IJAIR
International Journal of Agriculture Innovations and Research
Volume 8, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 15/12/2019 | Accepted on: 01/01/2020 | Published: 14/01/2020

Preparation and Characterization of Resveratrol Loaded Nanoemulsions

Qayyum Shehzad [1], Abdur Rehman [1], Ahmad Ali [1], Sohail Khan [1], Amer Ali Mahdi [1], Aiman Karim [1], Salman Khan [2], Fang Yang [1] and Wenshui Xia [1*]

[1] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

[2] School of Biotechnology, Jiangnan University, Wuxi, Jiangu, 214122, China.

*Corresponding author email id: xiaws@jiangnan.edu.cn

***Abstract* – Resveratrol (RES) is an important polyphenolic compound having a wide range of applications in functional foods but the main hurdle in its utilization is its instability and low solubility. In this study, different concentrations of RES (0%, 0.01%, 0.02%, 0.03% and 0.04%) were used with tuna fish oil to prepare the nanoemulsions stabilized by octenyl succinic anhydride-modified starch (OSA-MS) Hi-cap 100. Results indicated that the particle size and polydispersity index (PDI) of fresh and stored nanoemulsions showed irregular behavior. While the increase in size for stored samples was very little, which did not cause any physical instability to the nanoemulsions. The zeta potential (ZP) values of fresh and stored nanoemulsions were significantly increased with the increasing concentration of RES. Further, the rheological properties showed that the apparent viscosity increased with the increasing concentration of RES and a similar trend was observed in fresh and stored nanoemulsions. The confocal microscopy images confirmed very fine and evenly distributed oil droplets. Thus, results revealed that nanoemulsions containing 0.03% RES showed the best and optimum results, however, it could be used in dairy and beverages products.**

***Keywords* – Resveratrol, Nanoemulsions, Hi-cap 100, Physical Stability, Rheology.**

I. INTRODUCTION

Resveratrol (RES) (3, 5, 4'- *trans* - trihydroxystilbene) is one of the most extremely focused phenolic bioactive that abundantly found in grapes, berries, and peanuts. RES has been recognized as a health promotor for human beings (Khan, Yue et al. 2019). The consumption of RES improves the immune system and helps to avoid a number of diseases such as different kinds of cancers, cardiovascular diseases, diabetes, atherosclerosis, inflammation (Kumar, Kaur et al. 2017). In recent times, the incorporation of functional ingredients into food products has been gained much attention from the food industry in order to improve the nutritional requirements of consumers (Rezaei, Fathi et al. 2019). Lipophilic bioactive compounds, such as RES are very sensitive, easy to oxidize and less soluble in water (Borges, Ferreira et al. 2018). Hence, the above-mentioned limitations hinder its therapeutic potential as well as decrease its delivery in edible form (Zhang, Khan et al. 2019). Nanotechnology is an emerging science in the field of the pharmaceutical and food industry, which involves the encapsulation and manipulation of materials at nano-metric size (Rehman, Ahmad et al. 2019). Different types of encapsulation techniques such as micro fluidization, sonication, high-pressure homogenization, spray drying, spray cooling, inclusion complexation, solvent evaporation, extrusion, coacervation, co-crystallization, immobilization, phase inversion, and liposomes are being used in order to encapsulate the bioactive components (Rehman, Tong et al. 2019, Riaz, Iqbal et al. 2019).

Nanoemulsions are known as thermodynamically unstable colloidal systems comprising a size of less than 200 nm (Marwaha and Dabas 2019). The smallest particle size provides more transparency to the nanoemulsions; however, they are being well exploited into transparent food and beverage products. Nanoemulsions are fabricated through the mixing of two immiscible solvents (oil and water) with the help of an appropriate emulsifier in order to achieve a final homogenous dispersion (Li, Li et al. 2020). The properties of emulsifiers are very concerned with the preparation of nanoemulsions (Rehman, Ahmad et al. 2019). Mostly, there are two leading methods used to prepare nanoemulsions; high energy and low energy methods. High energy methods include high-pressure homogenization, micro fluidization, and ultrasonication, while low energy approaches include nanoemulsion inversion point, phase inversion temperature and spontaneous emulsification (Harwansh, Deshmukh et al. 2019). There are various factors that govern the stability of nanoemulsions such as type of lipid phase, type and concentration of emulsifier, emulsification method and storage circumstances (de (by) Oca-Avalos, Candal et al. 2017).

Octenyl succinic anhydride modified starches (OSA-MS) have good emulsifying and wall forming properties that provide stability against environmental stresses (i.e., temperature, pH, ionic strength and digestive tract) (Rehman, Tong et al. 2019). Hi-cap 100 is newly developed an OSA-MS. It has been applied as an emulsifier, stabilizer, and encapsulant in many latest studies (Hasani, Ojagh et al. 2018). It possesses good emulsification properties and required at a low quantity. The aim of this study was to develop a stable delivery system enriched with resveratrol and stabilized with Hi-cap 100, which could be further explored for the production of functional beverages and foods.

II. Material and Methods

Materials and Chemicals

The ≥98% pure resveratrol (RES) was purchased from Sangon Biotech Co. Ltd. Shanghai, China, and tuna fish oil was gifted by Novosana (Taicang) Ltd. Suzhou, Jiangsu, China. The OSA-MS Hi-cap 100 was obtained from National Starch, Bridge Water, USA. Nile Red dye was obtained from Sigma Aldrich Co. Ltd. Deisenhofen, Germany. All other chemicals used in this research were of analytical grade. Double distilled water was used for all experiments.

Preparation of Resveratrol Loaded Oil in Water (O/W) Nanoemulsion

The HI-cap 100 stabilized nanoemulsions were prepared by the method of (Sharif, Williams et al. 2017) with some modifications. A 5% (w/v) HI-cap 100 was dispersed into distilled water and kept stirred overnight at room temperature to ensure its complete hydration. Tuna oil was used as a carrier oil with different concentration of RES (0%, 0.01%, 0.02%, 0.03% and 0.04%). Coarse emulsions were prepared by subjecting the aqueous phase (90% w/w) and lipid phase (10% w/w) to high-speed homogenizer (Ultra-Turrax T25 IKA Janke and Kunkle, GmbH and Co KG, Germany) at 20,000 rpm for 3 minutes at room temperature. After that, the coarse emulsions were then passed through a high-pressure homogenizer (IKA-Labor Pilot 2000/4, IKA-Werke GmbH and Co. Staufen, Germany) at 80 MPa pressure with 7 processing cycles to get the fine droplets. The inlet and outlet temperature were controlled at 15 ^{0} C by using a heat exchanger.

Determination of Size and PDI (Polydispersity Index) of Nanoemulsions

The particle size of nanoemulsions was analyzed as mean droplet diameter by dynamic light scattering analyzer (Zeta sizer Nano ZS, Malvern Instruments, Malvern U.K.). Before measuring the particle size, all samples were diluted 200 times with distilled water and agitated well to avoid multiple light scattering effects. Size measurements of fresh and stored samples were carried out by selecting 12 number of runs and the outcomes expressed in nm. The refractive index value used for oil and water was 1.45 and 1.33. PDI was estimated by cumulant analysis. All analysis was carried out in triplicates.

Determination of Zeta Potential (ZP)

The ZP values of nanoemulsions were recorded by using zeta sizer (Zeta sizer Nano ZS, Malvern Instruments, Malvern, U.K.). Nanoemulsions were diluted (1:200) with distilled water and placed in washed and clear disposable zeta cells. The number of runs was set from 12 (minimum) to 100 (maximum) and ZP was measured from the electrophoretic mobility using the Smoluchowski equation. The ZP values were expressed in mV. All analysis was done in triplicates.

Determination of Rheological Properties

The rheological behavior of fresh and stored RES loaded nanoemulsions stabilized by HI-cap 100 were determined according to the method of (Sharif, Williams et al. 2017) with some modifications, using the Discovery Hybrid Rheometer (DHR-2, TA-Instruments, New Castle, DE, USA). The temperature was maintained at 25 °C and other parameters were set as; cone diameter at 40 mm, the angle at 2^0 and gap at 56 µm. For each analysis, ~1ml of nanoemulsion sample was placed in between the cone and plate fixture. The viscosity of RES loaded nanoemulsions was recorded by a steady-state flow program with the shear rate ranging from 0.1 S^{-1} to 500 S^{-1}. Experimental flow curves were fitted to a power-law model

$$\eta = K\gamma^{n-1} \qquad (1)$$

In this equation, η is the viscosity (Pa·s), K is the consistency index ($Pa \cdot s^n$), γ is the shear rate (S^{-1}), and n is the flow behavior index. The temperature was kept at 25 ^{0}C for all triplicate measurements.

Confocal Laser-Scanning Microscopy (CLSM) Of Nanoemulsion

CLSM was done by following the method of (Sharif, Williams et al. 2017) with some changes. Images of the fresh and stored samples were taken to illustrate the structural changes occurring during storage at room temperature. Nile red (1mg/ml ethanol) dye solution was prepared and excited with a 543 nm continuous-wave argon-ion laser. A Zeiss LSM 710 confocal microscope (Leica, Heidelberg, Germany) with a 40× oil immersion objective lens was used to capture the confocal images. The images generated had a size of 512×512 pixels. All images were taken and processed using the instrument software program (LSM 710 ZEN version 7.2.3, Germany).

Statistical Analysis

The obtained experimental data were further subjected to analysis of variance (ANOVA) and multiple comparison analysis was done by SPSS statistics (software version 19.0) to determine the difference between means at a significance level of $p < 0.05$.

III. Results and Discussions

Size and PDI of Fresh and Stored Nanoemulsions

All prepared nanoemulsions showed the size in the nanometric range (<200nm) as shown in Figure 1a. The addition of RES (0.01%) showed a slight decrease in size, whereas, at 0.02% RES, the size increased as compared to the control sample. On the other hand, nanoemulsion containing 0.03% RES exhibited the smallest particle size as compared to all other nanoemulsions. The PDI is an important parameter that governs the distribution of the particles in the nanoemulsion system and a narrow range of PDI exhibits system stability. PDI values less than 0.3 are considered good that make better stability. There is a continuous rise and fall in PDI values with the increasing concentration of RES (Table 1). Earlier, a similar trend was reported by (Sharif, Williams et al. 2017), that fabricated flax seed oil and eugenol nanoemulsion using OSA-MS as an emulsifier and was found to be stable even after one-month storage. The nanoemulsion with 0.03% RES showed the lowest PDI value as compared to others. All samples were stored for a period of one month at room temperature to check the effect of storage time on the stability of nanoemulsions particle size and PDI values (Figure 1b and Table 1). No phase separation or creaming was observed for all samples after a one-month storage period. All the nanoemulsions were still in nanometric range and their droplet diameter was increased from ~15 to 25 nm. Results revealed that nanoemulsion containing 0.03% RES showed the lowest increase in size during storage.

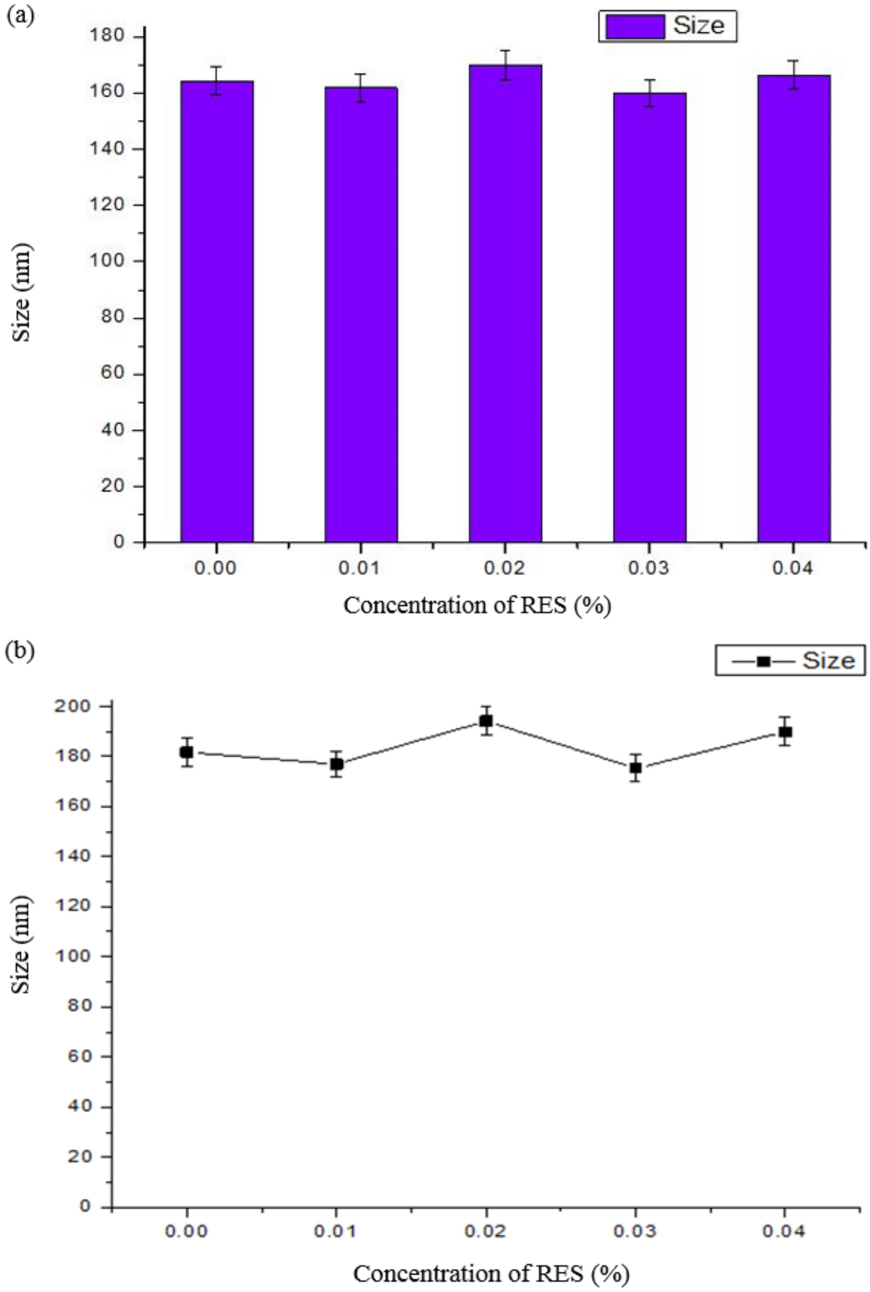


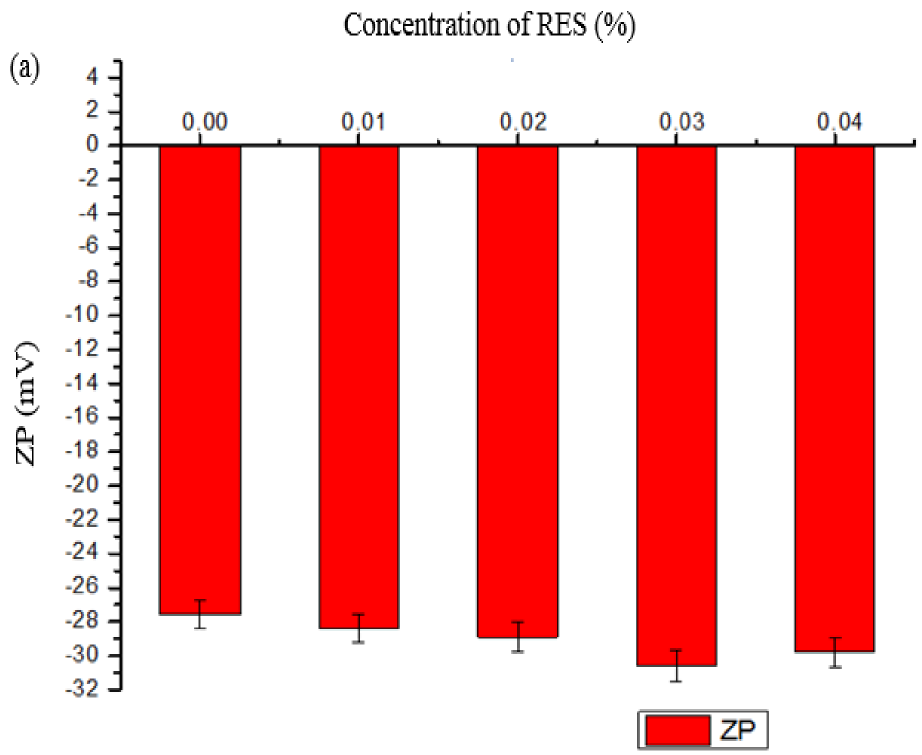
Fig. 1. Impact of different concentrations of RES on the (a) size of freshly prepared nanoemulsions (b) size of stored nanoemulsions.

Table 1. PDI values of the fresh and stored nanoemulsions with different concentrations of RES.

RES Concentrations	PDI (Fresh samples)	PDI (stored samples)
0% RES	0.135 ± 0.00405	0.173 ± 0.00519
0.01% RES	0.114 ± 0.00342	0.169 ± 0.00507
0.02% RES	0.161 ± 0.00483	0.195 ± 0.00585
0.03% RES	0.128 ± 0.00384	0.164 ± 0.00492
0.04% RES	0.138 ± 0.00414	0.177 ± 0.00531

Zeta Potential (ZP) of Fresh and Stored RES Nanoemulsions

ZP is an identical parameter that affects the stability of dispersed oil droplets and its values ranging from very high positive to very high negative are considered better for stability (Dinda, Biswal et al. 2013). Higher ZP means better electrostatic repulsion among the dispersed nanoparticles that enhances stability for a long time (Sharif, Goff et al. 2017). The results of ZP values ranged from -27.6 ± -0.852 to -30.6 ± -0.918 mV (Figure 2a). Negative values of ZP are because of the presence of negative charge on OSA-MS which seems during the esterification process. In present results, with the increasing RES content, an increase in ZP values was observed, which was significantly highest at 0.03% concentration of RES. After storage, a small reduction was observed in ZP values against the different concentrations of RES (Figure 2b). The slight drop in ZP values might be due to the formation of intermediate oxidation products, propanal, which further converted into propanoic acid and caused a reduction in pH (Polavarapu, Oliver et al. 2011).



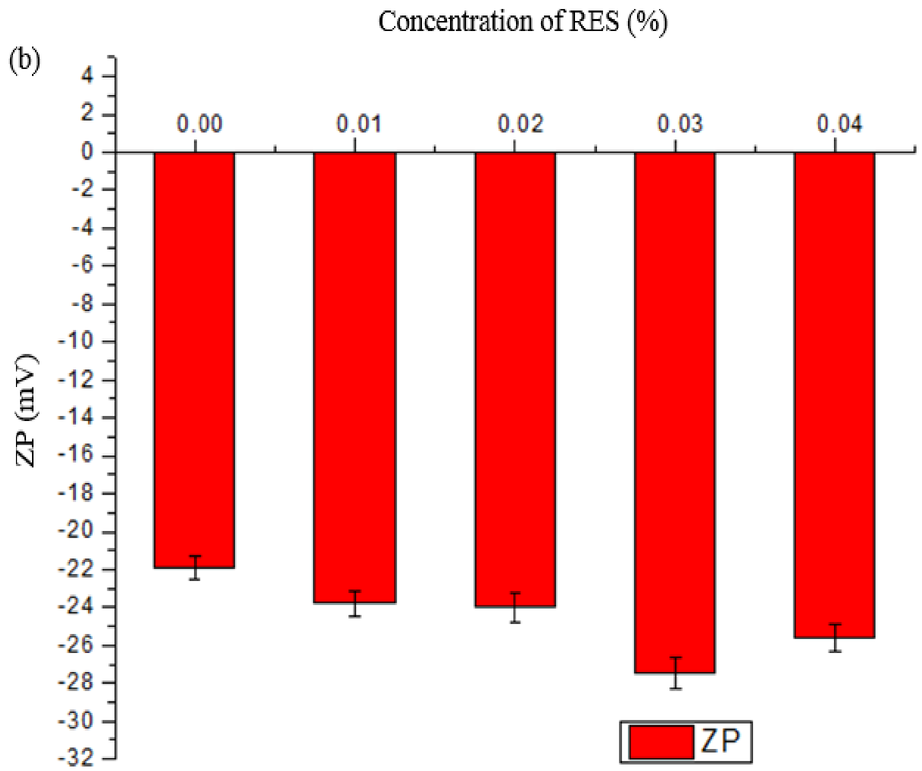


Fig. 2. Impact of different concentrations of RES on zeta potential of fresh (a) and stored (b) nanoemulsions.

Rheological Properties of Fresh and Stored Nanoemulsions

The rheological behavior of nanoemulsions is linked to their stability and final product applications. A direct correlation was found between the viscosity and the concentration of RES in nanoemulsions. Nanoemulsion contained 0.04% RES showed the highest value of viscosity Figure 3a. Data obtained was also subjected to the power-law model and found that all emulsions showed a shear-thinning behavior under shear rate. There exist three values ranges for n (flow behavior index): n<1 for shear-thinning fluid, n = 1 for a Newtonian fluid: n>1 for a shear-thinning fluid.

After storage, the viscosities of RES nanoemulsions against shear rate have been shown in Figure 3b. Shear-thinning property is a frequent phenomenon that could be observed when an emulsion-based system used in foods and beverages. It was fairly excellent for lowering the viscosity underflow during consumption (Liang, Xu et al. 2012). The nanoemulsions showed different degrees of shear-thinning behavior. The square of the correlation index (R^2) was >0.98 recommended the suitability of the power-law used to interpret the rheological properties of nanoemulsions. In all nanoemulsions formulation, *K* value decreased. However, flow behavior (*n*) increased for all nanoemulsion formulations, suggesting the shear-thinning nature of the nanoemulsions. The viscosities of all the nanoemulsions decreased after storage which was mainly due to the change in the mean droplet diameter. Overall, all the nanoemulsions showed good stability with no phase separation or creaming. Our findings are in accordance with previous research conducted by (Liang, Xu et al. 2012) in which they prepared peppermint oil loaded nanoemulsions. Size, ZP, and rheological studies showed that nanoemulsion with good physical characteristics can be prepared using a concentration of RES up to 0.03%.

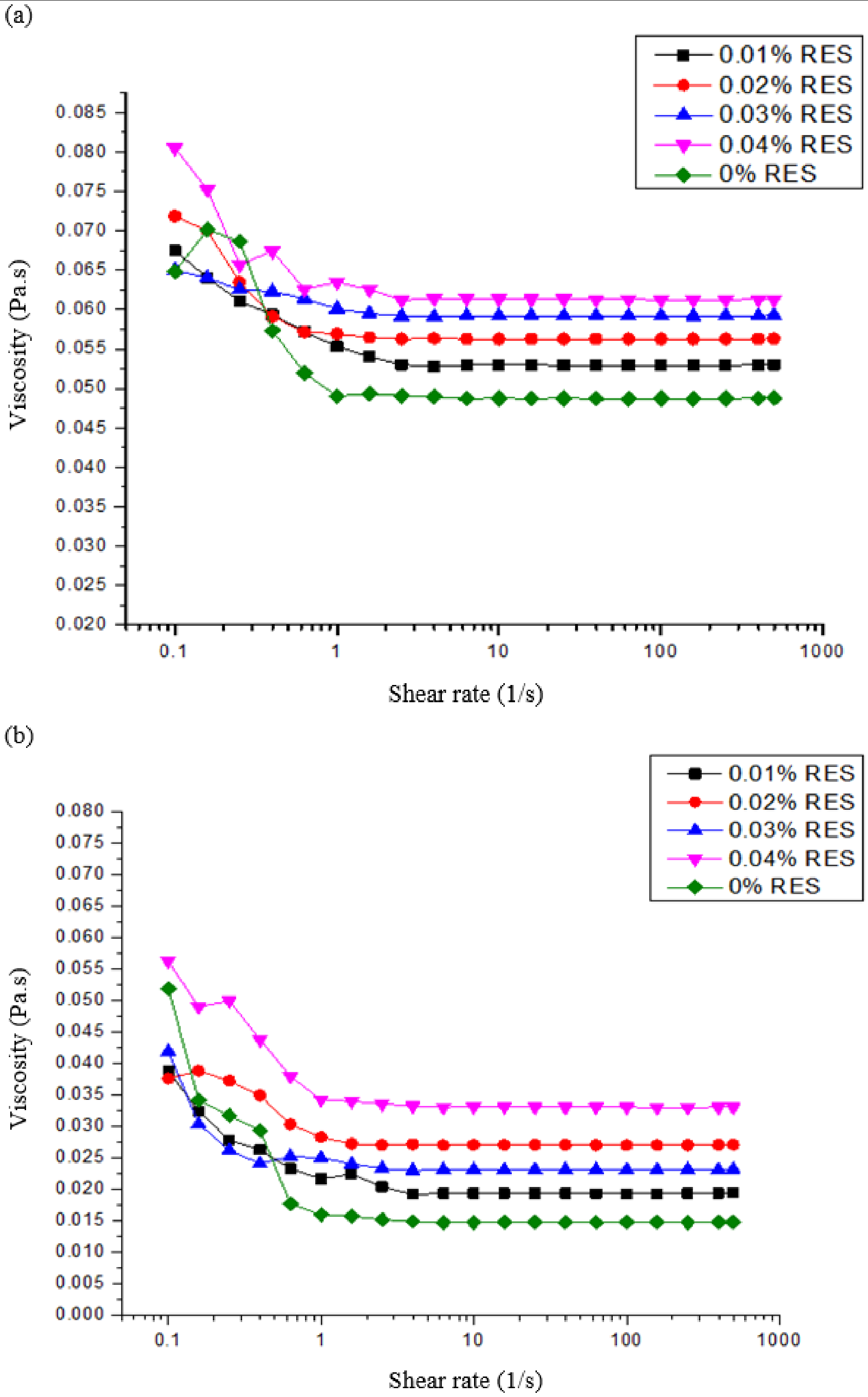
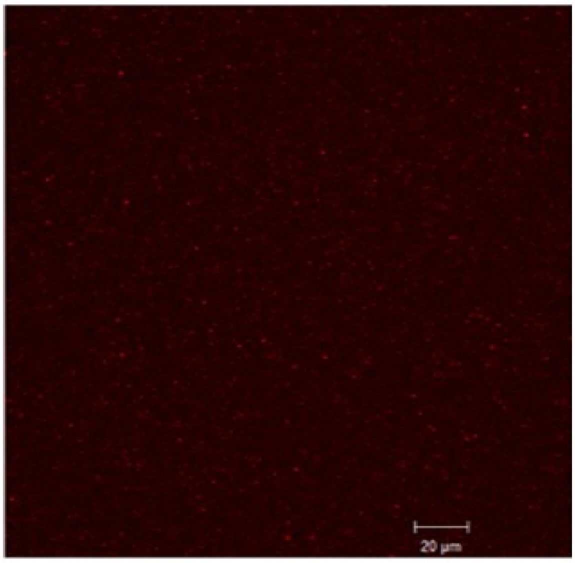


Fig. 3. Rheological properties of OSA-MS RES loaded nanoemulsion (a) fresh and (b) stored samples.

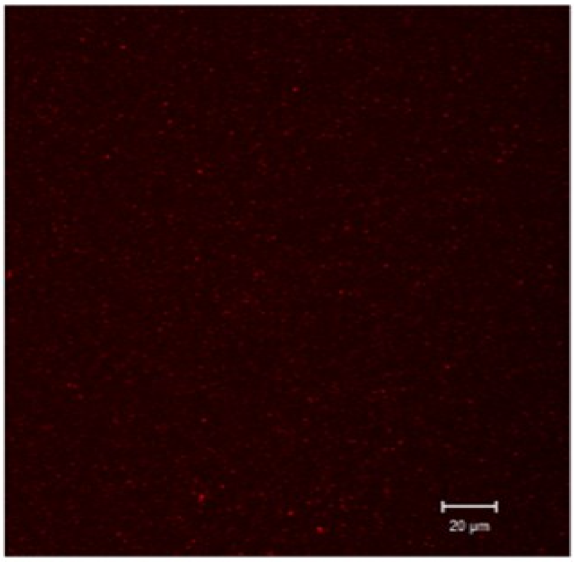
CLSM of Fresh and Stored Nanoemulsions

All fresh samples with different concentrations of RES showed very fine and evenly distributed oil droplets, indicating that high-pressure homogenizer is an efficient process to formulate nanoemulsions (Figure 4a). (Sharif, Williams et al. 2017) also observed very fine droplets of oil in water nanoemulsions stabilized by OSA-MS. However, due to the small droplet size, it was difficult to observe more details about droplet characteristics. After four-week storage at room temperature, all nanoemulsions showed an increase in size and nanoemulsion contained 0.03% RES showed very little increase in size as compared to other formulations.

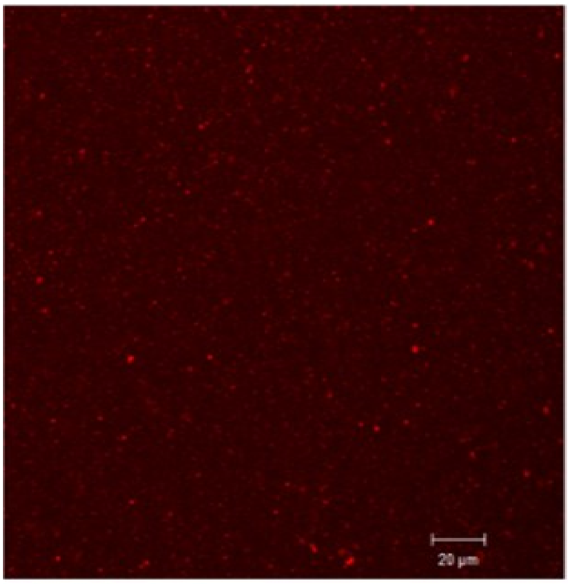
(a)



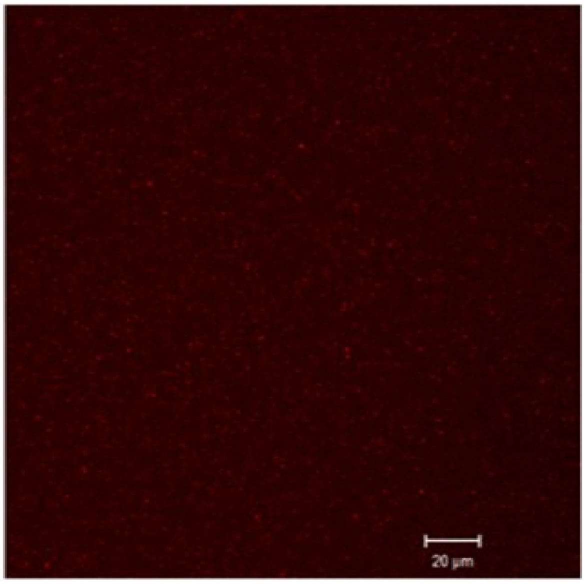
0% RES



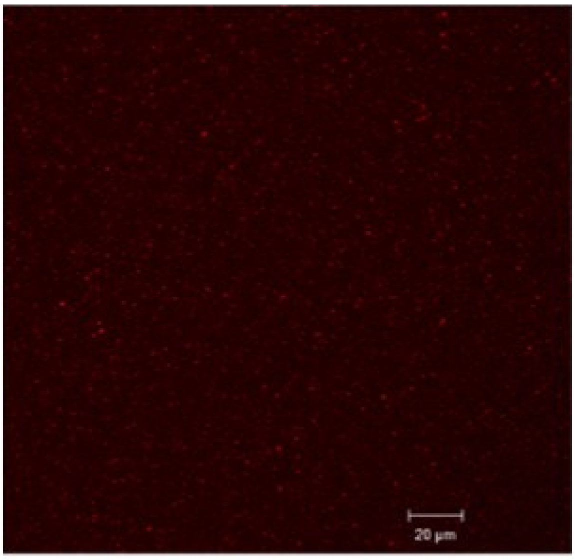
0.01% RES



0.02% RES

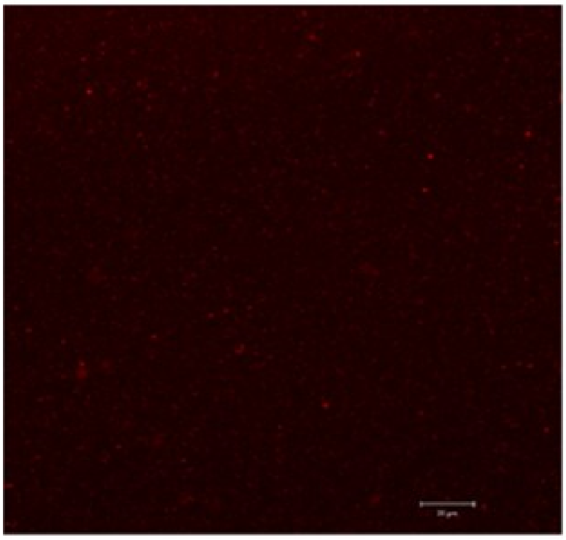


0.03% RES

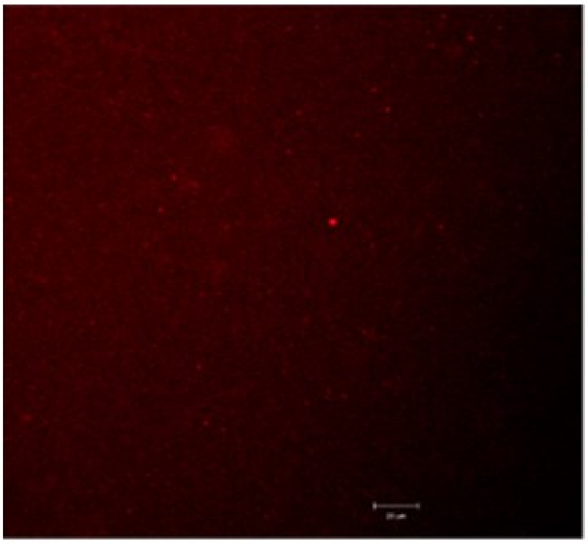


0.04% RES

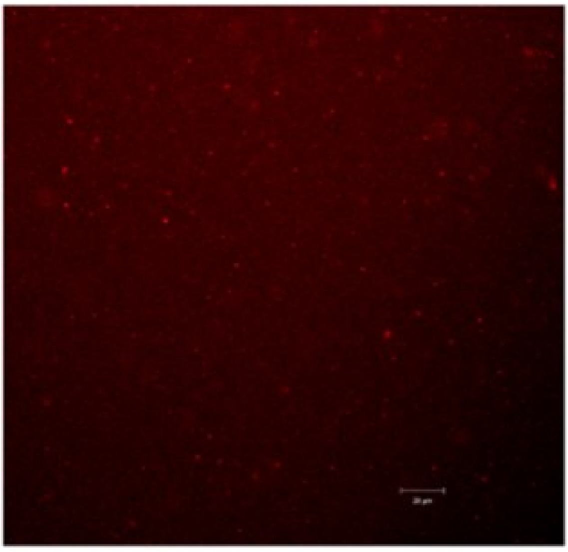
(b)



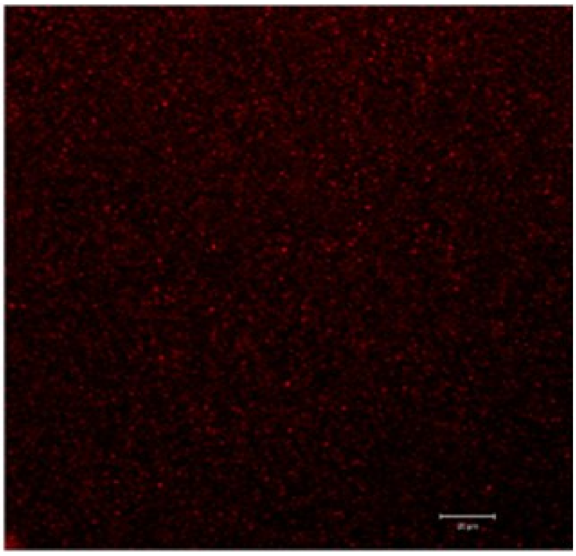
0% RES



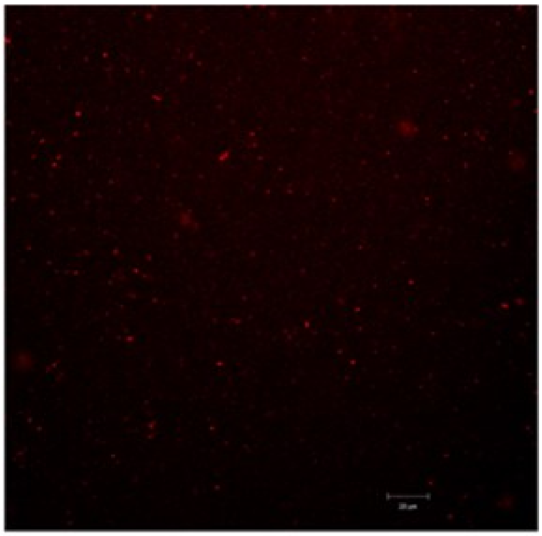
0.01% RES



0.02% RES



0.03% RES



0.04% RES

Fig. 4. CLSM of nanoemulsions containing different concentrations of RES (a) before and (b) after storage.

IV. Conclusion

Hi-cap 100 was used as an emulsifier to prepare the RES loaded nanoemulsions using different concentrations of RES. The mean droplet diameter of the samples showed that the nanoemulsion containing 0.03% RES had a minimum size. ZP values were negative for all the formulations and below 1n (flow behavior index). Confocal microscopy showed evenly distributed oil droplets. All nanoemulsions after storage at room temperature for one month were found to be stable. However, nanoemulsion with 0.03% RES showed the best and optimum results for all the analyses. The present findings could be helpful to develop RES loaded nanoemulsions and the production of functional foods and beverages.

Acknowledgment

This research was financially supported by the earmarked fund for China Agriculture Research System (CARS-45), and Collaborative innovation center of food safety and quality control in Jiangsu Province and 2014GB2C100 297, and national first-class discipline program of food science and technology (JUFSTR20180201), and the China Scholarship Council, Beijing, China.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Borges, S.C., et al. (2018). "Evaluation of the treatment with resveratrol-loaded nanoparticles in intestinal injury model caused by ischemia and reperfusion." Toxicology **396**: 13-22.
[2] de Oca-Avalos (from Oca-Avalos), J.M.M., et al. (2017). "Nanoemulsions: Stability and physical properties." Current Opinion in Food Science **16**: 1-6.
[3] Dinda, A., et al. (2013). "Formulation development and evaluation of paclitaxel loaded solid lipid nanoparticles using glyceryl monostearate." Journal of Applied Pharmaceutical Science **3**(8): 133.
[4] Harwansh, R.K., et al. (2019). "Nanoemulsion: Promising nanocarrier system for delivery of herbal bioactives." Journal of Drug Delivery Science and Technology.
[5] Hasani, S., et al. (2018). "Nanoencapsulation of lemon essential oil in Chitosan-Hicap system. Part 1: Study on its physical and structural characteristics." International journal of biological macromolecules **115**: 143-151.
[6] Khan, M.A., et al. (2019). "Alginate/chitosan-coated zein nanoparticles for the delivery of resveratrol." Journal of Food Engineering **258**: 45-53.
[7] Kumar, R., et al. (2017). "Ultrasound processed nanoemulsion: A comparative approach between resveratrol and resveratrol cyclodextrin inclusion complex to study its binding interactions, antioxidant activity and UV light stability." Ultrasonics sonochemistry **37**: 478-489.
[8] Li, Y., et al. (2020). "Preparation and digestibility of fish oil nanoemulsions stabilized by soybean protein isolate-phosphatidylcholine." Food hydrocolloids **100**: 105310.
[9] Liang, R., et al. (2012). "Physical and antimicrobial properties of peppermint oil nanoemulsions." Journal of agricultural and food chemistry **60**(30): 7548-7555.
[10] Marwaha, R.K. and A. Dabas (2019). "Bioavailability of nanoemulsion formulations vs conventional fat soluble preparations of cholecalciferol (D3)-An overview." Journal of clinical orthopaedics and trauma.
[11] Polavarapu, S., et al. (2011). "Physicochemical characterisation and oxidative stability of fish oil and fish oil-extra virgin olive oil microencapsulated by sugar beet pectin." Food chemistry **127**(4): 1694-1705.
[12] Rehman, A., et al. (2019). "Pectin polymers as wall materials for the nano-encapsulation of bioactive compounds." Trends in Food Science & Technology.
[13] Rehman, A., et al. (2019). "Carotenoid-loaded nanocarriers: A comprehensive review." Advances in colloid and interface science: 102048.
[14] Rezaei, A., et al. (2019). "Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers." Food hydrocolloids **88**: 146-162.
[15] Riaz, T., et al. (2019). "In vitro survival of Bifidobacterium bifidum microencapsulated in zein-coated alginate hydrogel microbeads." Journal of microencapsulation **36**(2): 192-203.
[16] Sharif, H.R., et al. (2017). "Physicochemical stability of β-carotene and α-tocopherol enriched nanoemulsions: Influence of carrier oil, emulsifier and antioxidant." Colloids and Surfaces A: Physicochemical and Engineering Aspects **529**: 550-559.
[17] Sharif, H.R., et al. (2017). "Physicochemical properties of β-carotene and eugenol co-encapsulated flax seed oil powders using OSA starches as wall material." Food hydrocolloids **73**: 274-283.
[18] Sharif, H.R., et al. (2017). "Influence of OSA-starch on the physico chemical characteristics of flax seed oil-eugenol nanoemulsions." Food hydrocolloids **66**: 365-377.
[19] Zhang, F., et al. (2019). "Co-encapsulation of α-tocopherol and resveratrol within zein nanoparticles: Impact on antioxidant activity and stability." Journal of Food Engineering **247**: 9-18.

AUTHOR'S PROFILE

First Author
Qayyum Shehzad, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Second Author
Abdur Rehman, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Third Author
Ahmad Ali, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Fourth Author
Sohail Khan, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Fifth Author
Amer Ali Mahdi, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Sixth Author
Aiman Karim, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Seventh Author
Salman Khan, School of Biotechnology, Jiangnan University, Wuxi, Jiangu, 214122, China.

Eighth Author
Fang Yang, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.

Ninth Author
Wenshui Xia, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu 1800, Wuxi, Jiangsu, 214122, China.