Food Chemistry 241 (2018) 79–85

Contents lists available at ScienceDirect

Food Chemistry

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

Effects of storage and yogurt matrix on the stability of tocotrienols encapsulated in chitosan-alginate microcapsules

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ARTICLE INFO

Keywords:
Alginate
Chitosan
Stability
Gelation
Oxidation
Self-assembled

ABSTRACT

Tocotrienol microcapsules (TM) were formed by firstly preparing Pickering emulsion containing tocotrienols, which was then gelled into microcapsules using alginate and chitosan. In this study, we examined the stability of TM during storage and when applied into a model food system, i.e. yogurt. During storage at 40 °C, TM displayed remarkably lower tocotrienols loss (50.8%) as compared to non-encapsulated tocotrienols in bulk oil (87.5%). When the tocotrienols were incorporated into yogurt, the TM and bulk oil forms showed a loss of 23.5% and 81.0%, respectively. Generally, the tocotrienols were stable in the TM form and showed highest stability when these TM were added into yogurt. δ-Tocotrienol was the most stable isomer in both forms during storage and when incorporated into yogurt. The addition of TM into yogurt caused minimal changes in the yogurt's color and texture but slightly altered the yogurt's viscosity.

1. Introduction

Apart from tocopherols, another member of the Vitamin E family, i.e., tocotrienols also possess excellent antioxidative properties. In fact, these micronutrients are able to protect the neuron system, lower blood cholesterol and help the human body to fight against cancer (Chen, Ma, Liang, Peng, & Zuo, 2011), therefore making them valuable compounds for potential food applications. However, tocotrienols are only present in limited plant sources such as palm oil, pumpkin, wheat germ and barley (Drotleff, Bohnsack, Schneider, Hahn, & Ternes, 2014; Durante, Lenucci, D'Amico, Piro, & Mita, 2014; Durante, Lenucci, Rescio, Mita, & Caretto, 2012). In addition to being susceptible to degradation and oxidation, the compounds possess very low bioavailability due to their lipophilic characteristic which make them hardly soluble in water (Fu, Che, Tan, & Teng, 2014). Considering these limitations of tocotrienols, continuous work has been carried out to seek for possible ways to protect the tocotrienols and at the same time, enhance their bioavailability.

Through microencapsulation, we could develop a biocompatible tool for the delivery of tocotrienol compounds. As such, calcium carbonate ($CaCO_3$) particles were utilized to encapsulate tocotrienols in the form of Pickering emulsion, which is a type of highly stable emulsion system stabilized by solid particles. The advantage of this type of emulsion is that it can eliminate the use of synthetic emulsifier, thus making it a safer product to be applied and consumed (Aditya, Hamilton, & Norton, 2017). Aside from the safety aspect, Pickering emulsion could be formed through low-energy homogenization process (Leong, Tey, Tan, & Chan, 2015). By utilizing the high shear homogenization, we were able to fabricate an oil-in-water Pickering emulsion containing palm tocotrienol-rich fraction (TRF) using $CaCO_3$ particles. The pH-sensitive nature of $CaCO_3$ allowed the resulting Pickering emulsion to be employed as the precursor for ionotropic gelation to fabricate hydrocolloid-based microcapsules.

Natural and readily available hydrocolloids, namely alginate and chitosan, were selected for the fabrication of tocotrienol microcapsules (TM). Because of its negative charge, alginate tends to gel rapidly under mild condition and readily crosslinks with Ca^{2+} ions to form the "egg-box" three-dimensional structure at acidic pH (Leong et al., 2015). Chitosan, on the other hand, aids in improving the stability and rigidity of this "egg-box" gel structure by binding to the carboxylate group ($-COO^-$) of alginate via its cationic amine group ($-NH^{3+}$) (Dima, Pătraşcu, Cantaragiu, Alexe, & Dima, 2016). Hence, we subjected the Pickering emulsion containing tocotrienols to the alginate gelation process and subsequent chitosan-coating to reduce the oxidization of

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http://dx.doi.org/10.1016/j.foodchem.2017.08.075
Received 1 July 2017; Received in revised form 6 August 2017; Accepted 22 August 2017
Available online 24 August 2017

the compounds. As a result of the microencapsulation process, we were able to obtain spherical microcapsules with considerably high encapsulation efficiency (84.9 ± 2.2%).

Generally speaking, oxidation has been the major factor that causes the loss of micronutrients and deterioration of oil quality (rancidity and off-flavour) (Sun-Waterhouse, Zhou, Miskelly, Wibisono, & Wadhwa, 2011). It is known that the reaction rate of oxidation would affect the shelf-life of oil product. Thus, by controlling the environmental conditions that govern the oxidation rate, we could thereby prolong the shelf-life of the TM. Therefore, the storage condition and the matrix surrounding the TM are important variables that could have a major impact on the stability of the TM. As far as we know, there is little published data comparing the stability of non-encapsulated (in the form of bulk oil) and encapsulated tocotrienols (in the form of alginate-chitosan microcapsules) during storage. Also, there has been no detailed investigation of their stability as affected by their interaction with the food matrix. Herein, we studied the change in tocotrienols content throughout a four-week storage period. Furthermore, in order to investigate the suitability of TM to be incorporated into food system, we chose yogurt as a model food system and added the TM into it to observe the changes in terms of tocotrienols content. The effect of TM on the yogurt's properties (pH, color, texture and viscosity) was also investigated in the present study.

2. Materials and method

2.1. Materials

Refined, bleached and deodorized (RBD) palm olein (*Elaeis guineenis* var. *tenera*) and palm tocotrienol rich fraction (TRF) were obtained from Moi Foods Malaysia Sdn. Bhd. (Selangor, Malaysia) and Supervitamins Company (Johor, Malaysia), respectively. Precipitated $CaCO_3$ nanoparticles, sodium alginate (Manugel GHB) and chitosan of molecular weight 100,000–300,000 were supplied by NanoMaterials Technology Company (Singapore), FMC Biopolymers (UK) and Fisher Scientific (USA), respectively. Standard consisting of tocotrienols and tocopherols (α-, β-, δ-, γ-) was purchased from LGC Standard (Teddington, UK). Sodium hydroxide (NaOH) was purchased from Friendemann Schmidt (Australia); whilst glacial acetic acid, hexane, methanol, acetonitrile and dichloromethane for HPLC analysis were supplied by Fisher Chemical (UK). Freeze dried yogurt starter (Yogourmet), containing three types of *Lactobacillus* strains (*L. casei*, *L. bulgaricus* and *L. acidophilus*), was obtained from Lyo-San Inc. (Canada).

2.2. Preparation of $CaCO_3$ dispersion

The emulsification and microcapsule formation procedures were conducted, with slight modifications, based on the method described by Leong et al. (2015). Initially, a coarse dispersion of $CaCO_3$ (5%, w/v) was prepared by homogenizing (5000 rpm, 30 min) precipitated $CaCO_3$ nanoparticles in deionized water using an Ultra Turrax rotor-stator homogenizer (IKA, Staufen, Germany). The final dispersion was obtained through high-pressure homogenization at 22,000 psi for 4 passes using Microfluidizer (M-110L, Microfluidics, MA, USA). The obtained dispersion was then diluted to 0.75% for subsequent formation of Pickering emulsion.

2.3. Formation of tocotrienol Pickering emulsion (TPE) template

Palm TRF (2%, w/w) was firstly dissolved in 10 mL RBD palm olein before being mixed with 0.75% $CaCO_3$ dispersion via high-shear homogenization (Silverson, MA, USA). After being homogenized at 5000 rpm for 15 min, the emulsion was then left to stand for 30 min so that the emulsion mixture would form two layers, with the tocotrienol-rich template on the upper layer. This resulting template layer was subsequently used for the gelation process to obtain gel microcapsules that were then coated with an extra layer of chitosan. By doing so, we expected the tocotrienols to be well-protected from being exposed to external environmental stresses, such as oxygen, moisture and heat.

2.4. Formation of tocotrienol microcapsules (TM)

The ionic gelation process was initiated by adding 2 mL of TPE into alginate solution (2%, w/v) under stirring, after which 1.0 M acetic acid was slowly added to reduce the mixture's pH. The mixing was stopped immediately once pH 4 was achieved. The mixture was then left to stand for 16 h to allow for the curing of microcapsules to occur. Then, the mixture was sieved (80 μm sieve) to collect the microcapsules. The TM were then washed with deionized water and dispersed in 0.1% calcium chloride for subsequent chitosan coating.

The chitosan solution (0.5% w/v) was prepared by dissolving exact amount of chitosan into 450 mL of deionized water, followed by the addition of 2 mL acetic acid; all while under agitation. The pH of the mixture was adjusted to pH 5.5–6.0 through the addition of 0.1 M NaOH. The mixture was then filtered and topped up to a total volume of 500 mL for layer-coating usage. Next, the previously collected TM were dispersed into the prepared chitosan solution at a volume ratio of 1:2 for 15 min under mild stirring. The solution was then sieved to collect the coated TM which were then washed using deionized water, and air dried. In order to study the effect of prolonged storage at elevated temperature, the dried TM were then kept in a few sets of amber bottles (with 100 mg in each bottle) and stored in an oven at 40 °C for four weeks. Bulk oil containing 2% TRF was also kept under the same condition to serve as the control sets. Each set of TM and bulk oil was analyzed on a weekly basis to measure the changes in their tocotrienols content.

2.5. Yogurt preparation

Yogurt was produced using the procedures stated by Ozturkoglu-Budak, Akal, and Yetisemiyen (2016). Fresh whole cow milk (1 L) was added with 0.1% (w/v) of TM, stirred and heated in a water bath at 90 °C for 5 min. The heated milk was cooled down to 45 °C and was later mixed with one sachet of the yogurt starter powder. The yogurt mix was then incubated a 43 °C until its pH dropped to pH 4.6. Original yogurt, bulk oil-added (0.1%, w/v) yogurt and TM-added (0.1%, w/v) yogurt were prepared and kept at 4 °C for four weeks to monitor their properties and tocotrienols content.

2.6. Yogurt analyses

2.6.1. pH and color

After each week of storage, the yogurts were taken out from the refrigerator and immediately subjected to analyses. The yogurt's pH was measured using a pH meter that was calibrated with standard buffers (pH 4 and pH 7) prior to use. The color attributes (L^*, a^* and b^*) of the yogurts were determined using a Chroma Meter CR-300 (Konica Minolta Co., Osaka, Japan) and their intensity was recorded accordingly.

2.6.2. Texture

A TA-XT2i texture analyzer (Stable Micro Systems, MA, USA) was used to measure the texture profile (firmness, consistency and cohesiveness) of the yogurts. The single compression cycle test was performed using a SMSP/0.5S probe with a penetration speed of 2 mm/s and test distance of 10 mm. The firmness is related to the force required to achieve the maximum depth; whilst the cohesiveness refers to the force needed to pull the probe away from the sample (Gutiérrez et al., 2016).