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Review on Factors Affecting Partitioning of Bioactive Compounds in Emulsions

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Functional foods are popular nowadays as people become more health conscious and thus demand for healthier foods. Functional foods usually contain phytonutrients which are plant bioactive compounds with many health benefits. These functional foods are available in many forms and one of the common forms is the emulsion-based encapsulation of bioactive compound. Encapsulation through emulsification can improve the stability of phytonutrients during storage and processing. However, the partitioning of phytonutrients may occur during the emulsion formation and storage, which can affect the chemical stability of the phytonutrients. Therefore, it is important to study the partitioning behaviour of bioactive compound within an emulsion and the factors affecting the partitioning. In this review, several influencing factors of bioactive partitioning in emulsions are discussed, which include oil loadings, surfactant types and concentrations, bioactive co-encapsulation and solid fat content. This review is crucial in developing more stable emulsion-based functional foods in the future.

Keywords: Bioactive, phytonutrient, partitioning, emulsion, encapsulation

INTRODUCTION

Phytonutrients are plant bioactive compounds that have many health benefits for humans. Nowadays, many functional foods that contain beneficial phytonutrients are produced. A functional food is defined as the food that can give benefits and provide additional nutrients to the body or reduce the risk of getting a certain disease (Roberfroid, 1999). Recently, functional food is very popular as consumers become more conscious about their health. These functional foods are beneficial in improving overall health, delaying the onset of age-related diseases, after illness recovery, reducing stress, for pregnancy and slimming, enhancing sports performance, treating a wide range of symptoms such as cough, cold, and arthritis, and also as a supplementation to a poor diet (Gupta and Prakash, 2014). The functional foods are added with nutrients in multiple ways, and one of them is through emulsification, where the bioactive compound is carried either in the oil or aqueous phase of emulsions.

Emulsion is a mixture of two or more liquids that normally do not mix together due to liquid-liquid phase separation, but is able to be mixed together as an emulsion by external forces such as homogenisation or addition of emulsifier that mixes the immiscible liquids together to become homogenous. The most common types of emulsion are oil-in-water (O/W) and water-in-oil (W/O) emulsions. Milk is an example of O/W emulsion, where fat droplets or oil are dispersed in water. On the other hand, butter or margarine represents W/O emulsion in which water droplets are dispersed

within the continuous phase of oil. These phases will separate as time goes by, but the separation process can be slowed down or the emulsion can be stabilized by several means such as addition of emulsifier or surfactant and physically by homogenisation. The large interfacial area between oil and water phases causes physical instability of the emulsion, which may result in high level of free energy. In an emulsion, the emulsifier added is held by a highly anisotropic and narrow region (1–40 nm thick) of oil-water interface that surrounds emulsion droplets (Farooq et al., 2021). The oil-water interface of an emulsion has a major effect on the lipid oxidation pathway.

As for emulsions containing phytonutrients, the bioactive compound is usually incorporated in oil before being mixed together with water or other liquid that is immiscible with oil. When the oil and water are mixed together or become an emulsion, there is a possibility for the phytonutrient in the oil to partition into the water phase. Partitioning of phytonutrient or bioactive compound within an emulsion is a process that occurs when the compound gets segregated from a phase to the other phase(s) within the emulsions. The partitioning at oil-water interface of an emulsion may give effect towards the effectiveness of some bioactive compound in inhibiting lipid oxidation in emulsion (Farooq et al., 2021). In addition, as time goes by, degradation of the phytonutrient will occur and the degradation rate may be different depending on the phase it resides. Therefore, it is important to understand the partitioning characteristics of the phytonutrient in order to obtain a chemically and physically stable carrier system for the bioactive compound. This review elaborates the partitioning characteristics of different bioactive compounds in emulsions that are varied in oil loadings, surfactant types and concentrations, type of bioactive compounds, bioactive co-encapsulation and solid fat contents, based on literatures of previous experimental studies.

FACTORS AFFECTING PARTITIONING OF BIOACTIVE COMPOUNDS IN EMULSION

Effects of oil loading

Based on a study by Huang et al. (1997), different oil loadings of an emulsion did affect the partitioning characteristics of the bioactive compound within it. Oil loading in an emulsion refers to the percentage of oil phase incorporated in the emulsion formulation. Bioactive compounds in emulsion systems with lesser oil ratio (1 corn oil:1 water ratio) partitioned more into the oil phase than in emulsions with higher oil ratio (9 corn oil:1 Tween 20 ratio). In the emulsion systems with 1 corn oil:1 water ratio, the bioactive compounds; methyl carnosate, carnosol and carnosic acid partitioned 96.2%, 94.5% and 91.6% respectively into the oil phase (Huang et al., 1997). Meanwhile in the emulsions with 9 corn oil:1 Tween 20 ratio, all the bioactive compounds partitioned much lesser into the oil phase; 70.1%, 70.4%, 13.9% for methyl carnosate, carnosol and carnosic acid respectively (Huang et al., 1997).

Effects of surfactant type and concentration

Surfactant or emulsifier is a molecule consisting of the water-soluble (hydrophilic or lipophobic) head and fat-soluble (lipophilic or hydrophobic) tail. In an emulsion, the function of surfactant incorporated is to stabilize the emulsion by preventing oil coagulation that causes separation of the oil and water phases of the emulsion. The surfactant prevents the newly formed droplets from combining and coagulating by surrounding the droplets' surface (Ngouémazong et al., 2015). In order to get the most stable emulsion, the right surfactant must be chosen. For different types of emulsion system, there will be different suitable surfactants as each surfactant has its own hydrophile-lipophile balance (HLB) value. The HLB of each surfactant is different and it will determine whether the surfactant is more hydrophilic or hydrophobic thus acting as a guide in choosing the right surfactant for an emulsion. For W/O emulsions, it is suitable to use surfactants with HLB value ranging from 3.5 to 6.0, while surfactants with HLB value between 8 to 18 are more suitable for O/W emulsions (Zheng et al., 2015).

Initially in diluted water (or oil) solutions, the emulsifier dissolves and exists as a monomer, but the molecules of the emulsifier begin to associate spontaneously to form aggregates (termed as micelles) after the concentration of the emulsifier exceeds critical micelle concentration (CMC) (Housaindokht and Pour, 2012). Other than that, the structural features of an emulsifier largely determine the emulsifier concentration, emulsion-stabilising effectiveness and emulsifying activity of any polymer (Ngouémazong et al., 2015).

Chwarz and Einonen (2022) had studied the effects of different emulsifier types (10% emulsified rapeseed oil and 2% whey proteins) on the partitioning of several berry anthocyanins in emulsions. From that study, it was reported that in samples containing 2% whey proteins, partitioning

of raspberry anthocyanin was weaker than lingonberry and blackcurrant anthocyanins. Meanwhile, there was no significant difference ($p < 0.05$) in partitioning of different berry anthocyanin fractions could be observed between different phases of samples with 10% emulsified rapeseed oil. In comparison between samples containing 2% whey proteins with samples containing 10% emulsified rapeseed oil with 2% whey proteins, lingonberry anthocyanins in samples containing only 2% whey proteins partitioned more into the water phase than lingonberry anthocyanin in samples with 10% emulsified rapeseed oil with 2% whey proteins. Meanwhile in another W/O system consisting 1 rapeseed oil:9 water ratio, all anthocyanins partitioned into the water phase and no anthocyanin was located in the oil droplets (Chwarz and Einonen, 2022).

There is a study by Stöckmann et al. (2000), who incorporated different types of emulsifierin O/W emulsions to examine the partitioning of gallic acid between the phases. Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), polyoxyethylene 20 cetyl ether (Brij 58) and partially hydrolysed soybean lecithin (PHSL) were used as the emulsifiers and the results showed that the gallic acid partitioned more into the oil phase as the solubilisation capacity of the emulsifier decreased (CTAB > Brij 58 > SDS > PHSL).

Meanwhile, Sytar et al. (2013) has reported that there was a significant difference in partitioning of propyl gallate (as the bioactive compound) at aqueous phase, oil phase and interface of emulsions when different emulsifier type was used. Propyl gallate partitioned more into the oil phase and interphase when Tween 65 was used as compared to Tween 80. Meanwhile, propyl gallate partitioned more into the aqueous phase when emulsifier Tween 80 was used instead of Tween 65. The cause of the partitioning behaviour might be due to the emulsifier's properties itself. Emulsions containing different types of emulsifiers e.g. sodium caseinate and Tween range (Tween 20, 40, 60 or 80), had different properties of crystallization behaviour, degree of supercooling and shear stability (Fuller et al., 2018). The properties were also affected by the presence of protein (Fuller et al., 2018).

In a study by Wan Mohamad et al. (2017), they showed that pre-treatment of whey protein isolate (WPI) as the emulsifier did affect the partitioning of β-carotene in the emulsion. There was a significant difference in partitioning of β-carotene using treated and untreated WPI as the emulsifier. There was no significant difference in β-carotene partitioning in emulsions with WPI treated with heat at 70 °C and 80 °C, but β-carotene significantly partitioned more into the aqueous phase of emulsion when treated with high-pressure at 600 MPa pressure at 20 °C. Treatment of WPI using heat or pressure had altered the protein structure as a result of protein denaturation that caused thickening of the interfacial layer (Wan Mohamad et al., 2017).

Besides the type of surfactant, it has been reported that concentration of surfactant in emulsions also affects the partitioning of carried bioactive compounds. Kiralan et al. (2014) studied stripped soybean O/W emulsion containing tocopherols added with different concentrations of surfactant Tween 20. An increasing concentration of Tween 20 from 0.1% to 1.0% caused increasing aqueous-phase concentration of the tocopherol homologues. Naturally, tocopherols are not soluble in water, but the study found that tocopherols partitioned into the aqueous phase due to the Tween 20 molecules forming co-micelles with tocopherols. The study also compared the partitioning of different tocopherols, where the δ-tocopherols (90%) partitioned the highest into the aqueous phase as compared to γ- (89%) and α-tocopherols (73%).

In another study by Zhang et al. (2019) also showed that the surfactant concentration affected the partitioning of bioactive compounds in whey protein emulsions. In the study, they compared the partitioning of resveratrol as the bioactive compound in emulsions with different concentrations of $CaCl_2$ as the emulsifier. Resveratrol has many nutritional properties such as antioxidant, anti-inflammatory, antibacterial, anti-apoptopic and anti-depressant effects (de Oliveira et al., 2018; Yang et al., 2015), but it has poor solubility in sunflower oil and aqueous solution (Cheng et al., 2020; Filip et al., 2003; Summerlin et al., 2015) and thus having limited bioavailability (Salehi et al., 2018). They found no resveratrol detected in the oil phase of the emulsions after the resveratrol was extracted with isooctane/isopropanol mixture. Although resveratrol is hydrophobic, it can form complexes with whey proteins such as with β-lactoglobulin, α-lactalbumin and bovine serum albumin (Cheng et al., 2018; Liang et al., 2008), forming a film-liked structure at the surface of oil droplets (Salehi et al., 2018; Wan et al., 2014; Wang et al., 2016). In addition, the partitioning of resveratrol at the oil-water interface increased as the $CaCl_2$ concentration increased (0, 0.2 and 1.6 mM). The addition of $CaCl_2$ caused reduction of water

droplets size, which in turn lowered the attractive force between water droplets and increased the adsorption density of the emulsifier, thus producing a more stable emulsion (Márquez et al., 2010).

Effects of bioactive type

In emulsion, bioactive compound is added to delay lipid oxidation reaction besides adding nutritional value to the emulsion. According to the ‘‘Polar Paradox” (Di Mattia et al., 2009; Porter, 1993; Porter et al., 1989), hydrophilic antioxidants tend to locate more at the oil-air interfaces where they have better protection against lipid oxidation compared to lipophilic antioxidants that partition more in the oil phase. The antioxidant partitioning at the interface might also affect the antioxidant activity (Sytar et al., 2013). In a study by Di Mattia et al. (2009), they investigated the effects of bioactive compounds (gallic acid, catechin and quercetin) with different concentrations (250 μM, 350 μM and 500 μM) on their partitioning. All of the three bioactive compounds are phenolics compounds that are naturally occurring substances with different polarity (Di Mattia et al., 2009). The results showed that they partitioned the highest in aqueous phase at the bioactive concentration of 250 μM. For catechin and quercetin, the partitioning of bioactive compounds in the oil phase decreased as their concentrations increased. However, there was not much difference in the partitioning observed for gallic acid. Meanwhile, gallic acid exhibited the highest percentage of bioactive compound partitioned into the aqueous phase, followed by catechin and quercetin. In terms of polarity, gallic acid is the most polar while quercetin is the least polar bioactive compound.

Sytar et al. (2013) also studied the partitioning behaviour of different bioactive compounds in emulsions using Tween 80 and Tween 65 as emulsifiers. For each of the bioactive compounds (ascorbyl palmitate, gallic acid, or α-tocopherols), there was no significant difference in partitioning of each respective bioactive compound in the aqueous phase, oil phase and interface when either Tween 80 or Tween 65 were used. However, for propyl gallate, there was a significant difference in the partitioning of the bioactive compound into the aqueous phase, oil phase and interface when different emulsifiers were used (Tween 80 or Tween 65). Meanwhile for comparing the partitioning of different bioactive compounds (ascorbyl palmitate, gallic acid, propyl gallate and α-tocopherols), it was shown that each of the bioactive compounds had different partitioning behaviours when either Tween 80 or Tween 65 were used respectively. The type of bioactive compounds had influenced their partitioning because each type has different hydrophobicity– ascorbyl palmitate, gallic acid, and propyl gallate were less hydrophobic than α-tocopherols (Sytar et al., 2013).

Similarly, Stöckmann et al. (2000) reported that the alkyls of a bioactive compound affected the partitioning of the compound in emulsions. In that study, they found that an increasing number of carbon atom in alkyl chain of gallates (as the bioactive compound) from methyl gallate to butyl gallate could cause the antioxidant to partition more into the oil phase than the aqueous phase. This can be related to the increasing solubility of hydrocarbon in non-polar solvent as the length of the hydrocarbon increases. Meanwhile for gallic acid, most of the antioxidant partitioned into the aqueous phase and only a trace of it partitioned into the oil phase.

Effects of bioactive co-encapsulation

In an emulsion, several bioactive compounds can be added at a time. Fang et al. (2019) examined the partitioning of α-tocopherol that was co-encapsulated with resveratrol/naringenin in emulsions stabilised by whey protein isolate (WPI). The results showed that different co-encapsulated bioactive compounds affected the partitioning of the main bioactive compound differently. At the oil-water interphase, α-tocopherol that was co-encapsulated with naringenin in an emulsion partitioned more at the interphase than the one with resveratrol. In addition, it partitioned more into the oil phase (~76%) compared to the water phase (~23%) due to the hydrophobic nature of α-tocopherol (Fang et al., 2019). Its hydro-solubility and stability can be improved using an effective carrier system such as encapsulation (Wang et al., 2016). Resveratrol on the other hand has a very low solubility in both oil and water that form protein-ligand complexes before emulsion formation. Therefore, resveratrol molecules are more likely to bind to WPI at the oil droplet interface (Wang et al., 2016). Meanwhile, resveratrol partitioned more than naringenin (either bound or free) in the aqueous phase, and it remained freely dissolved in the aqueous phase due to its very hydrophilic properties (Wang et al., 2016).

In another study, Cheng et al. (2020) investigated the partitioning behaviour of α-tocopherol and/or resveratrol as bioactive compounds in whey protein emulsions. α-tocopherol was found mostly in the oil phase due to its hydrophobic properties, while resveratrol that

is amphiphilic partitioned in the aqueous phase and oil–water interface by binding to whey proteins. The percentage of resveratrol at the oil-water interface was lower (44%) than that at the oil-protein water interface (~ 50%) due to higher interfacial percentage of WPI (~ 60%) (Cheng et al., 2020; Wang et al., 2016). The co-encapsulation of α-tocopherol and resveratrol in emulsion did not affect the percentage partitioning of both bioactive compounds. The addition of gum arabic at concentrations of 0.1%, 0.5% and 1.0% did not significantly influence the partitioning of α-tocopherol, however it significantly affected the resveratrol in the aqueous or oil phase. The addition of pectin at concentration of 0.1% and 0.5% also exhibited a similar trend to gum arabic.

Effects of solid fat content

In nature, fat can exist in two phases, either in liquid or solid form at room temperature. Solid fat usually contains only single bond in its structure, while liquid fat or oil contains one or more than one double bond. For example, palm oil has two phases of fat at room temperature; the solid stearin and liquid olein, which can be separated by fractionation process.

The partitioning of phytonutrient in an emulsion-based delivery system can also be influenced by solid fat content of the carrier oil. In a study by Wan Mohamad et al. (2018), palm stearin i.e. the solid fraction of palm oil, was mixed in an increasing ratio to the liquid palm olein to form an increasingly crystalline oil phase of emulsions. From the study, it was observed that the partitioning of β-carotene was affected by the solid fat content. The bioactive compound partitioned more into aqueous phase as the solid fat content increased by the increasing ratio of stearin. There was not much difference in physical properties between the palm olein and stearin, except for iodine value and slip melting point (Pande et al., 2012). The iodine value measures the unsaturation of an oil, which is determined by the uptake of halogen. Oil with greater iodine value has a higher degree of unsaturation and thus is more susceptible to oxidation. In addition to this, iodine value can give estimation towards the quality of a certain oil as the melting point and oxidative stability are related to the degree of unsaturation (Sanders, 2017).

β-carotene was used as the bioactive compound due to its highly hydrophobic components that can be trapped within colloidal crystalline matrices (oil phase with higher solid fat content) (Wan Mohamad et al., 2018). However, some of the bioactive molecules were expelled once the carrier lipid (in this case palm stearin) crystallizes because they could not be easily incorporated into the highly ordered structure of the crystalline phase. Therefore, β-carotene in the emulsion partitioned more into the aqueous phase when the solid fat content increased (by increasing the palm stearin ratio that resulted in decreasing the overall iodine value and degree of unsaturation).

CONCLUSION

In conclusion, partitioning of bioactive compounds in emulsions is affected by several factors including the oil loading, bioactive type, surfactant and and concentration, bioactive co-encapsulation and solid fat content. Bioactive compound in emulsion systems with lesser oil loading tend to partition more into the oil phase than those with higher oil ratio. Different types and concentrations of emulsifiers have different properties and emulsifying strength, hence may cause different effects on partitioning of specific bioactive compounds too. Pre-treatment of a protein emulsifier (such as WPI) can cause the bioactive compound to partition more into the aqueous phase due to protein denaturation. Besides that, different types of bioactive compounds also resulted in a different partitioning behaviour due to its polarity that affects the solubility of the bioactive compounds in oil and aqueous phase. Co-encapsulation of a bioactive compound with another bioactive compound also affects its partitioning in the emulsion since each bioactive compound has different physicochemical properties in nature. Other than that, an increasing solid fat content of emulsions may result in lipophilic bioactive compound (such as β-carotene) to partition more into the aqueous phase of the emulsions after being expelled from the more ordered structure of the crystalline oil phase.

Although various factors affecting the bioactive partitioning characteristics in emulsions have been discovered, it is difficult to find literatures reporting some other factors such as the type of emulsion i.e. comparison between O/W and W/O emulsions as the carrier systems for the same bioactive compound; or between macro- and nano-emulsions, and different types of bioactive compounds carried by the same system. Therefore, further study is required to look into the above important yet undiscovered factors for designing a stable carrier of bioactive compounds in producing various functional foods in the future.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SNH reviewed past researches and wrote the review paper. WAF and WMF checked and corrected the paper. All authors read and approved the final version.

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