Emulsion stabilizing capacity of sugar beet fibers compared to sugar beet pectin and octenyl succinate modified maltodextrin in the production of O/W emulsions: individual and combined impact

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A R T I C L E   I N F O

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Sugar beet pectin
OSA maltodextrin
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Creaming index

A B S T R A C T

Biopolymer-based stabilizers are becoming particularly favourable natural solutions for application in complex systems, such as O/W emulsions. Individual and combined impact of three polysaccharide-based stabilizers (sugar beet fibers, sugar beet pectin and OSA modified maltodextrin) on the formation and stability of corn oil-in-water emulsions was evaluated. The obtained emulsion droplet mean diameters ranged from 4.9 to 10.1 μm, indicating good emulsifying properties of the stabilizers used. The application of sugar beet fibers (SBF) resulted in emulsions with lower creaming index values compared to the sugar beet pectin (SBP) and OSA modified maltodextrin (OSAm) emulsions, hence it significantly affected emulsion stability. The interactions between applied polysaccharide-based stabilizers resulted in significant changes regarding emulsion creaming, zeta potential, droplet size and droplets’ size distribution. Overall, OSA–SBF combined impact is expressed through the production of emulsions having the most prominent overall characteristics in terms of surface weighted mean diameter, specific surface area and creaming index values. The OSA and SB combined influence on emulsion properties is characterized by relatively low values of droplet mean diameters and high level of droplet size uniformity. The possible competition in the process of adsorption on droplet surface is noticed in the experiments where combined SBF and SBP were used.

1. Introduction

The emulsion based systems, as some of the most challenging systems in food industry, are integral parts of various food and beverage products (Dickinson, 2015). The emulsions represent thermodynamically unfavourable systems stabilized using emulsifiers or texture modifiers which are able to inhibit coalescence of dispersed oil droplets within the aqueous phase (McClements, 2015). High number of previous reports highlights the promising application of biopolymer-based emulsifiers (amphiphilic proteins and polysaccharides) as particularly favourable natural substitutes (Agama-Acevedo & Bello-Perez, 2017; Ajar, Gençcelep, Sarıcaoğlu, & Turhan, 2016; Bai, Huan, Li, & McClements, 2017a; Dokić, Dokić, Dapčević, & Krstonišić, 2008; Moschakis, Murray, & Biliaderis, 2010).

Polysaccharides and proteins (biopolymer macromolecules), as essential functional ingredients and two main classes of food macromolecules, play a crucial role in defining stability and rheology characteristics of food colloids (Cao, Dickinson, & Wedlock, 1990; Pimentel-Moral, Ochoa-Pulido, Segura-Carretero, & Martinez-Perez, 2018). The corresponding macromolecules determine the texture, shelf-life and functional characteristics of most food products (Dickinson, 1992). The application of corresponding macromolecules in the established food matrices is determined by specific macromolecular functional characteristics, availability, product structure or price (Dickinson & Lorient, 2007). The ever-growing demand for „label-friendly” products strongly supports further research in the aim to introduce natural ingredients able to substitute synthetic additives (Bai et al., 2017a).

Nowadays, in order to tackle increasing food prices, numerous studies are dealing with various low-cost by-products of different agri-food industries aiming to explore and elaborate possible source materials regarding the corresponding biopolymer macromolecules which can be applied in food matrices (Alipour, Rezaei, Shabanpour, & Tabarsa, 2018; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Karnik & Wicker, 2018; Šorona-Simović et al., 2016). The investigated biopolymer-based emulsifiers, such as starch-based hydrocolloids, sugar beet pectin or fibrous polysaccharides, are reported to be able to...
form and stabilize emulsions in food and other applications (Agama-
Acevedo & Bello-Perez, 2017; Bai et al., 2017a; Chen et al., 2016; Doki-
it et al., 2008; Moschakis et al., 2010; Zhai, Lin, Liu, & Yang, 2018).

Starch-based hydrocolloids have been the subject of intensive re-
search and investigation for many years, probably the most intensive
compared to any other biopolymer (Dokić et al., 2008). In order to
satisfy consumers’ demand for naturally derived raw materials able to
perform functional benefits, several starch modifications have been
proposed (Dokić, Jakovljević & Dokić-Baucal, 1998; Fonseca-Florido
et al., 2018; Hadnadev, Dokić, Hadnadev, Pojić, & Torbica, 2014).
Oxentyl-succinate starches (OSAst) and maltodextrins (OSAm) have
been recognized as particularly promising agents in the production of
O/W emulsions (Agama-Acevedo & Bello-Perez, 2017; Dokić et al.,
2008). Amphiphilic nature of OSAm is acquired by introduction of
cold water soluble molecules, maltodextrins (Hadnadev et al.,
2014). The obtained maltodextrins are able to exhibit specific
surface-active and stabilizing properties (Agama-Acevedo and Bello-
Perez, 2017; Li et al., 2014). However, the reduction of starch mole-
cular mass has a negative influence on the thickness of the formed in-
terfacial (oil/water) layer, and hence the benefit of starch as a macro-
molecule is lost (Li et al., 2014). Therefore, further studies aiming to
diminish the corresponding negative effect are necessary as concluded
by previous researchers (Agama-Acevedo and Bello-Perez, 2017).

Sugar beet pectin (SBP) represents an emerging potential pectin
with significant structural differences compared to the other commonly
used pectins. SBP is not capable of forming gels, but has been confirmed
as an effective stabilizer of O/W emulsions (Chen et al., 2016; Leroux,
Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The reported emul-
sifying properties mostly originate from protein residues present within
the pectin structure and the presence of phenolic esters in the lateral
chains (Funami et al., 2007). Furthermore, SBP showed better emulsi-
fying properties compared to the other polysaccharides (e.g. gum
arabic, corn fiber gum) due to the more extended conformation of the
pectin molecule (Bai et al., 2017a; Leroux et al., 2003). However,
previous reports also suggested lower emulsion stabilization effect of
SBP due to the relatively thin hydrated layer unable to provide suffi-
cient steric effects. Therefore, the application of chelating agents or the
complexes with other polysaccharides have been proposed to increase
the stability of emulsions involving SBP (Nakauma et al., 2008).
Nevertheless, there is a lack of studies in the field of combined emul-
sifying impact of SBP and other polysaccharide hydrocolloids (e.g.
OSAm) capable of emulsion formation and stabilization.

Fibrous polysaccharide material usually represents the most
abundant by-product in agri-food industry where the biggest producer,
sugar beet processing industry, generates more than 107 tonnes of sugar
beet pulp, only in Western Europe (Dinand, Chanzy, & Vignon, 1996;
Šorona-Simović et al., 2016). Sugar beet pulp, a fibrous by-product, is
mainly used as cattle feed, but in the recent decades more applications
have been proposed (Maravič et al., 2018; Šorona-Simović et al.,
2017). The extraction of SBP as valuable emulsifying agent has found a
large market in the recent decades (Leroux et al., 2003). Bergentstahl
(1988) has highlighted the ability of polysaccharides to act as steric
stabilizers of surfactant-coated emulsion droplets, through poly-
saccharide adsorption at the formed emulsion droplet surface. How-
ever, the protein–polysaccharide complexes found in sugar beet fibers
(SBF) suggest further investigation of feasible application in the field of
emulsion-based systems (Dickinson, 2008).

In order to mitigate and compensate the distinctive negative effects
of above-mentioned biopolymer macromolecules on emulsion stability,
this research aims to investigate O/W emulsions of various composition
and complexity. Relatively small changes in the specific amount and
type of applied and combined biopolymers are assumed to significantly
affect physical characteristics and structure of the formed series of
emulsion systems. The stabilization effect of SBF and the effect of
possible interaction with relatively large (SBP) and small (OSAm) bio-
polymer-based stabilizers were evaluated. The corresponding stabilizers
are evaluated in terms of individual and combined impacts on the
formation and stability of corn oil-in-water emulsions prepared using a
high-shear homogenizer.

### 2. Materials and methods

Corn oil (Olitalia, Italia) purchased from a local supermarket was
used for the purpose of dispersed phase. SBP (Herbstreith & Fox KG,
Germany), OSAm (C’EmCap 12633, Cargill) and SBF (Nordic Sugar A/S
factory, Sweden) were kindly donated by the producers. SBF were
ground in a planetary ball mill (400 rpm, 30 min, PM 400, Retsch,
Germany) in order to reduce the particle size towards colloidal sizes.

Amino acid content in corresponding biopolymers was measured
according to the EN ISO 13903/2005 high-pressure liquid chromatog-
raphy method used for amino acid analyses (2005). The external
standard amino acid (AA) mixture calibration enabled peak identifi-
cation and was used to verify the results of HPLC analysis (Table 1).
Limit of quantification (LoQ) was determined by analysing 10 injections
of mixed standard solutions of AA and calculated as LoQ = 10xRelative
Standard Deviation of 10 measurements. For other validation para-
eters assessment, samples of corresponding biopolymer fortified by
adding reference standard of each AA at two concentration levels
(5xLoQ and 10xLoQ) were prepared. Corresponding samples were
prepared and analysed within two days, by different person per each
day, under same experimental conditions. The repeatability was re-
lected as standard deviation within results of group of samples ana-
lysed under repeatability conditions (same concentration level, same
person for sample preparation, during one day), while intra-laboratory
reproducibility was presented as standard deviation within all analysed
samples and considered different concentration levels, persons and time

A continuous phase solution was prepared in double-distilled water
24h before the emulsification process in order to obtain complete hy-
dration of the biopolymers used. According to the experimental plan
(Table 2), the corresponding amount of stabilizers (SBP, OSAm, SBF)
was calculated on the total emulsion mass.

Corn oil-in-water emulsions were prepared by one-step addition
homogenization of 10 wt% corn oil with 90 wt% aqueous continuous
phase by homogenizer Ultra-Turrax T25 basic, equipped with S 25 N-
18 G dispersing tool, for 10 min at 6500 min−1 on a constant tem-
perature of 25 ± 0.1 °C.

Droplet size and droplet size distribution were measured by the

1. Experimental plan

Response surface methodology (RSM) was applied to evaluate the effects of different stabilizers for various dependent responses. Central composite design (CCD) with three numeric factors on three levels was used. Experimental design consisted of twenty three randomized runs, including three replicates at the central point. Different concentrations of corresponding stabilizers (0%, 0.5% and 1% (w/w)) were investigated as independent variables. The experimental design and multiple linear regression analysis were performed using Design-Expert v.7 Trial (Stat-Ease, USA). The results were statistically tested by analysis of variance (ANOVA) with the significance levels of 5%. The adequacy of the models was evaluated by model p-values, coefficient of multiple determination (R²) and lack of fit testing.

3. Results and discussion

All experiments and subsequent characterization of obtained emulsions are conducted according to the experimental plan, and the values regarding corresponding responses are presented in Table 2. The statistical analysis of dependent responses highlighted the particular influence of input factors and their interactions (Table 6). As presented, all obtained models were statistically significant (p < 0.05) indicating good fitting of the experimental results.

The obtained oil droplet mean diameters ranged from 4.9 to 10.1 μm. As high-performance dispersing instrument (high emulsification efficiency and shear forces) was used in the process of emulsion production, corresponding droplet size range is expected. In addition, aiming to further investigate emulsion stability and physicochemical properties of adsorbed layers, emulsion zeta potential was measured and presented in Table 3. In order to completely evaluate all the effects of different stabilizers (and their possible interactions) on the observed emulsions characteristics, several of emulsion forming scenarios are supposed and illustrated (Fig. 1) and consequently elaborated.

3.1. OSA modified maltodextrin (Fig. 1a)

The results of the experiments where OSAm was used as a single stabilizer in the emulsification process (Run 5 and 21) indicate good emulsifying properties. The higher level (1%) of single OSAm addition in the conducted experiments provided emulsion oil droplets diameter in the range of 5.8–5.9 μm with the average value of 5.85 μm. However, significant increase in oil droplet diameter was noticed in the

### Table 2

The obtained results regarding corresponding responses (SBP – sugar beet pectin, SBF – sugar beet fibers, OSAm – octenyl succinate modified starch, SD – Surface Weighted Mean Diameter, CI – Creaning index, SSA – Specific Surface Area).

<table>
<thead>
<tr>
<th>Run</th>
<th>SBP (%)</th>
<th>SBF (%)</th>
<th>OSAm (%)</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>CI (%)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>CI (%)</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CI (%)</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>CI (%)</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CI (%)</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>CI (%)</td>
</tr>
<tr>
<td>12</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>SD (μm)</td>
</tr>
<tr>
<td>13</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>15</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>16</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>17</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>18</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>19</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>22</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
<tr>
<td>23</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>CI (%)</td>
</tr>
</tbody>
</table>

* Clear oil layer/cannot be considered as emulsion.

Zeta potential was measured by using Zetasizer Nano ZS (Malvern Instruments, UK). Emulsions were diluted to 1/10 in bi-distilled water prior to analysis and pH was set to 6.2. All measurements were conducted in triplicate.

For the purpose of stability experiments, emulsion samples were stored 24 h in 10 mL graduated glass cylinders at room temperature. Creaning of investigated emulsions was monitored visually and the extent of creaming was quantified by the creaming index CI (%) (Eq. (1)):

$$CI = \frac{H_c}{H_E} \times 100$$  

(1)

where $H_c$ is the volume of transparent serum layer at the bottom of the cylinder, and $H_E$ is the total emulsion sample volume.

Viscosity measurements, using the Ubbelohde capillary viscometer, were performed at constant temperature of 25 ± 0.1 °C. The samples tested were prepared according to the procedure previously described for continuous phase solutions formulation. Solutions' density was measured directly by using Densitostat 30PX, Density Meter (Mettler Toledo, USA). Relative ($\eta_{rel}$) and specific viscosity ($\eta_{sp}$) were determined according to Eq. (2) and Eq. (3):

$$\eta_{rel} = \frac{\eta - \eta_0}{\rho - \rho_0}$$  

(2)

$$\eta_{sp} = \eta_{rel} - 1$$  

(3)

where $\rho_1$ and $\rho_0$ represent density of tested solution and water, respectively, $t_1$ and $t_0$ represent efflux time of tested solution and water, respectively. Intrinsic viscosity [$\eta$] of tested macromolecules was determined according to Huggins (4) and Kraemer equation (5):

$$[\eta] = \lim_{c \to 0} \left( \frac{\eta_{sp}}{c} \right)$$  

(4)

$$[\eta] = \lim_{c \to 0} \left( \frac{\ln(\eta_{sp})}{c} \right)$$  

(5)
experiments where lower amount of single OSAm addition (0.5%) was used. The corresponding result suggested possible coalescence and aggregation of formed oil droplets as a result of the insufficient amount of stabilizer available to adsorb on the produced oil droplet surface. Moreover, in run 21, a thin layer of corn oil was visible on emulsion surface after 48 h of storage, suggesting high instability of produced emulsion due to the insufficient quantity of OSAm and low density of the formed surface layer. Furthermore, droplet size is influenced by the micelles disintegration rate, since it can be assumed that micelles are present in the continuous phase at both concentrations used (CMC = 0.05–0.08 g/100 cm³ – OSA starch solutions) (Krstonosić, Dokić, & Milanović, 2011). Nevertheless, the obtained SD values are significantly lower compared to the results of the previous researchers where OSAst was used (Dokić et al., 2008; Timgren, Rayner, Dejmek, Marku, & Sjöö, 2013). It is assumed that smaller droplet diameters were obtained due to the better steric stabilization effect of OSAm molecules (particle size: OSAm < < OSAst). More OSAm molecules are able to adsorb on the larger surface of oil droplets with smaller intermolecular hindrance, and consequently prevent droplet coalescence and other instability factors (Agama-Acevedo & Bello-Perez, 2017). The positive effect of OSAm on emulsion stability is confirmed by low ζ-potential values (Table 3) in all experiments where OSAm was used. The corresponding values indicate significantly higher OSAm adsorption on oil/water interface compared to SBP and SBF.

3.2. Sugar beet fibers (Fig. 1b, c and d)

Taking into account the low water solubility of the SBF, in order to discuss the following emulsification results, particle size distribution of grinded SBF was measured and calculated. The results obtained are presented in Fig. 2a. The addition of SBF resulted in the production of average emulsion droplets from 6.5 to 7.6 μm for Run 23 and 2, respectively. Even though smaller droplets were obtained in the emulsion containing lower amount of SBF (0.5%) a significantly lower Span value of emulsion prepared with 1% of SBF addition (2.144 < 4.541) indicated significantly narrower droplet size distribution. The higher SBF concentration significantly contributed to the transition of bimodal towards monomodal particle size distribution (Fig. 2b). Creaming index (CI) of all emulsions obtained with SBF (70–74%) was lower compared to the OSAm emulsions (90%) and SBP emulsions (86%), indicating a significant effect of SBF on emulsion stabilization. The ability of SBF to diminish emulsion creaming is mainly attributed to the specifically higher increase of continuous phase viscosity upon addition of corresponding hydrocolloid (Stokes Law). Moreover, formation of complex matrix of cellulose and other polysaccharides, which act as steric stabilizers for oil droplets, also significantly contributed to lower CI values. Since sugar beet polysaccharides consist mainly of low water soluble fibers, which tend to sediment in water solution, but are also able to adsorb oil droplets (AA lipophilic moieties) on particle surface, balance between oil droplet creaming and polysaccharide sedimentation occurred. The increased viscosity of continuous phase (Table 4), induced by higher amount of SBF added, also contributed to the narrower droplet size distribution within the single SBF addition experiments (Dokić et al., 2008).

Moreover, the corresponding emulsion stabilization, noticed in all emulsions containing SBF, could be an outcome of gel-like (cream) structure formation of hydrated SBF elemental units (fibers, polysaccharides and proteins) in which corn oil droplets are incorporated (Fig. 3). In the experiments 2 and 23, the stabilization phenomena occurred due to the adsorption of multiple corn oil droplets to the hydrophobic sites of SBF particles, originating from AA hydrophobic residues (Table 5). The ability of corn oil to interact with hydrophobic AA residues provides amphiphilic character to SBF (similar to SBP). The presence of both polar and non-polar parts in SBF emphasize a strong tendency to adsorb at corn oil–water interfaces, and consequently form stabilizing layer around oil droplets. Relatively low value obtained ζ-potential, regarding SBF emulsion, confirmed abovementioned
Table 4
Relative viscosity of corresponding continuous phase and intrinsic viscosity of macromolecules (SBP, sugar beet pectin; SBF, sugar beet fibers; OSAm, octenylsuccinate modified maltodextrin).

<table>
<thead>
<tr>
<th>SBP</th>
<th>Relative viscosity (Pa·s/Pas)</th>
<th>Intrinsic viscosity (cm²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Huggings Eq.</td>
<td>Kraemer Eq.</td>
</tr>
<tr>
<td>0.5 g/100 ml</td>
<td>2.95</td>
<td>300.14</td>
</tr>
<tr>
<td>1 g/100 ml</td>
<td>6.50</td>
<td>300.14</td>
</tr>
<tr>
<td>SBF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 g/100 ml</td>
<td>1.12</td>
<td>22.11</td>
</tr>
<tr>
<td>1 g/100 ml</td>
<td>1.28</td>
<td>22.11</td>
</tr>
<tr>
<td>OSAm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 g/100 ml</td>
<td>1.09</td>
<td>17.97</td>
</tr>
<tr>
<td>1 g/100 ml</td>
<td>1.21</td>
<td>17.97</td>
</tr>
</tbody>
</table>

Table 5
Amino acid content of sugar beet pectin (SBP) and sugar beet fibers (SBF).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>SBP g/100 g</th>
<th>SBF g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydrophilic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.26</td>
<td>1.75</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.15</td>
<td>0.33</td>
</tr>
<tr>
<td>Serine</td>
<td>0.42</td>
<td>0.29</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.33</td>
<td>1.2</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.19</td>
<td>0.27</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.67</td>
<td>1.17</td>
</tr>
<tr>
<td>hydrophobic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>0.38</td>
<td>0.96</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.52</td>
<td>0.44</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.22</td>
<td>0.46</td>
</tr>
<tr>
<td>Valine</td>
<td>0.06</td>
<td>0.36</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.11</td>
<td>0.29</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.09</td>
<td>0.46</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.36</td>
<td>0.51</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Total content</td>
<td>4.39</td>
<td>9.71</td>
</tr>
</tbody>
</table>

assumption (Table 3). Such behaviour is characteristic for combinations of polysaccharides and proteins that are able to fulfill both stabilizing and emulsifying roles (Dickinson, 2003). Therefore, it can be concluded that SBP matrix stabilized the emulsion by three possible scenarios: corn oil droplets incorporated into SBP “shells” (larger particles) (Fig. 1b), by “picking” effect of the smallest SBP particles (Fig. 1c) (Binks, 2002; Pickering, 1997) and by emulsifying abilities of lipophilic proteinous sites on SBP surface (1d). The observed effects highlighted the possibilities of SBP application in food industry as both stabilizing and gelling or thickening agent able to retard processes leading to emulsion instability and at the same time provide functional benefits to food.

3.3. Sugar beet pectin (Fig. 1e)

The results obtained in the experiments where SBP was used as a single emulsion stabilizer (Run 11 and 22) confirmed promising application of this sugar industry by-product in the field of O/W food systems production (Fig. 3). In contrast to the SBF experiments, droplet size decreased with the increase of SBP addition to the system within the applied range (higher impact of SBP on further viscosity increase). The smallest mean droplet size (4.9 μm) was produced at the highest level of SBP addition (1%), which also represents the smallest droplet size obtained in all conducted experiments. However, the SBP increase from 0.5% to 1% did not significantly contribute to the lower Span values and monomodal particle size distribution was obtained in all experiments. Decrease of emulsion droplet size with the increase of SBP concentration is explained as a consequence of smaller droplet surface area covered during the process of homogenization (Tcholakova, Denkov, & Damer, 2004) and reduced droplet re-coalescence within the homogenizer due to faster adsorption of SBP molecules on droplet surface (Jafari, Assadpoor, He, & Bhandari, 2008) and higher density of the adsorption layer. Further increase of SBP concentration is expected to significantly increase the continuous phase viscosity but also to decrease the mean droplet size (Bai et al., 2017b). The creaming index of the SBP emulsions was constant and no impact of hydrocolloid concentration was noticed.

3.4. Impact of OSA maltodextrin and SBF mixtures (Fig. 1f)

The combined impact of OSAm and SBF addition, at lower (Run 12) and higher level (Run 16), was used for investigation of emulsion stabilization properties. Mean droplet diameter was 5.9 and 8.5 μm for Run 12 and 16, respectively, suggesting significant influence of added macromolecules and their interactions on the obtained responses which is confirmed by low p-values (Table 6, p < 0.05). Moreover, evident decrease in CI values is noticed in all experiments where OSAm-SBF was used. The obtained CI values in experiments 12 and 16 (54% and 60%, respectively) represent the lowest values obtained in this study. Strong influence of SBF properties in the overall OSAm-SBF impact is noticed through significant increase in Span values and decrease in CI values. Furthermore, conversely to the Run 21 (single addition of OSAm (0.5%)), in the experiments where both OSAm and SBF were added at the lower levels, no phase separation occurred after 48 h. Moreover, the corresponding emulsion (Run 12) expressed the most prominent overall characteristics in terms of SD, SSA and CI values. In addition, ζ-potential value of the corresponding emulsion is significantly lower compared to emulsions where combined SBF-SBP and SBP-OSAm were used at the same level (Table 3). However, the higher level of SBF, in combination with OSAm, addition emphasized a strong impact of SBF on continuous phase viscosity increase, hence hindering the OSAm potential mobility throughout/in the continuous phase. Therefore, smaller contribution of OSAm impact (previously recognized through smaller droplet formation) in combined OSAm-SBF influence, resulted in overall larger emulsion droplets compared to the experiments conducted at OSAm-SBF lower levels. Dominant effect of increased

![Fig. 3. Light microscopy of different emulsions (40×): sugar beet pectin (a), sugar beet fibers (b) and octenylsuccinate maltodextrin (c) emulsion.](image-url)
viscosity on emulsion stabilization is enhanced by OSAm capacity to form smaller droplets able to incorporate in the SBF formed matrix (Fig. 1f). Therefore, close pack of the emulsion droplets, influenced by OSAm-SBF interaction, is negatively affecting creaming velocity by increasing specific droplet concentration and droplet crowding effects (Chanamai & McClements, 2000; Hunter, 1986). OSAm significantly contributed to the lower SD values at the medium level of addition. A small amount of sediment accumulated at the bottom phase in the experiments where combined OSAm and SBF were used (Run 3, 8, 12, 15, 16, 17, 18, 19, 20). The possible explanation for the corresponding phenomenon is the formation of encapsulated complexes of small OSAm-coated emulsion droplets within the network of larger SBF particles, which were overall more dense than the aqueous phase. The sedimentation of O/W polysaccharide complexes was also reported in the previous studies (Moschakis et al., 2010).

3.5. Impact of OSA maltodextrin and SBP mixtures (Fig. 1g)

The combined influence of OSAm and SBP on emulsion characteristics is investigated through higher and lower levels of OSAm–SBP addition (Run 1 and 14). The obtained emulsions are characterized by relatively low values of droplets’ SD ranging from 5.2 to 5.9 μm and, high level of droplet size uniformity with Span values from 1.671 to 1.932. Furthermore, CI values did not differ significantly from the values obtained by single addition of OSAm or SBP. Compared to the OSAm-SBF emulsions, smaller interaction of corresponding macromolecules is noticed. However, the positive effect of macromolecular interaction is emphasized through high-stability of obtained emulsions even at a lower level of addition (0.5%) where emulsion droplet distribution and sizes showed minor changes after 48 h of storage (Fig. 4). It is assumed that formation of strong macromolecular layer on droplet surface prevented possible oil droplet coalescence and aggregation (Dickinson, Phillips, Wedlock, & Williams, 1988) along with the significant viscosity increase induced mostly by SBP introduction. According to the intrinsic viscosity measurements (Fig. 5) and previously published results, the viscosity-average molecular mass was calculated using the Mark’s variation of Staudinger’s formula (Arslan, 1995; Dokić et al., 2008).

The calculated viscosity-average molecular mass of SBP and OSAm were 78337 Da and 5882 Da, respectively. Results regarding the SBP are in accordance to with producer’s specification (Herbstreith & Fox KG, Germany) but not in accordance with the previously published results reported by Bai et al. (2017a), 417 kDa, measured by size exclusion chromatography.

However, both obtained molecular mass values of OSAm and SBP support our assumption that smaller sized OSAm molecules are able to be entangled within the “cavities” of larger SBP molecules (Fig. 1g) and hence form stronger macromolecular layer on oil droplet surface. The corresponding positive effect of combined OSAm–SBP stabilizing effect could be further valorised in the pre-encapsulated emulsion preparations tailored for spray-drying or other high-speed drying technique where suitable carrier has to be introduced in the feed formulation (Agama-Acevedo & Bello-Perez, 2017).

3.6. Impact of SBF–SBP mixtures (Fig. 1h)

In the experiments where combined SBF and SBP (Run 7 and 10) were used as emulsion stabilizers, SD values ranged from 7.6 μm to 8.0 μm. Creaming index values did not significantly differ at lower and higher levels of combined stabilizers addition (74% and 75%, respectively). However, significant decrease in Span values was noticed with decreasing content of corresponding stabilizers (5.788 → 2.798).

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*p statistically significant at p < 0.05.

Fig. 4. Determination of intrinsic viscosity of sugar beet pectin (a), sugar beet fibers (b) and OSA modified maltodextrin (c).
increase of Span values with rising content of corresponding polysaccharides is most likely a result of significant viscosity increase and strong steric hindrance influenced by large polysaccharide molecules. It is assumed that smaller droplets formed within the SBF matrix are not sufficiently stabilized and hence susceptible to coalescence. The concept of oil droplet encapsulation is similar to the experiments where OSAm–SBF impact was investigated. However, significantly larger molecule of SBP (Bai et al., 2017b) is not able to incorporate the SBF matrix as good as small OSAm molecule (Dokić et al., 1998). This effect is also recognized through relatively higher ζ-potential values regarding the emulsions where combined SBF–SBP were used compared to the emulsions where SBF and SBP were combined with smaller macromolecule, OSAm (Table 3). As it can be noticed in the experiments where beside SBP and SBF, OSAm was introduced to the investigated system (Run 4, 8, 13, 15, 17), significant decrease in droplet size occurred. However, this effect was only expressed in the experiments where at least two factors were set to medium level, while other experiments (Run 3, 18, 19) showed opposite influence of OSAm addition. The possible explanation for this effect is the apparent competition of SBP with SBF lipophilic moiety, which occurred in the process of oil droplet surface adsorption. As already reported in several studies (Bai et al., 2017a; Chen et al., 2016; Leroux et al., 2003), proteinous moiety of SBP is responsible for emulsifying properties and adsorption on oil droplet surface, hence in the combined SBF–SBP effect both protein and polysaccharide content play a crucial role in emulsion stabilization.

proteinous moiety of SBF and SBP is presented through amino acid content in Table 5 and polysaccharide content is reported elsewhere (Maravić et al., 2018). Amino acid profiles of SBP and SBF are mostly similar (Fig. 6), however some differences can be noticed. As SBF represents raw material for the production of SBP (high pectin content (15–30%) on dry weight basis), significant amount of linked SBP is already present in the structure of introduced SBF. The production procedure of SBP from SBF influence AA profile by increasing relative content of Serin, Lysin and Arginin (hydrophilic AA) but also increasing relative content of Glycin and Tyrosin (hydrophobic AA). The presented differences highlight the potential covalent bonds between protein and polysaccharide molecules, hence forming biopolymer ‘conjugates’ which have been found to have effective emulsion-stabilizing properties (Dickinson, 2008). Besides the mentioned ‘conjugates’, SBF also includes excessive amount of cellulose and hemicellulose which are unable to exhibit amphiphilic performance and therefore inhibit the emulsification properties of corresponding material. Emulsion stabilization effect of cellulose and hemicellulose is recognized through viscosity increase, “pickering” effect and “shell” forming as previously discussed in Section 3.2.

4. Conclusions

The evaluation of corresponding polysaccharide stabilizers (SBP, SBF and OSAm) in terms of individual and combined impacts on the
formation and stability of corn oil-in-water emulsions is presented in this study. The results of the experiments where OSAm was used as emulsion stabilizer in the emulsification process indicate good emulsifying properties with the obtained values of droplet size significantly lower and ζ-potential significantly higher compared to the results of previous researchers where OSASt was used. The application of SBF significantly affected emulsion stability through significantly lower creaming index values (70–74%) compared to the OSAm emulsions (90%) and SBF emulsions (86%). The obtained AA profile of SBF confirms the presence of both polar and non-polar parts in SBF, suggesting a strong tendency to adsorb at corn oil-water interfaces and consequently form stabilizing layer around oil droplets. However, in the experiments where 1% of SBF was used as the single emulsion stabilizer the smallest mean droplet size of 4.9 μm was produced, which also represents the smallest droplet size obtained in all the conducted experiments. Furthermore, significant influence of macromolecules interactions on obtained responses is confirmed by low p-values. Overall OSAm-SBF combined impact is expressed through significant increase in Span values and decrease in CI values, and hence production of the emulsions having the most prominent overall characteristics in terms of SD, SSA and CI values. Emulsion stabilizing capacity of combined OSAm and SBF is characterized by relatively low values of droplets diameter and high level of droplet size uniformity. In the combined SBF–SBF effect both protein and polysaccharide content play a crucial role in emulsion stabilization. The protein moiety of SBF and SBF, presented through amino acid content, highlighted the differences in relative content of hydrophilic and hydrophobic amino acids. Better understanding of the corresponding complex system opens the opportunity for wide range application in “clean label” food industry aiming at fat replacement and specific targeted emulsion preparations. Presentated capacity of SBF, as emulsion promising stabilizer, confirmed hypotheses and suggested further research which including different emulsification techniques and rheological characterization of the obtained products.

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References