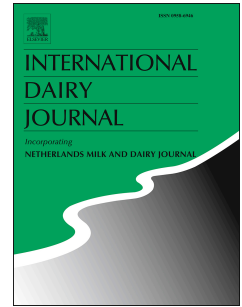


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The impact of Cheddar or white brined cheese with various maturity degrees on the processed cheese consistency: A comparative study

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1 **The impact of Cheddar or white brined cheese with various maturity degrees on the**  
2 **processed cheese consistency: A comparative study**

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ABSTRACT

This study focussed on the dependence on different emulsifying salt ternary mixture composition [disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20; number of phosphate units in the chain  $\approx 20$ ), trisodium citrate (TSC)] of hardness and gel strength of spreadable processed cheese (PC) manufactured from Cheddar and white brined cheeses. All PC samples were stored for 60 days ( $6 \pm 2$  °C). The hardest PC and samples with the highest gel strength were those produced from DSP and TSPP in a ratio 1:1. The hardness of all examined samples increased with the extending storage period, whilst their hardness and gel strength decreased with the rising maturity degree of the raw material utilised. Furthermore, higher values of gel strength were reported for the PC samples produced with Cheddar cheese in comparison with those made from white brined cheese.

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## 48 1. Introduction

49

50 Processed cheese (PC) is a viscoelastic dairy-based gel described also as a stable oil-  
51 in-water emulsion (Chen & Liu, 2012; Hanaei, Cuvelier, & Sieffermann, 2015; Lee, Buwalda,  
52 Euston, Foegeding, & McKenna, 2003). In traditional PC production, the main raw material is  
53 natural cheese of various degrees of maturity. The types of cheese predominantly used in  
54 different world areas vary (including Cheddar, Dutch-type, Swiss-type, mozzarella and white  
55 brined cheeses). In the English-speaking countries (e.g., Britain, USA, Canada, Australia,  
56 New Zealand) the main raw material for PC production is usually Cheddar (CDC) and  
57 mozzarella cheeses (typically, e.g., for New Zealand). On the other hand, in countries around  
58 the Mediterranean area, Balkan, the Near and Middle East, white brined cheeses (WBC)  
59 represent the most consumed cheese varieties and are therefore widely used as the main raw  
60 material for the production of PC (Černíková, Nebesářová, Salek, Řiháčková, & Buňka, 2017;  
61 Moatsou & Govaris, 2011). Many dairy ingredients (e.g., anhydrous butterfat, butter, cream,  
62 milk powder, whey, buttermilk, caseinates, coprecipitates) or non-dairy components (e.g.,  
63 stabilisers, preservatives, flavouring agents, hydrocolloids, acidifying agents) can be  
64 optionally added into the mixture of raw materials. Besides the ingredients in the formulation,  
65 physicochemical, technological, and microbiological factors could affect the properties of the  
66 final PC (Ferrão et al., 2016; Kapoor & Metzger, 2008).

67 The desired final smooth and homogeneous matrix of PC is formed by blending  
68 shredded natural cheese in the presence of emulsifying salts (ES; mainly, sodium salts of  
69 phosphates, polyphosphates, citrates or combinations of these), heated under partial vacuum  
70 and constant shear, commonly in a temperature range of 90 to 100 °C. ES are essential  
71 components in the formulation. The addition of ES results in ion exchange of calcium and  
72 sodium ions and, subsequently, casein dispersion. The dispersed proteins (sodium

73 paracaseinates) can serve as effective surface active substances and emulsify the dispersed  
74 free fat globules. The control and stabilisation of the pH level and an influence on the  
75 formation of the final casein network over holding time at the melting temperature and/or  
76 over cooling are some additional roles of ES (Buňka et al., 2014; Chen & Liu, 2012; Dimitreli  
77 & Thomareis, 2009; Sádliková et al., 2010 Salek, Černíková, Maděrová, Lapčík, & Buňka,  
78 2016 ).

79 Furthermore, the consistency of PC can be affected by many factors, including, e.g.:

80 (i) raw material composition – the type and chemical profile of the natural cheese applied  
81 (dry-matter, fat, protein, and calcium ion contents, and maturity degree), composition and  
82 concentration of ES, addition of other optional dairy and non-dairy ingredients and also the  
83 pH of the mass to be melted; and (ii) processing and storage conditions – agitation speed,  
84 target melting temperature and holding time, cooling rate and also storage temperature.

85 Nowadays, hydrocolloids, regularly used in the production of PC, are important components  
86 affecting the consistency of PC (Dimitreli & Thomareis, 2007; Shirashoji, Jaeggi, & Lucey,  
87 2006).

88 CDC is a ripened hard cheese and its body has a near white or ivory through to light  
89 yellow or orange colour. In addition, its texture can be described as firm, smooth and waxy.  
90 Furthermore, gas-holes are absent, whereas some openings and splits are acceptable. The  
91 ripening period to develop typical flavour and body characteristics is normally from 5 weeks  
92 up to 2 years (at 7–15 °C), depending on the extend of maturity required (Codex standard  
93 263-1966; Codex Alimentarius Commission, 2013). In contrast, WBC are produced from  
94 curds that are not subjected to any elevated heating (cooking) after coagulation (such as is the  
95 case for CDC, Dutch- or Swiss-type cheeses). Their flavour is slightly acid and salty that  
96 sometimes turns to rancid and piquant. Moreover, the cheese mass has no rind, no gas-holes  
97 or other openings, except for some small mechanical openings and its texture appears to be

98 soft but sliceable. Thus, they are consumed after several days or up to some months of  
99 ripening in brine of various NaCl concentrations (10–18 g 100 g<sup>-1</sup>) (Hayaloglu, 2016;  
100 Moatsou & Govaris, 2011).

101 The impact of ES composition on the consistency of PC produced from different  
102 natural cheeses (the main raw material), particularly Edam (Buňka et al., 2014; Salek et al.,  
103 2015), Cheddar (Brickley, Auty, Piraino, & McSweeney, 2007), mozzarella (Chavhan,  
104 Kanawjia, Khetra, & Puri, 2015; Chen & Liu, 2012; Khetra, Chavhan, Kanawjia, & Puri,  
105 2015; Salek et al., 2017;) and Swiss-type (Salek et al., 2016) cheeses has been previously  
106 reported. Nevertheless, research providing a direct comparison of the spread properties of PC  
107 produced under identical processing parameters and similar experimental design from two  
108 technologically very different varieties of natural cheese (CDC and WBC), and additionally  
109 with various levels of maturity, has not been performed to date. Therefore, the main aim of  
110 the present study was to compare the influence of two different varieties of natural cheese  
111 with varying levels of maturity in combination with the different composition of ES on the  
112 textural properties and the gel strength of spreadable PC during a 60-day storage period ( $6 \pm 2$   
113 °C). Disodium hydrogenphosphate (DSP, Na<sub>2</sub>HPO<sub>4</sub>), tetrasodium diphosphate (TSPP,  
114 Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>), sodium salt of polyphosphate with mean length  $n \approx 20$  (P20) and trisodium citrate  
115 (TSC, Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) were used in four types of ternary mixtures of ES. The pH of the tested  
116 samples was adjusted to the target values within the interval of 5.60–5.80, corresponding to  
117 the standard pH values of PC spreads.

118

## 119 **2. Materials and methods**

120

### 121 *2.1. Materials*

122

123 Commercially available CDC blocks [dry-matter content 62 g 100 g<sup>-1</sup>; fat in dry-  
124 matter content, 50 g 100 g<sup>-1</sup>; 4, 8, 12 and 16 weeks of maturity (storage at 10 ± 2 °C); 1.8 g  
125 100 g<sup>-1</sup> NaCl content], WBC blocks [Akawi-type cheese; dry-matter content 48 g 100 g<sup>-1</sup>; fat  
126 in dry-matter content 38 g 100 g<sup>-1</sup>; 2, 4, 8, 16 and 24 weeks of maturity (storage at 10 ± 2 °C);  
127 7.4 g 100 g<sup>-1</sup> NaCl content] and butter (dry-matter content 84 g 100 g<sup>-1</sup>, fat content 82 g 100 g<sup>-1</sup>)  
128 <sup>1</sup>) were purchased wholesale in the Czech Republic. The same batch of the individual cheese  
129 varieties was used in the whole experiment.

130 When Cheddar is used as raw material in Central Europe (a minority, but some  
131 producers use it), the maturity is generally approximately 8 weeks. Therefore, the half of this  
132 period and twice this period (i.e., 4–16 weeks) were chosen as an appropriate storage interval  
133 for the experiment. According to the authors' knowledge, the selected maturity interval also  
134 covers usual storage times for raw material for PC in some other countries. In the case of  
135 white brine cheese, our practical experience from the Near and Middle East shows us that the  
136 storage period could be longer than the usual 2 or 3 months. This is valid also for raw material  
137 for PC. Therefore, approximately half year was chosen as the longest storage period. DSP,  
138 TSPP and P20 were obtained from Fosfa PLC Company (Břeclav, Czech Republic); TSC,  
139 HCl, and NaOH were purchased from Sigma Aldrich Inc. (Schnelldorf, Germany). Water was  
140 also added to adjusting to the required dry matter content of the model PC.

141

## 142 2.2. *Manufacturing procedure of the processed cheese samples*

143

144 The composition of raw materials of the PC samples was calculated to achieve final  
145 products with 40 g 100 g<sup>-1</sup> dry matter content and 50 g 100 g<sup>-1</sup> fat in dry matter content.  
146 Moreover, four ternary mixtures of ES (TSC:TSPP:P20, DSP:TSC:P20, DSP:TSPP:P20 and  
147 DSP:TSPP:TSC) were prepared. For all four ternary mixtures the ES were blended in 12

148 percentage ratios (100:0:0; 50:50:0; 0:100:0; 40:40:20; 40:20:40; 20:40:40; 50:0:50; 0:50:50;  
149 40:0:60; 20:20:60; 0:40:60; 0:0:100 – the percentages of the components were calculated on  
150 the total weight of the ES; where total weight was 100%). The total concentration of the  
151 applied ES was 3 g 100 g<sup>-1</sup> of the total weight of the melt. Fig. 1 illustrates the experimental  
152 design.

153 A Vorwerk Thermomix TM blender cooker (2 L capacity; Vorwerk & Co Thermomix  
154 GmbH, Wuppertal, Germany) with indirect heating was employed for the production of the  
155 model PC samples in laboratory scale (the same device was used previously by Lee et al.,  
156 2004) and Nagyová et al., 2014). The manufacturing procedure was described in detail in  
157 Salek et al., 2015, 2016, 2017. The processing conditions [melting temperature 90 °C held for  
158 1 min (total melting time: 10–12 min) at approximately 2750 rpm] were the same for all the  
159 formulations. The pH of the samples was adjusted (target values within the interval of 5.60–  
160 5.80) using acid or alkali (1 mol L<sup>-1</sup> HCl or NaOH). To maintain the dry-matter content at the  
161 desired target value (40 g 100 g<sup>-1</sup>), the addition of water was decreased (based on the amount  
162 of the added acid/alkali). Finally, the hot molten PC mass was poured into cylindrical plastic  
163 containers (55 mm diameter, 50 mm height) and sealed. Thereafter, the samples were left to  
164 cool and were stored under refrigeration conditions (6 ± 2 °C) until further analysis.

165 Analyses were performed on the 2<sup>nd</sup>, 9<sup>th</sup>, 30<sup>th</sup> and 60<sup>th</sup> day of storage; rheological  
166 analysis was undertaken on the 30<sup>th</sup> day after the production. Each PC sample tested was  
167 produced in duplicate (CDC – 4 maturity levels × 4 types of ternary mixtures × 12 percentage  
168 ratios × 2 repetitions = 384 lots; WBC – 5 maturity levels × 4 types of ternary mixtures × 12  
169 percentage ratios × 2 repetitions = 480 lots) resulting in 864 lots in total. The raw materials  
170 applied and processing parameters were arranged to imitate industrial conditions. The samples  
171 had the same target parameters (dry matter content and pH-value) and were produced under  
172 the same conditions (e.g., the equipment, the target melting temperature, the holding time, the



173 same packaging, the cooling time, the storage time and temperature). Therefore, the model  
174 PCs manufactured were fully comparable.

175

### 176 2.3. *Chemical analysis of the cheese and the processed cheese samples*

177

178 In the processed cheese samples, the dry matter (DM) content and the pH-values were  
179 determined. DM content was gravimetrically analysed according to ISO 5534 (ISO, 2004) by  
180 drying the samples at  $102 \pm 2$  °C to constant mass. The pH values were determined at ambient  
181 temperature by inserting a glass tip electrode of a calibrated pH-meter (pH Spear, Eutech  
182 Instruments, Oakton, Malaysia) directly into the cheese at three randomly chosen locations.

183 In the cheese (CDC and WBC), the determination of free amino acid (FAA) content  
184 was undertaken in accordance with the process previously performed by Buňková et al.  
185 (2009), Hladká et al. (2014) and Pachlová et al. (2011) using the AAA 400 amino acid  
186 analyser (Ingos, Prague, Czech Republic). The FAA content was calculated as a sum of 22  
187 individual FAAs and the content of similar substances ( $\gamma$ -aminobutyric acid, alanine, aspartic  
188 acid, asparagine, arginine, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine,  
189 isoleucine, leucine, tyrosine, lysine, methionine, ornithine, phenylalanine, proline, serine,  
190 threonine, valine; results were expressed in  $\text{g kg}^{-1}$ ). Additionally, prior to the particular  
191 determination each natural cheese was lyophilised (Christ Alpha 1–4, Christ, Osterode,  
192 Germany) twice. Furthermore, each lyophilised sample was extracted twice and each extract  
193 was loaded on the column in triplicate ( $n = 12$ ).

194

### 195 2.4. *Hardness measurements of the processed cheese samples*

196

197 The selected textural properties of the model PC samples were evaluated using a  
 198 texture analyser TA.XTplus (Stable Micro Systems Ltd., Godaming, UK) equipped with a 20  
 199 mm in diameter cylindrical aluminium probe. The analysis was performed by penetration into  
 200 the sample (depth 10 mm and trigger force 5 g; deformation rate was 2 mm s<sup>-1</sup>) at 6 ± 2 °C  
 201 (the measurement was carried out within the containers). From the force/time curves, the  
 202 hardness value were obtain as the maximum force (N) observed during penetration (n = 6).

203

#### 204 2.5. *Rheological measurements of the processed cheese samples*

205

206 A dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany)  
 207 equipped with a parallel plate-plate geometry having a 35 mm diameter was used for the  
 208 determination of the PC viscoelastic properties. The rheological tests were carried out at 20.0  
 209 ± 0.1 °C and a gap of 1 mm was applied. Amplitude sweeps were performed to determine the  
 210 linear viscoelastic regions at which the frequency sweep of the samples was obtained. During  
 211 the tests, of the storage  $G'$  (elastic modulus) and  $G''$  (viscous modulus) moduli were measured  
 212 at frequencies between 0.01 and 100.00 Hz and subsequently the complex modulus ( $G^*$ ) was  
 213 calculated as the complex sum of  $G'$  and  $G''$ .

214 The Winter and Chambon (1986) critical gel model was implemented for the changes  
 215 evaluation in the samples viscoelastic properties as a function of frequency:

$$216 \quad G^*(\omega) = A_F \cdot \omega^{\frac{1}{z}} \quad (1)$$

217 where where  $A_F$  (Pa s<sup>1/z</sup>) represents the gel strength,  $\omega$  is the frequency (Hz) and  $z$   
 218 (dimensionless) corresponds to the interaction factor. The recorded values were the mean of at  
 219 least eight replicates (n = 8).

220

#### 221 2.6. *Data analysis*

222

223 The experimental data obtained were analysed using the Unistat<sup>®</sup> 6.5 (Unistat,  
224 London, UK) statistical software. Kruskal-Wallis and Wilcoxon tests were applied for the  
225 evaluation of the results. Significance was considered as  $P < 0.05$ . For the estimation of  $A_F$   
226 the method of Marquardt-Levenberg, a nonlinear regression analysis method ( $A_F > 0$  and  $z >$   
227  $0$ ) was implemented. Correlation analysis was also carried out using Spearman correlation  
228 coefficient.

229

### 230 3. Results and discussion

231

#### 232 3.1. Free amino acids content in Cheddar and white brined cheese

233

234 The total FAA concentrations of the CDC after 4, 8, 12 and 16 weeks were 2.75,  
235 22.18, 41.72 and 48.66 g kg<sup>-1</sup>, respectively. In comparison, in the WBC the total FAA  
236 concentrations after 2, 4, 8, 16 and 24 weeks of ripening were 8.47, 18.74, 37.96, 58.91 and  
237 78.19 g kg<sup>-1</sup>, respectively. With the prolonging of the ripening period (regardless of the  
238 natural cheese applied as the main raw material) more intensive proteolysis occurred ( $P <$   
239  $0.05$ ). Hydrolytic processing of caseins was faster in WBC in comparison with that in CDC ( $P$   
240  $< 0.05$ ). A possible reason could lie in different cultures (microorganisms), which are used in  
241 the production of the above mentioned cheese. According to Fox, Guinee, Cogan, and  
242 McSweeney (2000) and Pachlová et al. (2011) it could be assumed that the amount of intact  
243 casein present in the natural cheese (main raw material) correlates with the concentration of  
244 FAA. The mean length of casein fragments after hydrolysis and so the amount of intact casein  
245 remaining could significantly influence the consistency of the PC (Diana, Rafecas, Arco, &  
246 Quílez, 2014; Petrella et al., 2015; Salek et al., 2017).

247

248 *3.2. Chemical analysis of the processed cheese samples*

249

250 The dry matter content (DM) of all PC samples tested (regardless of the type of  
251 cheese, the ripening period, the storage time and the composition of ES used) ranged in the  
252 interval 40.21 to 40.83 g 100 g<sup>-1</sup> ( $P \geq 0.05$ ). The composition of ES ternary mixture  
253 significantly influences the value of pH ( $P < 0.05$ ) (Nagyová et al, 2014; Salek et al., 2015,  
254 2016, 2017). Therefore, the pH-values were adjusted during manufacturing (see subsection  
255 2.2). At the beginning of the storage, the pH-values of all PC without regard to the studied  
256 factors were in the interval of 5.61 to 5.84. During the 60-day storage the pH-values slightly  
257 but significantly increased by 0.1 – 0.2 ( $P < 0.05$ ). Similar DM content and the pH-value are  
258 essential for successful comparison of consistency of the final products (Lee & Klostermeyer,  
259 2001; Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Piska & Štětina, 2004; Sádliková et al.,  
260 2010).

261

262 *3.3. Textural and rheological properties of the processed cheese samples*

263

264 For the recent study, two approaches for evaluation of PC consistency were chosen.  
265 Firstly, hardness (N) was used as the parameter describing sample behaviour under large  
266 deformation. Subsequently, the testing was enhanced by using of rheological measurements  
267 (gel strength – A<sub>F</sub>; Pa), for studying of sample changes under small shear deformation.

268 The effect of three factors influencing processed cheese consistency was observed: (i)  
269 the composition of ternary mixtures of four ES; (ii) the ripening period of the main raw  
270 material – natural cheese; and (iii) the storage time up to 60 days. All these factors were  
271 studied using samples manufactured using two very different varieties of cheese – CDC and

272 WBC for a comparison of the effect of varying the main raw material. The results of the  
273 sample response on the large (hardness) and small (gel strength) deformations are shown in  
274 Figs. 2–7.

275 When ES were singly added (regardless the type of natural cheese used, the maturity  
276 level and the length of the storage period), increase of hardness and gel strength (Figs. 2–7) of  
277 PC with sodium salts of polyphosphate (P20) was observed in comparison with the samples  
278 with the other ES (DSP, TSPP and TSC;  $P < 0.05$ ).

279 Sodium salt of polyphosphate probably can strongly bind calcium into complexes  
280 resulting in casein dispersion enhancement. Polyphosphates possess the strongest binding  
281 capability to bivalent ions in comparison with monophosphates, diphosphates and/or citrates  
282 (Buňka et al., 2012; Shirashoji et al., 2006). Additionally, the use of TSC resulted in PC  
283 samples with similar values of hardness to those made with TSPP ( $P \geq 0.05$ ). Thus, TSC does  
284 not provide the ability to create new networks (Mizuno & Lucey, 2007). The results obtained  
285 are in accordance to those previously reported by El-Bakry, Duggan, O’Riordan, and  
286 O’Sullivan (2011), Nagyová et al. (2014), and Salek et al. (2015, 2016, 2017), who also used  
287 TSC.

288 Figs. 2, 6A and 7A illustrate the dependence of hardness and gel strength on the  
289 composition of the mixture of DSP, TSPP and P20. Regardless the other studied factors (the  
290 ripening time of raw material, the storage time and the type of cheese), there is a specific ratio  
291 of DSP and TSPP of approximately 1:1 under which the hardness and also the gel strength  
292 increased in comparison with the other mixtures of the tested ES ( $P < 0.05$ ). This  
293 phenomenon was observed especially when the relative content of P20 was under 50%.  
294 However, this observation was not noticed when the relative ratio of P20 was over 50%. The  
295 above mentioned effect of the specific ratio of DSP:TSPP (including the dependence of the  
296 relative ratio of P20) was previously detected when Dutch-type, Swiss-type and mozzarella-

297 type cheeses were applied (Salek et al., 2015, 2016, 2017). The explanation lies in (i) the high  
298 ability of TSPP to support forming casein gels and (ii) the small size of DSP and its capability  
299 to bind onto caseins and increase their hydration. When the relative amount of TSPP is too  
300 low, the gel is too weak due small number of interaction between caseins. On the other hand,  
301 when the relative concentration of TSPP is too high, the gel is also too weak because calcium  
302 ions are strongly bonded and are not possibly “used” during the protein network forming  
303 (Buňka et al., 2012; Kaliappan & Lucey, 2011; Mizuno & Lucey, 2007). The same effect ( $P <$   
304  $0.05$ ) was observed also when PC with the ternary mixtures of DSP:TSPP:TSC (Figs. 3, 6B  
305 and 7B). In the other samples, where DSP, TSPP and P20 were used, it was observed that  
306 with the rising relative amount of P20 in the mixture, the hardness and gel strength of the  
307 samples increased ( $P < 0.05$ ; under the same levels of the other factors tested). The latter  
308 trend was noticed in all samples with this ternary mixture, without regard to the other tested  
309 factors (see above).

310 The next studied ternary mixture was the combination of DSP, TSPP and TSC. The  
311 results of consistency parameters tested are presented in Figs. 3, 6B and 7B. In some PC, the  
312 effect of TSPP on hardness and gel strength was slightly higher in comparison with the  
313 influence of TSC. In the remaining samples, the parameter tested was similar ( $P \geq 0.05$ ) or the  
314 trend was opposite without any clear reason. The results obtained about the practical  
315 similarity of the effect of TSPP and TSC on the consistency of PC are in accordance with  
316 those previously reported by El-Bakry et al. (2011), Nagyová et al. (2014), and Salek et al.  
317 (2015, 2016, 2017). Beside that discussed above with respect to the specific 1:1 ratio of DSP  
318 and TSPP, the changes of hardness and gel strength were controlled by the relative amount of  
319 TSPP and/or TSC. When the relative amount of TSPP and/or TSC was increased in  
320 comparison with DSP, hardness and gel strength increased and vice versa ( $P < 0.05$ ). With  
321 regard to the fact that TSC does not provide the ability of creating new networks (Mizuno &

322 Lucey, 2007), we could assume that the ability of ion exchange, and subsequently the  
323 intensity of caseins dispersion, are the main factors influencing hardness and gel strength in  
324 samples with this ternary mixture containing predominantly TSC. DSP possesses lower  
325 capacity of ion exchange in comparison with TSPP and/or TSC (Kapoor & Metzger, 2008;  
326 Molins, 1991). The effect of the specific ratio of DSP and TSPP 1:1 was decreasing when the  
327 relative concentration of TSC raised ( $P < 0.05$ ). The latter mentioned trend was expected  
328 because the relative amount of the effectively and synergistically acting mixture (DSP and  
329 TSPP, 1:1) was decreasing. In most of the samples, where only TSPP and TSC were used in a  
330 ratio of 1:1, the increase of hardness and gel strength values of PC were observed ( $P < 0.05$ ;  
331 compared with samples that also contained DSP, but excluding samples where DSP and TSPP  
332 were in ratio of approximately 1:1). The same situation was in the ternary mixture of TSC,  
333 TSPP and P20 (Figs. 5, 6D and 7D). The synergistic effect of the mixture of 50% TSPP and  
334 50% TSC can also be expected; however, clear explanation was not found in the literature.  
335 The same phenomenon were also observed in the studies of Salek et al. (2015, 2016, 2017)  
336 where Dutch-type, Swiss-type and mozzarella type cheeses as the main raw material and the  
337 same ternary mixtures of ES were used.

338 Besides the effect of the specific ratios of DSP:TSPP and TSC:TSPP (described in  
339 detail above), the influence of the composition of the other ternary mixtures (DSP:TSC:P20  
340 and TSC:TSPP:P20) on hardness (Figs. 4 and 5) and gel strength (Figs. 6C,D and 7C,D) of  
341 the samples was regulated by the ability of individual ES to ion exchange and therefore casein  
342 dispersion. The ion exchange capability of used ES was increasing in the following order:  
343 DSP > TSPP  $\approx$  TSC > P20. The latter relationships between selected ES have been published  
344 in several articles, e.g., Dimitreli and Thomareis (2009), El-Bakry et al. (2011), Nagyová et  
345 al. (2014), Shirashoji et al. (2006), and are also in accordance with our results (Figs. 2–7).

346 When the relative amount of ES with better ability to exchange ions was higher, the values of  
347 hardness and gel strength increased and vice versa ( $P < 0.05$ ).

348 In all four times of storage (2, 9, 30 and 60 days) and in all four types of ternary  
349 mixtures, hardness and gel strength of PC (Figs. 2–7) decreased with the rising ripening  
350 period of the CDC and also the WBC ( $P < 0.05$ ; samples with the same time of storage and  
351 the same composition of ES were compared). The explanation of these phenomena lies in  
352 proteolysis and “shortening” of caseins in cheeses during ripening. The results of FAA  
353 analysis unambiguously showed that in CDC, and also in WBC, intensive proteolytic  
354 reactions took place (see section 3.1). When the mean length of proteins in cheese decreases,  
355 hardness and gel strength of PC is also lower and vice versa (Brickley et al., 2007; Buňka et  
356 al., 2013; Salek et al., 2015, 2016, 2017). Based on our results, we can assume that ripening  
357 period influenced the absolute values of hardness of the samples but the relations between  
358 four ES in the ternary mixtures and especially its effect on product consistency remained  
359 unchanged.

360 The hardness of all PC produced increased during the whole 60-day storage ( $P < 0.05$ )  
361 regardless of (i) the type and composition of ternary mixture; (ii) the ripening period of CDC  
362 and WBC; or (iii) the type of cheese – CDC or WBC. The absolute values of PC samples  
363 hardness (Figs. 2 and 3) increased, whereas the effect of ES was practically the same.  
364 Hardness of PC could increase, e.g., due to hydrolysis of phosphates, possible changes in the  
365 forms of binding of the salts present and thus a change in their dissociative characteristics and  
366 also due to possible changes in the crystalline modifications of milk fat (Awad & Sato, 2002;  
367 Kapoor & Metzger, 2008; Molins, 1991).

368 In all four types of ternary mixtures and in all days when analyses were performed,  
369 hardness and gel strength (Fig. 7) of CDC were higher ( $P < 0.05$ ) in comparison with WBC  
370 (Figs. 2–5). This difference in the hardness and gel strength values development could be due



371 to different chemical composition (including pH, calcium content, NaCl content, residual  
372 lactose content, etc.) and cheesemaking process of CDC and WBC (Piska & Štětina, 2004;  
373 Purna, Pollard, & Metzger, 2006). The intensity of proteolysis played also important role.

374 At the end of our work, correlation analysis between the values of hardness and the  
375 values of gel strength were done. The Spearman correlation coefficients for the tested ternary  
376 mixtures (calculated individually for each ternary mixture type and CDC and WBC) ranged in  
377 the interval of 0.794 to 0.922 ( $P < 0.05$ ). This finding confirmed again that the results  
378 obtained by the equipment using large uniaxial deformation and by the equipment using small  
379 shear deformation of material were in good accordance with each other.

380 With respect to our previous studies (Salek et al., 2015; 2016; 2017) and our recent  
381 work, we are able to infer that the general trend of the effect of the ternary mixtures  
382 composition is the same when different natural cheeses are used as raw material. Five types of  
383 natural cheese (Dutch-type, Swiss-type, mozzarella-type, Cheddar-type and white brined-  
384 type), which are used through the whole world, were tested. In all five cheese varieties the  
385 mechanisms of function of the above mentioned ternary mixtures were practically very  
386 similar regardless of the ripening period of the cheese utilised.

387

#### 388 **4. Conclusions**

389

390 The impact of the CDC and WBC maturity and different compositions of ternary  
391 mixtures of ES on the hardness and gel strength of PC during 60-days of storage was  
392 investigated. With raising storage period of the PC samples, an increase in hardness was  
393 observed. On the other hand, the hardness and gel strength of the samples decreased with  
394 prolonging of cheese ripening period for both cheeses (CDC and WBC) used as the main raw  
395 material. The hardest samples were those composed of DSP:TSPP (1:1). However, when the

396 relative amount of DSP and TSPP (in the ratio of 1:1) were replaced by TSC or P20, the  
397 influence of the latter mentioned ratio diminished. Furthermore, higher values of hardness and  
398 gel strength were reported for the PC samples produced with CDC in comparison with those  
399 made from WBC.

400

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402

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407

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## Figure legends

**Fig. 1.** Scheme of the experimental design with model processed cheeses manufactured using white-brined-type and Cheddar-type cheese in various time of storage and the different percentage ratios of the four types of ternary mixtures comprising DSP:TSPP:P20, DSP:TSPP:TSC, DSP:TSC:P20 and TSC:TSPP:P20 (abbreviations: DSP,  $\text{Na}_2\text{HPO}_4$ ; TSPP,  $\text{Na}_4\text{P}_2\text{O}_7$ ; P20, sodium salt of polyphosphate with mean length  $n \approx 20$ ; TSC, trisodium citrate). The model samples were tested after 2, 9, 30 and 60 days of storage.

**Fig. 2.** The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, tetrasodium diphosphate and sodium salt of polyphosphate) during 60-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ ); processed cheese were sampled after 2 (■), 9 (■), 30 (□) and 60 (■) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

**Fig. 3.** The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, tetrasodium diphosphate and trisodium citrate) during 60-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ ); processed cheese were sampled after 2 (■), 9 (■), 30 (□) and 60 (■) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different



time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

**Fig. 4.** The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (disodium phosphate, trisodium citrate and sodium salt of polyphosphate) during 60-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ ); processed cheese were sampled after 2 (■), 9 (■), 30 (□) and 60 (■) days of storage]. Processed cheeses were made from white-brined-type (WBC) cheese after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

**Fig. 5.** The dependence of processed cheese hardness (N) on the relative amount (%) of three emulsifying salts (trisodium citrate, tetrasodium diphosphate and sodium salt of polyphosphate) during 60-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ ); processed cheese were sampled after 2 (■), 9 (■), 30 (□) and 60 (■) days of storage]. Processed cheeses were made from white-brined-type cheese (WBC) after different time of storage (A, 2 weeks; B, 4 weeks; C, 8 weeks; D, 16 weeks; E, 24 weeks) and Cheddar-type cheese (CDC) after different time of storage (F, 4 weeks; G, 8 weeks; H, 12 weeks; I, 16 weeks). Please note the differences in y-axis values and hence the placement of panel I.

**Fig. 6.** The dependence of gel strength ( $\text{Pa s}^{1/2}$ ) of processed cheese on the relative amount (%) of three emulsifying salts (A, DSP:TSPP:P20; B, DSP:TSPP:TSC; C, DSP:TSC:P20; D, TSC:TSPP:P20; where DSP, disodium phosphate; TSPP, tetrasodium diphosphate; P20, sodium salt of polyphosphate; TSC, trisodium citrate) after 30-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ )]. Processed cheeses were made from white-brined-type (WBC) cheese after different times of storage (■, 2 weeks; ■, 4 weeks; □, 8 weeks; ▨, 16 weeks; ■, 24 weeks).

**Fig. 7.** The dependence of gel strength ( $\text{Pa s}^{1/2}$ ) of processed cheese on the relative amount (%) of three emulsifying salts (A, DSP:TSPP:P20; B, DSP:TSPP:TSC; C, DSP:TSC:P20; D, TSC:TSPP:P20; where DSP, disodium phosphate; TSPP, tetrasodium diphosphate; P20, sodium salt of polyphosphate; TSC, trisodium citrate) after 30-day storage at  $6 \pm 2$  °C [results expressed as means ( $n = 6$ )]. Processed cheeses were made from Cheddar-type cheese (CDC) after different time of storage (■, 4 weeks; ■, 8 weeks; □, 12 weeks; ■, 16 weeks).

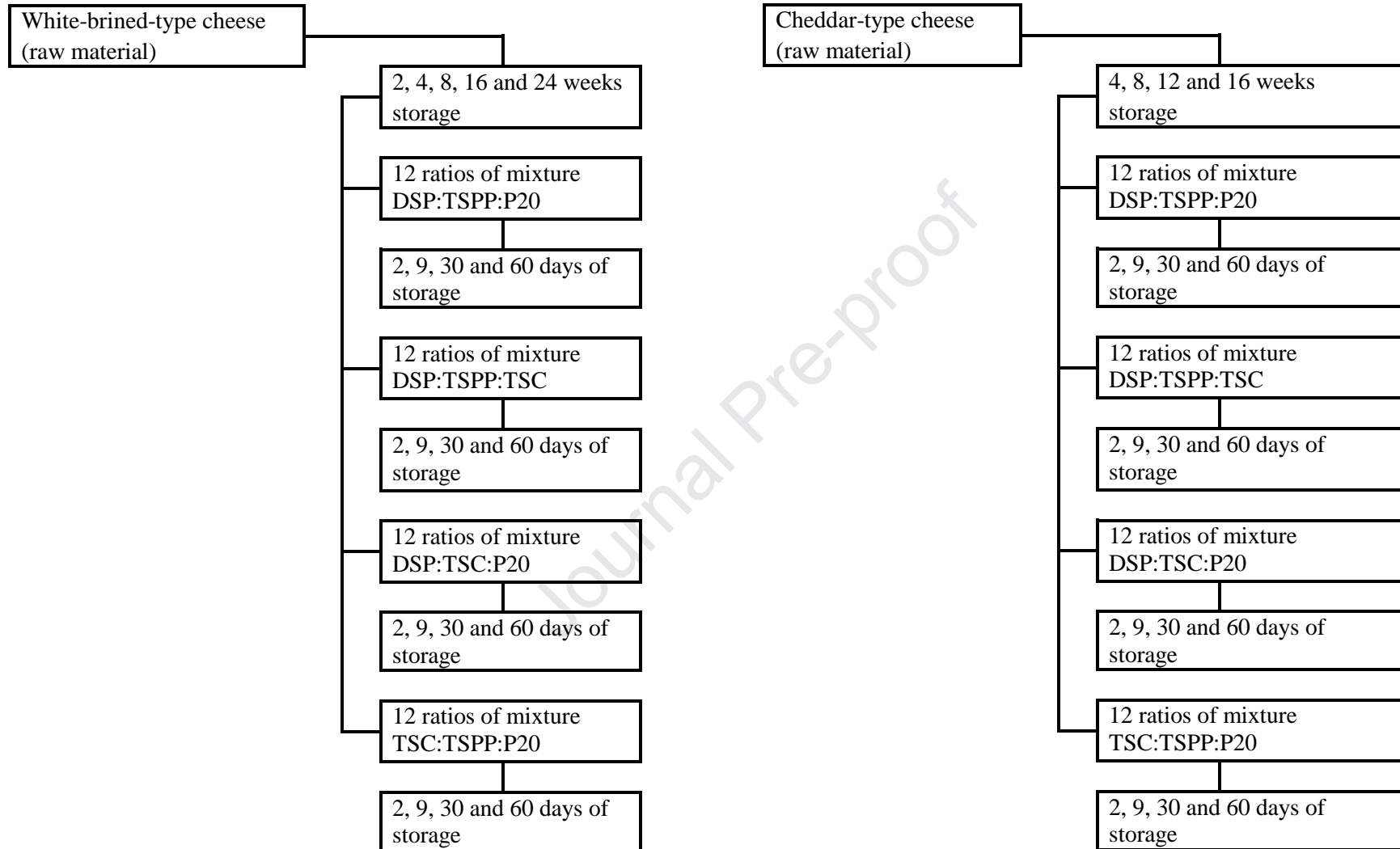


Figure 1

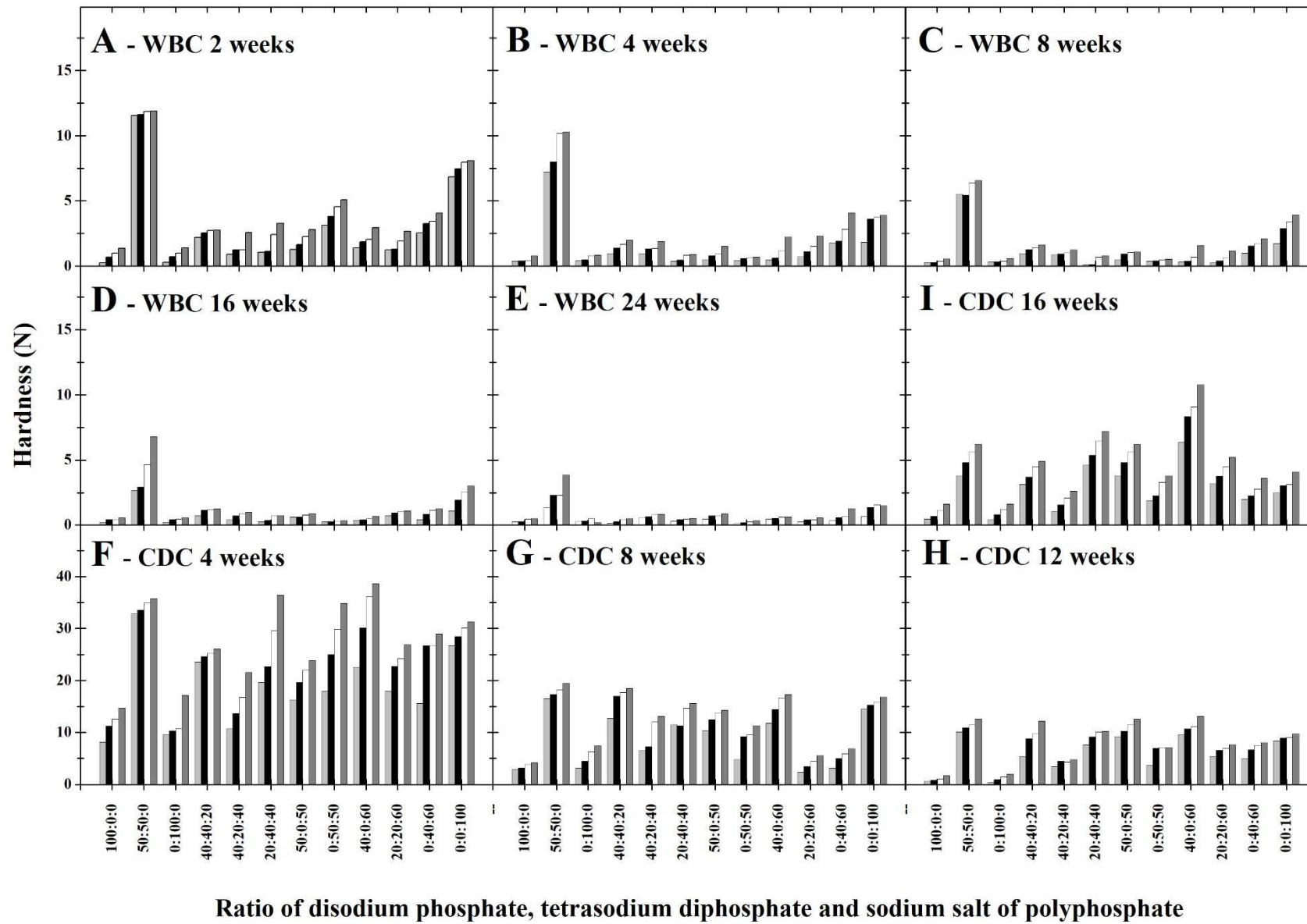


Figure 2

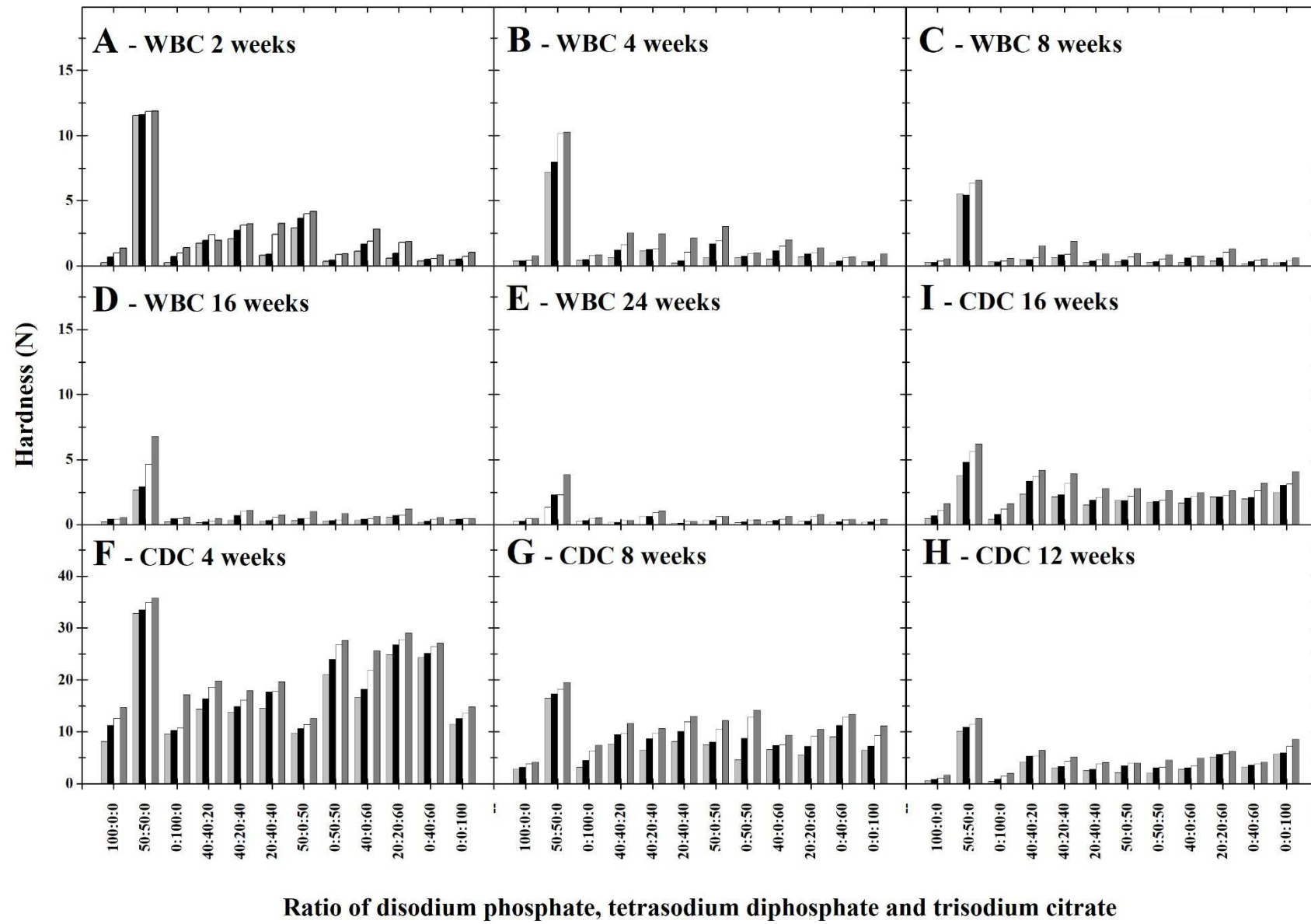


Figure 3

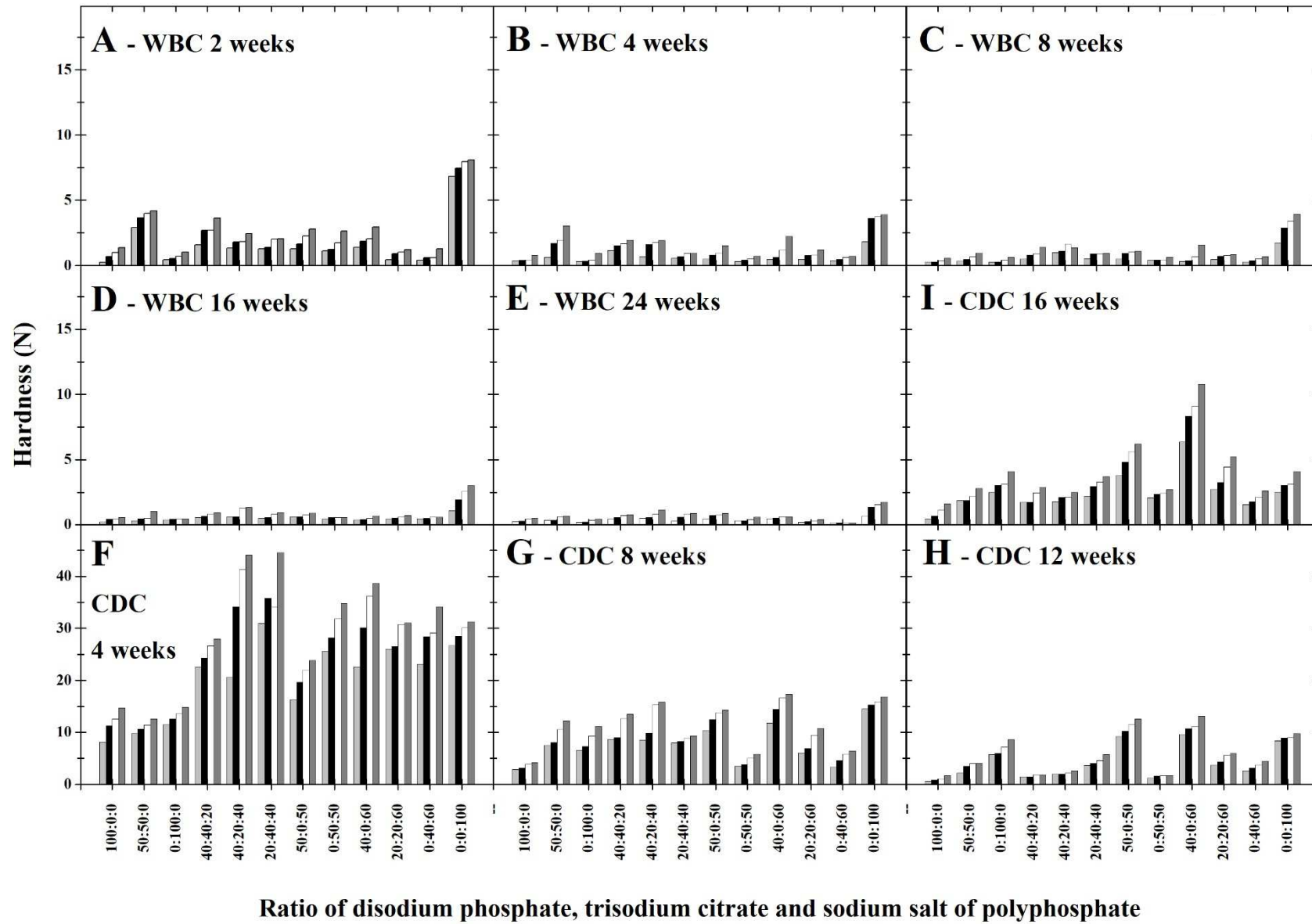


Figure 4

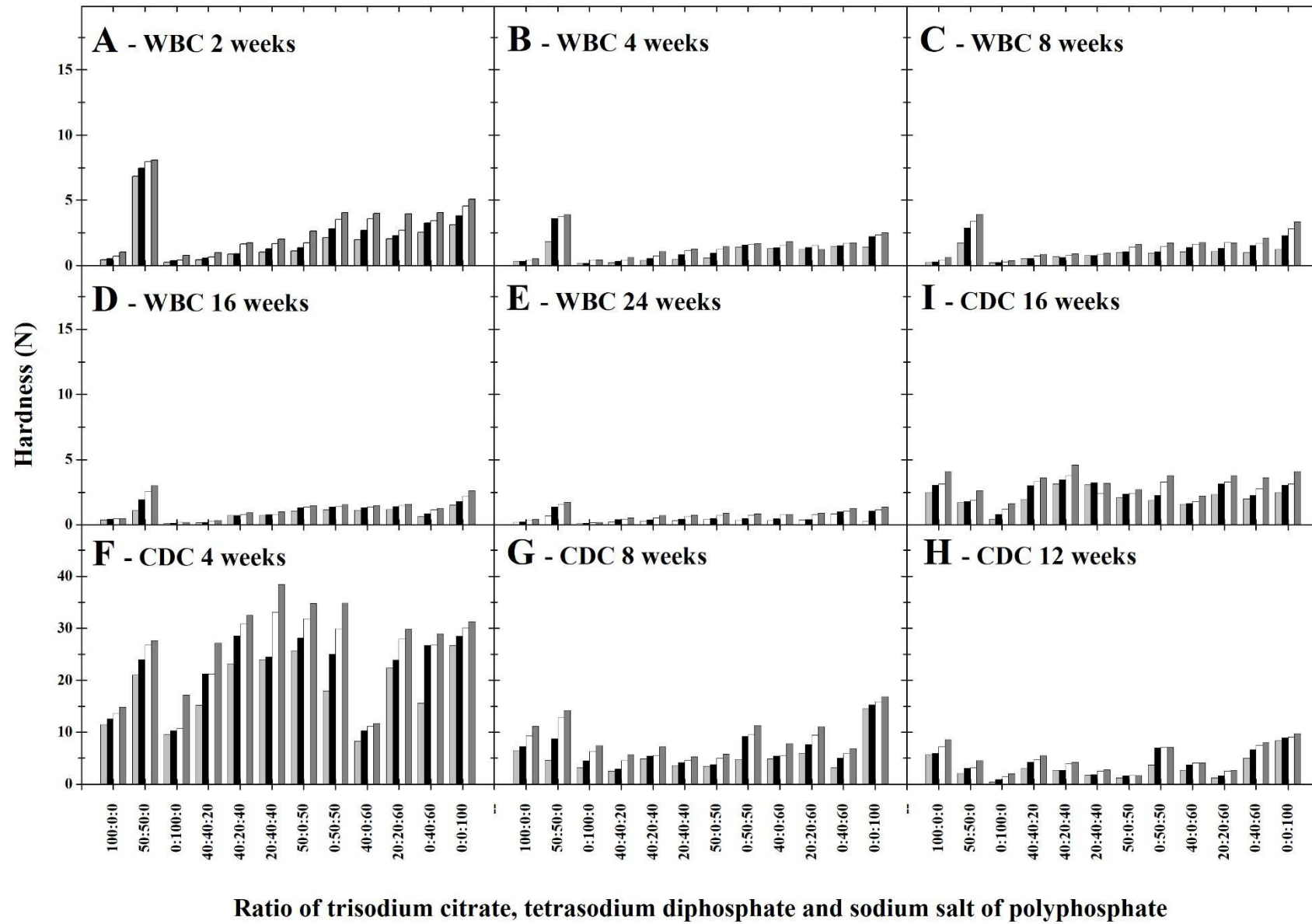


Figure 5

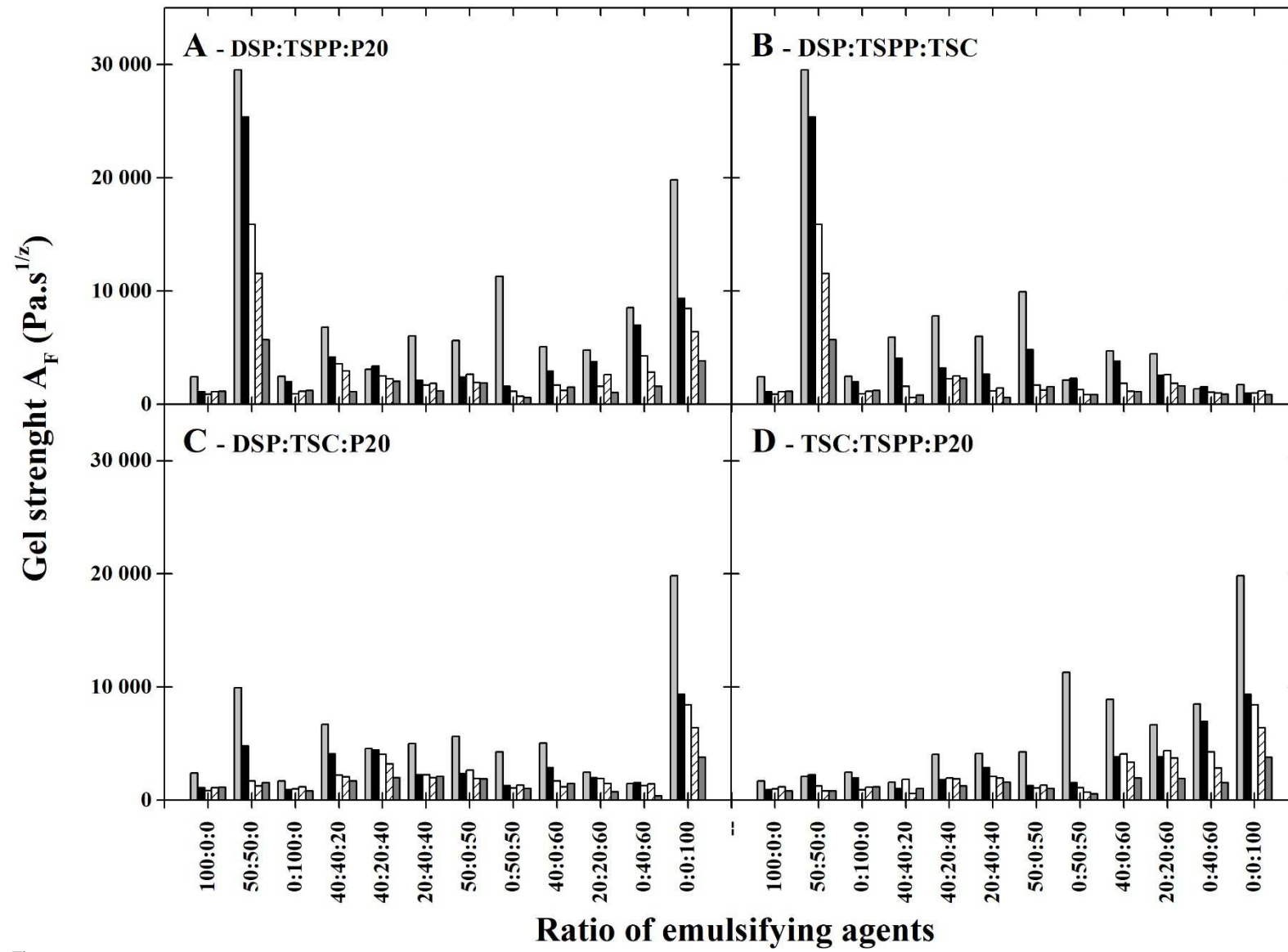


Figure 6



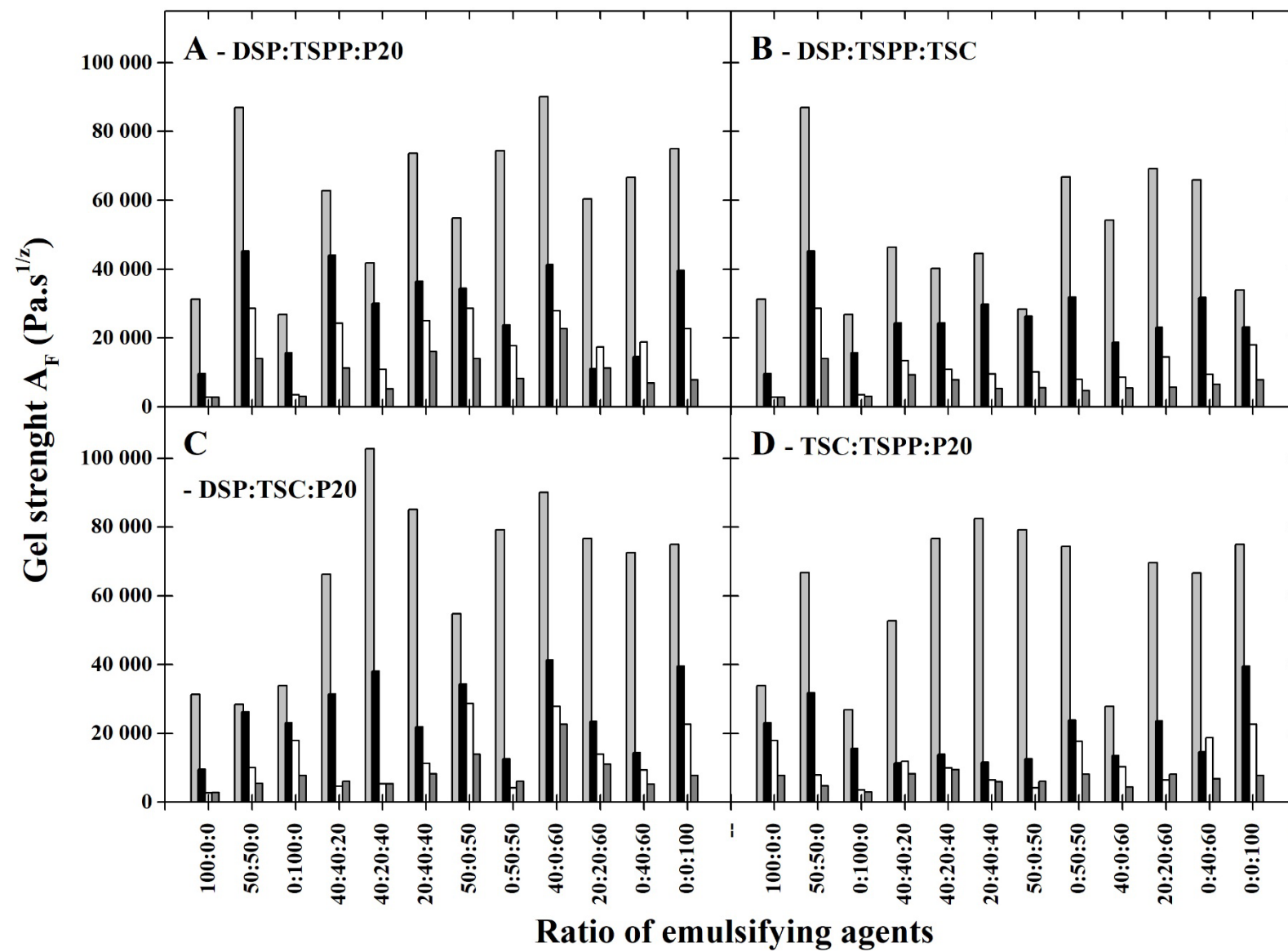


Figure 7

**Credit Author Statement**

Richardos Nikolaos Salek: Methodology; Investigation; Writing - Review & Editing; Visualization; Investigation; Writing - Original Draft; Revisions of the manuscript

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František Buňka: Conceptualization; Supervision; Writing - Review & Editing; Project administration; Visualization; Revisions of the manuscript