HNUE JOURNAL OF SCIENCE
Natural Sciences 2024, Volume 69, Issue 2, pp. 98-109
This paper is available online at http://hnuejs.edu.vn/ns
DOI: 10.18173/2354-1059.2024-0024

STUDY ON PREPARATION AND CHARACTERIZATION OF SACHI SEED OIL BASED EMULSION CONTAINING COLLAGEN PEPTIDES

Nguyen Thuy Chinh[1], Cung Dinh Duc[2], Nguyen Ngoc Quynh[2], Thai Hoang[1], Nguyen Phi Hung[3], Pham Thi Thu Trang[4] and Vu Quoc Trung[5,*]

[1]*Institute for Tropical Technology, Vietnam Academy of Science and Technology, Hanoi city, Vietnam*
[2]*Faculty of Chemical Technology, Hanoi University of Industry, Hanoi city, Vietnam*
[3]*Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi city, Vietnam*
[4]*Faculty of Chemistry, Vietnam Military Medical University, Hanoi city, Vietnam*
[5]*Faculty of Chemistry, Hanoi National University of Education, Hanoi city, Vietnam*
[*]Corresponding author: Vu Quoc Trung, email: trungvq@hnue.edu.vn.

Received May 25, 2024. Revised June 18, 2024. Accepted June 25, 2024.

Abstract. In this study, collagen peptides were carried by a Sacha inchi (sachi) seed oil-based emulsion with Tween 80 under optimal conditions: 26.26% sachi seed oil, 26.06% Tween 80, and 47.68% distilled water. Collagen peptides, varying from 5 - 30% of the total emulsion mass, were dispersed in the aqueous phase before being introduced into the emulsion. Using the sachi seed oil-based emulsion to carry collagen peptides helps prevent the hydrolysis of collagen peptides by acids and enzymes in the digestive system. The characteristics of the emulsion were evaluated using dynamic light scattering, infrared spectroscopy, and ultraviolet-visible spectroscopy. The emulsions loaded with collagen peptides have average droplet sizes ranging from 83.57 - 286.93 nm. Emulsions carrying 5 - 10% collagen peptides exhibit a high stability for up to 30 days. These results demonstrate the potential application of sachi seed oil emulsions carrying collagen peptides in functional foods and healthcare products.

Keywords: collagen peptides, sachi seed oil, emulsion, stability.

1. Introduction

Collagen is widely used for its beauty benefits and improving skin health. However, the natural collagen content in food is shallow. Furthermore, the molecular size of collagen is often larger than naturally produced collagen in the body, making it easily excreted upon

absorption, reducing its effectiveness as a collagen supplement [1], [2]. With its high compatibility with the human body, collagen has been utilized in various medical fields [2], [3]. Extracting collagen from fish by-products is currently of significant interest due to the outstanding characteristics of fish collagen such as high absorption rate, lack of religious barriers, safety, etc [1], [4]-[6]. To enhance collagen absorption, collagen is hydrolyzed into collagen peptides with short amino acid chains [7]-[11]. Collagen peptides, due to their good solubility in water, are better absorbed [7] and exhibit various valuable biological activities such as antioxidant properties [9], wound healing [10], and bone regeneration [11]. Collagen peptides can be degraded by acids and enzymes in the digestive tract. Therefore, developing a suitable nano-delivery system to package collagen peptides to protect and maintain their biological activity in the acidic environment of the stomach is crucial. Hou et al. extracted collagen peptides from catfish skin and incorporated them into an emulsion system for application in wound healing and diabetes treatment [12]. Collagen peptides were extracted in acetic acid combined with ultrasound and hydrolyzed in a one-stage and two-stage process to obtain collagen peptides with molecular weights of 37 kDa and 728 Da, respectively. The emulsion system carrying hydrolyzed collagen with a molecular weight of 728 Da had an average droplet size of 15.3 nm, a dispersity of 0.215, and a zeta potential of -63.0 mV, showing the highest thermal stability and preservation stability. Collagen hydrolysate is accompanied by a soybean oil phase, lecithin emulsifier, Tween 80 surfactant, and water phase. In in-vivo experiments on mice, the emulsion containing collagen peptides was shown to reduce fasting blood sugar levels by up to 46.75% and maintain the largest wound healing area by 95.53%. These emulsions have a great potential for development as functional foods or wound-healing drugs [12]. Sylwia et al. developed a water-in-oil emulsion containing collagen and hyaluronic acid to enhance skin permeability [13]. The emulsions exhibit high stability and potential applications in various fields such as food, cosmetics, pharmaceuticals, and technology [14]-[16].

In emulsion systems, the oil phase plays an important role in the stability properties of the emulsion system. Sacha inchi oil, extracted from the seeds of the Sacha inchi plant (Plukenetia Volubilis L.), is safe, odorless, and contains high levels of essential fatty acids (ω-3, ω-6, ω-9), with linoleic acid accounting for 34-37% and linolenic acid accounting for 42-51% of the total fatty acids present [17]-[22], making it a potential oil phase agent for emulsion systems. The Sacha inchi oil extracted from crops in Vietnam has high levels of fatty acids such as linoleic (42.62%), linolenic (36.32%), and oleic (11.64%) [23]. Pure cold-pressed Sacha inchi oil is suggested as an industrial oil suitable for skin care products without further refinement, as indicated by its physical, chemical, and biological properties [24].

Our previous study confirmed Sacha inchi oil as a suitable oily phase for formulating nano-sized emulsion systems [25]. However, this emulsion system has not been extensively studied in terms of its loading capacity for loading collagen peptides. In another report, these emulsion systems consisting of collagen peptides, Sacha inchi oil, and a surfactant/co-surfactant mixture (Tween 80/propylene glycol) were optimized [26]. Research on formulating emulsion systems using Sacha inchi oil in the presence of Tween 80 for loading collagen peptides has not been extensively explored. Therefore, this study focuses on the

preparation and evaluation of the characteristics of emulsion systems from Sacha inchi oil/Tween 80 loading collagen peptides.

2. Content

2.1. Experiments

2.1.1. Materials

The ingredients and chemicals used in this study include Sacha inchi seed oil (or sachi oil, SO, Vietnam) with omega-3, omega-6, and omega-9 content reaching 45%; surfactant Tween 80 (polysorbate 80, China) with fatty acid content ≥ 58%; distilled water (99.9%, Vietnam); collagen peptides were extracted from fish scales as detailed in reference [26].

2.1.2. Preparation of emulsions from Sacha inchi seed oil loading collagen peptides

The composition of the emulsion system was optimized using response surface methodology and a Box-Behnken model consisting of 26.26% SO, 26.06% Tween 80, and 47.68% distilled water [25]. In this study, the optimal ratio of the emulsion system was kept constant, while the collagen peptide content varied from 5-30% in comparison with the total mass of the emulsion. Emulsion samples with collagen content of 5%, 10%, 15%, 20%, and 30% were designated as C1, C2, C3, C4, and C5, respectively.

The process of preparing emulsion from SO loaded with collagen peptides at various ratios of collagen peptides is described as follows: First, 5 clean and dried bottoms were prepared and marked in order as samples 1, 2, 3, 4, 5. The total mass of the emulsion sample to be prepared is 5 g. The SO and Tween 80 components were weighed and mixed evenly for 5 minutes before being ultrasonicated for 30 minutes at 40 ^{o}C. After ultrasonication, the SO and Tween 80 mixture was stirred for 5 minutes using a vortex mixer (Dlab, China). At the same time, the collagen peptide solution was prepared by dissolving collagen peptides in distilled water. Then, slowly add the collagen peptide solution to the bottoms containing the SO-Tween 80 mixture above. The mixture was then stirred for 5 minutes and ultrasonicated for 30 minutes at room temperature. This process was repeated 3 times to achieve a homogeneous mixture. The emulsion samples were stored in the refrigerator for 24 hours to stabilize the system. At the end of the process, 5 samples of thick, milky-white structures were obtained. Subsequently, these emulsion samples were stored at room temperature to evaluate their stability.

2.2. Characterization

Samples of emulsions from SO loading collagen peptides were analyzed by infrared spectroscopy (IR) using an iS10 infrared spectrophotometer (USA) in the wavenumbers range from 4000 - 400 cm^{-1}, with 32 scans, and a resolution of 8 cm^{-1}. The ultraviolet-visible (UV-Vis) spectra of the samples were recorded using a Libra S80 UV-Vis spectrophotometer (Biochrom, UK) in the wavelength range of 200 to 800 nm. The emulsion samples were diluted 1000 times for UV-Vis absorption and transmission spectra recording [26], [27]. Droplet size distribution, average droplet size, and Zeta potential analysis were carried out based on photon correlation spectroscopy (PCS) and

dynamic light scattering (DLS) on an SZ-100Z2 nanoparticle size analyzer (Horiba, Japan). The samples were diluted 100 times to measure the average droplet size and Zeta potential [26], [27].

2.3. Results and discussion

2.3.1. Characteristics of SO emulsion systems loading collagen peptides

The average droplet size of the SO emulsions containing collagen peptides was determined using the DLS technique. The droplet size distribution graph of the SO emulsions loading collagen peptides is presented in Figure 1. It can be seen that the SO emulsion samples containing collagen peptides have droplet sizes distributed from 75 - 610 nm. These are considered as the sizes of nano and sub-micron emulsion systems [15], [16]. Table 1 presents the average droplet size and polydispersity index (PDI) of the SO emulsion systems containing collagen peptides. The SO emulsions loading collagen peptides have relatively small average droplet sizes, ranging from 83.57 - 286.93 nm. These emulsions all have a PDI > 0.2 indicating they are polydisperse systems. To achieve monodisperse systems, filtration membranes can be used to obtain more uniformly sized emulsion particles.

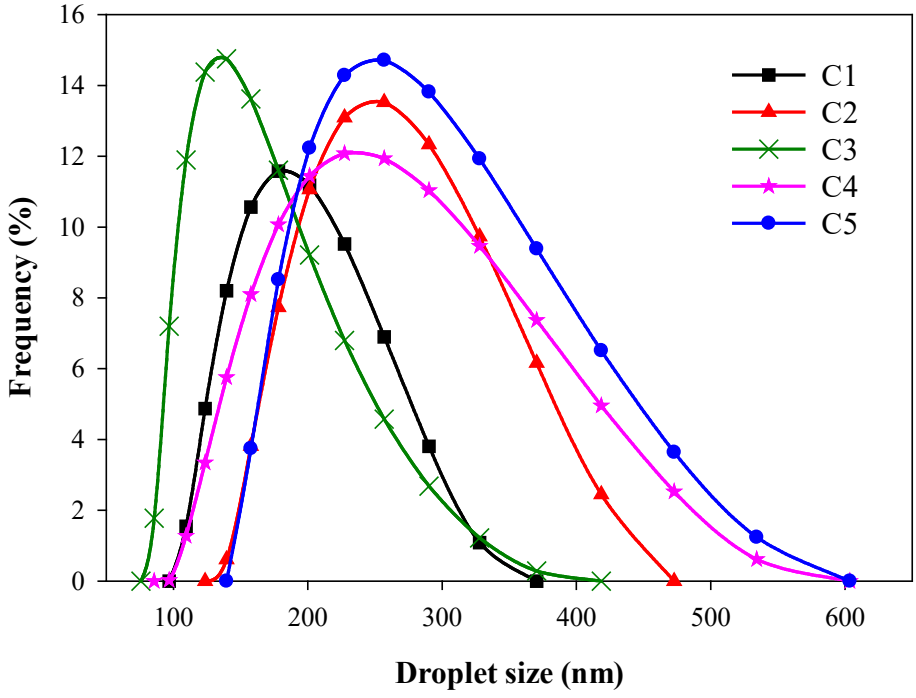

Figure 1. Droplet size distribution of SO emulsion systems loading collagen peptides

The results in Table 1 also show that the average droplet size of the emulsions significantly depends on the collagen peptide content. As the collagen peptide content in the sample increases, the average droplet size of the emulsion systems also increases. For instance, the droplet size of sample C1 is 83.57 ± 5.54 nm. When the collagen peptide content is increased from 10 - 30%, the average droplet size of the emulsion system increases from 157.80 to 286.93 nm. This could be due to an excess of collagen in the

emulsion system causing a significant increase in droplet size. Samples C1 and C2 with smaller average droplet sizes would be more suitable for application studies of SO emulsions carrying collagen peptides.

The Zeta potential and electrophoretic mobility of emulsions made from sacha inchi seed oil containing collagen peptide are listed in Table 2. It can be observed that all droplets in the emulsion systems exhibit a negative charge. The Zeta potential values of samples C1, C2, C3, and C4 are all less than -30 mV, specifically -34.23 ± 1.55 mV, -31.11 ± 0.74 mV, -32.00 ± 3.97 mV, and -32.43 ± 8.30 mV, respectively, indicating the stability of these emulsion systems when dispersed in water. The electrophoretic mobility of the samples is directly proportional to the Zeta potential. The less stable sample C5 may be due to an excessive amount of collagen peptide present.

Table 1. The average droplet size of SO emulsion systems loading collagen peptides

Sample	Average droplet size (nm)	PDI
C1	83.57 ± 5.54	0.541 ± 0.055
C2	157.80 ± 1.06	0.454 ± 0.073
C3	159.87 ± 1.55	0.383 ± 0.123
C4	219.57 ± 9.76	0.275 ± 0.242
C5	286.93 ± 7.57	0.356 ± 0.100

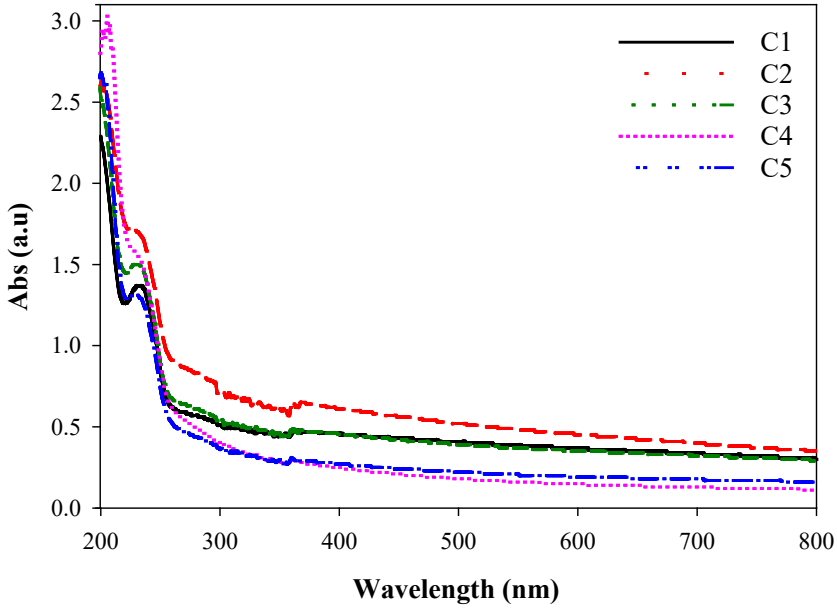

Figure 2. Absorption UV-Vis spectra of SO emulsion systems loading collagen peptides

Figure 2 illustrates the UV-Vis absorption spectra of SO emulsions containing different ratios of collagen peptides. The absorption peak between 200 nm to 300 nm in the UV-Vis spectra of emulsions is characteristic of the π-π interaction of SO and collagen peptides [28], [29]. Sample C5, with the lowest absorption, indicates lower stability, possibly due to a lower content of collagen peptides that loaded in the SO emulsion.

Comparing the UV-Vis light transmittance capabilities of SO emulsions containing collagen peptides, it is evident that these emulsions have a transmittance capacity ranging from 44% - 76% (Figure 3). The transmittance decreases as increasing the collagen peptide content in the emulsion samples, aligning with the analysis of the average droplet sizes presented in Table 2.

Table 2. Zeta potential and electrophoretic mobility of SO emulsion systems loading collagen peptides

Sample	Zeta potential (mV)	Electrophoretic mobility (cm^2/Vs)
C1	-34.23 ± 1.55	$-2.65x10^{-4} \pm 2.7x10^{-5}$
C2	-31.11 ± 0.74	$-2.54x10^{-4} \pm 1.2x10^{-5}$
C3	-32.00 ± 3.97	$-2.27x10^{-4} \pm 2.1x10^{-5}$
C4	-32.43 ± 8.30	$-2.51x10^{-4} \pm 6.5x10^{-5}$
C5	-28.13 ± 3.77	$-2.18x10^{-4} \pm 2.9x10^{-5}$

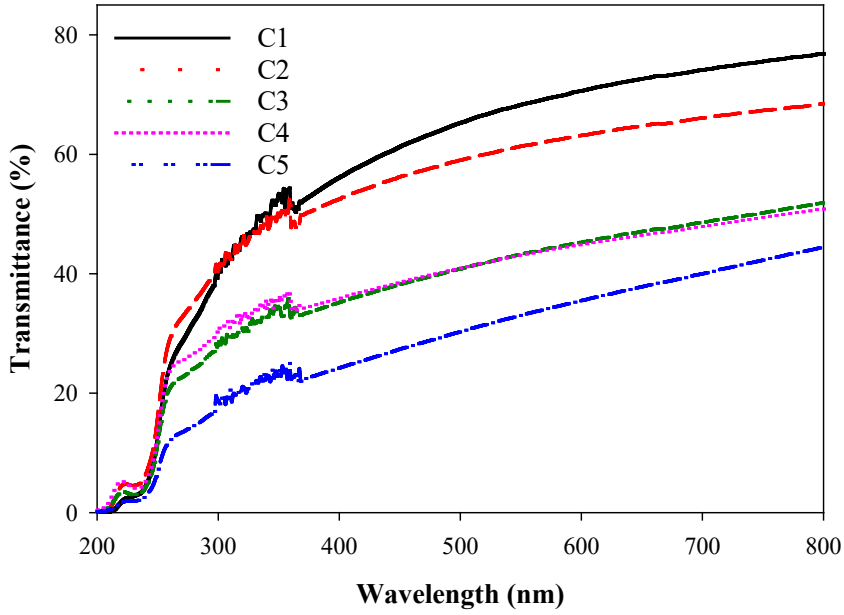

Figure 3. Transmittance UV-Vis spectra of SO emulsion systems loading collagen peptides

The IR spectra of SO emulsions containing different contents of collagen peptides are shown in Figure 4. The appearance of characteristic peaks in the vibration of functional groups in SO, Tween 80, and collagen peptides can be observed. Specifically, the peak at 3306 cm^{-1} is characteristic of the stretching vibration of O-H and N-H bonds; peaks at 2916, 2848, 1455, and 1403 cm^{-1} are characteristic of the stretching and deformation vibrations of C-H bonds; the peak at 1741 cm^{-1} is characteristic of the stretching vibration of C=O bonds; the peak at 1634 cm^{-1} corresponds to the stretching

vibration of C=C bonds; the peak at 1548 cm^{-1} is attributed to the deformation vibration of O-H, N-H bonds; peaks at 1025 - 1249 cm^{-1} are characteristic of the stretching vibration of C-O and C-C bonds [26], [28], [30]. The variation in the collagen peptide content does not significantly affect the vibration of characteristic functional groups in the emulsions.

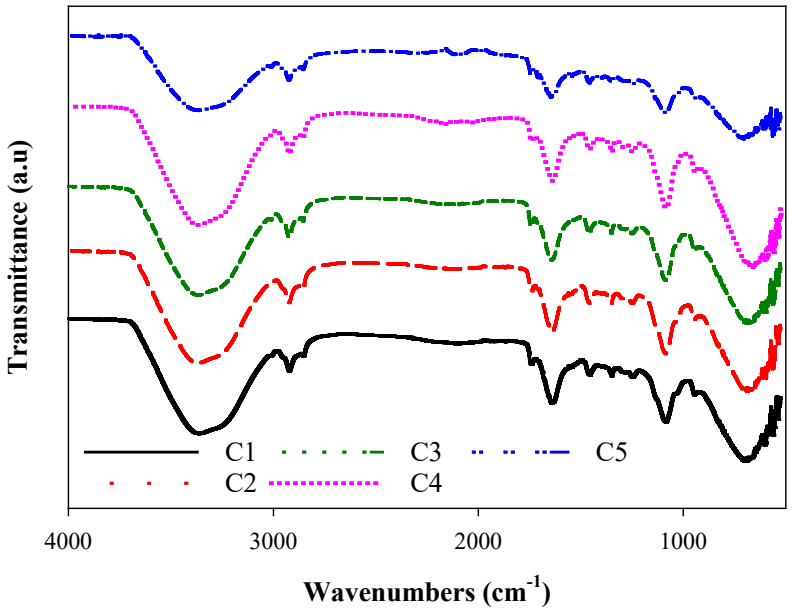

Figure 4. IR spectra of SO emulsion systems loading collagen peptides

2.3.2. Stability of SO emulsion systems loading collagen peptides

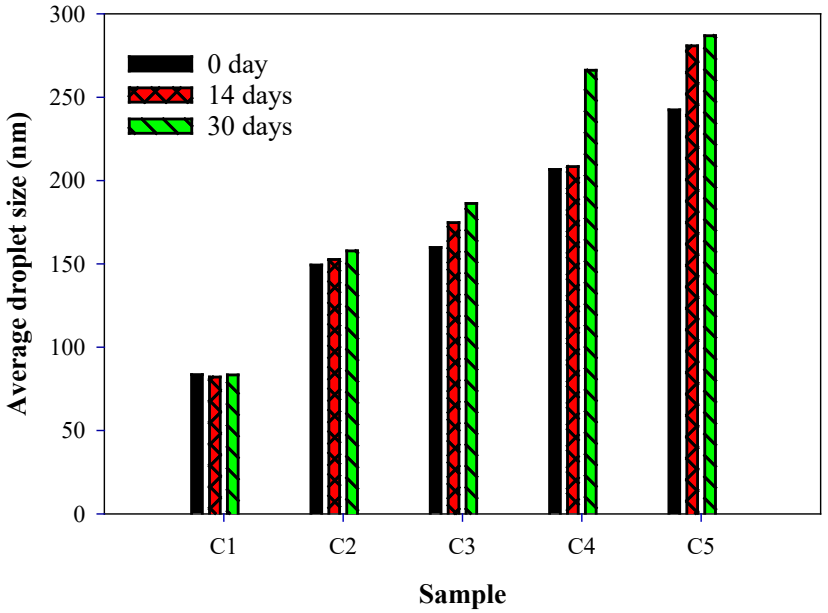

Figure 5. The average droplet size of SO emulsion systems loading collagen peptides at the time of 0, 14, and 30 days

The stability of SO emulsion systems loading collagen peptides was evaluated over 30 days at room temperature based on the analysis of average droplet size, Zeta potential, and UV transmittance at a wavelength of 800 nm. The average droplet size of emulsions C1, C2, C3, C4, and C5 at 0, 14, and 30 days is presented in Figure 5. Upon observation of Figure 5, it can be seen that samples C1 and C2 remain stable after 30 days. The average droplet size of samples C3, C4, and C5 slightly increases, especially at the time of 30 days. This is because samples C3, C4, and C5 are less stable, and prolonged storage at room temperature leads to the aggregation of droplets, increasing the droplet size of the emulsions. Additionally, the excess collagen peptide content can cause phase separation, further contributing to samples C3, C4, and C5 instability. Comparing the UV transmittance of emulsions from sacha inchi oil containing collagen peptide at a wavelength of 800 nm in Figure 6, it is evident that samples C1 and C2 maintain the highest transmittance among the emulsions studied, with minimal differences indicating their relative stability throughout the study period.

Table 3. Zeta potential (mV) of SO emulsion systems loading collagen peptides at the time of 0, 14, and 30 days

No.	Sample	0 day	14 days	30 days
1	C1	-34.23 ± 1.55	-33.33 ± 0.98	-34.87 ± 3.71
2	C2	-31.11 ± 0.74	-32.40 ± 6.25	-32.20 ±3.40
3	C3	-32.00 ± 3.97	-31.87± 0.28	-28.63 ± 0.70
4	C4	-32.43 ± 8.30	-32.87± 1.46	-29.35 ± 0.78
5	C5	-28.13 ± 3.77	-29.30 ± 2.29	-28.53 ± 2.33

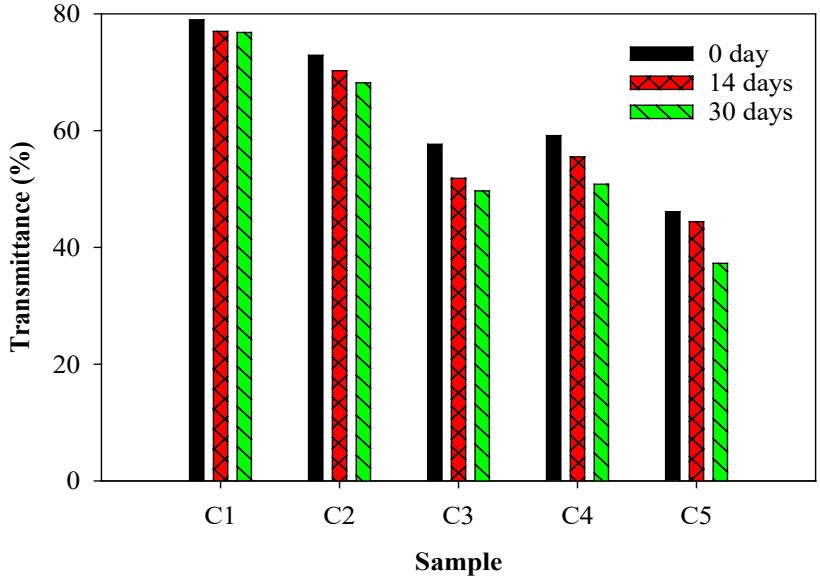

Figure 6. UV transmission at 800 nm of SO emulsion systems loading collagen peptides at the time of 0, 14, and 30 days

Table 3 presents the results of determining the Zeta potential of SO emulsions consisting of collagen peptides at 0, 14, and 30 days. It can be observed that the Zeta potential of samples C1 and C2 remains stable after 30 days and is consistently below -30 mV, indicating good stability of these two emulsions. The Zeta potential of samples C3, C4, and C5 shows more variation, suggesting lower stability compared to C1 and C2. Based on the results obtained, it is evident that samples C1 and C2 exhibit high stability and small particle size. Therefore, the appropriate collagen peptide content in the emulsions is ≤ 10%.

3. Conclusions

Collagen peptides have been loaded in the emulsions containing Sacha inchi seed oil, and Tween 80 surfactant under optimal manufacturing conditions. The average droplet size of the emulsions reached below 300 nm. The functional groups characteristic of the components in the emulsions have been confirmed by IR analysis. The emulsions are capable of absorbing UV light in the wavelength range of 200-300 nm. Increasing the collagen peptide content reduces the UV-Vis transmittance of the samples. Emulsions containing 5% and 10% collagen peptides exhibit stability for 30 days, surpassing those with 15-30% collagen peptides. The results suggest that emulsions carrying 5-10% collagen peptides have potential applications as oral supplements in functional foods or topical creams.

Acknowledgment. This research is funded by the Vietnam Academy of Science and Technology under grant number UDSXTN.06/23-24.

REFERENCES

[1] Subhan F, Hussain Z, Tauseef I, Shehzad A & Wahid F, (2021). A review of recent advances and applications of fish collagen. *Critical Reviews in Food Science and Nutrition*, 61(6), 1027-1037. https://doi.org/10.1080/10408398.2020.1751585.

[2] Wang Y, Wang Z & Dong Y, (2023). Collagen-based biomaterials for tissue engineering. *ACS Biomaterials Science & Engineering,* 9(3), 1132-1150. https://doi.org/10.1021/ acsbiomaterials.2c00730.

[3] Rezvani GE, Nourbakhsh N, Akbari KM, Zare M & Ramakrishna S, (2021). Collagen-based biomaterials for biomedical applications. *Journal of Biomedical Materials Research B Appl Biomater*, 109(12), 1986-1999. https://doi.org/10.1002/ jbm.b.34881.

[4] Gaikwad S & Kim MJ, (2024). Fish by-product collagen extraction using different methods and their application. *Marine Drugs*, 22(2), 60. https://doi.org/10.3390/ md22020060.

[5] Sarkar MSI, Hasan MM, Hossain MS, Khan M, Islam AA, Paul SK, Rasul MG & Kamal M, (2023). Exploring fish in a new way: A review on non-food industrial applications of fish. *Heliyon*, 9(12), e22673. https://doi.org/10.1016/j.heliyon. 2023.e22673.

[6] Tran TM, Nguyen TC, Vu QM, Nguyen TTT, Tran DMT, Vu QT, Ha VH & Thai H, (2019). Effect of fish scale collagen on some characteristics and drug release of carrageenan/collagen/allopurinol film. *Vietnam Journal of Science and Technology*, 57 (3B), 1-8. http://dx.doi.org/10.15625/2525-2518/57/3B/14044.

[7] Watanabe KM, Shimizu M, Kamiyama S, Taguchi T, Sone H, Morimatsu F, Shirakawa H, Furukawa Y & Komai M, (2010). Absorption and effectiveness of orally administered low molecular weight collagen hydrolysate in rats. *Journal of Agricultural and Food Chemistry*, 58, 835-841. https://doi.org/10.1021/jf9031487.

[8] Liu D, Liang L, Regenstein JM & Zhou P, (2012). Extraction and characterization of pepsin-solubilised collagen from fins, scales, skins, bones, and swim bladders of bighead carp (*Hypophthalmichthysnobilis*). *Food Chemistry*, 133, 1441-1448. https://doi.org/10.1016/j.foodchem.2012.02.032.

[9] Ding J, Li Y, Xu J, Su X, Gao X & Yue F, (2011). Study on the effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. *Food Hydrocolloids l*, 25, 1350-1353. https://doi.org/10.1016/j.foodhyd.2010.12.013.

[10] Zhang Z, Wang J, Ding Y, Dai X & Li Y, (2011). Oral administration of marine collagen peptides from Chum Salmon skin enhances cutaneous wound healing and angiogenesis in rats. *Journal of the Science of Food and Agriculture,* 91(12), 2173-2179. https://doi.org/10.1002/jsfa.4435.

[11] Yamada S, Yamamoto K, Nakazono A, Matsuura T & Yoshimura A, (2021). Functional roles of fish collagen peptides on bone regeneration. *Dental Materials Journal,* 40(6), 1295-1302. https://doi.org/10.4012/dmj.2020-446.

[12] Hou NT & Chen BH, (2023). Preparation of nanoemulsions with low-molecular-weight collagen peptides from sturgeon fish skin and evaluation of anti-diabetic and wound-healing effects in mice. *Pharmaceutics*, 15(9), 2304. https://doi.org/10.3390/ pharmaceutics15092304.

[13] Kupper S, Chomiczewska IK & Szumała P, (2017). Collagen and hyaluronic acid hydrogel in water-in-oil microemulsion delivery systems. *Carbohydrate Polymers*, 175, 347-354. https://doi.org/10.1016/j.carbpol.2017.08.010.

[14] Shukla T, Upmanyu N, Agrawal M, Saraf S, Saraf S & Alexander A, (2018). Biomedical applications of microemulsion through dermal and transdermal route. *Biomedicine & Pharmacotherapy*, 108, 1477-1494. https://doi.org/10.1016/j.biopha. 2018.10.021.

[15] Magrode N, Poomanee W, Kiattisin K & Ampasavate C, (2024). Microemulsions and nanoemulsions for topical delivery of tripeptide-3: from design of experiment to anti-sebum efficacy on facial skin. *Pharmaceutics*, 16 (4), 554. https://doi.org/10.3390% 2Fpharmaceutics16040554.

[16] Nguyen TC & Thai H, (2023). Review: emulsion techniques for producing polymer based drug delivery systems. *Vietnam Journal of Science and Technology*, 61 (1), 1-26. https://doi.org/10.15625/2525-2518/17666.

[17] Nur ARMR & Lai KL, (2022). Sacha Inchi (*Plukenetia Volubilis* L.): recent insight on phytochemistry, pharmacology, organoleptic, safety and toxicity perspectives. *Heliyon*, 8 (9), e10572. https://doi.org/10.1016/j.heliyon.2022.e10572.

[18] Alexandra V, Frank LRO, Adriana VO, Dayana BR, Ana MM & Fernando RE, (2021). Sacha Inchi Seed (*Plukenetia volubilis* L.) Oil: Terpenoids, *Terpenes and Terpenoids - Recent Advances*, (Eds) Shagufta P and Areej MAT, Intechopen, London, United Kingdom.

[19] Ankit G, Beenu T, Manvesh KS & Vivek S, (2022). Sacha inchi (*Plukenetia volubilis* L.): An emerging source of nutrients, omega-3 fatty acid, and phytochemicals. *Food Chemistry*, 373 (B), 131459. https://doi.org/10.1016/j.foodchem.2021.131459.

[20] Norhazlindah MF, Jahurul MHA, Norliza M, Shihabul A, Shahidul I, Nyam KL & Zaidul ISM, (2023). Techniques for extraction, characterization, and application of oil from sacha inchi (*Plukenetia volubilis* L.) seed: a review. *Journal of Food Measurement and Characterization, 1*7, 904–915. https://doi.org/10.1007/s11694-022-01663-0.

[21] Sunan W, Fan Z & Yukio K, (2018). Sacha inchi (*Plukenetia volubilis* L.): Nutritional composition, biological activity, and uses. *Food Chemistry*, 265, 316-328. https://doi.org/10.1016/j.foodchem.2018.05.055.

[22] Muangrat R, Veeraphong P & Chantee N, (2018). Screw press extraction of Sacha inchi seeds: Oil yield and its chemical composition and antioxidant properties. *Journal of Food Processing and Preservation*, 42(6), e13635. https://doi.org/ 10.1111/jfpp.13635.

[23] Mai HC, Nguyen DC, Nguyen PTN & Bach LG, (2020). Physico-chemical properties of sacha inchi (*Plukenetia volubilis* L.) seed oil from Vietnam. *Asian Journal of Chemistry,* 32, 335-338. https://doi.org/10.14233/ajchem.2020.22233.

[24] Chirinos R, Zuloeta G, Pedreschi R, Mignolet E, Larondelle Y & Campos D, (2013). Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds, and antioxidant capacity. *Food Chemistry*, 141 (3), 1732-1739. https://doi.org/10.1016/j.foodchem.2013.04.078.

[25] Dam XT, Cung DD, Nguyen NQ, Pham TT, Thai H, Nguyen PH & Nguyen TC, (2024). Optimizing the preparation of nanoemulsions based on sacha inchi (*Plukentia volubilis* L.) seed oil by response surface methodology. *Vietnam Journal of Science and Technology*, in press.

[26] Vu TTL, Vu TNL, Mai DH, Nguyen XT, Nguyen PH, Ngo TCQ, Dam XT &Thai H, (2024). Optimizing preparation of fish scale collagen peptide/sacha inchi (*Plukenetia volubilis* L.) seed oil nanoemulsion. *ChemistrySelect*, 9 (11), e202303659. https://doi.org/10.1002/slct.202303659.

[27] More SK & Pawar AP, (2020). Preparation, optimization, and preliminary pharmacokinetic study of curcumin encapsulated turmeric oil microemulsion in zebrafish. *European Journal of Pharmaceutical Sciences*, 155, 105539. https://doi.org/10.1016/j.ejps. 2020.105539.

[28] Yeon JJ, Petra KH & Schaaf US, (2019). Collagen peptide-loaded W_1/O single emulsions and $W_1/O/W_2$ double emulsions: influence of collagen peptide and salt concentration, dispersed phase fraction and type of hydrophilic emulsifier on droplet stability and encapsulation efficiency. *Food & Function*, 10, 3312. https://doi.org/10.1039/C8FO02467G.

[29] Bui VH, Nguyen TAS & Tran KV, (2023). Study on physiological and biochemical parameters of sacha inchi leaf (*Plukenetia volubilis* L.) and the process of making tea bags in Vietnam. *HNUE Journal of Science, Natural Sciences*, 68 (3), 162-174. DOI: 10.18173/2354-1059.2023-0072

[30] Xiaoqiu S, Jinyu W, Shuaitao L & Yifei W, (2022). Formation of sacha inchi oil microemulsion systems: effects of non-ionic surfactants, short-chain alcohols, straight-chain esters, and essential oils. *Journal of the Science of Food and Agriculture*, 102 (9), 3572-3580. https://doi.org/10.1002/jsfa.11703.