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## **Impact of lipophilic surfactant on the stabilization of water droplets in sunflower oil**

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## Abstract

The food industries are continuously facing enormous challenges in the formation of stable w/o emulsions. The stability of the w/o emulsions is a key factor when being used as primary emulsions for the formation of double emulsions (DE). Long-term stability of the primary emulsions in a DE is still a non-solved challenge. Therefore, a mixture of various surfactants or the chemical composition of Polyglycerol Polyricinoleate (PGPR) as a surfactant for stabilizing the emulsion is a scientific interest to alleviate this problem. In order to stabilize the water droplets, two lipophilic surfactants, namely PGPR: Sorbitan monolaurate (Span 20), PGPR: Sorbitan monooleate (Span 80) blends in various ratios, and various total surfactant quantities were used. The properties of the emulsions have been evaluated in terms of structure, droplet size, stability, and viscosity. The influence of three different providers of PGPR with different chemical compositions on the water droplets is also investigated. Results show that the provider of PGPR significantly affects the properties of the emulsions. PGPR from providers A and B result in emulsions with similar droplet sizes (12 and 13  $\mu\text{m}$  respectively) and stability using the rotor-stator device at 10000 rpm, whereas PGPR from provider C yields emulsions with the lowest droplet size (1.7  $\mu\text{m}$ ). The mass Spectrometric analysis shows that the C PGPR contains lower molecular units with fewer impurities. Therefore, the interfacial tension of the C PGPR exhibits significantly lower values compared to the other two, leading to the production of droplets with the lowest size. Furthermore, the emulsion properties are shown to be affected by the type or concentration of the surfactants. Overall, blends of PGPR: Span and the PGPR provider can tailor the physicochemical properties of w/o emulsions.

## Keywords

Water-in-oil emulsion; polyglycerol polyricinoleate; emulsion stability; interfacial tension; viscosity.

## Novelty Impact Statement

- I. The effect of three different providers' PGPR (a lipophilic surfactant) on the emulsion properties and stability.
- II. The small molecular weight of PGPR shows a lowering interfacial tension, which provides smaller emulsion droplet size and in consequence higher viscosity and higher stability.
- III. Proposed optimum compositions of PGPR-Span 20 or Span 80 blends for obtaining the best stable structure of w/o emulsions.

## 1. Introduction

Microstructured liquid products for the delivery of functionality are known to be an emerging trend in various industries, such as food, pharmaceutical, and cosmetics. Specifically for the food industry, this microstructured liquid can affect the rheological profile of the final product or can increase the functionality by encapsulating bioactive compounds or by reducing the fat content of the final product [1-5]. The majority of the published work in this field is dealing with structures having an aqueous continuous phase (oil-in-water emulsions, o/w) [4-9].

As far as the water-in-oil (w/o) emulsions are concerned, they are mostly being studied at the solid or semi-solid state [10-12]. However, a limited amount of studies is dealing with the production of liquid w/o emulsion [9, 11]. The encapsulation capacity of a double emulsion (w/o/w) very much depends on the primary emulsion which is w/o. W/o emulsions are usually thermodynamically unstable systems, like all emulsions [13]. A plausible approach to overcome the inadequate stability is to explore the interactions between oil, water, and surfactant at the water/oil interface. This may lead to the production of stable emulsions and the development of new products.

The hydrophilic and lipophilic surfactants play a major role in the establishment of the stable size distribution of the water droplets [14]. Most of the studies are using one hydrophilic and one lipophilic surfactant to stabilize the interface [15, 16]. The lipophilic surfactants (HLB below 8) promote the stability of the oil-based emulsions by adsorbing at the water-oil interface to form a rigid film surrounding the water droplets, leading to the reduction of the interfacial tension and, ultimately, to the production of stable emulsions [17].

One of the most powerful lipophilic surfactants that are being commonly used in the food industry is polyglycerol polyricinoleate (PGPR) and it is being formed by the esterification of castor oil fatty acid with polyglycerol [18]. The main application of PGPR is in chocolate products where it is being used as an agent to reduce viscosity [19, 20]. Specifically, PGPR is known to decrease chocolate's yield stress and to increase the tolerance of the chocolate to the thickening effect caused by traces of water sometimes introduced into the chocolate during enrobing operations [20, 21]. These lead to the possibility to handle chocolate mass at lower temperatures and remove the air bubbles [22]. However, one of the major pitfalls of PGPR is that large amounts of it need to be incorporated into the emulsion in order to make it stable. According to recent studies, the minimum amount of 4 to 8% wt. PGPR is sufficient in producing stable emulsions [23, 24]. PGPR addition is being determined by legislation: acceptable daily intake (ADI) of 7.5 mg/kg body weight per day for PGPR [25].

Considering these drawbacks, mixing PGPR with other food-grade small molecular weight surfactants can serve as a solution to reduce its concentration in the final product while retaining stability. In this regard, sorbitan monolaurate (Span 20) and sorbitan monooleate (Span 80) may be beneficial for producing stable emulsions. Several studies have shown the potential use of Span 80 and Span 20 for the production of w/o emulsions [26]. However, the final emulsions are, in most cases, not sufficiently stable. So far not much research has been carried out on the impact of the chemical composition of PGPR in the water-in-oil emulsion stability and the optimum blend composition with other surfactants.

Hence, the aims of the present work are to better understand (i) the effect of three different providers' PGPR (a lipophilic surfactant) on the emulsion properties, and (ii) the optimum compositions of PGPR-Span 20 or Span 80 blends for obtaining the best stable structure of w/o emulsions.

## 2. Materials and methods

### 2.1 Materials

Sunflower oil was purchased from a local supermarket and used as the oil phase without further processing. Sunflower oil was chosen as the oil phase as it is cost-effective oil and it is being extensively

used in the food industry. The lipophilic surfactants were sorbitan monolaurate (Span 20) and sorbitan monooleate (Span 80), both purchased from Sigma- Aldrich (St Louis, USA). Their Hydrophilic-to-lipophilic balance (HLB) was 8.6 and 4.3 respectively, according to the manufacturer. Different providers of the lipophilic surfactant polyglycerol polyricinoleate (PGPR) were incorporated in the emulsions in order to gain a deeper understanding of the effect of the surfactant on the physical properties of the emulsions (Provider A, HLB= 4; Provider B, HLB= 1.6; Provider C, HLB= 3.1). The precise amount of lipophilic surfactant was being dissolved in the oil and stirred with a magnetic stirrer for 20 min at 600 rpm (AREC hot plate stirrer, VELP Scientifica, Italy).

## 2.2. Emulsion preparation

In order to stabilize the water droplets, two lipophilic surfactant-blends were used (PGPR: Span 20 blends or PGPR: Span 80 blends) in various mass ratios (1:0, 3:1, 1:1, 1:3, and 0:1) and the total surfactant content of the emulsions varied between 3 and 5% wt. The final water to oil ratio was: 40:60. This ratio of water to oil and the surfactant ratio was chosen due to wide applications and previous studies [11, 23, 24, 27]. Table 1 summarizes the different emulsions tested during the present study. After the complete dissolution of all the surfactants in oil, the oil phase was being stirred by a magnetic stirrer at 200 rpm while the aqueous phase was added slowly (manually) approximately 200 ml/min. The emulsions were further processed by the rotor-stator system at 10000 rpm for 5 min (L5M-A, Silverson, Chesham, UK). The final volume of each emulsion was 2 L and all prepared at 25° C. Finally, in order to observe the influence of the PGPR provider on the physical properties of the emulsions, three providers of PGPR (A, B, C) were added in the oil phase of emulsions containing 5% wt. total lipophilic surfactant content. The blend that had been used in that case was PGPR: Span 20 1:3. In this case, the produced emulsions were the most unstable ones, making it easy to identify the differences between the 3 tested PGPRs. In all cases, the final produced emulsion was 2 L.

## 2.3 Droplet size analysis

The droplet size distribution of the emulsions was determined using the laser scattering technique (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK). The refractive indices of sunflower oil and water were taken as 1.473 and 1.330, respectively, while the obscuration range was fixed between 8% and 20%. The stirrer function was applied to 1000 rpm to avoid the flocculation of the water droplets during the measurement period. A dilution of 1:100 with sunflower oil was performed during the measurement. The Mie theory was used for the analysis.

The size distribution was expressed as the volumetric mean diameter ( $d_{50}$ ). The polydispersity index was also evaluated used the following equation:

$$Span = \frac{D_{90}-D_{10}}{D_{50}} \quad (Eq. 1)$$

Where  $D_{10}$ ,  $D_{50}$ , and  $D_{90}$  are diameters at 10%, 50%, and 90% cumulative volume respectively.

The volumetric mean diameter has been used as mean droplet size due to a target application, where fat needs to be replaced volumetrically [27]. At least three measurements were performed on freshly prepared and after 20 days stored emulsions at 25° C and the results are expressed as the average value.

## 2.4 Light microscopy

Samples of freshly prepared and after 20 days stored emulsions were observed using an optical microscope (Olympus BX51, Essex, UK) at 25° C to observe or visualize the actual situation in the different samples. The emulsion was being diluted for observable separation between droplets with oil (1:6) having the same composition of surfactant as the emulsion's continuous phase to avoid any alteration of the droplet sizes. A small drop was placed on the glass slide and gently covered with a coverslip. Several pictures were taken from random sample positions representing the overall appearance of the emulsions.

### 2.5 Emulsion stability

The stability of emulsions upon storage at 25° C was performed with intervals of 24 hours and a total time of 20 days. Emulsion samples (approximately 40 mL) were put into test tubes and stored at 25 °C. The upper height of separation after storage was being recorded for all the tested emulsions. The stability is presented in the serum Index (SI), which is calculated using the following equation:

$$SI \% = \frac{H_s}{H_e} * 100 \quad (Eq. 2)$$

where  $H_s$  is the height of the serum layer and  $H_e$  is the total height of the emulsion. A lower SI, therefore, represents a more stable emulsion.

### 2.6 Interfacial tension

The Du Nouy ring method using a KSV Sigma 701 tensiometer (KSV Instruments Ltd., Finland) was used for the characterization of the interfacial tension of the dispersed (water) and the continuous phase at ambient temperature (25° C). Each phase was left to equilibrate for 1 hour, before each measurement.

### 2.7 Emulsion rheology

Rheological measurements of the emulsions were performed on a stress-controlled rheometer (Physica MCR 301, Anton Paar, Graz, Austria) equipped with a double-gap geometry (DG-26.7). The temperature was kept constant ( $25.0 \pm 0.1$  °C) using a water bath. To achieve thermal and structure equilibrium, 15 ml of the sample was left at rest in the measurement system for 5 min prior to analysis. The apparent viscosity was determined versus the imposed shear rate from 0.1 to 100 s<sup>-1</sup>. 10 points per decade were measured while the whole measuring time was 10 min. The viscosity measurements are reported as the average of at least three different samples in order to assure reproducibility. All rheological measurements were completed at freshly prepared emulsions.

### 2.8 Electrospray Ionization-Mass Spectrometry

Electrospray ionization mass spectrometric analyses were performed using an LCQ Advantage ion trap mass spectrometer (Thermo Fischer Scientific, UK). Instrument settings were optimized using a sodium trifluoroacetate standard mixture. Standard and sample solutions were introduced into the electrospray source by direct infusion at a flow rate of 3 µL min<sup>-1</sup>. Prior to analysis, PGPR samples were diluted 6 fold using dimethyl sulfoxide and stirred for 1 minute. Mass spectra were acquired in positive ion mode over 1 minute with a scan frequency of 0.5 Hz.

### 2.9 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA) and an F-test was applied in order to compare the mean values of selected properties at 95% level of confidence.

## Results and discussion

### 3.1 Effect of the PGPR providers on the emulsion properties

The physical properties of PGPR are crucial not only for stabilizing the water/oil interface but also for the properties of the produced emulsions. In general, the properties of PGPRs significantly vary between suppliers. This phenomenon might affect the final properties of the product.

After using identical emulsification process and mechanical treatment, the average droplet size ( $D_{50}$ ), the polydispersity of the storage emulsions, and the storage stability of emulsions after 20 days of storage using different PGPR providers (A, B, C) were measured to gain insight in these mechanisms and are presented in Table 2, while the droplet size distribution of these emulsions is presented in Fig. 1.

Table 2 shows that emulsions containing PGPR Provider A or B have higher droplet size, span, and greater instability compared to the emulsions stabilized by PGPR Provider C. Specifically, the average droplet size of Provider A and B emulsions is found to be statistically the same (roughly 13  $\mu\text{m}$ ), while for the Provider C emulsions is found to be in the submicron range, roughly 1.7  $\mu\text{m}$  using same emulsification process and conditions. The  $D_{50}$  values exhibit the same trend as the span (Eq. 1). The span of Provider A, B emulsions do not significantly differ (roughly 21), indicating a bi-modal and multi-modal droplet size distribution, respectively. On the other hand, for the Provider C emulsions, the span value is very low compared to the other two and close to 1 [28]. Hence, it can be said that PGPR Provider does affect the emulsions droplet size perhaps due to different interfacial tension, HLB values, and impurities. PGPR Provider A and B result in emulsions with higher serum index (SI=5-%) compared to those stabilized by PGPR Provider C, which also indicates that the stability of emulsions with PGPR provider C is significantly different from the other two due to smaller droplet size and interfacial tension as shown Table 2. From Table 2, it is observed that Provider C emulsion is resulting the highest viscosity values at a shear rate of  $0.1 \text{ s}^{-1}$  due to the smallest droplet size.

The small average droplet size of Provider C emulsions is accompanied by high stability due to low interfacial tension and smaller droplet size. The smaller droplets are more stable in the oil phase as they move with lower sedimentation velocity. Additionally, the lower interfacial tension of PGPR Provider C benefits the quicker droplet breakup [29]. The higher viscosity of the Provider C emulsions further assists in the prevention of droplet coalescence and creaming, which is in accordance with other studies [26, 30, 31].

Another factor that could have affected the anchoring of the emulsifier molecule into the aqueous phase is the molecular weight. To gain this deeper understanding, electrospray ionization-mass spectrometry analyses have been carried out and the spectra for each PGPR are presented in Fig. 2. Mass spectrometry analysis of three purchased PGPR samples revealed a varying abundance of discrete PGPR species. As shown in Fig. 3, sample C contained a higher proportion of low molecular weight (500 - 1000 Da) species. The small molecular weight species adsorb faster in the interface and reduces the interfacial energy to deform and break the droplet further. The interfacial tension of different oil-water interfaces has been shown in Table 2, and it confirms that PGPR provider C exhibits the lowest interfacial tension at the equilibrium condition among all others.

Specifically,  $m/z$  805.6 is the most prominent ion in sample C, corresponding to the dehydrated polymer [ $n_3m_2 + \text{Na}^+$ ]; where  $n$  denotes a glycerol moiety and  $m$  a ricinoleate residue. Whereas in samples A and B  $m/z$  1103.7 is the most abundant ion; characterized as dehydrated [ $n_3m_3 + \text{Na}^+$ ]. The higher proportion of this larger PGPR species is likely responsible for the increase in interfacial tension when compared to sample C. Furthermore, Sample A contained many impurities which could not be identified as PGPR species; potentially owing to the reduced surfactant efficacy [32]. Therefore, PGPR Provider C was found to produce emulsions with enhanced stability and it has been exploited for the following experiments (3.2).

### 3.2 Effect of the lipophilic surfactants blend on the emulsion droplet characteristics

As stated previously, the surfactant composition plays a significant role on the droplet size of the emulsions. Various studies have pointed out the insufficient water droplet coverage by PGPR, Span 20,

and Span 80 [23, 33]. Hence, in this study the stabilization of the interface by PGPR: Span 20 or PGPR: Span 80 blends on ratios varying between 1:0, 3:1, 1:1, 1:3, or 0:1 and with a total surfactant content of 3 or 5% wt. is being investigated (Table 1). Table 3 summarizes the mean droplet size ( $D_{50}$ ) and the span of the produced fresh emulsions and corresponding emulsion after 20 days of storage time.

From Table 3 it can be seen that the ratio of PGPR: Span 20 or 80 together with the total surfactant content yield emulsions with various droplet sizes. It is clearly seen that none of the surfactants alone have the capability to produce small droplets and high storage stability. Emulsions containing only PGPR exhibit a droplet size of 2.9  $\mu\text{m}$  and 1.9  $\mu\text{m}$  for 3 and 5 wt% surfactant concentration, respectively. Emulsion with only Span-20 or Span-80 with 3 wt% show significantly higher droplet sizes of 38  $\mu\text{m}$  and 40  $\mu\text{m}$ , respectively. A similar impact has been observed for 5 wt% surfactant concentration with a slightly lower drop size of 30  $\mu\text{m}$  (Span-20) and 32  $\mu\text{m}$  (Span-80), respectively. However, any combination of PGPR and Span-20/Span-80 shows significant improvement in producing small droplets and storage stability. Among all combinations, 75% PGPR with Span-20 exhibits the best result to achieve lower droplets and higher stability.

The bimodal distribution (in Figure 5) of the emulsions containing higher amounts of Span 20 or Span 80 could be attributed to the fact that PGPR has the capability to create a robust interface due to its lower molecular weight compared to Span [26, 29, 34]. When the emulsification process begins, PGPR is moving faster to the interface, and because the amount of PGPR is being substituted by Span 20 or Span 80, there is a distinctive increase of the droplet size for the 1:1 and 1:3 PGPR: Span ratios.

The microscopic images of the emulsions stabilized by various ratios of PGPR:Span 20 immediately after production and after 20 days of storage are shown in Fig. 4. It is noted that emulsions containing PGPR: Span ratio 3:1 are composed of submicron droplets while flocculation phenomena cannot be observed. On the other hand, the systems composed of PGPR: Span ratio 1:1 and 1:3 show larger droplets and more pronounced flocculation than the emulsions with lower Span concentration.

The droplet size distribution of the emulsions stabilized by various ratios of PGPR: Span-20 immediately after production are shown in Fig. 5. It is noted that emulsions containing PGPR: Span ratio 3:1 and 1:1 are observed to be a monomodal distribution and consistently small droplets are found. On the other hand, the systems composed of PGPR: Span ratio 1:0, 1:3, and 1:0 show bimodal or multimodal size distribution with a wider span (quantitative values are shown in Table 3). As far as the emulsions stabilized only by Span 20 or Span 80 are concerned, their droplet size and polydispersity are the highest among the tested samples. Specifically, the  $D_{50}$  of the Span 20 stabilized emulsions reaches values up to 38  $\mu\text{m}$ . This enhances the theory that these are not efficient surfactants [23].

To gain a deeper understanding of the stabilization mechanism of the water-oil interface, the interfacial tension of the different surfactant mixtures was evaluated and presented in Table 3. As it can be seen, by increasing the amount of PGPR at the oil phase, the interfacial tension decreases. This has been seen before [35] and it is suggested that the addition of PGPR at the interface creating a lower interfacial tension, and as such there is a reduction in time required to deform a droplet and break up within the homogenization process. This is observed as an overall reduction in emulsion droplet size. What is more, interfacial tension values of mixtures with high Span concentration (e.g. 1:3, 0:1) exhibit are higher than those with a large amount of PGPR (e.g. 0:1, 3:1). That phenomenon indicates that PGPR moves faster than Spans at the water-oil interface, leading to its better stabilization.

By increasing the total surfactant content from 3 to 5% wt., the droplet size (Table 3), and the distribution (Fig 5a & Fig 5c) significantly decreased, only for the emulsions containing higher amounts of Span 20 or 80 (e.g. PS80 1:3). PGPR is an excellent surfactant for the oil system. The droplets of the emulsions containing higher amounts of PGPR favor the rapid migration of PGPR to the water/oil. In this case, the 5% wt. surfactant content emulsions have higher amounts of PGPR in their system, leading



to the formation of more stable emulsions with lower droplet sizes and mono-modal distributions (Fig. 5).

In Table 3, the average droplet size, and the span of the emulsions after a 20-day storage period are being depicted. It is obvious, that emulsions stabilized with higher PGPR: Span 20/ Span 80 ratio showed remained unaffected by that, as their  $D_{50}$  is the same. For instance, the  $D_{50}$  of the P: S80 3:1 (5% wt. total surfactant content) fresh emulsions is 0.7  $\mu\text{m}$  while after the end of the storage period it is slightly increased (0.9  $\mu\text{m}$ ). Emulsions stabilized only by Span 20 or Span 80 are the least stable, showing a 20% increase in the  $D_{50}$ . Moreover, in w/o emulsions stabilized with Span 80, other authors observed a relatively low dilatational elasticity at relatively high emulsifier concentration despite the strong stability of the system [26]. In summary, the type of surfactant, composition, and amount of lipophilic surfactant can alter the droplet characteristics of the final emulsions.

### *3.3 Effect of the lipophilic surfactant on the emulsion stability*

The stability mechanisms of the O/W and W/O emulsions are different from each other. O/W emulsions are stabilized by the shared effect of steric and electrostatic repulsions, while for the W/O emulsions, the dominant force is only the steric force due to the low electrical conductivity of the continuous phase. In this study, the stability of the emulsions after 20 days of storage at ambient temperature was recorded and is summarized in Fig. 6.

Worth noting is the increase of the serum index by increasing the concentration of Span 20 or Span 80. This could be attributed to the effect that the elastic network that is being formed has at the interface. Specifically, the reason for the more structured interface when using PGPR can be associated with the hydrophobic chain-chain interaction between PGPR and sunflower oil, which was more compatible than interactions between Span 80 and oil chains [26, 33, 36]. PGPR shows more compatibility with oil since this oil is less hydrophobic. The chain compatibility between surfactant and oil was described elsewhere [37].

After 20 days storage period, all the emulsions are phase-separated to some degree except the 3:1 ratio, regardless of the Span type. In general, the phase separation of emulsions prepared with 3% total surfactant content was much faster than in the case of the emulsions containing 5% total surfactant content, in which the SI was lower than 50%. These results are in accordance with the trends observed in the particle size distribution and the images obtained through optical microscopy.

The viscosity of the emulsions as a function of different PGPR: Span ratios are being tested and presented in Fig. 7. All the emulsions with 3% wt. total surfactant concentration, independent of the PGPR: Span ratio, exhibit a nearly Newtonian-like behavior as their viscosity is practically independent of the shear rate (Fig. 7A, B). This Newtonian-like behavior is typical for w/o emulsions stabilized with Span 80 and PGPR as has been previously reported [29].

When the total surfactant content is being increased to 5% wt., the emulsions stabilized by higher concentrations of PGPR (PS20 3:1 and PS80 3:1), exhibit a shear-thinning behavior due to smaller particle size. All other surfactant ratios at 5% wt, the viscosity of the emulsions exhibits Newtonian behavior due to relatively larger droplet size (Table 3). Moreover, by increasing the total surfactant concentration, the viscosity increases in all the tested cases over the range of the studied shear rate range (0.1 to 100  $\text{s}^{-1}$ ).

For all emulsions, increasing the PGPR concentration, their viscosity increases significantly from roughly 0.12 Pa.s to 0.25 Pa.s (no Span). The increase of PGPR to the emulsion is not only increased the viscosity of the continuous media but also got adsorbed over the surface of the droplets resulting in a decrease in sedimentation rate, collision frequency, and an increase in electrostatic as well as steric stability [19, 38].

As far as the effect of the Span type on the rheological profiles of the produced emulsions, it can be seen from Fig. 7 that emulsions containing Span 80 display higher viscosity values than those containing

Span 20. Span 80 has the ability to reduce more interfacial tension than Span 20. The lower interfacial tension leads to the requirement of less shear force to deform the droplets and higher stability [39, 40]. To sum up, parameters such as the rheological profile of the emulsion are being affected not only by the type of surfactant but also by the total surfactant concentration. PGPR to Span 80 ratio 3:1 with both 3% and 5% wt. total surfactant content led to stable emulsions with the smaller droplet size.

## Conclusions

This study demonstrated that different providers of PGPR affect the physical properties of the emulsions. The decreased values of the interfacial tension of a surfactant, leads to the production of smaller water droplets with high stability against gravitational separation, compared to the surfactants with higher interfacial tension values. The composition of these samples was elucidated through ESI-MS, which revealed a correlation between interfacial tension and the proportion of low molecular weight PGPR species. Furthermore, emulsions with varying types (PGPR and Span20/ Span 80) and concentration (3-5% wt.) of surfactants have a significant favorable influence on the physical properties and stability of the emulsion compared to any of the investigated single surfactant. Parameters such as the droplet size, stability, and rheological profile of the emulsion are being affected not only by the type of surfactant but also by the total surfactant concentration and combination. It was concluded that the stability in emulsion with the addition of PGPR was due to robust interface and lowering interfacial tension, which provides smaller droplet size and in consequence higher viscosity and higher stability. In conclusion, blends of PGPR: Span and the PGPR provider can support the tailoring of the physicochemical properties of w/o emulsions.

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## Captions of Tables

**Table 1.** The composition of the emulsions used for the present study is listed below, where PGPR from provider C is used as a surfactant with/without a combination of Span-20 or Span-80.

**Table 2.** Physical properties ( $D_{50}$ , span, serum index, and viscosity) of PS20 1:3 emulsions obtained using different PGPR providers.

**Table 3.** Volumetric mean droplet size ( $D_{50}$ ), corresponding span value and interfacial tension of different fresh and stored emulsions (20 days of storage).

**Table 1.**

Emulsions Names	PGPR* : Span ratio	Span type	Total surfactant content
P:S20 1:0	1:0	N/A	3 and 5% wt. for all cases
P:S20 3:1	3:1	Span 20	
P:S20 1:1	1:1	Span 20	
P:S20 1:3	1:3	Span 20	
P:S20 0:1	0:1	Span 20	
P:S80 3:1	3:1	Span 80	
P:S80 1:1	1:1	Span 80	
P:S80 1:3	1:3	Span 80	
P:S80 0:1	0:1	Span 80	

\* Three different providers of PGPR (A, B, C) are used.

**Table 2.**

PGPR provider	D <sub>50</sub> fresh emulsions (um)	Span fresh emulsions (-)	D <sub>50</sub> after 20 days of storage (um)	Span stored emulsions (-)	SI (%) after 20 days of storage	Equilibrium interfacial tension (mN/m)	Viscosity at 0.1 s <sup>-1</sup> shear rate (Pas)
A	12.5 <sup>b</sup> (±1)	<sup>b</sup> (±1.9)	25.5 <sup>b</sup> (±2)	35.5 <sup>b</sup> (±1.6)	52 <sup>b</sup> (±3)	17.9 <sup>b</sup> (±0.1)	0.13 <sup>a</sup> (±0.01)
B	13.4 <sup>b</sup> (±1)	23.4 <sup>b</sup> (±1.4)	28.8 <sup>b</sup> (±2)	38.9 <sup>b</sup> (±0.3)	50 <sup>b</sup> (±2)	17.8 <sup>b</sup> (±0.3)	0.16 <sup>b</sup> (±0.01)
C	1.7 <sup>a</sup> (±0.1)	1.2 <sup>a</sup> (±1)	3.1 <sup>a</sup> (±0.1)	1.7 <sup>a</sup> (±1)	25 <sup>a</sup> (±1)	12.2 <sup>a</sup> (±0.1)	0.19 <sup>c</sup> (±0.01)

Mean values followed by the same letters in the same column are not significantly different ( $P > 0.05$ ).

**Table 3.**

Emulsions	3% wt. total surfactant					5% wt. total surfactant			
	D <sub>50</sub> fresh emulsions ( $\mu\text{m}$ )	Span fresh emulsions (-)	D <sub>50</sub> stored emulsions ( $\mu\text{m}$ )	Span stored emulsions (-)	Equilibrium interfacial tension (mN/m)	D <sub>50</sub> fresh emulsions ( $\mu\text{m}$ )	Span fresh emulsions (-)	D <sub>50</sub> stored emulsions ( $\mu\text{m}$ )	Span stored emulsions (-)
P:S20 1:0	2.9 <sup>d</sup>	1.2 <sup>e</sup>	4.5 <sup>d</sup>	29 <sup>d</sup>	3.1 <sup>a</sup>	1.9 <sup>c</sup>	20 <sup>d</sup>	2.8 <sup>d</sup>	26 <sup>e</sup>
P:S20 3:1	1.0 <sup>a</sup>	1 <sup>a</sup>	1.2 <sup>a</sup>	1 <sup>a</sup>	6.7 <sup>c</sup>	0.8 <sup>a</sup>	1 <sup>a</sup>	0.9 <sup>a</sup>	1 <sup>a</sup>
P:S20 1:1	1.5 <sup>b</sup>	2 <sup>b</sup>	2.4 <sup>b</sup>	2 <sup>b</sup>	10.3 <sup>e</sup>	1.4 <sup>c</sup>	1 <sup>a</sup>	1.6 <sup>b</sup>	2 <sup>b</sup>
P:S20 1:3	1.7 <sup>d</sup>	13 <sup>d</sup>	3.1 <sup>c</sup>	17 <sup>d</sup>	12.2 <sup>f</sup>	1.8 <sup>c</sup>	10 <sup>c</sup>	2.3 <sup>c</sup>	13 <sup>d</sup>
P:S20 0:1	38.2 <sup>e</sup>	35 <sup>f</sup>	42.3 <sup>e</sup>	38 <sup>f</sup>	16.6 <sup>g</sup>	30.2 <sup>d</sup>	29 <sup>e</sup>	37 <sup>e</sup>	31 <sup>f</sup>
P:S80 3:1	0.9 <sup>a</sup>	1 <sup>a</sup>	1.0 <sup>a</sup>	1 <sup>a</sup>	4.3 <sup>a</sup>	0.7 <sup>a</sup>	1 <sup>a</sup>	0.9 <sup>a</sup>	1 <sup>a</sup>
P:S80 1:1	1.8 <sup>b</sup>	1 <sup>a</sup>	2.0 <sup>b</sup>	1 <sup>a</sup>	9.8 <sup>b</sup>	1.2 <sup>b</sup>	1 <sup>a</sup>	1.3 <sup>b</sup>	1 <sup>a</sup>
P:S80 1:3	2.3 <sup>c</sup>	4 <sup>c</sup>	2.5 <sup>b</sup>	7 <sup>c</sup>	18.4 <sup>d</sup>	1.6 <sup>c</sup>	3 <sup>b</sup>	2.2 <sup>c</sup>	6 <sup>c</sup>
P:S80 0:1	40.2 <sup>e</sup>	63 <sup>g</sup>	45.7 <sup>e</sup>	68 <sup>g</sup>	10.9 <sup>e</sup>	32.6 <sup>d</sup>	60 <sup>f</sup>	37.8 <sup>e</sup>	62 <sup>g</sup>

Mean values followed by the same letters in the same column are not significantly different ( $P > 0.05$ ).



## Captions of Figures

**Fig. 1.** Droplet size distribution of PS20 1:3 fresh emulsions obtained using PGPR from different providers: A (▲), B (◆), and C (●).

**Fig. 2.** Variation of PGPR species in samples A, B and C as determined by ESI-MS.

**Fig. 3.** ESI-MS Spectra from analysis of three discrete PGPR samples (A, B, C represent three different PGPR providers).

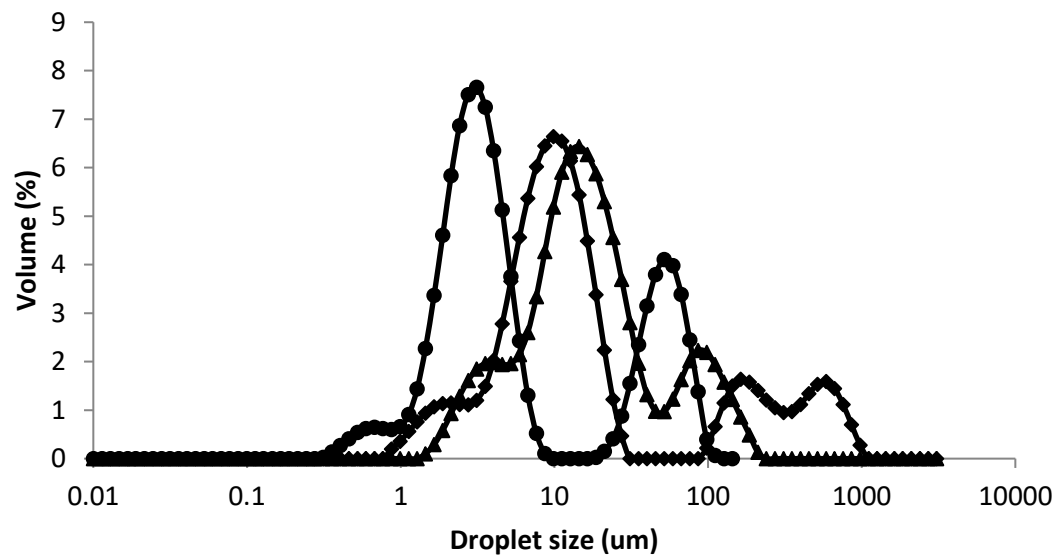
**Fig. 4.** Typical micrographs of emulsions containing 3% wt. total surfactant content and different ratios of PRGR and Span 20.

**Fig. 5.** Droplet size distribution of emulsions containing 3% wt. (A, B) and 5% wt. (C,D) total surfactant content and different ratios of PRGR and Span 20 (A, C) or Span 80 (B, D): 1:0 (■), 3:1 (●), 1:1 (\*), 1:3 (◆), and 0:1 (▲).

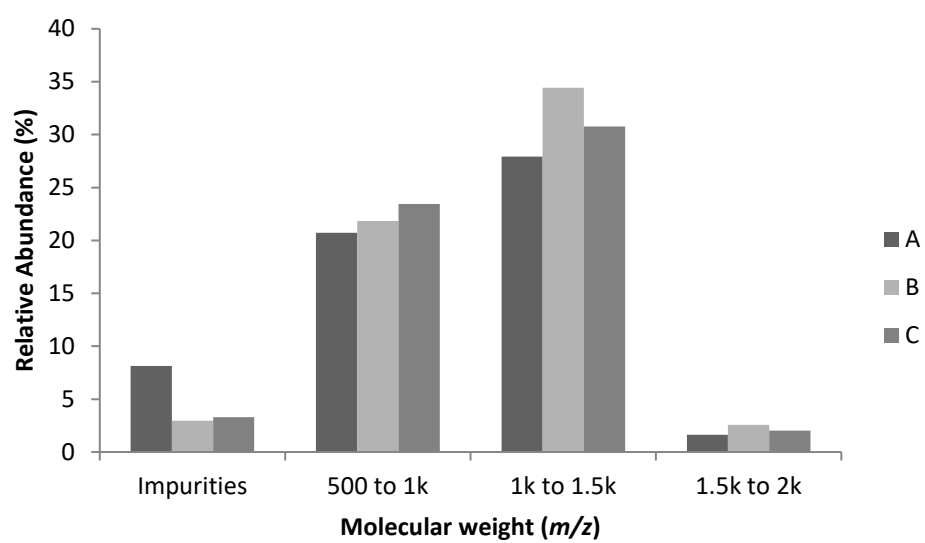
**Fig. 6.** Serum index (SI) of emulsions at storage time of 20 days containing 3% wt. (black) and 5% wt. (grey) total surfactant content and different ratios of PRGR and Span.

**Fig. 7.** Viscosity curves of emulsions containing 3% wt. (A, B) and 5% wt. (C,D) total surfactant content and different ratios of PRGR and Span 20 (A, C) or Span 80 (B, D): 1:0 (■), 3:1 (●), 1:1 (\*), 1:3 (◆), and 0:1 (▲).

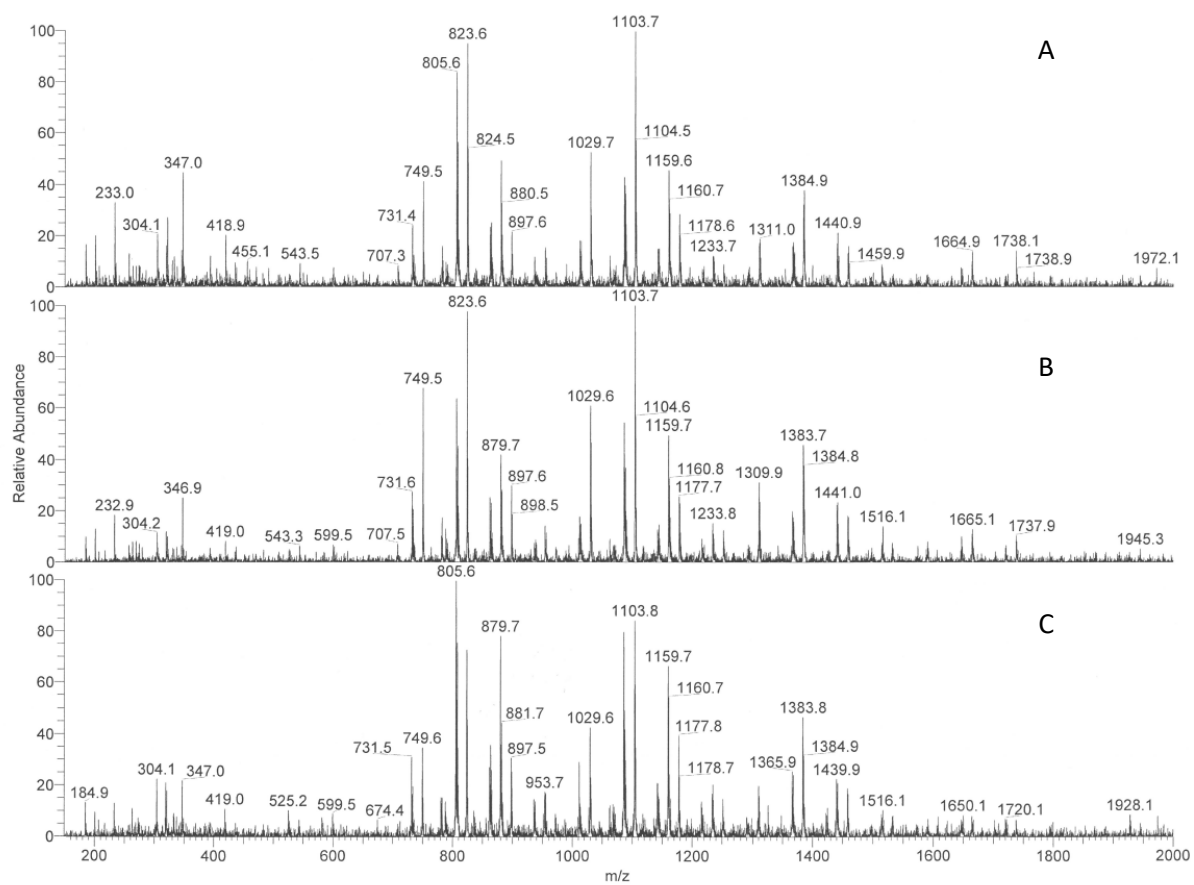
Fig. 1.



**Fig. 2.**



**Fig. 3.**



**Fig. 4**

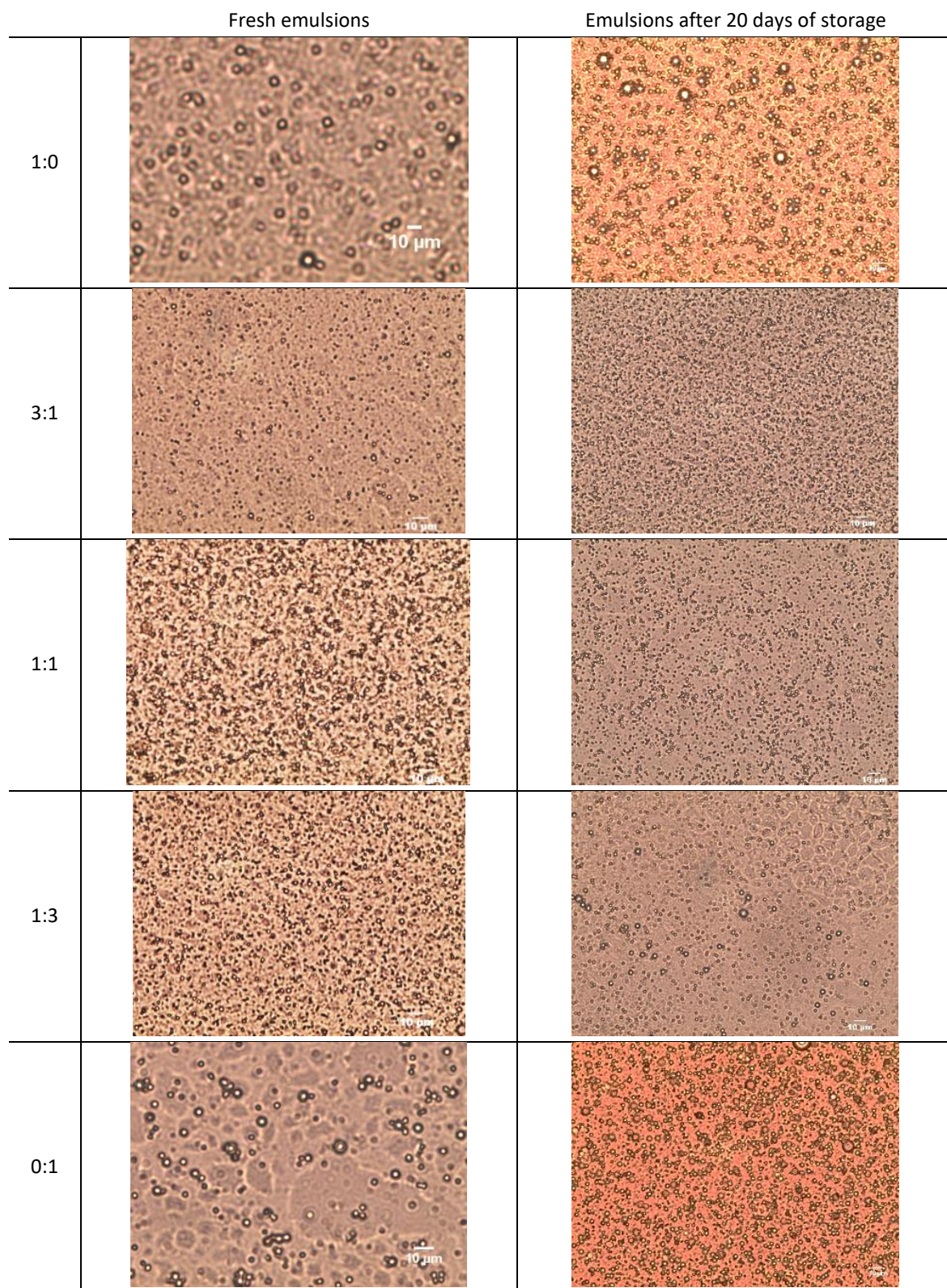


Fig. 5.

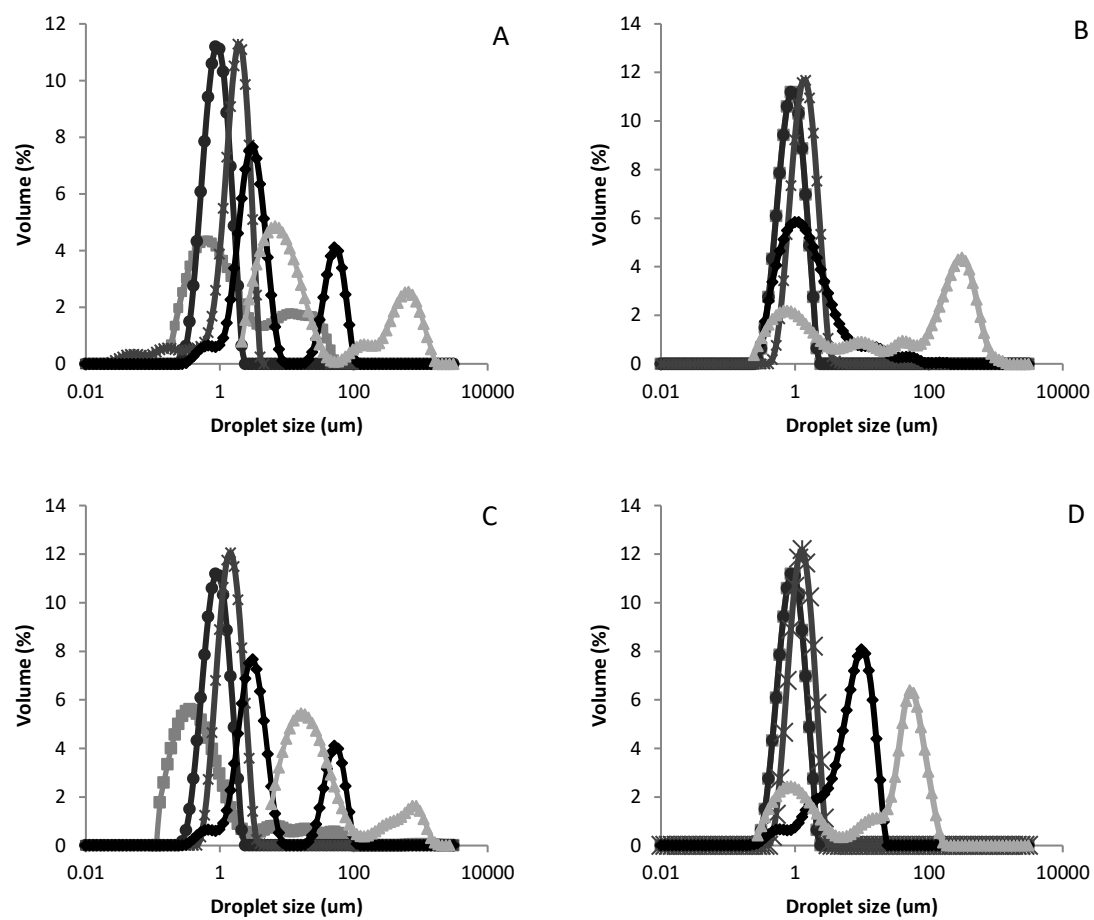


Fig. 6.

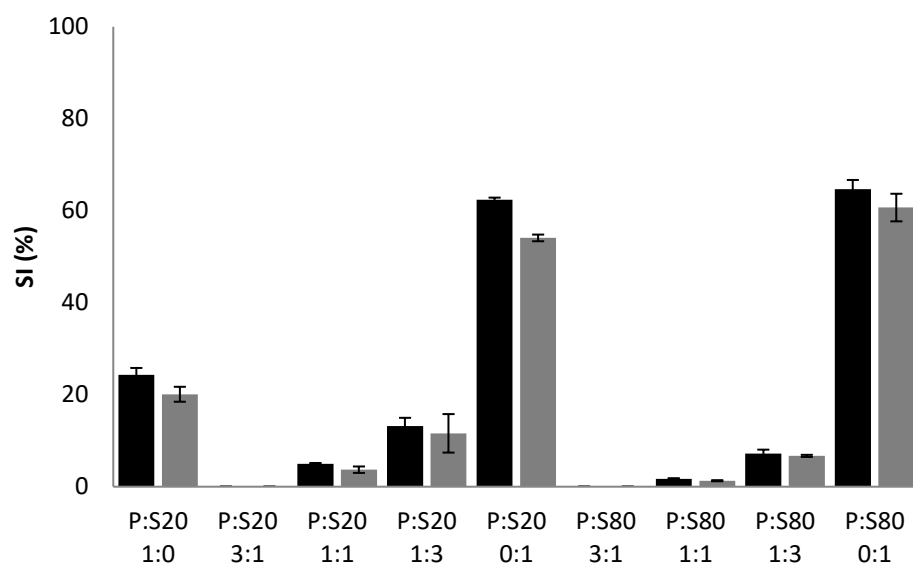


Fig. 7.

