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VEGETABLE OIL BASED EMULSIONS IN MILK

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ABSTRACT

Milk and dairy products represent an important part of functional food in the market. Based on their positive health and nutritional benefits, they have gained popularity and their consumption as well as production is on the rise in the last few decades. As a result of this trend, milk-based products are being used for the delivery of bioactive food ingredients. This study is devoted to the formulation of stable emulsions containing grape seed oil dispersed with several emulsifiers (Tween 80, monocaprylin, and lecithin) in milk. Photon correlation spectroscopy was used to evaluate the characteristics of the emulsions in terms of mean droplet size, droplet size distribution and polydispersity index. Emulsions were prepared using 2% and 5% w/w grape seed oil, and 3%, 5%, or 8% w/w emulsifier, and these were homogenized at two different rates of 1050 and 13400 rpm. Parameters influencing emulsion particle size and particle size distribution were identified, which included emulsifier type, its HLB value, oil type (virgin, refined), homogenization rate and the fat content in the milk. Homogenization at 13400 rpm for 10 min. produced fine emulsions with small mean particle sizes and monomodal distribution of droplets. Regarding emulsifier type, the smallest droplet sizes were obtained with formulations containing Tween 80 (250-315 nm), whereas lecithin primarily accounted for the monomodal particle size distributions.

Keywords: grape seed oil; photon correlation spectroscopy; milk; encapsulation; emulsions

INTRODUCTION

Oil in water (o/w) emulsions are systems suitable for encapsulating lipophilic substances in various food products, such as milk, yoghurt, ice-cream, etc. **(Shahidi and Bailey 2005, Chee et al., 2005, 2007)**. A suitable method of emulsification thus leads to fortifying selected foods with bioactive substances of a hydrophobic nature. The substances mostly used are, due to their biological effects, polyphenols, fatty acids, phytosterols and carotenoids **(McClements et al., 2007)**. Accessible sources of these bioactive substances include minor and non-typical vegetable oils. These are usually obtained under mild conditions with a view to preserving the specific properties of the original oils. They are usually only available in a limited amount, and include oils from grape seeds, almonds, borage, avocados, macadamia nuts, mangos, Camelina, marigolds and pumpkins **(Shahidi and Bailey 2005)**.

Grape seed oil represents a raw material of significant dietetic value. It is characterized by a high content of nutritionally important compounds, such as essential fatty acids, tocotrienols and polyphenols **(Bail et al., 2008; Madawala, 2012)**. It is a side-product of the wine industry and is made from pomace, i.e. pressed grapes used for wine. The seeds are removed from the pomace, and used to obtain oil by pressing or extracting the same with the use of solvents. Pressed oils are of higher quality, with the superior-quality oils being obtained by cold pressing in hydraulic presses. However, this method produces lower yields. The actual amount of oil obtained depends on many factors. Crucial to this are soil and climate conditions, as well as the characteristics of the variety utilized (the seeds of white grapes contain more oil than those of blue grapes). Its favourable properties make it a popular material in gastronomy, the cosmetics industry and in the production of paints and varnishes **(Burg and Zemánek, 2012)**.

In recent years, the functional foods market has witnessed a rise in the popularity of milk-based products, especially milk drinks. In addition to the preferences of consumers, their advantage lies in the fact that milk is a rich source of nutrients **(Sharma, 2005)**. Therefore, milk products are a suitable food matrix for encapsulating bioactive substances. By dispersing vegetable oils in milk, a system can be created that combines the benefits of both its components.

The aim of the work presented here was to develop stable grape seed oil emulsions in milk, and to evaluate their behaviour as regards the composition of said formula.

MATERIAL AND METHODOLOGY

Emulsion preparation

The model emulsions were prepared via the method of mechanical homogenisation, with the use of two devices possessing different speeds of revolution - a Heidolph mechanical overhead stirrer (1050 rpm; type RZR 2020, Heidolph Instruments GmbH) and an Ultra-Turrax disperser (13400 rpm; type T 25 digital, IKA Labortechnik). All the emulsions so prepared contained as a the basis the original, natural emulsion phase of milk with the fat contents of 0.5%, 1.5% and 3.5%, hereinafter referred to as the water phase (Laclel, Kunín Dairy). The dispersed phase of the emulsion was formed by the one of grape seed oil samples that had been processed in various ways; either extra virgin, cold-pressed, unrefined (Saint

George's), or refined (M+H, Míča a Harašta). Due to the inherent emulsifying properties of milk, emulsions of both types of oil were first prepared without emulsifiers. In order to improve their final stability, food emulsifiers were later added to the formula; these comprised lecithin (Mogador), monocaprylin (MAG C8:0) **(Janiš et al., 2000)** and Tween 80 (Polyethylene glycol sorbitan monooleate, Sigma-Aldrich).

In the first set of experiments a total of 60 emulsions were made with 2% oil concentration and varied emulsifier concentration (3%, 5%, 8% w/w). All the emulsions prepared were visually observed for stability immediately after emulsification and again after one day of being stored at the temperature of 25 °C. Emulsions with a composition that ensured stability and the lowest concentration of emulsifiers were selected out of this group.

Afterwards, these selected emulsions were prepared with 5% concentration of grape seed oil, i.e. with the highest possible concentration that enables creation of a homogeneous emulsion while preserving the original emulsifier concentration. Only the most stable emulsions were selected for analysis of particle size and particle size distribution. Visually registered phase separation was chosen as the stability indicating parameter, which pointed to disintegration of the system representing an undesirable situation from the perspective of consumers.

The following procedure was used with the Heidolph overhead stirrer. Two beakers were filled with the appropriate amounts of emulsifier, and the water and oil phase. These were subsequently warmed in a water bath at a temperature of 85 °C. The formula for emulsion with 5% oil concentration is provided in Table 1**.** The water phase of milk alone, or of the emulsifier dissolved in milk, was gradually added into the oil phase, under stirring. After all the components had blended together, the emulsion was homogenised for 10 minutes at the constant speed of 1050 rpm.

Preparing emulsions with the use of the Ultra-Turrax disperser was carried out as follows: the components of water and oil phase were weighed and filled into the dispersion test tube and then warmed in a water bath. The amounts needed for preparing the emulsions with 5% oil concentration are given in Table 2.

Once the temperature of the water bath had reached 85 °C, the mixture was homogenized for 10 minutes at the speed of 13400 rpm.

Emulsion analysis by photon correlation spectroscopy (PCS)

In the selected emulsions, the size (z-average diameter) and particle size distribution were analysed by photon correlation spectroscopy with the use of the Zetasizer Nano ZS device (Malvern Instruments). The emulsion analysed was first homogenised by manually shaking it. A measured sample was then put into a plastic cuvette - 1 ml of distilled water was filtered through a 0.22 μm (Millipore) filter while 3 μl of emulsion were added to it using a pipette. In order to ensure the constant temperature of the sample, the cuvette was covered with a lid and heated to 25 °C. The following measurement parameters were set on the device - viscosity of the dispersion medium: 0.8872 cP, refractive index of the dispersion medium: 1.330 and refraction index of the dispersed phase: 1.450. The measured data were processed by the Zetasizer software.

RESULTS AND DISCUSSION

Out of 60 emulsions prepared in the first set of experiments (including control emulsions without any added emulsifier), 24 samples were proposed for the experiment itself on the bases of primary selection. These were subsequently evaluated visually and analysed by photon correlation spectroscopy.

Visual observation

Emulsions were evaluated immediately after emulsification and again after one day of being stored at 25 °C. The parameters monitored included visual appearance, colour and stability of the emulsions.

As the formula contained milk, all the emulsions were of noticeable milky white colouring. The stable emulsions were homogeneous, without any signs of phase separation. Instability in the emulsions prepared was revealed by gradual separation of phases. This means that so-called creaming was observed, i.e. a layer of oil accumulated in the top part of the emulsion system due to its lower density.

Table 1 Formula for emulsion prepared with a stirrer and 5% (w/w) grape seed oil

Emulsifier concentration [%]	Emulsifier [g]	Water phase [g]	Oil phase [g]
0	-	47.5	2.5
3	1.5	46.0	2.5
5	2.5	45.0	2.5
8	4.0	43.5	2.5

Table 2 Formula for emulsion prepared with an Ultra-Turrax and 5% (w/w) grape seed oil

Emulsifier concentration [%]	Emulsifier [g]	Water phase [g]	Oil phase [g]
0	-	19.0	1.0
3	0.6	18.4	1.0
5	1.0	18.0	1.0
8	1.6	17.4	1.0

Visual observation revealed that each kind of the milk tested demonstrated sufficient capacity for emulsification and stabilization, which enabled creation of homogeneous emulsion with 5% concentration of grape seed oil (refined or raw) within a single day. It was possible to form emulsion without emulsifiers thanks to the presence of the surface-active agents - casein and whey protein - in the milk serum. In order to increase stability, the basic formula consisting of milk and 5% (w/w) grape seed oil was enriched with one of the food emulsifiers (monocaprylin, lecithin, Tween 80). The 3% concentration of emulsifiers enabled creation of a homogeneous emulsion in the system, with the exception of monocaprylin.

Photon correlation spectroscopy

Many physical, chemical and sensory properties of food emulsions depend on the size and nature of the particles they contain. The important characteristics of emulsion particles also include the parameters determined in this work. Namely, these were the mean particle size and size distribution, which both influenced the properties, stability and application of emulsions.

All the emulsions prepared can be classified as typical food emulsion systems, whose particle size varies in dependence to the composition - between 250 and 1,000 nm **(Shahidi and Bailey, 2005)**. As analysis revealed, the milk samples tested showed a mean particle size of 200 to 350 nm and can be hence ranked as macroemulsions; i.e. the size characteristics in the emulsions prepared concur with expectations.

The first set of experiments showed that in order to create emulsions with the required stability it is optimal to carry out homogenization with the use of the Ultra-Turrax disperser for 10 minutes at 13400 rpm. In this way, stable emulsions can be obtained with a low mean size of particles and monomodal size distribution, which complies with the theoretical expectation of a higher efficiency of homogenization caused by more intensive stirring **(Akoh and Min, 2008)**.

When the z-average diameter of emulsion particles was evaluated more thoroughly, the initial theoretical premise was confirmed, i.e. the size of emulsion particles depends on the type of emulsifier used. As is obvious from Figure 1, 3% concentration of both lecithin and Tween 80 led to a visible decrease in particle size z-average in comparison with the control emulsions prepared without any emulsifier, irrespective of what kind of milk was used. When 3% concentration of Tween 80 was used, the size of emulsion particles was reduced most noticeably. In samples with 8% concentration of monocaprylin, an opposite tendency was observed (with the exception of the emulsion in which whole milk was used as the dispersion environment) - namely, the particle size grew in comparison with the control emulsions. This diversity of results received from the emulsions tested can be explained by the specific values of the hydrophilic-lipophilic balance (HLB) of particular emulsifiers. Tween 80 with HLB 15 is a hydrophilic surfactant with high solubility in the water phase, which is why it efficiently stabilizes o/w systems, as was manifested in the resulting small size of emulsion particles. Nevertheless, monocaprylin (HLB ≈ 6), due to its larger lipophilic part, dissolves in water less readily, hence is better for stabilizing w/o emulsions. The HLB value for soya lecithin equals 8 and is on the border between both the situations mentioned above **(Bartovská and Šišková, 2005)**. In conformity with the theory of hydrophilic-lipophilic balance, the size of the emulsion particles prepared with this emulsifier, represents the transition between the two previous systems.

Figure 1 also shows that the rising amount of milk fat in the system results in larger sized particles. Deflection from this behaviour was again seen in emulsions with 8%

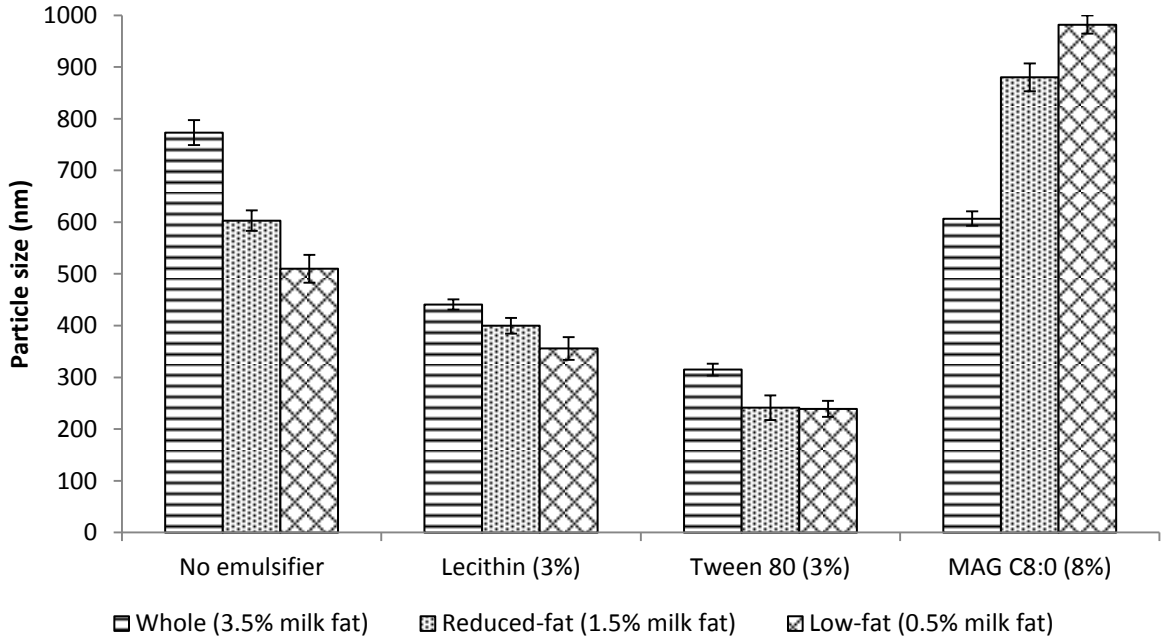

Figure 1 Impact of using various emulsifiers on particle size in 5% concentration of unrefined grape seed oil

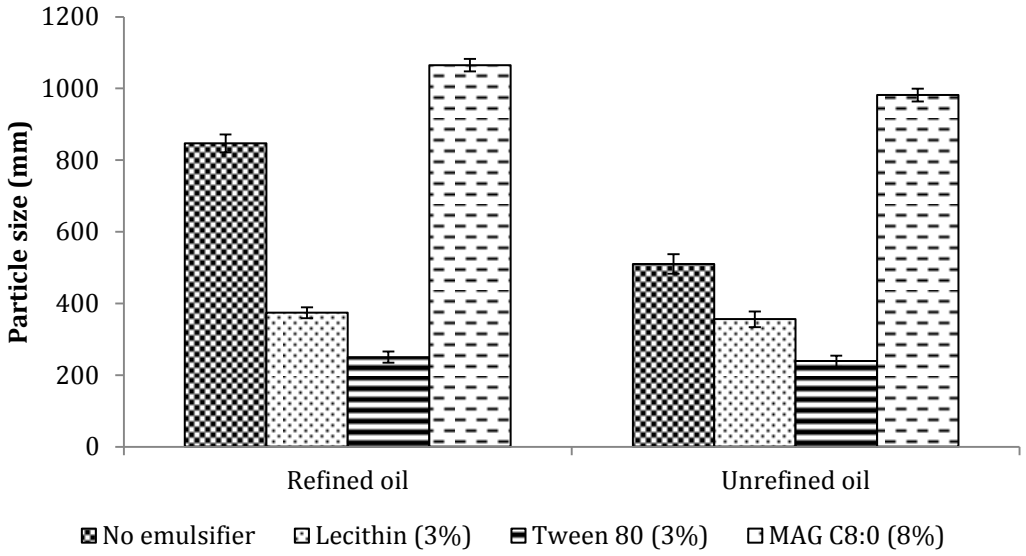

Figure 2 Impact of the kind of grape seed oil (5% w/w) on particle size in low-fat milk serving as the continuous phase of emulsion

concentration of monocaprylin, where an opposite tendency was observed.

Adding grape seed oil (5% w/w) into used kinds of milk resulted, with no exception, in increasing the size of the emulsion particles. An interesting finding arose from comparing the sizes of particles in two different grape seed oils: unrefined extra virgin oil formed emulsion particles of smaller size than refined oil (see Figure 2). This result could be related to the presence of surface-active agents, free fatty acids, proteins and phospholipids, which are present in raw oil, and thus can contribute to better emulsification. In refined oil, the content of these substances is reduced to the minimum concentrations.

The influence of the emulsifiers used on particle size and distribution in the prepared emulsions containing fat-reduced milk is shown in Figure 3. The development of the recorded distribution curves proves that using 3% concentration of Tween 80 resulted in a significant decrease in the mean value of particle sizes in comparison with control emulsions. As in the previous cases, unrefined oil formed emulsions with particles of a smaller z-average. Although the distribution curves were mostly bimodal, one fraction was strongly predominant. Emulsions with 3% lecithin concentration primarily registered one particle population. In comparison with control samples that did not contain emulsifiers, the z-average of particles was smaller. In this group, refined oil also formed emulsions with larger particles. In emulsions with 8% monocaprylin the distribution curves were multimodal, which proves the high polydispersity in the system. Compared to the other emulsifiers, monocaprylin did not cause a decrease in the size of the emulsion particles.

For comparative purposes, photon correlation spectroscopy was used to analyse not only the emulsions prepared but also the kinds of milk used. The received distribution curves in low-fat milk and whole milk could be regarded as monomodal. Of the two identified populations of particles present in reduced-fat milk, the size of a smaller fraction was just within the measuring range of the device being of 5 μm. Consequently, it can be

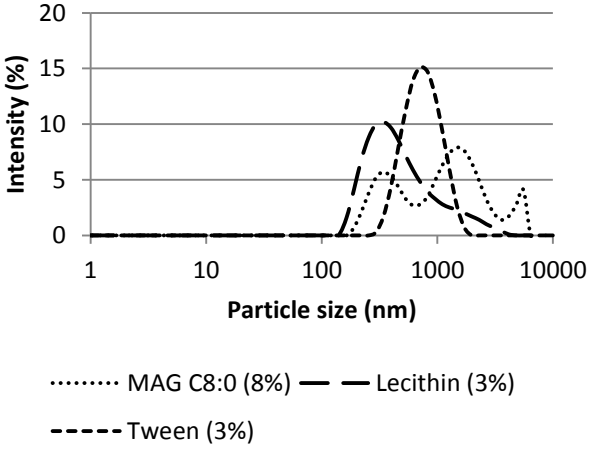

Figure 3 Influence of an emulsifier on the particle size distribution of selected emulsions

assumed that the samples of low-fat and whole might also be polymodal. However, the instrument is not able to detect the particle size >5 µm. The findings from the analyses of milk samples also revealed that the fat content influences the resulting size of particles. Alongside a rising volume of fat, the z-average increased.

CONCLUSION

This experimental work focused on preparing stable emulsions of milk and grape seed oil with the use of lecithin, monocaprylin and Tween 80 as food emulsifiers. The emulsions prepared were subsequently evaluated visually and *via* photon correlation spectroscopy. The experiments conducted revealed that in order to create a model emulsion with small mean particle sizes and monomodal size distribution, the optimal method is homogenization taking 10 minutes at 13400 rpm. The resultant size of particles in the emulsions prepared was most significantly influenced by the type of emulsifier, its respective HBL value, the fat content in the milk and the method of oil processing. In terms of impact on particle size, the optimal emulsifier appeared to be Tween 80. When it was used in 3% concentration, the z-average of the particle size was in the narrowest interval of all the sets of emulsions tested (250-315 nm). With regard to particle size distribution in emulsions, the most suitable emulsifier was lecithin, as it enabled creation of homogeneous emulsions, which was proven by the presence of predominantly monomodal distribution curves. Lecithin as a part of the formula can thus contribute to the nutritional quality of the given system.

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