in fermented milk. *Food Microbiology*, *42*, 117–121.

Che Marzuki, N. H., Abdul Wahab, R., & Abdul Hamid, M. (2019). An overview of nanoemulsion: concepts of development and cosmeceutical applications. *Biotechnology and Biotechnological Equipment*, *33*(1), 779–797.

Che Mohd Hashim, A. J., & Musa, R. (2014). Factors influencing attitude towards halal cosmetic among young adult urban muslim women: a focus group analysis. *Procedia - Social and Behavioral Sciences*, *130*, 129–134.

Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Ashari, S. E., Basri, H., & Ismail, M. (2017). Predicting the optimum compositions of a transdermal nanoemulsion system containing an extract of *Clinacanthus nutans* leaves *(L.)* for skin antiaging by artificial neural network model. *Journal of Chemometrics*, *31*(7), 1–13.

Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Ashari, S. E., & Ismail, M.

(2016). Design and development of a nanoemulsion system containing extract of *Clinacanthus nutans (L.)* leaves for transdermal delivery system by D-optimal mixture design and evaluation of its physicochemical properties. *RSC Advances*, *6*, 67378–67388.

Che Sulaiman, I. S., Mahiran, B., Kim, W. C., Ashari, S. E., Fard Masoumi, H. R., & Ismail, M. (2015). In vitro antioxidant, cytotoxic and phytochemical studies of *Clinacanthus nutans Lindau* leaf extracts. *African Journal of Pharmacy and Pharmacology*, *9*(34), 861–874.

Chen, M., Ji, H., Zhang, Z., Zeng, X., Su, W., & Liu, S. (2019). A novel calcium-chelating peptide purified from *Auxis thazard* protien hydrolysate and its binding properties with calcium. *Journal of Functional Foods*, *60*, 1–8.

Chen, W. C., Tseng, T. S., Hsiao, N. W., Lin, Y. L., Wen, Z. H., Tsai, C. C., Yu, C. L., Hui, H. L., & Tsai, K. C. (2015). Discovery of highly potent tyrosinase inhibitor, T1, with significant anti-melanogenesis ability by zebrafish in vivo assay and computational molecular modeling. *Scientific Reports*, *5*(1), 1–8.

Chi, C. F., Cao, Z. H., Wang, B., Hu, F. Y., Li, Z. R., & Zhang, B. (2014). Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. *Molecules*, *19*(8), 11211–11230.

Costa, A. M., Bueno, K. T. L., Rosa, A. P. C. da, & Costa, J. A. V. (2019). The antioxidant activity of nanoemulsions based on lipids and peptides from *Spirulina sp. LEB18*. *LWT-Food Science and Technology*, *99*, 173–178.

Da Costa, S., Basri, M., Shamsudin, N., & Basri, H. (2014). Stability of positively charged nanoemulsion formulation containing steroidal drug for effective transdermal application. *Journal of Chemistry*, *2014*, 1–8.

Dal Mas, J., Zermiani, T., Thiesen, L. C., Silveira, J. L. M., Da Silva, K. A. B. S., De Souza, M. M., Malheiros, A., Bresolin, T. M. B., & Lucinda-Silva, R. M. (2016). Nanoemulsion as a carrier to improve the topical anti-inflammatory activity of stem bark extract of *Rapanea ferruginea*. *International Journal of Nanomedicine*, *11*, 4495–4507.

Daliri, E. B. M., Oh, D. H., & Lee, B. H. (2017). Bioactive peptides. *Foods*, *6*(32), 1–21.

Dayan, N. (2005). Pathways for Skin Penetration. *Cosmetics & Toiletries*, *120*(6), 67–76.

De Azevedo Ribeiro, R. C., Barreto, S. M. A. G., Ostrosky, E. A., Da Rocha-Filho, P. A., Veríssimo, L. M., & Ferrari, M. (2015). Production and characterization of cosmetic nanoemulsions containing *Opuntia ficus-indica (L.)* mill extract as moisturizing agent. *Molecules*, *20*(2), 2492–2509.

De Morais, D. V., De Carvalho Costa, M. A. P., Bárbara, M. F. S., De Lima Silva,

F., Moreira, M. M., Delerue-Mato, C., Dias L. A. G., Estevinho, M. L. M., & De Carvalho, C. A. L. (2018). Antioxidant, photoprotective and inhibitory activity of tyrosinase in extracts of *Dalbergia ecastaphyllum*. *PLoS ONE*, *13*(11), 1–16.

Delmas, T., Piraux, H., Couffin, A. C., Texier, I., Vinet, F., Poulin, P., Cates, M. E., & Bibette, J. (2011). How to prepare and stabilize very small nanoemulsions. *Langmuir*, *27*(5), 1683–1692.

Desmelati, Sumarto, Dewita, Dahlia, Syafrijal, & Sari, P. A. (2020). Determination of nano-collagen quality from sea cucumber *Holothuria scabra*. *IOP Conference Series: Earth and Environmental Science*, *430*(1).

Dias, D. de O., Colombo, M., Kelmann, R. G., Kaiser, S., Lucca, L. G., Teixeira, H. F., Limberger, R. P., Veiga Jr., V. F., & Koester, L. S. (2014). Optimization of copaiba oil-based nanoemulsions obtained by different preparation methods. *Industrial Crops and Products*, *59*, 154–162.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, *23*(6), 1473–1482.

Donsì, F., Annunziata, M., Vincensi, M., & Ferrari, G. (2012). Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. *Journal of Biotechnology*, *159*(4), 342–350.

El Abbassi, A., Khalid, N., Zbakh, H., & Ahmad, A. (2014). Physicochemical characteristics, nutritional properties, and health benefits of argan oil: a review. *Critical Reviews in Food Science and Nutrition*, *54*(11), 1401–1414.

El Hussein, S., Muret, P., Berard, M., Makki, S., & Humbert, P. (2007). Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (ex-vivo study). *Experimental Dermatology*, *16*, 830–836.

Elhalil, A., Tounsadi, H., Elmoubarki, R., Mahjoubi, F. Z., Farnane, M., Sadiq, M., Abdennouri, M., Qourzal, S., & Barka, N. (2016). Factorial experimental design for the optimization of catalytic degradation of malachite green dye in aqueous solution by Fenton process. *Water Resources and Industry*, *15*, 41–48.

Fang, J. Y., & Leu, Y. L. (2006). Prodrug strategy for enhancing drug delivery via skin. *Current Drug Discovery Technologies*, *3*(3), 211–224.

Farahin, A. W., Yusoff, F. M., Basri, M., Nagao, N., & Shariff, M. (2019). Use of microalgae: Tetraselmis tetrathele extract in formulation of nanoemulsions for cosmeceutical application. *Journal of Applied Phycology*, *31*(3), 1743–1752.

Fard Masoumi, H. R., Basri, M., Samiun, W. S., Izadiyan, Z., & Chaw, J. L. (2015). Enhancement of encapsulation efficiency of nanoemulsion-containing aripiprazole for the treatment of schizophrenia using mixture

experimental design. *International Journal of Nanomedicine*, *10*, 6469–6476.

García-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. *Biotechnology Advances*, *18*(7), 549–579.

Ghanbari, R., Ebrahimpour, A., Abdul-Hamid, A., Ismail, A., & Saari, N. (2012). *Actinopyga lecanora* hydrolysates as natural antibacterial agents. *International Journal of Molecular Sciences*, *13*(12), 16796–16811.

Ghanbari, R., Zarei, M., Ebrahimpour, A., Abdul-Hamid, A., Ismail, A., & Saari, N. (2015). Angiotensin-I converting enzyme (ACE) inhibitory and anti-oxidant activities of sea cucumber *(Actinopyga lecanora)* hydrolysates. *International Journal of Molecular Sciences*, *16*, 28870–28885.

Ghosh, S., & Coupland, J. N. (2008). Factors affecting the freeze-thaw stability of emulsions. *Food Hydrocolloids*, *22*(1), 105–111.

Gilaberte, Y., Prieto-Torres, L., Pastushenko, I., & Juarranz, Á. (2016). *Anatomy and function of the skin*. *Nanoscience in Dermatology*. Elsevier Inc.

Goebel, A., & Neubert, R. H. H. (2008). Dermal peptide delivery using colloidal carrier systems. *Skin Pharmacology and Physiology*, *21*(1), 3–9.

Golubovic-Liakopoulos, N., Simon, S. R., & Shah, B. (2011). Nanotechnology use with cosmeceuticals. *Seminars in Cutaneous Medicine and Surgery*, *30*(3), 176–180.

Gorouhi, F., & Maibach, H. I. (2009). Role of topical peptides in preventing or treating aged skin. *International Journal of Cosmetic Science*, *31*, 327–345.

Gottipati, R., & Mishra, S. (2010). Process optimization of adsorption of Cr(VI) on activated carbons prepared from plant precursors by a two-level full factorial design. *Chemical Engineering Journal*, *160*(1), 99–107.

Guo, Y., Michael, N., Madrigal, J. F., Aguirre, C. S., & Jauregi, P. (2019). Protein hydrolysate from *Pterygoplichthys disjunctivus*, armoured catfish, with high antioxidant activity. *Molecules*, *24*(1628).

Gurpreet, K., & Singh, S. K. (2018). Review of nanoemulsion formulation and characterization techniques. *Indian Journal of Pharmaceutical Sciences*, *80*(5), 781–789.

Halla, N., Fernandes, I. P., Heleno, S. A., Costa, P., Boucherit-Otmani, Z., Boucherit, K., Rodrigues, A. E., Ferreira, I. C. F. R., & Barreiro, M. F. (2018). Cosmetics preservation: a review on present strategies. *Molecules*, *23*(7), 1–41.

Hashim, P., & Mat Hashim, D. (2013). A review of cosmetic and personal care products: halal perspective and detection of ingredient. *Pertanika Journal*

*of Science and Technology*, *21*(2), 281–292.

Hidajat, M. J., Jo, W., Kim, H., & Noh, J. (2020). Effective droplet size reduction and excellent stability of limonene nanoemulsion formed by high-pressure homogenizer. *Colloids and Interfaces*, *4*(1), 5.

Honary, S., & Zahir, F. (2013a). Effect of zeta potential on the properties of nano-drug delivery systems- a review (part 1). *Tropical Journal of Pharmaceutical Research*, *12*(2), 255–264.

Honary, S., & Zahir, F. (2013b). Effect of zeta potential on the properties of nano-drug delivery systems- a review (part 2). *Tropical Journal of Pharmaceutical Research*, *12*(2), 265–273.

Honey-Escandón, M., Arreguín-Espinosa, R., Solís-Marín, F. A., & Samyn, Y. (2015). Biological and taxonomic perspective of triterpenoid glycosides of sea cucumbers of the family Holothuriidae (Echinodermata, Holothuroidea). *Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology*, *180*, 16–39.

Huang, S. W., Frankel, E. N., Aeschbach, R., & German, J. B. (1997). Partition of selected antioxidants in corn oil-water model systems. *Journal of Agricultural and Food Chemistry*, *45*(6), 1991–1994.

Hwang, J. Y., Shyu, Y. S., Wang, Y. T., & Hsu, C. K. (2010). Antioxidative properties of protein hydrolysate from defatted peanut kernels treated with esperase. *LWT-Food Science and Technology*, *43*(2), 285–290.

Ismail, A., Azlan, A., Hock, E. K., Prasad, K. N., & Kin, W. K. (2013). *Antioxidant Assays: Principles, Methods and Analyses* (1st ed.). Universiti Putra Malaysia Press.

Jadhav, S. B., Kumar, K. C., Bandichhor, R., & Bhosale, P. N. (2016). Analysis development of RP UPLC-TOF/MS, stability indicating method for omeprazole and its related substances by applying two level factorial design ; and identification and synthesis of non-pharmacopoeial impurities. *Journal of Pharmaceutical and Biomedical Analysis*, *118*, 370–379.

Jaiswal, M., Dudhe, R., & Sharma, P. K. (2015). Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*, *5*(2), 123–127.

Jakubczyk, A., Karas, M., Rybczynska-Tkaczyk, K., Zielinska, E., & Zielinski, D. (2020). Current trends of bioactive peptides- new sources and therapeutic effect. *Foods*, *9*.

Jaslina, N. F., Faujan, N. H., Mohamad, R., & Ashari, S. E. (2020). Effect of addition of PVA /PG to oil-in-water nanoemulsion kojic monooleate formulation on droplet size : three-factors response surface. *Cosmetics*, *7*(73).

Jaworska, M., Sikora, E., Zielina, M., & Ogonowski, J. (2013). Studies on the

formation of o/w nano-emulsions, by low-energy emulsification method, suitable for cosmeceutical applications. *Acta Biochimica Polonica*, *60*(4), 779–782.

Jemil, I., Jridi, M., Nasri, R., Ktari, N., Ben Salem, R. B. S., Mehiri, M., … Nasri, M. (2014). Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis A26*. *Process Biochemistry*, *49*(6), 963–972.

Jintapattanakit, A. (2018). Preparation of nanoemulsions by phase inversion temperature (PIT) method. *Pharmaceutical Sciences Asia*, *45*(1), 1–12.

Jordan, M., Nayel, A., Brownlow, B., & Elbayoumi, T. (2012). Development and evaluation of tocopherol-rich argan oil-based nanoemulsions as vehicles possessing anticancer activity. *Journal of Biomedical Nanotechnology*, *8*, 944–956.

Kala, M., Shaikh, M. V., & Nivsarkar, M. (2016). Development and optimization of psychological stress model in mice using 2 level full factorial design. *Journal of Pharmacological and Toxicological Methods*, *82*, 54–61.

Karkouch, I., Tabbene, O., Gharbi, D., Mlouka, M. A. Ben, Elkahoui, S., Rihouey, C., Limam, F. (2017). Antioxidant , antityrosinase and antibio film activities of synthesized peptides derived from *Vicia faba* protein hydrolysate : a powerful agents in cosmetic application. *Industrial Crops & Products*, *109*, 310–319.

Karthik, P., Ezhilarasi, P. N., & Anandharamakrishnan, C. (2017). Challenges associated in stability of food grade nanoemulsions. *Critical Reviews in Food Science and Nutrition*, 57(7), 1435-1450.

Katkade, M. B., Syed, H. M., Andhale, R. R., & Sontakke, M. D. (2018). Fatty acid profile and quality assessment of safflower *(Carthamus tinctorius)* oil. *Journal of Pharmacognosy and Phytochemistry*, *7*(2), 3581–3585.

Kaul, S., Gulati, N., Verma, D., Mukherjee, S., & Nagaich, U. (2018). Role of nanotechnology in cosmeceuticals: a review of recent advances. *Journal of Pharmaceutics*, 1–19.

Kaur, K., Kaur, J., Kumar, R., & Mehta, S. K. (2017). Formulation and physiochemical study of α-tocopherol based oil in water nanoemulsion stabilized with non toxic, biodegradable surfactant: sodium stearoyl lactate. *Ultrasonics Sonochemistry*, *38*, 570–578.

Keng, P. S., Basri, M., Zakaria, M. R. S., Abdul Rahman, M. B., Ariff, A. B., Abdul Rahman, R. N. Z., & Salleh, A. B. (2009). Newly synthesized palm esters for cosmetics industry. *Industrial Crops and Products*, *29*(1), 37–44.

Khotimchenko, Y. (2018). Pharmacological potential of sea cucumbers. *International Journal of Molecular Sciences*, *19*(5), 1–42.

Kim, M. J., Doh, H. J., Choi, M. K., Chung, S. J., Shim, C. K., Kim, D. D., & Choi, H. G. (2008). Skin permeation enhancement of diclofenac by fatty acids. *Drug Delivery*, *15*(6), 373–379.

Kim, M., & Jeong Park, K. (2016). Molecular mechanisms of skin aging and rejuvenation. In *World's Largest Science, Technology & Medicine* (pp. 75–100). INTECH.

Ko, H. H., Chiang, Y. C., Tsai, M. H., Liang, C. J., Hsu, L. F., Li, S. Y., & Lee, C. W. (2014). Eupafolin, a skin whitening flavonoid isolated from *Phyla nodiflora*, downregulated melanogenesis: role of MAPK and AKT pathways. *Journal of Ethnopharmacology*, *151*, 386–393.

Kolarsick, P. A. J., Kolarsick, M. A., & Goodwin, C. (2011). Anatomy and physiology of the skin. *Journal of the Dermatology Nurses' Association*, *3*(4), 203–211.

Kong, W. Y., Salim, N., Masoumi, H. R. F., Basri, M., Da Costa, S. S., & Ahmad, N. (2018). Optimization of hydrocortisone-loaded nanoemulsion formulation using D-optimal mixture design. *Asian Journal of Chemistry*, *30*(18), 2424–2430.

Kumar, M., Bishnoi, R. S., Shukla, A. K., & Jain, C. P. (2019). Techniques for formulation of nanoemulsion drug delivery system: a review. *Preventive Nutrition and Food Science*, *24*(3), 225–234.

Kusumadjaja, A. P., & Dewi, R. P. (2010). Determination of optimum condition of papain enzyme from papaya var java *(Carica papaya). Indonesian Journal of Chemistry*, *5*(2), 147–151.

Lavers, I. (2017). Exploring skin anatomy, function and site-specific treatment options. *Journal of Aesthetic Nursing*, *6*(4), 172–180.

Lee, M. H., Kim, Y. K., Moon, H. S., Kim, K. D., Kim, G. G., Cho, H. A., Han, S. K. (2012). Comparison on proximate composition and nutritional profile of red and black sea cucumbers *(Apostichopus japonicus)* from ulleungdo (island) and dokdo (island), Korea. *Food Science and Biotechnology*, *21*(5), 1285–1291.

León-López, A., Fuentes-Jiménez, L., Hernández-Fuentes, A. D., Campos-Montiel, R. G., & Aguirre-Álvarez, G. (2019). Hydrolysed collagen from sheepskins as a source of functional peptides with antioxidant activity. *International Journal of Molecular Sciences*, *20*(16), 3931.

Li, Y., Jin, H., Sun, X., Sun, J., Liu, C., Liu, C., & Xu, J. (2019). Physicochemical properties and storage stability of food protein-stabilized nanoemulsions. *Nanomaterials*, *9*(25), 1–14.

Low, S. (2017). The rising demand for halal cosmetics in Malaysia. Retrieved January 30, 2018, from https://www.star2.com/style/2017/10/13/rising-demand-halal-cosmetics/

Mahdi, E. S., Noor, A. M., Sakeena, M. H., Abdullah, G. Z., Abdulkarim, M. F., & Sattar, M. A. (2011). Formulation and in vitro release evaluation of newly synthesized palm kernel oil esters-based nanoemulsion delivery system for 30% ethanolic dried extract derived from local *Phyllanthus urinaria* for skin antiaging. *International Journal of Nanomedicine*, *6*, 2499–2512.

Manninen, A. H. (2009). Protein hydrolysates in sports nutrition. *Nutrition & Metabolism*, *6*(1), 38.

Marrakchi, S., & Maibach, H. I. (2007). Biophysical parameters of skin: map of human face, regional, and age-related differences. *Contact Dermatitis*, *57*(1), 28–34.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C. B., & Graves, S. M. (2006). Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter*, *18*(41), 635–666.

Mat Hadzir, N., Basri, M., Abdul Rahman, M. B., Salleh, A. B., Raja Abdul Rahman, R. N. Z., & Basri, H. (2013). Phase behaviour and formation of fatty acid esters nanoemulsions containing piroxicam. *American Association of Pharmaceutical Scientists*, *14*(1), 456–463.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, *9*(5), 305–313.

McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter*, *7*, 2297–2316.

Mendis, E., Rajapakse, N., Byun, H. G., & Kim, S. K. (2005). Investigation of jumbo squid *(Dosidicus gigas)* skin gelatin peptides for their in vitro antioxidant effects. *Life Sciences*, *77*(17), 2166–2178.

Mohamad, N. R., Abd Gani, S. S., Abdul Wahab, R., & Zaidan, U. H. (2019). Development of nanoemulsion incorporated with *Hibiscus sabdariffa* for cosmeceutical application. *Malaysian Applied Biology*, *48*(2), 77–81.

Mohiuddin, A. K. (2019). Heavy metals in cosmetics: the notorious daredevils and burning health issues. *American Journal of Biomedical Science & Research*, *4*(5), 332–337.

Monfalouti, H. El, Guillaume, D., Denhez, C., & Charrouf, Z. (2010). Therapeutic potential of argan oil: a review. *Journal of Pharmacy and Pharmacology*, *62*(12), 1669–1675.

Mukul, S., Surabhi, K., & Atul, N. (2011). Cosmeceuticals for the skin: an overview. *Asian Journal of Pharmaceutical and Clinical Research*, *4*(2), 1–6.

Mulcahy, E. M., Park, C. W., Drake, M. A., Mulvihill, D. M., & O'Mahony, J. A. (2016). Improvement of the functional properties of whey protein hydrolysate by conjugation with maltodextrin. *International Dairy Journal*,

*60*, 47–54.

Murthy, R. S. R., Arora, N., & Agarwal, S. (2012). Review article latest technology advances in cosmaceuticals. *International Journal of Pharmaceutical Sciences and Drug Research*, *4*, 168–182.

Musa, S. H., Basri, M., Fard Masoumi, H. R., Shamsudin, N., & Salim, N. (2017). Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis : in vitro diffusion and in vivo hydrating action. *International Journal of Nanomedicine*, *12*, 2427–2441.

Nagda, D., Rathore, K. S., Bharkatiya, M., Sisodia, S. S., & Nema, R. K. (2010). Bucky balls: a novel drug delivery system. *Journal of Chemical and Pharmaceutical Research*, *2*(2), 240–248.

Nakchum, L., & Kim, S. M. (2016). Preparation of squid skin collagen hydrolysate as an antihyaluronidase, antityrosinase, and antioxidant agent. *Preparative Biochemistry and Biotechnology*, *46*(2), 123–130.

Nasri, M. (2016). Protein hydrolysates and biopeptides: production, biological activities, and application in foods and health benefits. a review. *Advances in Food and Nutrition Research*, *81*, 109–159.

Naylor, E. C., Watson, R. E. B., & Sherratt, M. J. (2011). Molecular aspects of skin ageing. *Maturitas*, *69*(3), 249–256.

Ng, S. H., Basri, M., Abdul Rahman, M. B., Abdul Rahman, R. N. Z. R., Salleh, A. B., & Ismail, Z. (2011). Phase behavior and formulation of palm oil esters o/w nanoemulsions stabilized by hydrocolloid gums for cosmeceuticals application. *Journal of Dispersion Science and Technology*, *32*(10), 1428–1433.

Ngan, C. L., Basri, M., Lye, F. F., Fard Masoumi, H. R., Tripathy, M., Abedi Karjiban, R., & Abdul-Malek, E. (2014). Comparison of box–behnken and central composite designs in optimization of fullerene loaded palm-based nano-emulsions for cosmeceutical application. *Industrial Crops and Products*, *59*, 309–317.

Ngan, C. L., Basri, M., Tripathy, M., Abedi Karjiban, R., & Abdul-Malek, E. (2015). Skin intervention of fullerene-integrated nanoemulsion in structural and collagen regeneration against skin aging. *European Journal of Pharmaceutical Sciences*, *70*, 22–28.

Nielsen, P. M., Petersen, D., & Dambmann, C. (2001). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, *66*(5), 642–646.

Noman, A., Xu, Y., AL-Bukhaiti, W. Q., Abed, S. M., Ali, A. H., Ramadhan, A. H., & Xia, W. (2018). Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate

obtained from Chinese sturgeon *(Acipenser sinensis)* by using papain enzyme. *Process Biochemistry*, *67*, 19–28.

Nursid, M., Marraskuranto, E., Kuswardini, A., & Winanto, T. (2019). Screening of tyrosinase inhibitor, antioxidant and cytotoxicity of dried sea cucumber from tomini bay, Indonesia. *Pharmacognosy Journal*, *11*(3), 555–558.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, *16*(6), 12871–12890.

Padma Preetha, J., & Karthika, K. (2009). Cosmeceuticals- an evolution. *International Journal of ChemTech Research*, *1*(4), 1217–1223.

Pan, X., Fang, Y., Wang, L., Shi, Y., Xie, M., Xia, J., & Hu, Q. (2019). Covalent interaction between rice protein hydrolysates and chlorogenic acid: improving the stability of oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, *67*(14), 4023–4030. research-article.

Pan, X., Fang, Y., Wang, L., Xie, M., Hu, B., Zhu, Y., & Hu, Q. (2019). Effect of enzyme types on the stability of oil-in-water emulsions formed with rice protein hydrolysates. *Journal of the Science of Food and Agriculture*, *99*(15), 6731–6740.

Panich, U., Sittithumcharee, G., Rathviboon, N., & Jirawatnotai, S. (2016). Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem Cells International*, *2016*, 1–14.

Pavlicevic, M., Maestri, E., & Marmiroli, M. (2020). Marine bioactive peptides - an overview of generation, structure and application with a focus on food sources. *Marine Drugs*, *18*(424).

Petit, N., Dyer, J. M., Clerens, S., Gerrard, J. A., & Domigan, L. J. (2020). Oral delivery of self-assembling bioactive peptides to target gastrointestinal tract disease. *Food and Function*, *11*(11), 9468–9488.

Pillaiyar, T., Manickam, M., & Namasivayam, V. (2017). Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 403–425.

Polanco-Lugo, E., Dávila-Ortiz, G., Betancur-Ancona, D. A., & Chel-Guerrero, L. A. (2014). Effects of sequential enzymatic hydrolysis on structural, bioactive and functional properties of *Phaseolus lunatus* protein isolate. *Food Science and Technology*, *34*(3), 441–448.

Pongkai, P., Saisavoey, T., Sangtanoo, P., Sangvanich, P., & Karnchanatat, A. (2017). Effects of protein hydrolysate from chicken feather meal on tyrosinase activity and melanin formation in B16F10 murine melanoma

cells. *Food Science and Biotechnology*, *26*(5), 1199–1208.

Pongsumpun, P., Iwamoto, S., & Siripatrawan, U. (2020). Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochemistry*, *60*, 1–10.

Prakot, P., Chaitanawisuti, N., & Karnchanatat, A. (2015). In vitro anti-tyrosinase activity of protein hydrolysate from spotted babylon *(Babylonia areolata)*. *Food and Applied Bioscience Journal*, *3*(2), 109–120.

Primozic, M., Duchek, A., Nickerson, M., & Ghosh, S. (2017). Effect of lentil proteins isolate concentration on the formation, stability and rheological behavior of oil-in-water nanoemulsions. *Food Chemistry*, *237*, 65–74.

Proksch, E. (2018). pH in nature, humans and skin. *Journal of Dermatology*, *45*(9), 1044–1052.

Rajurkar, N. S., & Hande, S. M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, *73*(2), 146.

Rao, Q., Kamdar, A. K., & Labuza, T. P. (2016). Storage stability of food protein hydrolysates- a review. *Critical Reviews in Food Science and Nutrition*, *56*(7), 1169–1192.

Ray, A. (2017). Halal cosmetics market. Retrieved January 29, 2018, from https://www.alliedmarketresearch.com/halal-cosmetics-market

Regenstein, J. M., Chaudry, M. M., & Regenstein, C. E. (2003). The kosher and halal food laws. *Comprehensive Reviews in Food Science and Food Safety*, *2*(3), 111–127.

Regti, A., Laamari, M. R., Stiriba, S.-E., & El Haddad, M. (2017). Use of response factorial design for process optimization of basic dye adsorption onto activated carbon derived from Persea species. *Microchemical Journal*, *130*, 129–136.

Ren, G., Sun, Z., Wang, Z., Zheng, X., Xu, Z., & Sun, D. (2019). Nanoemulsion formation by the phase inversion temperature method using polyoxypropylene surfactants. *Journal of Colloid and Interface Science*, *540*, 177–184.

Rezaee, M., Basri, M., Raja Abdul Rahman, R. N. Z., Salleh, A. B., Chaibakhsh, N., & Fard Masoumi, H. R. (2014). A multivariate modeling for analysis of factors controlling the particle size and viscosity in palm kernel oil esters-based nanoemulsions. *Industrial Crops and Products*, *52*, 506–511.

Robinson, P. K. (2015). Enzymes: principles and biotechnological applications. *Essays in Biochemistry*, *59*, 1–41.

Roh, J. S., Han, J. Y., Kim, J. H., & Hwang, J. K. (2004). Inhibitory effects of active compounds isolated from safflower *(Carthamus tinctorius L.)* seeds for melanogenesis. *Biological and Pharmaceutical Bulletin*, *27*(12), 1976–1978.

Roselan, M. A., Ashari, S. E., Faujan, N. H., Mohd Faudzi, S. M., & Mohamad, R. (2020). An improved nanoemulsion formulation containing kojic monooleate: optimization, characterization and in vitro studies. *Molecules*, *25*(11), 1–23.

Ruiz, M. A., Clares, B., Morales, M. E., Cazalla, S., & Gallardo, V. (2007). Preparation and stability of cosmetic formulations with an anti-aging peptide. *Journal of Cosmetic Science*, *58*(2), 157–171.

Safari, R., & Yaghoubzadeh, Z. (2020). Antioxidant activity of bioactive peptides extracted from sea cucumber *(Holothuria leucospilata). International Journal of Peptide Research and Therapeutics*, *26*(4), 2393–2398.

Saharudin, S. H., Ahmad, S., & Basri, M. (2016). Role of xanthan gum on physicochemical and rheological properties of rice bran oil emulsion. *International Food Research Journal*, *23*(4), 1361–1366.

Sahu, R., Saxena, P., & Johnson, S. (2014). *Heavy metals in cosmetics*. *Centre for Science and Environment* (Vol. 46). India.

Sainsbury, F., Zeng, B., & Middelberg, A. P. J. (2014). Towards designer nanoemulsions for precision delivery of therapeutics. *Current Opinion in Chemical Engineering*, *4*, 11–17.

Salamanca, C. H., Barrera-Ocampo, A., Lasso, J. C., Camacho, N., & Yarce, C. J. (2018). Franz diffusion cell approach for pre-formulation characterisation of ketoprofen semi-solid dosage forms. *Pharmaceutics*, *10*(3), 1–10.

Salim, N., Basri, M., Rahman, M. B. A., Abdullah, D. K., & Basri, H. (2012). Modification of palm kernel oil esters nanoemulsions with hydrocolloid gum for enhanced topical delivery of ibuprofen. *International Journal of Nanomedicine*, *7*, 4739–4747.

Samson, S., Basri, M., Fard Masoumi, H. R., Abedi Karjiban, R., & Abdul Malek, E. (2016). Design and development of a nanoemulsion system containing copper peptide by D-optimal mixture design and evaluation of its physicochemical properties. *RSC Advances*, *6*(22), 17845–17856.

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: a review. *Food Quality and Safety*, *1*, 29–46.

Santos, S. D. dos, Martins, V. G., Salas-Mellado, M., & Prentice, C. (2011). Evaluation of functional properties in protein hydrolysates from bluewing searobin *(Prionotus punctatus)* obtained with different microbial enzymes. *Food and Bioprocess Technology*, *4*, 1399–1406.

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: a review. *Peptides*, *31*(10), 1949–1956.

Sbroggio, M. F., Montilha, M. S., Figueiredo, V. R. G. de, Georgetti, S. R., & Kurozawa, L. E. (2016). Influence of the degree of hydrolysis and type of enzyme on antioxidant activity of okara protein hydrolysates. *Food Science and Technology*, *36*(2), 375–381.

Segger, D., Abmus, U., Brock, M., Erasmy, J., Finkel, P., Fitzner, A., & Weser, G. (2008). Multicenter study on measurment of the natural pH of the skin surface. *International Journal of Cosmetic Science*, *30*, 75–76.

Shahinozzaman, M., Ishii, T., Takano, R., A. Halim, M., Hossain, M. A., & Tawata, S. (2018). Cytotoxic desulfated saponin from Holothuria atra predicted to have high binding affinity to the oncogenic kinase PAK1: a combined in vitro and in silico study. *Scientia Pharmaceutica*, *86*(32), 1–13.

Shakeel, F., & Ramadan, W. (2010). Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. *Colloids and Surfaces B: Biointerfaces*, *75*, 356–362.

Shen, L., & Tang, C. H. (2012). Microfluidization as a potential technique to modify surface properties of soy protein isolate. *Food Research International*, *48*, 108–118.

Shoaib, M. H., Tazeen, J., Merchant, H. A., & Yousuf, R. I. (2006). Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. *Pakistan Journal of Pharmaceutical Sciences*, *19*(2), 119–124.

Sicuro, B., Piccinno, M., Gai, F., Abete, M. C., Danieli, A., Dapra, F., Mioletti, F., & Vilella, S. (2012). Food quality and safety of mediterranean sea cucumbers *Holothuria tubulosa* and *Holothuria polii* in southern adriatic sea. *Asian Journal of Animal and Veterinary Advances*, *7*(9), 851–859.

Sila, A., & Bougatef, A. (2016). Antioxidant peptides from marine by-products: isolation, identification and application in food systems. a review. *Journal of Functional Foods*, *21*, 10–26.

Silva, H. D., Cerqueira, M. A., & Vicente, A. A. (2015). Influence of surfactant and processing conditions in the stability of oil-in-water nanoemulsions. *Journal of Food Engineering*, *167*, 89–98.

Singh, Y., Meher, J. G., Raval, K., Khan, F. A., Chaurasia, M., Jain, N. K., & Chourasia, M. K. (2017). Nanoemulsion: concepts, development and applications in drug delivery. *Journal of Controlled Release*, *252*, 28–49.

Sonklin, C., Laohakunjit, N., & Kerdchoechuen, O. (2018). Assessment of antioxidant properties of membrane ultrafiltration peptides from mungbean meal protein hydrolysates. *PeerJ*, (7), 1–20.

Sripokar, P., Benjakul, S., & Klomklao, S. (2019). Antioxidant and functional properties of protein hydrolysates obtained from starry triggerfish muscle using trypsin from albacore tuna liver. *Biocatalysis and Agricultural Biotechnology*, *17*, 447–454.

Sroyraya, M., Hanna, P. J., Siangcham, T., Tinikul, R., Jattujan, P., Poomtong, T., & Sobhon, P. (2017). Nutritional components of the sea cucumber *Holothuria scabra*. *Functional Foods in Health and Disease*, *7*(3), 168–181.

Standards Malaysia. (2008). Islamic consumer goods- part 1: cosmetic and personal care- general guidelines (Vol. MS 2200: P). Retrieved from https://law.resource.org/pub/my /ibr/ms.2200.1.e.2008.pdf

Sugumar, S., Ghosh, V., Nirmala, M. J., Mukherjee, A., & Chandrasekaran, N. (2014). Ultrasonic emulsification of eucalyptus oil nanoemulsion: antibacterial activity against *Staphylococcus aureus* and wound healing activity in wistar rats. *Ultrasonics Sonochemistry*, *21*(3).

Suter, F., Schmid, D., Wandrey, F., & Zülli, F. (2016). Heptapeptide-loaded solid lipid nanoparticles for cosmetic anti-aging applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *108*, 304–309.

Syed Azhar, S. N. A., Ashari, S. E., & Salim, N. (2018). Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. *International Journal of Nanomedicine*, *13*, 6465–6479.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, *108–109*, 303–318.

Tanzadehpanah, H., Asoodeh, A., & Chamani, J. (2012). An antioxidant peptide derived from ostrich *(Struthio camelus)* egg white protein hydrolysates. *Food Research International*, *49*, 105–111.

Thanasukarn, P., Pongsawatmanit, R., & McClements, D. J. (2004). Influence of emulsifier type on freeze-thaw stability of hydrogenated palm oil-in-water emulsions. *Food Hydrocolloids*, *18*(6), 1033–1043.

Tirok, S., Scherze, I., & Muschiolik, G. (2001). Behaviour of formula emulsions containing hydrolysed whey protein and various lecithins. *Colloids and Surfaces B: Biointerfaces*, *21*, 149–162.

Tobin, D. J. (2017). Introduction to skin aging. *Journal of Tissue Viability*, *26*, 37–46.

Tonglairoum, P., & Opanasopit, P. (2015). Effect of nutrient formulations on permeation of proteins and lipids through porcine intestine in vitro. *Tropical Journal of Pharmaceutical Research*, *14*(7), 1161–1166.

Tsai, M. J., Fu, Y. S., Lin, Y. H., Huang, Y. Bin, & Wu, P. C. (2014). The effect of

nanoemulsion as a carrier of hydrophilic compound for transdermal delivery. *PLoS ONE*, *9*(7), 1–7.

Uchida, R., Ishikawa, S., & Tomoda, H. (2014). Inhibition of tyrosinase activity and melanine pigmentation by 2-hydroxytyrosol. *Acta Pharmaceutica Sinica B*, *4*(2), 141–145.

Venkatesan, J., Anil, S., Kim, S. K., & Shim, M. S. (2017). Marine fish proteins and peptides for cosmeceuticals: a review. *Marine Drugs*, *15*(5), 1–18.

Vig, K., Chaudhari, A., Tripathi, S., Dixit, S., Sahu, R., Pillai, S., & Singh, S. R. (2017). Advances in skin regeneration using tissue engineering. *International Journal of Molecular Sciences*, *18*(789), 1–19.

Villareal, M. O., Kume, S., Bourhim, T., Bakhtaoui, F. Z., Kashiwagi, K., Han, J., & Isoda, H. (2013). Activation of MITF by argan oil leads to the inhibition of the tyrosinase and dopachrome tautomerase expressions in B16 murine melanoma cells. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–9.

Vogeley, C., Esser, C., Tüting, T., Krutmann, J., & Haarmann-Stemmann, T. (2019). Role of the aryl hydrocarbon receptor in environmentally induced skin aging and skin carcinogenesis. *International Journal of Molecular Sciences*, *20*(1–21).

Wahgiman, N. A., Salim, N., Abdul Rahman, M. B., & Ashari, S. E. (2019). Optimization of nanoemulsion containing gemcitabine and evaluation of its cytotoxicity towards human fetal lung fibroblast (MRC5) and human lung carcinoma (A549) cells. *International Journal of Nanomedicine*, *14*, 7323–7338.

Wan Mohtar, W. A. A.-Q. I., Abdul Hamid, A., Abd-Aziz, S., Syed Muhamad, S. K., & Saari, N. (2014). Preparation of bioactive peptides with high angiotensin converting enzyme inhibitory activity from winged bean *Psophocarpus tetragonolobus (L.) DC.* seed. *Journal of Food Science and Technology*, *51*(12), 3658–3668.

Wang, Y. H., Wan, Z. L., Yang, X. Q., Wang, J. M., Guo, J., & Lin, Y. (2016). Colloidal complexation of zein hydrolysate with tannic acid: Constructing peptides-based nanoemulsions for alga oil delivery. *Food Hydrocolloids*, *54*, 40–48.

Wanjari, N., & Waghmare, J. (2015). A review on latest trend of cosmetics-cosmeceuticals. *International Journal of Pharma Research & Review*, *4*(5), 45–51.

Wooster, T. J., Golding, M., & Sanguansri, P. (2008). Impact of oil type on nanoemulsion formulation and ostwald ripening stability. *Langmuir*, *24*(22), 12758–12765.

Wu, Y., Tian, Q., Li, L., Khan, M. N., Yang, X., Zhang, Z., Hu., X., & Chen, S.

(2013). Inhibitory effect of antioxidant peptides derived from *Pinctada fucata* protein on ultraviolet-induced photoaging in mice. *Journal of Functional Foods*, *5*, 527–538.

Yukuyama, M. N., Ghisleni, D. D. M., Pinto, T. J. A., & Bou-Chacra, N. A. (2016). Nanoemulsion: process selection and application in cosmetics- a review. *International Journal of Cosmetic Science*, *38*, 13–24.

Zemour, K., Labdelli, A., Adda, A., Dellal, A., Talou, T., & Merah, O. (2019). Phenol content and antioxidant and antiaging activity of safflower seed oil *(Carthamus tinctorius L.). Cosmetics*, *6*(55), 1–11.

Zhang, L., & Falla, T. J. (2009). Cosmeceuticals and peptides. *Clinics in Dermatology*, *27*, 485–494.

Zhang, Z., & McClements, D. J. (2018). Overview of nanoemulsion properties: stability, rheology, and appearance. In *Nanoemulsions* (pp. 21–49). Elsevier Inc.

Zhuang, Y. L., Zhao, X., & Li, B. F. (2009). Optimization of antioxidant activity by response surface methodology in hydrolysates of jellyfish *(Rhopilema esculentum)* umbrella collagen. *Journal of Zheijiang University SCIENCE B*, *10*(8), 572–579.

Zielińska, A., & Nowak, I. (2014). Fatty acids in vegetable oils and their importance in cosmetic industry. *Chemik*, *68*(2), 103–110.

Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 279–309.

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Aqilah Noor binti Bahari was born in Raub, Pahang on 20th March 1987. She had her primary school at Methodist Girls School, Raub Pahang from 1994 until 1999. She continues her secondary school at SMKA Dong, Raub from 2000 to 2002 and SMKA Al-Ihsan, Kuantan Pahang from 2003 to 2004. Later, she completed her matriculation level at Malacca Matriculation College majoring in Biological Sciences. In 2006, she was accepted to further her studies at Universiti Putra Malaysia (UPM) and graduated for her first degree in Bachelor Science-Petroleum Chemistry in 2009. In the same year, she pursued her Master of Science degree in chemistry majoring catalysis under supervision of Professor Taufiq Yap Yun Hin and graduated in 2012 with Master of Science- Catalysis. Her MSc was supported by graduate research fellowship (GRF) by Universiti Putra Malaysia and Bajet Mini 2009 by Ministry of Higher Education, Malaysia. In 2015, she continued her study in the same university (Halal Products Research Institute) and enrolled in Doctor of Philosophy programme in Halal Products Development under supervision of Dr. Norazlinaliza Salim. During her PhD, she was awarded MyPhD under MyBRAIN15 programme by Ministry of Higher Education, Malaysia.

## LIST OF PUBLICATIONS

### Research Papers

**Aqilah Noor Bahari,** Nazamid Saari, Norazlinaliza Salim and Siti Efliza Ashari (2020). Response factorial design analysis on papain-generated hydrolysates from *Actinopyga lecanora* for determination of antioxidant and antityrosinase activities. *Molecules 2020*, 25, 2663.

**Aqilah Noor Bahari,** Norazlinaliza Salim, Siti Efliza Ashari and Nazamid Saari (2021). Development of *Actinopyga lecanora* hydrolysates-based nanoemulsion: *In vitro* penetration and cytotoxicity studies. (Submitted).

### Conference

**Aqilah Noor Bahari,** Nazamid Saari, Norazlinaliza Salim and Siti Efliza Ashari. Design and development of nanoemulsion system containing hydrolysates from *Actinopyga lecanora. International Conference of Analytical Sciences 2018 (SKAM 31),* 17-19 August 2018, Kuantan Pahang. (Oral presenter).

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