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Encapsulation of Vitamin E using Maltodextrin/Sodium Caseinate/Selenomethionine and its Release Study

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Vitamin E is important for human and animal as they promote health benefits. Vitamin E is also widely used as food additives, pharmaceuticals, and cosmetics industries. However, vitamin E from natural sources is easy to degrade, labile to heat and oxygen, insoluble in water and also low in absorption hence, poor bioavailability. Encapsulation helps in protecting vitamin E against the unfavorable environment to be release at targeted sites. In this study, encapsulation of vitamin E was incorporated with selenomethionine and the effects on physicochemical properties were investigated. The release of vitamin E was evaluated in simulated gastrointestinal tract (GIT). Vitamin E was encapsulated in a solution of maltodextrin (DE 13 - 17) and sodium caseinate with a ratio of 3 : 2 : 1 (maltodextrin:vitamin E:sodium caseinate) and the amount of selenomethionine are ranging from 1.6 to 10.6 mg. The powder form of the particle was obtained by using a freeze-dried technique. From the results, good solubility and moisture content were obtained from the formulation with 5.6 mg selenomethionine which was attributed to the differences in concentration and hydrophobicity of solute. The lower size was obtained but high in PdI value, indicating sufficient stability to aggregation under that formulation. At 5.6 mg of selenomethionine, encapsulated vitamin E with maltodextrin and sodium caseinate resulted in 87 % and 42 % vitamin E release, after 30 min in simulated gastic fluid (SGF) and simulated intestinal fluid (SIF) solution. This attributes to the different absorption under different pH. Overall, encapsulated vitamin E with selenomethionine was successfully obtained with better properties and performance, as an attempt for improving vitamin E delivery and bioavailability.

1. Introduction

Vitamin E is an essential nutrient for humans and animals that promotes numerous health benefits as it can act as lipid-soluble antioxidant and non-antioxidant food additive. It is also widely used in pharmaceutical and cosmetic industries (Yang and McClements. 2013). However, vitamin E, which is derived from natural sources is easily degraded by hostile environment after being extracted from plant tissue. It is also sensitive (chemically instable) to heat and oxygen which in turn enables it to irreversibly transformed into quinone (Yoo et al. 2006). More crucially, vitamin E is also poorly soluble in water (Yang and McClements. 2013). So it is not well-absorbed specifically when it has to pass through the gastrointestinal tract (GIT). Its bioavailability is also decreased (Yoo et al. 2006).

Encapsulation technique is applied to encapsulate and protect bioactive compounds like polyphenols, micronutrients, enzymes, antioxidants and nutraceuticals against adverse environment and also to aid their release at targeted sites (Ezhilarasi et al. 2013). In addition, encapsulation technique can protect these bioactive compounds from UV light, eradicate incompatibilities with other materials, improve solubility in aqueous systems, or mask unpleasant taste or odor.

Selenium (Se) is nutritionally an essential trace mineral in human diet. Se has the ability to control the formation of free radicals upon reactive oxygen species (ROS). In order to preserve the stability of selenium, many studies reported the use of proteins, polyphenols, polysaccharides, melatonin, ATP as well as monosaccharides (Zhang et al., 2015). Se content in animal products is derived from their consumed diet (usually in the form of L-

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selenomethionine or L-selenocysteine) (Stahl et al., 2002). Compared to vitamin E, selenomethionine has different properties. In addition to being soluble in water, selenomethionine is also easily absorbed and highly bioavailable in human body (Edens, 2002).

Theoretically, the desired nanocapsule for the drug delivery systems needs to be able to protect the core materials from strong acidic environment. It means that the carrier must be resistant against acidic pH. Once the nanocapsule reaches the stomach, it should maintain its effectiveness as a carrier against the acidic environment as well as maintaining a low absorption rate. However, when it reaches the small intestine, it should sustain the release of the core materials at a specific rate (Yoo et al., 2006).

The aim of this study is to encapsulate vitamin E incorporated with selenomethionine in a wall material formula consists of maltodextrin and sodium caseinate, to characterize their solubility, moisture content, particle size, and size distribution properties and to evaluate their release in simulated GIT, as an attempt to improve vitamin E delivery and bioavailability.

2. Materials and methods

2.1 Materials

Maltodextrin (DE 13 - 17) was purchased from Sigma-Aldrich, USA. Vitamin E was chosen in this study as bioactive compounds and was purchased from Super Vitamins Sdn. Bhd, Malaysia. The manufacturer reported that this vitamin E is isolated from local palm oil and contained 22.9 % tocopherol and 77.1 % tocotrienols. Sodium caseinate was obtained from Acros, USA. L-selenomethionine was ordered from Calbiochem, USA. Other chemicals were analytical grade.

2.2 Encapsulated vitamin E preparation

The samples were prepared according to Farias et al. (2007) with slightly modified. In this study, oil-in-water (O/W) emulsions were prepared by homogenizing lipid phase with an aqueous phase in the ratio of 3 : 2 : 1. The lipid phase was vitamin E, dissolved with and without the presence of selenomethionine, while the aqueous phase consisted of distilled water, 5.01 g maltodextrin and 1.67 g of sodium caseinate. The mixture was gently stirred using stirrer hotplate (IKA ® WERKE C-MAG HS 7, MY). The mixture was left for five minutes for emulsification processes and it was freeze-dried using freeze-dryer (ALPHA 1 - 2 LD plus, CHRIST, Germany) at -41°C, ~4 x 10^{-4} mbar to form a dried sample. These dried samples were then collected and blended to form a powder. This powder was stored in a seal plastic at -20 °C for further analysis.

2.3 Water solubility

The solubility of powder in water was obtained by referring to Barbosa et al. (2005). The powder was dissolved in water (0.4 % w/v) and was gently stirred until all solid-solubilized. The powder was considered soluble when the time of solubilization was not greater than 5 min. The time obtained was recorded.

2.4 Moisture content determination

This analysis was conducted according to the method Jaya and Das (2004) with minor modifications. Approximately, 5 g of a sample was used and heated at the temperature of 105 ºC. Determination of moisture content was performed in triplicates and the average was recorded. Sample preparation for moisture determination has to be handled carefully in order to avoid evaporation and to prevent adsorption.

2.5 Particle characterization

The samples were characterized in terms of particle size and size distribution. The particle size analysis measurements were performed using Zetasizer Nano-ZS (Malvern Instruments Ltd, Malvern, UK.). For the measurement, a total of 1 mL sample was transferred into vial prior to being analysed using Zetasizer for their particle and size distribution.

2.6 In vitro release determination

Each sample passed through a two level of different pH designing for the simulated gastrointestinal tract (GIT) model; they were including stomach and intestines. The in vitro release study was performed by referring to a previous study (Song et al. 2009) with little modification. The samples (150 mg) were placed in 25 mL of simulated fluids in different pH value in which 1.2 for simulated gastric fluid, SGF (36.5 % HCl plus 0.2 % NaCl, adjusted using HCl range from 0.1 to 1 M), and 7.4 for simulated intestinal fluid, SIF (0.6 g KH_2PO_4 plus 3.5 g K_2HPO_4, adjusted using NaOH range from 0.1 to 1 M). Then, the samples were placed in the incubator shaker at temperature, 37 °C with a total speed at 100 rpm. After 30 min time interval, the samples were collected and further to separate using centrifuge (MED. Instrument Centrifuge MPW-352) at 3000 rpm for 10 min. The same amount of simulated fluid then was supplied to replace the discharged sample at given time interval. Then, the samples were measured using UV-Vis spectrophotometer (Jenway, UK) at the wavelength of 285 nm. All simulations were prepared without the use of digestive enzymes. The release of vitamin E was calculated based on following equation:

$$\text{Release} = (R_t - R_0) / R_t \times 100 \quad (1)$$

Where, R_t represents vitamin E release at t time and R_0 refers to their reading at 0 times.

2.7 Statistical analysis

Each experiment was performed at least three replicates per sample, and all the results were reported in average and standard deviation of 12 measurements.

3. Results and discussion

3.1 Water solubility

Water solubility means the maximum concentration of solute used to dissolve in water while hydrophobicity means lacking in water attraction. As the saturation of solute's concentration in water increases, the hydrophobicity of that solute will usually decrease as the relationship between solubility and hydrophobicity is opposite to each other. Table 1 shows the results obtained for different formulations used in this study. From the table, it can be observed that the water solubility for control (C) is 1.40 ± 0.54 minutes and the water solubility for the samples (S1, S2, and S3) are 0.43 ± 0.05, 0.37 ± 0.04 and 0.29 ± 0.08 minutes. The results clearly showed that the time taken for control is higher compared to all other samples. However, it is noted that as the amount of selenomethionine increases, the solubility of the sample also increases. It can be deduced that the addition of selenomethionine increases the saturation of concentration of solute in water. Accordingly, the hydrophobicity of that solute will decrease thus resulting in high solubility. Increasing the amount of selenomethionine will also decrease the ratio of vitamin E retained in the encapsulation. The hydrophobicity of encapsulated vitamin E decreases which in turn results in higher water solubility. This study is in agreement with Arvisenet et al. (2002) and Tapanapunnitikul et al. (2008) which reported that lower lipid flavor retention can be significantly attributed to either lower solubility or lower hydrophobicity.

Table 1: The results obtained for different formulation

Sample	Composition	Water solubility (min)	Moisture content (%)
C	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate	1.40 ± 0.54	7.67 ± 0.22
S1	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 1.6 mg Selenomethionine	0.43 ± 0.05	7.49 ± 0.46
S2	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 5.6 mg Selenomethionine	0.37 ± 0.04	7.06 ± 0.31
S3	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 10.6 mg Selenomethionine	0.29 ± 0.08	6.33 ± 0.58

3.2 Moisture content determination

Table 1 shows that the percentage of moisture content for encapsulated vitamin E for control is 7.67 ± 0.22 % while the percentage for S1, S2, and S3 are 7.49 ± 0.46, 7.06 ± 0.31, and 6.33 ± 0.58 %. As seen in the table, the control has higher moisture content than other formulations and it is noted that the moisture content decreases whenever the amount of selenomethionine increases. This is due to the different crust formation of the formulations after addition of different amounts of selenomethionine. As a result, water diffusion through the wall materials would vary, therefore affecting water evaporation and rendering of the powders with different moisture contents. The determination of moisture content is important for the study of storage stability of the powders as lower water content could reduce the degradation of encapsulated oil. Previous studies showed that food that has been dried to less than 2 to 3 % of moisture content is susceptible to lipid oxidation. However, if

the moisture content is lower than 7 %, water diffusion from the food matrix could also reduce. Consequently, the effect of moisture content on physical and chemical characteristics of encapsulated oils and the accessibility of oxygen to oil through the pores of wall material could also decrease (Takeungwongtrakul et al., 2015). In reference to this study, it can be considered that the percentage of moisture content of encapsulated vitamin E is acceptable and is improved by the addition of selenomethionine.

3.3 Size and Polydispersity index (PdI)

We performed test on the effect of selenomethionine on particle characteristics of encapsulated vitamin E. Four formulations of encapsulated vitamin E were prepared in this study at an initial ratio of 3 : 2 : 1. The formulations were labeled C, which stands for control (maltodextrin + sodium caseinate + vitamin E) and S (1 - 3), referring to the sample formulations containing the different amounts of selenomethionine. The influence of selenomethionine on particle size and polydispersity index (PdI) of encapsulated vitamin E was measured in comparison to the control (with no selenomethionine), as presented in Table 2. From the table, it can be observed that the size obtained for control is 4.56 ± 0.38 μm while the size obtained for S1, S2, and S3 are 4.72 ± 0.42, 3.00 ± 0.55 and 5.26 ± 0.46 μm. The results showed that the largest particle size obtained is from S3, the moderate size is S1 and the control while the smallest size is obtained by S2. It is noted that a stable particle size is obtained when the formulation is added with 5.6 mg of selenomethionine while other formulations increase in size whenever the amount of selenomethionine increases.

On the other hand, the size distribution for control is 0.40 ± 0.14 and the size distribution for S1, S2, and S3 are 0.27 ± 0.22, 0.81 ± 0.16 and 0.36 ± 0.04. From the table, it can be seen that all formulations have small size distribution except for S2 which has a PdI value of 0.81 ± 0.16. Lower PdI value indicates smaller size distribution while higher PdI value indicates bigger size distribution. A zero value is used to indicate the smallest PdI value while a value of one is the largest PdI value (Yuan et al., 2008). It is noted that the size distribution obtained is sufficiently stable for aggregation after addition of different amounts of selenomethionine.

Table 2: The results obtained for different formulation

Sample	Composition	Size (μm)	Polydispersity index (PdI)
C	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate	4.56 ± 0.38	0.40 ± 0.14
S1	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 1.6 mg Selenomethionine	4.72 ± 0.42	0.27 ± 0.22
S2	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 5.6 mg Selenomethionine	3.00 ± 0.55	0.81 ± 0.16
S3	5.01 g maltodextrin 3.34 g Vitamin E 1.67 g Sodium caseinate 10.6 mg Selenomethionine	5.26 ± 0.46	0.36 ± 0.04

3.4 Release determination

Figure 1 shows the percentage of vitamin E release for formulations with and without the presence of selenomethionine. It can be seen that the highest percentage of vitamin E release is from formulation C, whereas the lowest percentage of vitamin E release is from S3. It is presumably due to the higher ratio of vitamin E retained in the wall material of formulation C compared to formulation S3. It is observed that at the first 30 min, vitamin E release from formulation C is approximately 98 % and sustained release until 180 min in the solution, whereas for formulations S1, S2, and S3, it is found that the release obtained are 81, 87 and 61 %. Among all samples, formulation S2 has the ability to encapsulate and protect more vitamin E compared to S1 and S3 as the release obtained is almost the same with the control. To further confirm, the study was continued with in vitro release of encapsulated vitamin E in simulated intestinal fluid (SIF).

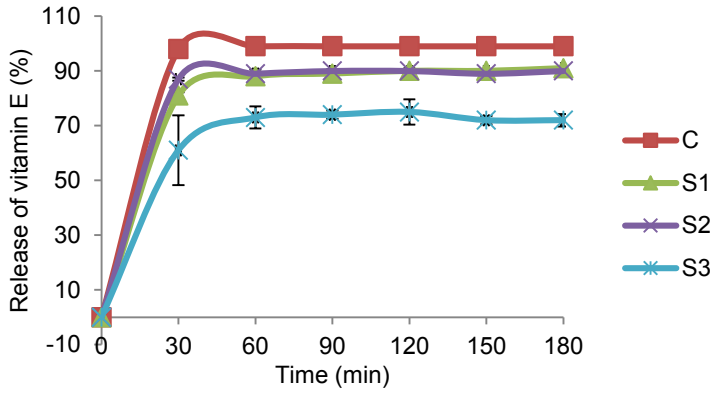

Figure 1: Vitamin E release in simulated gastric fluid (SGF) at pH 1.2

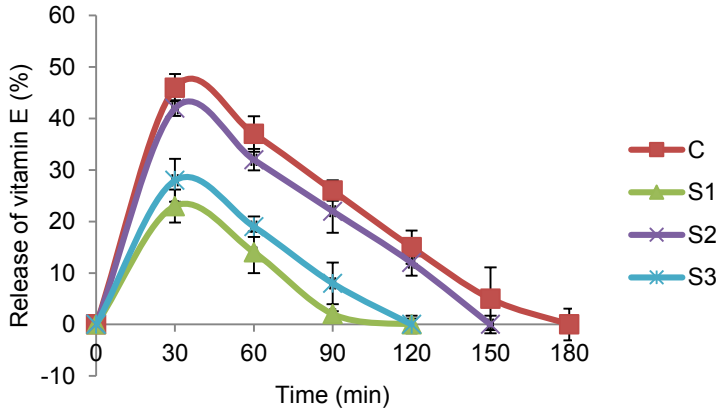

Figure 2: Vitamin E release in simulated intestinal fluid (SIF) at pH 7.4

Figure 2 shows the percentage of controlled release for encapsulated vitamin E with and without addition of selenomethionine at pH 7.4. It can be seen that at first 30 min, vitamin E release from formulation C is 46 % and continues to decrease after 150 min in SIF solution. However, for formulations S1, S2, and S3, their release are 23, 42, and 28 % and they go lower after 150 min in SIF solution. The zero values in the graph are obtained when the samples were completely removed from the solution. As highlighted before, the desired nanocapsule for the drug delivery systems must be able to sustain their release at a specific rate within the small intestine (Yoo et al. 2006). It is observed that an addition of 5.6 mg of selenomethionine into formulation S2 resulted in high vitamin E retained as compared to S1 and S3. The study showed that the reduction of particle size, size distribution, viscosity, and hydrophobicity may significantly impact the absorption, reaction and bioavailability of vitamin E in the stomach. The reduction in particle size could increase the surface-to-volume ratio which consequently increases their reaction towards other components as their mechanical, electrical and optical properties also change. The solubility of bioactive compounds determines the release rate and release properties from a polymeric matrix system. The more soluble are the compounds, the faster the release rate and release kinetic will be as higher dissolution rate will be obtained (Ezhilarasi et al. 2013).

4. Conclusions

To conclude, it is found that vitamin E was successfully encapsulated in a formulation containing maltodextrin and sodium caseinate, with and without selenomethionine at a ratio of 3 : 2 : 1. The encapsulation of vitamin E with 5.6 mg selenomethionine resulted in good solubility and moisture content. The size obtained for encapsulated vitamin E with 5.6 mg selenomethionine was smaller than other formulations and the size distribution obtained was below than 1. In relation to release determination, the total amount of vitamin E obtained in simulated gastric fluid (SGF) was higher for formulation S2 as compared to formulation S1 and S3,

and there was also significant difference in vitamin E release from all formulations in simulated intestinal fluid (SIF), which may be attributed to the different effects of selenomethionine on pH and vitamin E release. Overall, this study provided insights on the impact of selenomethionine which attempts to improve emulsion-based delivery systems for vitamin E.

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