



Influence of Inulin and Isomalto-oligosaccharides as Thickener on the Stability of Vitamin C Containing $W_1/O/W_2$ Double Emulsion

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Abstract. Encapsulation with a $W_1/O/W_2$ double emulsion (DE) system is a method that could protect vitamin C or other active ingredients from external influences thus increasing their stability and bioavailability. The DEs were prepared using hydrogenated coconut oil (HCNO) and middle chain triglycerides (MCT) coconut oil stabilised by PgPr and Tween 20/ PgPr to strengthen the inner and outer interfacial layers, respectively. The prebiotic polysaccharides such as inulin and isomalto-oligosaccharides (IMO) of 1-5% were added to the outer aqueous phase as thickeners. It turned out that the addition of thickeners induced DE destabilisation. The increased thickeners' concentrations enhanced the detrimental effect on the stability of the DEs. Furthermore, the MCT based DEs demonstrated a higher rate of destabilisation compared to the HCNO based DEs. The gelled form of HCNO acted as an effective barrier that hindered the movement of aqueous phase between the internal and external phases. The effect of inulin addition on the destabilisation of DE was less detrimental compared to IMO due to its higher viscosity. The thickeners seemed to be incompatible with the emulsifiers thus destabilising the DEs. The DE stability of higher than 70% and encapsulation efficiency of more than 70% achieved using HCNO oil after 4-day storage seemed promising to be further developed as low-fat and nutritious food. Tailoring the stability of interfacial layers of the Vitamin C containing DEs with the prebiotic thickeners seemed crucial to bring the fortified products into commercialisation.

Keywords: Destabilisation, Double Emulsion, HCNO, Inulin, Isomalto-oligosaccharide, MCT, Vitamin C

1. Introduction

Vitamin C, or ascorbic acid, is a water-soluble nutrient present in numerous fruits and vegetables and commonly taken as a supplement. It holds significance for connective tissue well-being, as it plays a vital role in collagen synthesis [1]. Vitamin C also acts as a strong antioxidant, aiding in shielding the body from harm caused by free radical and helps control the immune system [1]. Nevertheless, Vitamin C readily breaks down when exposed to water-based environments, elevated pH levels, the presence of oxygen, and metal ions [1, 2, 3] thus encapsulation of Vitamin C is highly required to protect its bioavailability and stability to prolong its shelf life.

DE water-in-oil-in-water ($W_1/O/W_2$) has been one of the nutraceutical carriers which encapsulates the nutrients in the dispersed phase as well as controls their release [4-7]. Multiple emulsions are intricate polydispersed arrangements where both water-in-oil (W/O) and oil-in-water (O/W) emulsions coexist simultaneously. Frequently, they are created through a two-stage procedure and typically stabilised using a blend of surfactants with both water-attracting and water-repelling properties. Attaining stable multiple emulsions hinges on the proper proportion of these surfactants. Moreover, the presence of two oil-water interfacial layers has made the stability to be more difficult to attain compared to the single emulsion. Recently, various techniques have been employed to enhance the DE stability for food applications, such as the addition of electrolyte and gelling agent in the inner aqueous phase [4, 8], gelation of lipid phase [7], thickening of the outer aqueous phase using low concentration of pectin, gum arabic, carboxy methyl cellulose/ CMC, and salt soluble protein [5, 9, 10, 11], and pickering stabilisation using rice husk silica/ chitosan particles [12, 13]. The addition of Na-CMC of 1-3% in the external aqueous phase of W/O/W without the presence of external emulsifiers has significantly increased the viscosity and the stability of the multiple emulsions [10]. Schuch et.al. [9] added several polymeric stabilisers in the outer water phase of W/O/W emulsion without added external emulsifiers

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and found out only CMC with the lowest interfacial activity tended to increase the DE stability. Therefore, employing biopolymers does not ensure an augmented stability for the DE depending on many other factors [5, 9, 14]. Utilizing biopolymers as stabilising agents in DEs offers various benefits. For instance, biopolymers are considerably less prone to diffusing and transferring between the internal and external aqueous phase, as opposed to small-molecule emulsifiers; biopolymers can form stabilising network structures such as viscoelastic solutions and gels in the aqueous phase; and surface-active biopolymers can effectively maintain the stability of the outer interfacial layer in W/O/W emulsions [14]. Several attempts have been carried out to encapsulate Vitamin C in the inner aqueous phase of the W/O/W multiple emulsions [8, 11, 15, 16] or spray dried of vitamin C loaded W/O/W to prolong the shelf life of Vitamin C [17].

The growing global demand for natural and health-conscious foods has underscored the paramount importance of developing fortified foods enriched with bioactive compounds. Bioactive compounds such as vitamin and dietary fibers seemed to be promising food ingredients since they could not only enhance the physicochemical and sensory attributes of food products, but also increase the delivery of nutraceuticals [18]. Inulin and isomalto-oligosaccharides (IMO) are belongs to prebiotics and dietary fibers which confer advantageous health impacts on the overall wellness of the host, and they commonly serve as thickeners augmenting the physical stability of emulsion based food products which is of high importance during transportation and storage [19-21]. A low concentration of inulin up to 10% has been exploited to improve the rheological properties as well as textural and sensory properties of O/W emulsion based food products by elevating the thickness of the external phase [19, 22, 23]. IMO could also serve as a viable alternative sweetener for individuals with diabetes [21]. However, there has been no investigation yet using IMO as stabiliser or thickener to increase the emulsion stability. Therefore, the aim of this study is to investigate the influence of low concentration inulin and IMO addition on the DE stability. The incorporation of inulin or IMO as dietary fibers and prebiotics would further increase the added value for the production of highly nutritious and DE based food products with reduced calorie content.

2. Materials and Methods

2.1 Materials

Hydrogenated coconut oil (HCNO) comprised of 0.5% caproic acid, 5% caprylic acid, 6% capric acid, 45% lauric acid, 20% myristic acid, 11% palmitic acid, 12% stearic acid, and trace amounts of oleic, linoleic, and linoleic acids; middle chain triglycerides (MCT) consisting of 60% caprylic acid (C8) and 40% capric acid (C10); Vitamin C/ ascorbic acid (Sigma-Aldrich, UK); Tween-20 (Merck, Germany), Polyglycerol Polyricinoleate/ PgPr 4120 (Palsgaard, Denmark); inulin (96.8% dry matter content consisting of 90% inulin and 10% fructose, glucose, sucrose; average chain length 9/ MW of ~1460 g/mole; ash content < 0.2%); isomalto-oligosaccharides/ IMO (99.5% oligosaccharides and 0.5% glucose, Dextrose Equivalent 33.55/ MW of ~3 g/mole); and demineralised water.

2.2 Preparation of W₁/O primary emulsion

Inner aqueous phase (W₁) containing 25% (w/w) Vitamin C was prepared by dissolving ascorbic acid powder in the demineralised water. Subsequently, the mixture was stirred using a magnetic stirrer at a speed of 100 revolutions per minute for around 3 minutes until homogeneous. The oil phase was prepared by mixing HCNO or MCT with 6% PgPr (w/w) using a magnetic stirrer at 800 rpm for about 7 minutes. The water phase containing a portion of 30% (w/w) was then evenly distributed within the oil phase through the use of a rotor-stator apparatus (IKA T25 digital ULTRATURRAX, Germany) at 20,000 rpm for 6 minutes. In particular of HCNO derived W₁/O emulsion, both aqueous and oil phases were brought to 60°C prior to emulsification.

2.3 Preparation of W₁/O/W₂ DE

The external aqueous phase (W₂) was prepared by mixing emulsifiers and thickeners at certain amounts, as depicted in Table 1. Tween 20 of 0.5% (w/w) and Tween 20 0.5%/ PgPr 0.5% (w/w) relative to the outer aqueous phase (W₂) were selected to stabilised HCNO and MCT based DEs, respectively. This selection was designated based on the most stable DEs according to our previous study. Inulin and isomalto-oligosaccharides of 1%, 3%, and 5% (w/w) relative to the outer aqueous phase (W₂) were added subsequently in order to examine how they impact the kinetic stability of the DEs. The mixtures were stirred with a magnetic stirrer at a speed of 300 revolutions per minute for duration of 7 minutes. The fractions of internal emulsion (W₁/O) dispersed into the external phase were 30% (w/w) and 40% (w/w) for HCNO and MCT oil, respectively. The emulsification

was performed utilizing a rotor-stator device (IKA T25 digital ULTRATURRAX, Germany) at a speed of 8,000 revolutions per minute for duration of 3 minutes. The resulting DE was transferred into a vial with dimensions of 25 mm inner diameter and 95 mm height, and it was maintained at room temperature (~28-30°C) for the stability test up to 4 days.

Table 1. Variation of thickener used for the DE

DE	Oil	External Emulsifier	Thickener
HF0 (control)			-
HF1			Inulin 1%
HF2			Inulin 3%
HF3	HCNO	Tween 20 (0.5%)	Inulin 5%
HF4			IMO 1%
HF5			IMO 3%
HF6			IMO 5%
MF0 (control)			-
MF1			Inulin 1%
MF2			Inulin 3%
MF3	MCT	Tween 20/ PgPr (0.5/ 0.5%)	Inulin 5%
MF4			IMO 1%
MF5			IMO 3%
MF6			IMO 5%

2.4 Assessment of the stability of DE

The kinetic stability of the DE was evaluated using the provided equation (1). The emulsion layer was indicated by a milky appearance which was distinctly seen from the watery rich phase. The DE stability was monitored up to 4 days.

$$\%S = \frac{h_t}{h_0} \times 100\% \quad (1)$$

whereas h_t represents the height of the DE at a specific time and h_0 represents the initial height of the DE.

2.5 Determination of DE viscosity

The DE viscosity was determined utilizing a Brookfield DV-III Ultra Rheometer (Brookfield Engineering Labs Inc., MA) equipped with a SC-21 spindle. A volume of 8 ml of recently prepared DE was introduced into the small sample adapter. The viscosity of the DE was read from the display at the rotation speed of 10 rpm.

2.6 Determination of release profile of Vitamin C and encapsulation efficiency

Fifteen grams of $W_1/O/W_2$ DEs prepared with MCT and HCNO thickened by 1% inulin was poured into the 20 cm long of visking tubing dialysis bag (diameter= 14.3 mm; MWCO= 10,000 to 14,000 Da), tightly tied on both ends, and slowly positioned within a beaker filled with 500 ml of demineralised water as the release medium and stirred at a speed of 100 rpm using a magnetic stirrer. An aliquot 2.5 ml of the release medium was taken at certain time intervals up to 4 days and was analysed for the ascorbic acid content using iodometric titration techniques [3]. The release profile of Vitamin C into the release medium was created by plotting the fraction release of Vitamin C versus time. The fraction of Vitamin C released (%FR) into the release medium was calculated as the ratio of Vitamin C released into the medium at a certain time (M_t) relative to the total amount of Vitamin C in the medium when they were all released (M_∞). The encapsulation efficiency (%EE) was the percentage of Vitamin C which was still encapsulated within the inner aqueous phase (W_1). All release experiments were conducted at room temperature.

$$\%FR = \frac{M_t}{M_\infty} \times 100\% \quad (2)$$

$$\%EE = 100\% - \%FR \quad (3)$$

3. Results and Discussion

3.1 The influence of thickeners on the stability of $W_1/O/W_2$ DEs

The destabilisation of DEs prepared using coconut oils, i.e. HCNO and MCT with time up to 24 hours could be seen in Fig. 1 and Fig. 3, respectively. The stability of DEs was demonstrated up to 24 hours, since it exhibited minimal changes afterwards. It turned out that the DEs were destabilising with time and the destabilisation was more remarkable in MCT based DE in comparison to the corresponding HCNO based DE. Interestingly, the stability of HCNO based DEs abruptly decreased for the first 3 hours and tended to be leveled off, while the destabilisation of MCT based DEs seemed to be delayed for the first 3 hours prior to continuous decrease. The stability of DEs prepared with HCNO was sufficiently high of over 70% in contrast with those prepared with MCT of which stability mainly below 70% after 24 hours. This was confirmed by the macroscopic stability of HCNO and MCT based DEs monitored after 4-day-storage as depicted in Fig. 2 and Fig. 4, respectively whereby the addition of thickeners such as inulin and isomalto-oligosaccharides (IMO) significantly induced the DEs destabilisation.

After 4-day-storage, it seemed that the stability of all MCT based DEs dropped below 50%, whereas the stability of HCNO based DEs stayed above 70% although the primary emulsion W_1/O fraction used was lower in the HCNO based DE compared to the MCT based DE. The higher stability of HCNO based DEs was conferred by the higher melting point of HCNO of about 31-33°C compared to MCT oil of which melting point ranged about 5-7°C. This huge discrepancy was due to the presence of entirely medium chain triglycerides in MCT in contrast to HCNO which comprised of more than 40% long chain triglycerides. HCNO tended to be more viscous and gelled at room temperature in contrast to MCT which was liquid and prone to coalescences leading to DE instability. This was corroborated by the viscosity measurements as depicted in Table 2, whereby the HCNO based DE generally showed a higher viscosity compared to the corresponding MCT based DE. Raising the viscosity of the lipid phase could potentially decrease the rates of diffusion between the two aqueous phases and mitigate the destabilisation of the inner water phase [7].

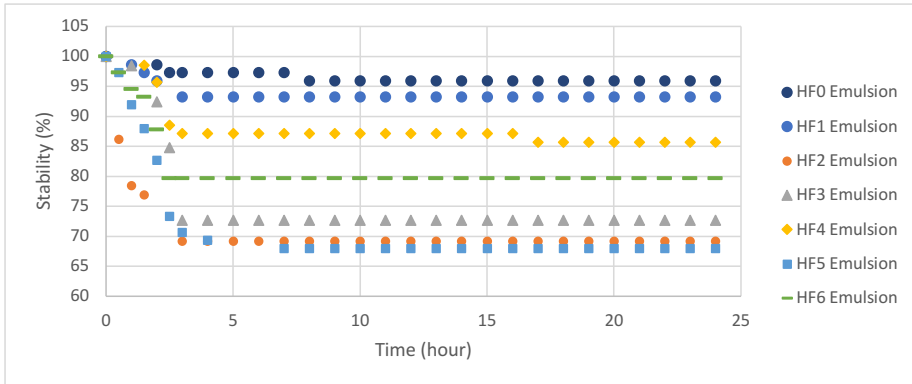


Fig. 1. Stability of DEs prepared using HCNO and various thickeners monitored until 24 hours

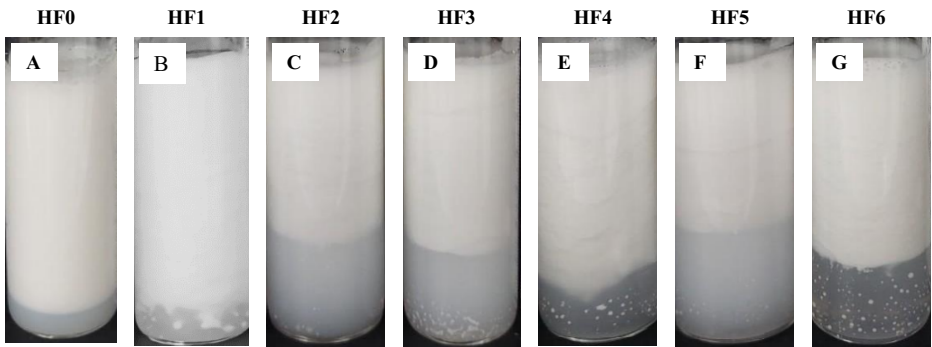


Fig. 2. Macroscopic stability of DEs prepared using HCNO and various thickeners after 4-day storage. A) no thickener; B) inulin 1%; C) inulin 3%; D) inulin 5%; E) IMO 1%; F) IMO 3%; G) IMO 5%

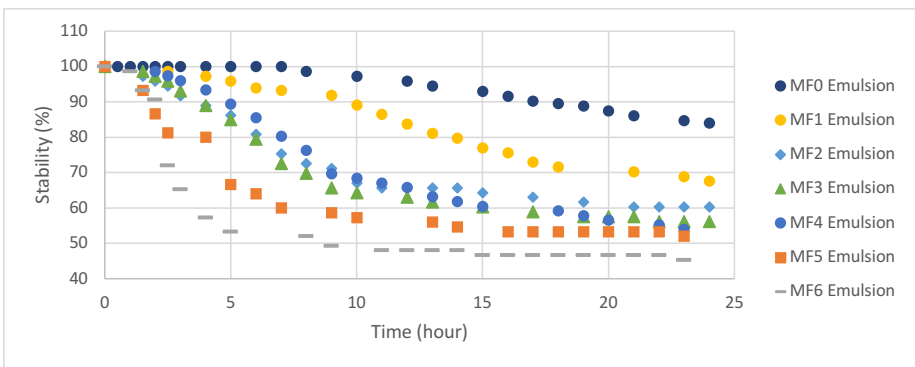


Fig. 3. Stability of DEs prepared using MCT and various thickeners monitored until 24 hours

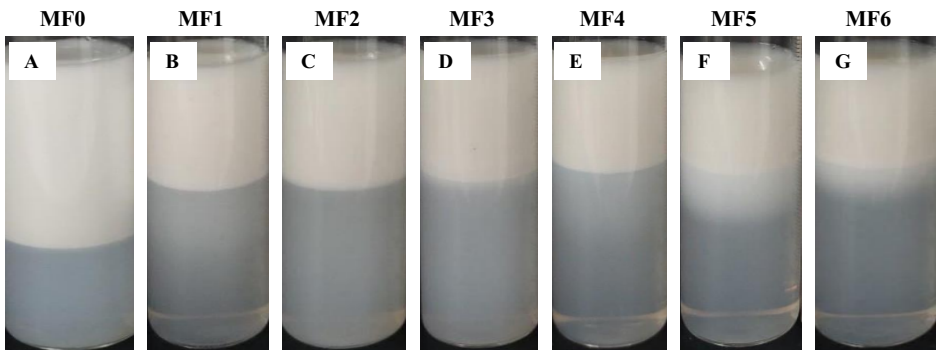


Fig. 4. Macroscopic stability of DEs prepared using MCT and various thickeners after 4-day storage. A) no thickener; B) inulin 1%; C) inulin 3%; D) inulin 5%; E) IMO 1%; F) IMO 3%; G) IMO 5%

The inclusion of IMO had a more pronounced effect on reducing the stability of the DE compared to inulin addition after 24 hours (Fig. 1 vs. Fig. 3) although the difference was barely seen macroscopically after 4-day-storage (Fig. 2 vs. Fig. 4). Inulin is a long chain with higher molecular weight whereas IMO is composed of short chain, therefore inulin would create a more viscous solution once dissolved in water compared to IMO. This was supported by the viscosity data presented in Table 2 indicating that the addition of inulin increased the viscosity of DEs more sharply in comparison to IMO addition both in HCNO as well as MCT based DEs. The increased viscosity improved the overall DE stability by delaying the flocculation and coalescences amongst the oil globules thus reducing the creaming rate [6]. The phase separation induced upon the addition of 1-5% thickeners could be mainly due to the creaming of less dense emulsion phase and sedimentation of the aqueous phase [6, 12, 14]. A multiple emulsion such as $W_1/O/W_2$ DE is a more intricate arrangement characterised by the existence of two interfacial layers between oil and water. The stability is quite difficult to attain and the instability mechanisms could be very complex. Rather than enhancing the stability of DE, the incorporation of thickeners indeed induced the destabilisation. Moreover, the destabilising rate was increasing as the added thickeners' concentrations were increased, which was more pronounced in MCT based DEs. This strongly implied the incompatibility between thickeners and emulsifiers.

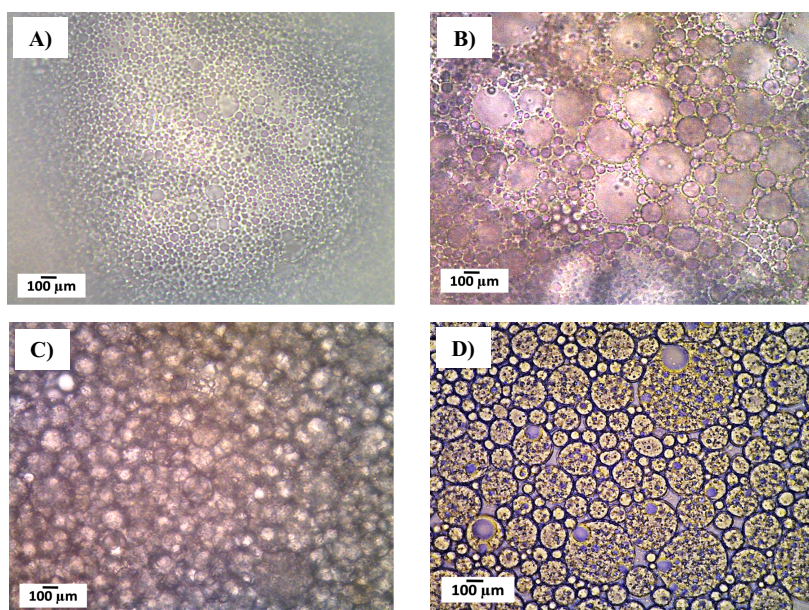


Fig. 5 Microscopic structure of DEs prepared using 1% inulin. (A) HCNO after preparation; (B) HCNO after 4 day storage; (C) MCT after preparation; (D) MCT after 4 day storage

Two different mechanisms were proposed to explain the destabilisation of HCNO and MCT based DEs in this study. In case of MCT DE, it seemed plausible that the thickeners incorporation might induce the diffusion of external aqueous phase into the internal aqueous phase. This might be triggered by the concentration gradients between the internal and external aqueous phases. This caused a quite high viscosity of DEs, which conferred the constant stability for the first 3 hours as could be seen in Fig. 3. However, the increased fraction of inner aqueous droplets would then result in the agglomeration and coalescence of the inner aqueous phase [17, 24]. This phenomenon was clearly seen in the microscopic structure as depicted in Fig. 5. There was inner aqueous droplet enlargement which also extend the size of oil globules in contrast to the HCNO based DE. Furthermore, the inner aqueous phase might be expelled out into the external aqueous phase leading to phase separation between the DE and the aqueous rich phase. The increased phase separation could be also caused by the detachment of

emulsifiers mainly adsorbed in the outer interfacial layer due to some interaction or competition between the thickeners and the emulsifiers [14, 24]. This mechanism was supported by the viscosity data, whereby viscosity was quite high upon the addition of 1% thickeners due to the migration of external water phase into the internal water phase, followed by the abrupt decrease in viscosity upon the addition of 3% and 5% thickeners because of the movement of the internal water phase towards the external water phase leading to the remarkable phase separation.

Table 2. Viscosity of freshly-prepared DE

DE	Oil	Thickeners	Viscosity (cP)
HF0	HCNO	-	3200
HF1		Inulin 1%	150
HF2		Inulin 3%	1630
HF3		Inulin 5%	3220
HF4		IMO 1%	60
HF5		IMO 3%	1090
HF6		IMO 5%	1790
MF0	MCT	-	540
MF1		Inulin 1%	1320
MF2		Inulin 3%	350
MF3		Inulin 5%	440
MF4		IMO 1%	1290
MF5		IMO 3%	330
MF6		IMO 5%	70

In case of HCNO based DEs, the oil droplets were much smaller compared to the MCT oil globules and the coalescence of internal aqueous droplets was barely seen. The smaller oil globule size increased the stability of all HCNO based DEs. The destabilisation mechanism was directly correlated with the desorption of the emulsifiers from the outer interfacial layer due to interaction of competition with the thickeners promptly inducing the phase separation. This also explained the abrupt decrease of the DE at the very beginning prior to leveling off (Fig. 1). The lowest viscosity was measured upon the addition of 1% thickeners due to the occurrence of instant phase separation and the viscosity increased with increased thickeners' concentrations. Interestingly, the phase separation occurred in DE prepared with HCNO and 1% inulin (HF1) was very less and comparable with that without inulin addition (Fig. 1 and Fig. 2). The increased of thickener's concentration tended to decrease the stability of DE albeit an increase in its viscosity which strongly implied that there could be incompatible interactions between Tween/PgPr with inulin or IMO preventing the thickeners from adsorbing onto the oil droplet surfaces in a coordinated manner, leading to ineffective stabilisation. Such incompatible interactions would diminish the efficiency of emulsion stabilisation, stemming from hydrophobic interactions and steric stabilisation. Inulin was supposed to confer a higher extent of steric stabilisation compared to IMO due to its bulky structure. However, they would be easily aggregated in the aqueous phase leading to phase separation if they could not be adsorbed on the oil surfaces. Furthermore, emulsion stabilisation due to electrostatic interaction seemed to be less dominant since all components used were uncharged. There have been many factors affecting the stability of DEs including the homogenisation techniques, processing parameters, and formulation variables which have to be optimised to gain more insight on the stability of Vitamin C encapsulated DEs.

3.2 Release profile of Vitamin C

The comparative release profiles of Vitamin C, encapsulated in DEs prepared with HCNO and MCT, both thickened with 1% inulin, are illustrated in Figure 6. The Vitamin C release from HCNO based DEs was much delayed compared to MCT based DEs. The burst release of Vitamin C into the release medium within the first 300 minutes was more pronounced in MCT based DE with a fraction release of nearly 50%, which was nearly 3 times higher than that in HCNO based DE. The encapsulation efficiencies of Vitamin C after 4-day storage were ~30% and ~70% for MCT and HCNO based DEs, respectively. This was comparable with the entrapment of Vitamin C in gelled W/O/W of about 76% after 15 days analysed using a different approach [8]. Once more, these results affirm the superior stability of DEs based on HCNO, which effectively encapsulate Vitamin C within the internal water phase. The HCNO based DE could better entrap and protect Vitamin C by hindering the diffusion of Vitamin C from the internal aqueous phase into the external aqueous phase due to its rigid oil

structure in comparison with the MCT structure. There are still many factors affecting the DE stability and the vitamin C release which need further investigation.

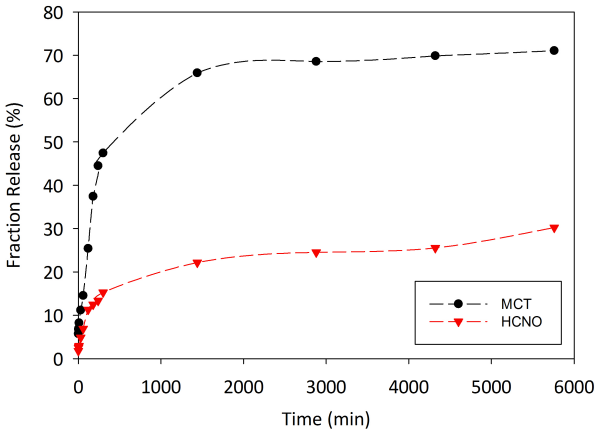


Fig. 6 Release profiles of Vitamin C entrapped in the DEs thickened by 1% inulin

4. Conclusions

The addition of thickeners such as inulin and IMO did induce the destabilisation of the DEs prepared using HCNO and MCT coconut oil. The mechanisms seemed to be different in case of MCT and HCNO based DE. The aqueous migration between the inner and outer water phase appeared to play a significant role which tremendously destabilised the overall MCT based DEs. At the very beginning, the outer aqueous phase might diffuse into the internal aqueous phase thus enhancing the viscosity of the DE. However, the stability of DEs remarkably dropped with time and with the increase of thickeners' concentrations due to phase separation which could be induced by the coalescence of inner aqueous phase and also by the detachment of the outer emulsifiers. In case of HCNO based DEs, the semi solid HCNO appeared to slow down the diffusion of aqueous phase between the two aqueous compartments thus preventing the high instability of the DEs. The destabilisation was probably induced by the detachment of the outer emulsifiers due to some incompatible interactions or competition leading to phase separation. Instead of thickening the continuous phase, the viscosity of external water phase was continuously decreased with increasing concentrations of thickeners indicating the occurrence of phase separation in MCT based DEs. On the other hand, the increased thickeners' concentrations did increase the viscosity of HCNO based emulsions but did not significantly increase the overall stability. Moreover, the addition of inulin decreased the destabilisation of the DE compared to IMO due to its higher viscosity. The quite high stability of HCNO based DEs containing Vitamin C of over 70% with the Vitamin C entrapment of ~70% after 4-day storage seemed promising to be further developed as functional foods. Enhancing the stability of oil-water interfacial layers using various ingredients, combined thickeners, along with managing the preparation techniques of aqueous and lipid phases of the $W_1/O/W_2$ DE, has consistently presented a challenge. This challenge holds significant importance in achieving commercially viable, safe, and health-conscious food products.

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