Industrial Crops and Products xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Physicochemical, oxidative and anti-oxidant stabilities of kenaf seed oil-in-water nanoemulsions under different storage temperatures

Ai Mun Cheong [a], Chin Ping Tan [b], Kar Lin Nyam [a,*]

[a] Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia
[b] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form 21 October 2016
Accepted 24 October 2016
Available online xxx

Keywords:
Kenaf seed (*Hibiscus cannabinus* L.) oil
Particle size
Polydispersity index (PDI)
Zeta-potential
Fatty acid compositions
Total phenolic content

ABSTRACT

Kenaf seed oil-in-water nanoemulsions stabilised by sodium caseinate, Tween 20 and β-cyclodextrin complexes were produced using high pressure homogeniser. This formulation has been shown to possess good lipid digestion and increased bioaccessibility of tocopherols and total phenolic contents. However, its physicochemical and oxidative stability during storage was unknown. Therefore, the main objectives of this study were to evaluate the effects of three storage temperatures (4 °C ± 2 °C, 25 °C ± 2 °C and 40 °C ± 2 °C) on the physicochemical, oxidative and antioxidant stability of formulated kenaf seed oil-in-water nanoemulsions. The results showed that nanoemulsions stored at 4 °C had maintained the highest stability with the highest zeta-potential value (−36.6 mV), lowest changes of PDI and pH over 12 weeks of storage. It also presented the lowest reduction of polyunsaturated fatty acids (PUFA) over the course of storage period. In contrast, nanoemulsions that stored at 40 °C exhibited lowest stability with the lowest zeta-potential (−27.3 mV). Sediment was observed in 8 weeks of storage and it had the highest reduction of PUFA. Total phenolic contents in nanoemulsions that stored at 4 °C and 25 °C showed decreasing trend during the storage period, except for nanoemulsions that stored at 40 °C showed a significant increase ($p < 0.05$) in the first week of storage, but subsequently also displayed decreasing trend. The overall results showed that nanoemulsions that stored at 4 °C and 25 °C were stable for up to 8 weeks of storage. Nanoemulsions that stored under accelerated storage temperature of 40 °C were stable for 1 week, which is equivalent to 28 days at room temperature (RT) based on Arrhenius equation. The results of this study could provide better understanding of the storage stability of kenaf seed oil-in-water nanoemulsions under different storage temperatures. It could be served as a predictive model to estimate its shelf-life.

1. Introduction

In this health-conscious era, there is increasing demand by consumers for functional food products, including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Lin et al., 2014). This is because the consumption of MUFA and PUFA in a long term has been proven to reduce plasma LDL-cholesterol levels and lower the risk of cardiovascular disease (Czernichow et al., 2010; Smith et al., 2003). Kenaf seed (*Hibiscus cannabinus* L.) oil contains high amount of MUFA and PUFA, mainly oleic acid (31.8%–33.0%) and linoleic acid (32.19%–33.6%) and total unsaturated fatty acids of 65.9%–70.55% (Chew et al., 2016; Ng et al., 2013). In addition, kenaf seed oil also contains significant amount of phytosterols, tocopherols and polyphenols (Nyam et al., 2009). Owing to its health functional properties, kenaf seed oil has the potential promise to be used as edible oil, in the application of functional foods and nutraceutical products. Despite the potential health benefits of kenaf seed oil, its application as functional foods and nutraceutical products are limited due to its poor water solubility as a result of different polarity of oil (non-polar) and water (polar). In addition, its poor water solubility decreases the absorption rate in the gastrointestinal tract, hence reducing bioavailability (McClements, 2011).

Nanoemulsion-based delivery systems are promising encapsulation technique as it have been shown to increase bioavailability of lipophilic bioactive compounds in the *in vivo* study (Gong et al., 2012) owing to its small particle size ranging from 10 to 200 nm (Wulff-Pérez et al., 2009). The small particle size with large surface area of nanoemulsions can improve the solubility rate of kenaf seed oil containing lipophilic bioactive compounds in the aqueous phase It also enhances the absorption across the epithelial cells of small intestine to improve the bioavailability (McClements,

* Corresponding author.
E-mail address: nyamkl@ucsiuniversity.edu.my (K.L. Nyam).

http://dx.doi.org/10.1016/j.indcrop.2016.10.047
0926-6690/

2011). Therefore, extensive researches have been carried out in attempting to formulate oil-in-water (O/W) nanoemulsions with improved physicochemical stability. In the previous research, a stable kenaf seed O/W nanoemulsion has been successfully produced by using food grade sodium caseinate (SC), Tween 20 (T20) and β-cyclodextrin (β-CD). The synergistic effect of these ternary emulsifiers have exhibited to improve physical stability to environmental stress (Cheong and Nyam, 2016; Cheong et al., 2016c). Study of releasing rate and bioaccessibility of kenaf seed O/W nanoemulsions during *in vitro* digestion have shown good lipid digestion in the simulated intestinal condition and good bioaccessibility of antioxidants (Cheong et al., 2016a). Similar study on the *in vitro* digestion of kenaf seed O/W nanoemulsions with different formulations have also shown to improve bioaccessibility (Cheong et al., 2016b).

To date, no study has been done on the physical and oxidative stabilisation of kenaf seed O/W nanoemulsions during storage. Nanoemulsions/nanodispersions stabilised by food grade/natural emulsifier(s) often experience low physical stability such as coalescence, flocculation, creaming or phase separation, compared to synthetic emulsifiers. It may be the reason for limited study found on the physicochemical and oxidative stability of nanoemulsions-stabilised by food grade emulsifier(s) with more than 1 month of accelerated storage temperature (40–50 °C) (Gharibzahedi et al., 2012; Osborn and Akoh, 2004). Some studies only reported the chilled storage condition for up to 3 months (Tan and Nakajima, 2005). Therefore, the main objectives of this study were to evaluate the effects of three storage temperatures on the physicochemical, oxidative stability and total phenolic acid contant (TPC) of kenaf seed O/W. Storage at 4 °C, 25 °C and 40 °C represent chilled, room temperature (RT) and accelerated storage conditions, respectively. The results from these three different storage conditions were compared with fresh sample to study their changes of physicochemical, oxidative and antioxidant activity over the course of storage period. According to Arrhenius equation, every increase of 10 °C doubles the rate of most chemical reactions, including the oxidative reactions (Ngan et al., 2014). Hence, 60 days of storage at 40 °C in this study can be considered equivalent to 240 days of storage at RT. In commercial application, emulsions may be stored at different temperatures prior consumed by consumers (Rao and McClements, 2011). Therefore, the results of this study may provide helpful indication for food industry in selecting better storage conditions of nanoemulsion-stabilised by similar ternary emulsifiers.

2. Materials and methods

2.1. Materials

Kenaf (*Hibiscus cannabinus* L.) seeds were obtained from the Malaysian Agricultural Research and Development Institute (MARDI) (Selangor, Malaysia). Beta-cyclodextrin (β-CD) was purchased from Zibo Qianhui Fine Chemical Co., Ltd. (Shandong, China). Sodium caseinate (SC) was purchased from a local food ingredient supplier (VIS Food Tech Ingredient Supplies, Malaysia). All chemicals used were of analytical grade (Merck, Darmstadt, Germany). Ultrapure water (Millipore Corp., Bedford,MA) was used in the study.

2.2. Solvent extraction of kenaf seed oil

Prior to solvent extraction of kenaf seed (*Hibiscus cannabinus* L.) oil, dried kenaf seeds were ground into fine powders using food processor grinder (Panasonic, Japan). Kenaf seed oil was extracted with hexane using Soxhlet extractor (Favorit, Thailand) at 60 °C for 3 h. Hexane solvent was then evaporated off at 241 mbar, 55 °C using Multivapor (BUCHI Multivapor P-6, Buchi, Switzerland) for oil recovery. Immediately, kenaf seed oil was transferred to schott bottle that wrapped with aluminium foil to prevent photo-oxidation. Subsequently, oil was purged with nitrogen gas to remove residual solvent and to minimize the oxidation. Oil was kept in the freezer −18 °C until further used.

2.3. Kenaf seed oil-in-water nanoemulsions preparation

The preparation of kenaf seed O/W nanoemulsions have been optimised and described in Cheong and Nyam (2016). Briefly, aqueous phase was prepared by mixing pre-dissolved sodium caseinate (5.79 wt%) and Tween 20 (2.76 wt%) with ultrapure water (40 wt%) at 45 °C. Sodium azide (0.02 wt%) was added into the aqueous phase to prevent microbial growth. Kenaf seed oil (10 wt%) was added drop-wise into aqueous phase containing sodium caseinate (SC) and Tween 20 (T20) while magnetically stirred at 45 °C on hotplate. Once the oil was completely incorporated into aqueous phase, the coarse O/W emulsion was allowed to stir for another 10 min. Subsequently, coarse O/W emulsion was subjected to pre-homogenisation by using high shear mixer (Ultra-Turrax, IKA UK) at 8600 rpm for 3 min to form primary emulsions. Meanwhile, β-CD (1.45 wt%) was pre-dissolved in ultrapure water (40 wt%) at 70 °C and added immediately to the primary emulsion. The primary emulsion was then further homogenised at 28,000 psi for 4 cycles using high pressure homogeniser (Nano DeBEE, BEE International, USA) to produce secondary nanoemulsion (Cheong and Nyam, 2016). All nanoemulsions were prepared in duplicate.

2.4. Storage conditions

The freshly prepared nanoemulsios were distributed into separate schott bottle and purged with nitrogen gas prior tight closure. Duplicate sets of nanoemulsion samples were stored in the dark at chiller (4 °C ± 2 °C) for 12 weeks, room temperature (25 °C ± 2 °C) and incubator (40 °C ± 2 °C) for 8 weeks. Duplicate bottles of samples were taken from each of the storage conditions for physical and chemical analysis at schedules times. Analysis for samples that stored at 40 °C was carried at fresh, first week, fourth week, and eighth week of storage. Samples that stored at 25 °C were analysed at fresh, second week, fourth week, and eighth week of storage. Samples that stored at 4 °C were analysed at fresh, second week, fourth week, eighth week and twelfth week of storage. Since the chemical reaction in the accelerated storage temperature of 40 °C was assumed to be doubled, hence samples stored at 40 °C were analysed earlier at the beginning of storage as it might show the largest change in the first week, followed by fourth and eighth week. The results from these three different storage temperatures were compared against fresh samples to compare the oxidative stability at different storage temperatures.

2.5. Extraction of oil from nanoemulsions

For each of the duplicate samples (130 mL), oil was extracted with 650 mL of isooctane and isopropanol mixture (3:2, v/v), followed by vortexed for 10 s three times before subjected to centrifugation at 1000 × *g* for 2 min (Hu et al., 2003). The extracted oils were subjected to chemical analysis.

2.6. Characterisation of nanoemulsions

2.6.1. Particle size and polydispersity index (PDI)

The mean particle size (z-averages) and PDI were measured by using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Prior to the measurement, each sample was diluted with ultra-

Please cite this article in press as: Cheong, A.M., et al., Physicochemical, oxidative and anti-oxidant stabilities of kenaf seed oil-in-water nanoemulsions under different storage temperatures. Ind. Crops Prod. (2016), http://dx.doi.org/10.1016/j.indcrop.2016.10.047