

1 **Rheological surface properties of commercial citrus**
2 **pectins at different pH and concentration**

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27 **Abstract**

28 *The interfacial activity of commercial low-methoxy (LM) and high-methoxy (HM)*
29 *pectins from citrus peel was investigated at air-water interfaces by focusing on the role*
30 *of their molecular weight (MW) and degree of methoxylation (DM). A pendant drop*
31 *tensiometer was used to carry out transient interfacial tension measurements and small*
32 *amplitude oscillations. Different pectin concentrations (ranging between 0.00001*
33 *g/100g and 5 g/100g) and pH conditions (4 and 6) were used during the tests. It was*
34 *observed that citrus pectins are characterised by interesting surface properties that*
35 *could allow potential practical uses. Experimental results evidenced that MW affects*
36 *the diffusion of molecules towards the interface, whereas other investigated parameters*
37 *(i.e. surface tension, adsorption rate, dynamic moduli) seem more dependent on DM*
38 *and a clear dependence on MW was not observed. pH conditions modify intermolecular*
39 *interactions in the bulk and surface layer, even if their effects are related to the fraction*
40 *and distribution of carboxylic groups along the chain. As a consequence, a complex*
41 *dependence on investigated parameters was observed and no clear relationship was*
42 *obtained. Nevertheless, among tested commercial samples LM pectins exhibited the*
43 *most interesting properties and this behaviour seems related to the intermolecular*
44 *interactions occurring among them.*

45

46 **Keywords:** *interfacial properties; dilatational rheology; pectin; molecular weight;*
47 *methoxylation degree*

48

49

50 1. INTRODUCTION

51 Pectin is a complex mixture of polysaccharides that consist mainly of galacturonic acid
52 (GalA) (at least 65% according to the definition of "pectin" given by the Joint
53 FAO/WHO Expert Committee on Food Additives (JECFA, 2009)) even if the fine
54 structure of the single components can be significantly different (Willats et al., 2006).
55 Three main pectic polysaccharide domains are identified (Willats et al., 2006):
56 Homogalacturonan (HG), where GalA units are covalently α (1 \rightarrow 4) linked to form a
57 linear backbone; Rhamnogalacturonan I (RGI) consisting of the repeating disaccharide
58 [\rightarrow 4)- α -D-GalA-(1 \rightarrow 2)- α -L-Rha-(1 \rightarrow)] to which glycan units are connected to
59 rhamnose component; Rhamnogalacturonan II (RGII) having a HG backbone where
60 complex side chains are connected to GalA units.

61 The GalA units of the pectin chain are variously methylesterified and this significantly
62 affects the structural properties of this biopolymer. As a consequence, the degree of
63 methoxylation (or methylesterification, DM) is used to classify pectin into high-
64 methoxyl (HM) pectins, containing more than 50% esterified carboxyl groups
65 (DM>50), and low-methoxyl (LM) pectins, with <50% esterified carboxyl groups
66 (DM<50) (May, 1990).

67 Pectin is produced, at industrial level, by extraction from apple pomace and citrus peels,
68 even though other sources are available such as sugar beet and sunflowers head
69 residues, cocoa husks, potato pulp, soy hull, etc. (Thakur et al., 1997; Yancheva et al.,
70 2016).

71 It is mainly used for its gelling ability in the food industry in jams, fruit preparations,
72 desserts and confectionery, and as a viscosity enhancer and stabilizer in beverages and,
73 moreover, as a stabilizer of acid milk drinks and in dairy products (Dickinson et al.,
74 1998; May, 1990; Schmidt et al., 2017; Yancheva et al., 2016).

75 In fact, HM pectin can form a three-dimensional network through hydrogen bonds and
76 hydrophobic interactions between the methyl-ester groups whereas LM pectin gelation
77 occurs mainly thanks to ionic linkages with divalent ions (typically calcium ions) (de
78 Cindio et al, 2016).

79 It is considered as a "safe" food ingredient, therefore there is no maximum acceptable
80 daily intake and it is only recommended to use it at a level not higher than the necessary
81 amount, according to good manufacturing practice (de Cindio et al., 2016). Moreover,

82 pectin is a source of dietary fibre and therefore can be considered a healthy ingredient
83 due to the ability to lower cholesterol as well as having several beneficial effects on the
84 colon (Willats et al, 2006).

85 Even if the food use is probably the most important, it is worth noticing the growing use
86 of this biopolymer also in different areas, such as pharmaceutical, cosmetic or
87 biomedical products (de Cindio et al., 2016). Pectin was used as structuring agent of
88 aqueous phase in emulgels and bigels for cosmetic use (Lupi et al., 2015; Lupi et al.,
89 2016); in nasal drug carriers as mucoadhesive polymer owing to hydrogen bonding with
90 mucin; in oral drug delivery owing to its ability to be resistant to enzymes in the
91 gastrointestinal tract and to be degraded by the intestinal microflora; in gels to be used
92 as wound healing patches, etc. (for further detail see Munarin et al. (2012)).

93 In addition to the "bulk" properties, recently, pectin interfacial properties has been
94 studied with the aim of investigating their potential ability to modify the structure of
95 interfacial layers (air/water or oil/water) and to be used as natural emulsifier (Alba &
96 Kontogiorgos, 2017). It was observed that pectin displays emulsifying properties
97 (Leroux et al, 2003; Ngouémazong et al., 2015; Schmidt et al., 2015) in addition to the
98 well-known thickening effect of the dispersing water phase (which improves emulsion
99 stability). Literature results evidenced interesting interfacial properties in sugar beet
100 pectin (Leroux et al., 2003; Yapo et al., 2007) and, more recently, in citrus pectin
101 (Schmidt et al., 2015; Schmidt et al., 2017).

102 The origin of these properties is not very clear and it was attributed to different
103 structural factors such as the acetyl content and protein fraction (particularly high in
104 sugar beet pectin) (Leroux et al., 2003), low molecular weight (Akhtar et al, 2002; Yapo
105 et al, 2007), degree of esterification (Akhtar et al., 2002), internal charge distribution
106 (Lutz et al, 2009), ferulic acid content (Alba & Kontogiorgos, 2017) etc.

107 It is worth noticing that also contradictory results have been obtained in a recent study
108 by Schmidt et al. (2015). In fact, they did not observe a direct relationship between low
109 molecular weight and emulsifying activities whereas they found a relevant influence of
110 DM contradicting the findings of Akhtar et al. (2002) who observed only minor effects
111 of this parameter.

112 Although further studies seem still necessary to better understand these effects, the
113 oil/water interfacial effects of pectin have been widely investigated in recent years; on

114 the contrary, few studies are available on the foaming ability and air/water interfacial
115 properties of these polysaccharides.

116 Foam is a dispersion of a gaseous phase in a solid or liquid dispersing medium
117 (Sadahira et al., 2014). In the food industry, owing to the peculiar characteristics
118 (mainly related to texture) given by aeration, foams are widely used; beer, bread,
119 whipped cream, confectionery products, sorbets, are just a few examples of aerated
120 foods from different areas (Campbell & Mougeot, 1999). Surface-active agents are
121 necessary to make easier the aeration step and to improve foam stability (Rodríguez
122 Patino et al., 2008). Among them, a mixture of proteins and polysaccharides are
123 frequently adopted, although only few polysaccharides have proven to have a real (even
124 if often limited) surface activity whereas often they only modify the protein surface
125 layer properties (Baeza et al., 2006; Rodríguez Patino et al., 2008).

126 According to recent studies (Gromer et al., 2009; Liu et al., 2016; Perez et al., 2011;
127 Yancheva et al., 2016), it seems that pectin from different sources (sugar beet, soya hull,
128 waste rose petals, citrus peels and apple pomace) exhibit interesting surface properties.
129 Nevertheless, few data describing surface rheological properties are available, and less
130 information is available when commercial products (i.e. not specifically modified) are
131 considered (see for instance Perez et al. (2011)).

132 Although a quantitative correlation between interfacial properties and foam
133 macroscopic characteristics is still not clear, it is known, at least from a qualitative point
134 of view, that layer microstructure affects foam production and stability (Maldonado-
135 Valderrama & Patino, 2010; Martin et al., 2002). Starting from these considerations, in
136 the present work, four commercial citrus pectins, with different DM and molecular
137 weight, were investigated at air/water interface using different pH and concentration
138 with the aim of exploring their potential ability to build a viscoelastic layer.

139

140 **2. MATERIALS AND METHODS**

141 *2.1 Materials*

142 Four commercial citrus pectins (kindly supplied by Silvateam Food Ingredients srl,
143 Italy) at high and low methylation degree are used in this work (Table 1).

144 Pectins were characterised through their degree of methoxylation and molecular weight.

145 DM was determined by the pectin producer (i.e. Silvateam Food Ingredients srl)
146 according to the titration procedure recommended by JECFA (2009) and described in
147 detail in Migliori et al. (2011). The average viscometric molecular weight (MW) was
148 determined using a viscometric method based on the relationship between MW and the
149 intrinsic viscosity that, in turn, was estimated by measuring the viscosity of pectin
150 solutions at different dilution rates; the procedure is described in detail by Lupi et al.
151 (2015).

152 With the aim of investigating the potential effects of degree of methylesterification and
153 molecular weight on interfacial behavior, two HM samples (HM 145 and HM 104) with
154 similar DM (65.1 and 65.3, respectively) and different average molecular weights were
155 chosen. In the same way, two LM samples (LM 123 and LM96) with similar DM (42.9
156 and 41, respectively) and molecular weights close to those of HM samples were used. It
157 is worth noticing that commercial products were used in this work, therefore it was not
158 possible to have an accurate control on molecular weight and only samples with
159 "similar" values were individuated.

160 Pectin solutions were prepared by dissolving the proper amount of polysaccharide in a
161 citrate buffer at the investigated pH (4 and 6), keeping constant the ionic strength (I) at
162 100 mM. These pH values were chosen because they are within the typical range of a
163 number of food foams (e.g. mousses, ice creams, whipped creams, meringues, different
164 types of dairy-based systems, etc.) where pectin could be used, alone or in combination
165 with other agents such as protein.

166 The buffer was prepared with a twice-distilled water obtained from a Milli-Q
167 purification system (Millipore, USA), and it was checked for contaminants before each
168 experiment, measuring the surface tension of the buffer solution at the air/water
169 interface at room temperature. All the reagents used were purchased from Sigma
170 Aldrich.

171 Pectins were dispersed in the buffer solution at room temperature ($22\pm 1^\circ\text{C}$) stirring for
172 12 hours by using a heating magnetic stirrer (AREX, Velp scientific, Italy), to promote
173 the complete solubilisation of the polysaccharide in buffer. Afterwards the interfacial
174 measurements were performed.

175

176 *2.2 Total protein content in pectin*

177 In order to assess the presence of protein residues, resulting from the extraction step of
178 the pectin from the raw materials, a Bradford protein assay kit was used. The
179 absorbance was read at 595 nm with a spectrophotometer S-3100 (SCINKO, Korea) and
180 bovine serum albumin was used as standard substance (Funami et al., 2007).

181 Data were reported in Table 1 as the mean and standard deviation of the triplicate.
182 Protein content determined with Bradford test was quite low in all samples, as expected
183 for citrus pectin, and in agreement with the literature data for other commercial products
184 (e.g. Schmidt et al. (2017)). The values were very close each to other and only sample
185 HM145 evidenced a value slightly lower than the others.

186

187 *2.3 Surface pressure isotherms and adsorption kinetics*

188 The equilibrium surface tension (γ) of the investigated solutions at the air –water
189 interface was evaluated by using an automated pendant drop tensiometer (FTA200, First
190 Ten Angstroms, USA) equipped with the *fta32 v2.0* software (Biresaw et al., 2008).
191 According to previous works (Seta et al., 2012; Seta et al., 2013; 2014), the drop profile
192 was monitored up to a maximum time of 180 min. at room temperature ($22\pm 1^\circ\text{C}$) and a
193 quasi-equilibrium value was assumed when the interfacial tension did not change by
194 more than 0.5 mN/m in 30 min (Seta et al., 2012). The surface pressure value, π , over
195 time, was computed as the difference between the surface tension of the buffer, γ_0
196 (equal to 70.5 ± 0.5 mN/m and 71.0 ± 0.7 for pH 4 and pH 6, respectively), and the
197 value measured for the tested solutions, γ .

198 The main phenomena, potentially involved in the adsorption process of proteins and
199 polysaccharides are: macromolecule diffusion from the bulk towards the interface,
200 adsorption/penetration at the interfaces and unfolding and molecular rearrangement
201 (Camino et al., 2009; Seta et al., 2012). The analysis of surface pressure as a function of
202 time can give information on the role of these different steps during the adsorption.

203 According to the literature, during the first step, at low surface pressure ($\pi < 10$ mN/m)
204 when diffusion is rate limiting, the modified form of the Ward-Torday equation can be
205 used to evaluate the diffusion rate (Camino et al., 2009):

$$206 \quad \pi = C_0KT \left(\frac{D_{dif} \cdot t}{\Pi} \right)^{\frac{1}{2}} \quad (1)$$

207 where π is the interfacial pressure at any time t , C_0 is the bulk concentration in the
 208 aqueous phase, K is the Boltzmann constant, T is the absolute temperature, D_{dif} is the
 209 diffusion coefficient and Π the Pi Greco value. When plotting the interfacial pressure as
 210 a function of $t^{1/2}$ a diffusion rate (K_{dif}) can be obtained (Seta et al., 2012):

$$211 \quad K_{dif} = C_0 K T \left(\frac{D_{dif}}{\Pi} \right)^{\frac{1}{2}} \quad (2)$$

212 After the first adsorption step, the (Graham & Phillips, 1979) approach was used to
 213 evaluate the potential unfolding/rearrangement phenomena of adsorbed pectins and a
 214 semi-empirical first-order equation was used, determining the rate constant, k_i (Seta et
 215 al., 2012):

$$216 \quad \ln \frac{\pi_f - \pi_t}{\pi_f - \pi_0} = k_i t \quad (3)$$

217 Where π_f , π_0 and π_t are the surface pressure at the end of the adsorption process, at $t = 0$,
 218 and at any time t , respectively. The application of Eq. (3) can evidence, according to the
 219 specific system, the potential presence of two linear regions with different slopes: the
 220 first one is related to the molecular adsorption, whereas the second one is related to the
 221 rearrangement of adsorbed molecules (Camino et al., 2012; Camino et al., 2009; Perez
 222 et al., 2011; Seta et al., 2012). Therefore, two different constants (k_{ads} and k_r) can be
 223 estimated to describe adsorption and rearrangement phenomena. This analysis was
 224 carried out, for all samples, at saturation concentration.

225

226 *2.4 Surface dilatational rheological properties*

227 The rheological properties of pectin film at the air/water interface were evaluated
 228 carrying out small amplitude oscillations in dilatational regime, using the previously
 229 described pendant drop tensiometer. A pectin solution drop was formed in air and the
 230 interfacial area, A , was subjected to infinitesimal compression and expansion of the
 231 drop in a sinusoidal way in order to obtain the surface dilatational moduli, E' (storage
 232 modulus) and E'' (loss modulus). Dynamic data can be reported, also, in terms of
 233 complex modulus, E^* , and phase angle, δ :

$$234 \quad E^* = \sqrt{(E')^2 + (E'')^2} \quad \delta = \arctan\left(\frac{E''}{E'}\right) \quad (4)$$

235 Details on interfacial dynamic tests can be found in (Ravera et al., 2010; Seta et al.,
236 2012).

237 Time sweep tests were carried out in the linear viscoelastic region by applying
238 deformation amplitudes of 6-10% at different frequencies ranging between 0.005 Hz
239 and 0.1 Hz until a steady value was obtained (maximum variation of 3% in 600 s was
240 accepted). Preliminary amplitude sweep tests (data not shown) were performed at the
241 lowest and largest tested frequency to estimate amplitude values suitable to guarantee
242 linear conditions. Steady values of complex modulus (E^*) and phase angle (δ) were
243 finally reported as a frequency function for samples at saturation concentration.

244

245 *2.5 Statistical data treatment*

246 Obtained parameters and experimental data were compared with a statistical analysis
247 (ANOVA, MS Excel 2016, Microsoft, USA) and differences among compared samples
248 were considered significant at p-value < 0.05 (interval of confidence of 95%).

249

250

251 **3. RESULTS AND DISCUSSION**

252 *3.1 Surface pressure isotherms*

253 Surface pressure isotherms are shown in Fig. 1a and 1b for samples investigated at pH 4
254 and pH 6, respectively. A sigmoidal trend, typical of active biopolymers and surfactants
255 (Seta et al., 2012) and similar to that observed for other commercial pectins (Perez et
256 al., 2011), is obtained for all samples at both pH values. A plateau region,
257 corresponding to the saturation level, is observed, in both cases, at a concentration of 1
258 g/100g for all samples except for LM96 that reaches a saturation condition
259 approximately at 2 g/100g, at both pH values.

260 The π_{eq} saturation value of pectins at pH 6 is significantly higher than that observed at
261 pH 4 (Table 1) even if the magnitude of difference is important mainly for sample
262 HM145. The effect of pH is related to the protonation of carboxylic groups which
263 increases with decreasing pH: Lutz et al. (2009) observed that the increase in
264 protonation yields less negative values of ζ -potential improving the intermolecular
265 interactions. This phenomenon depends on DM, which is a measure of esterified and de-
266 esterified groups, but also on charge distribution within the pectin that is related to the

267 distribution of carboxylic groups: lower DM and block-wise distribution seem to yield
268 lower ζ -potential values (Lutz et al., 2009). The same authors investigated surface
269 properties of the same samples and they observed a lower surface tension (i.e. a higher
270 surface pressure) for pectins with lower ζ -potential. This result was attributed to the
271 differences in the internal arrangement of carboxylic groups which, affecting
272 intermolecular interactions, seem to modify surface adsorption: lower chain-chain
273 interactions seem to improve adsorption at the interface probably because in these
274 conditions molecules can move more freely.

275 It is worth noticing that pectins investigated in the present work are commercial
276 products where a random (and not controlled) distribution of carboxylic groups is
277 expected, even if there can be differences among them and this could explain some
278 experimental results.

279 In the present work, higher π_{eq} values, increasing with pH, are observed for LM samples
280 (see Table 1), this seems in agreement with the literature results evidencing a decrease
281 in ζ -potential with decreasing DM (and suggesting, consequently, improved surface
282 adsorption). On the other hand, even if HM pectins are characterised by lower pressures,
283 sample HM145 is affected in an important way by pH modifications, suggesting a
284 change in surface charge; this could be explained speculating that a less random
285 distribution of carboxylic groups is present in HM145 yielding as a result a more
286 marked dependence on pH. This is in agreement with data from Lutz et al. (2009) who
287 did not observe any change in ζ -potential for an HM commercial pectin (with random
288 distribution) in the pH range from 4 to 7.

289

290 *3.2 Adsorption kinetics of biopolymers at saturation*

291 The evolution of surface pressure at bulk concentration able to saturate the air-water
292 interface was studied for all the pectins and data, arranged according to eq. 1, are shown
293 in Fig 2. It can be seen that the surface pressure increases with time, according to a
294 gradual increment of biopolymer adsorbed at the air-water interface (Perez et al., 2011).

295 It is worth noticing that samples with higher molecular weight (i.e. LM123 and HM
296 145) are characterised by an initial period where no change in π is observed (called
297 *induction* or *lag time*). This result is in agreement with the literature data for pectin
298 (Perez et al., 2011) and protein (Miller et al., 2000) evidencing a potential delay in

299 obtaining a minimum surface coverage of macromolecules related to macromolecular
300 flexibility in the bulk and ability to undergo conformational changes during adsorption.
301 Following the data analysis already described, the diffusion rate (Table 2) was
302 computed from data at low surface pressure ($\pi < 10$), corresponding to the initial period
303 where the diffusion of the biopolymer is the main phenomenon.
304 Obtained values evidence that the rate increases with decreasing molecular weight and
305 this behaviour is coherent with the typical trend found for surface active molecules
306 (Perez et al., 2011). Moreover, for HM 145 and LM 123 the diffusion rate increases
307 with the pH value in agreement with the effects of pH on intermolecular interactions,
308 previously discussed, whereas for samples with lower MW no significant change is
309 observed.
310 After the initial diffusion of pectin at the interface, the adsorption (or penetration) and
311 the rearrangement (or aggregation) become the controlling steps of the process,
312 therefore, following the (Graham & Phillips, 1979) approach (Eq. 3), adsorption rate
313 and rearrangement rates were computed and are reported in Table 2.
314 When k_{ads} values at pH 4 are considered, no statistical difference can be observed
315 among all the samples except for HM145 that exhibits the lowest k_{ads} , even if the DM is
316 the same as HM104; this unexpected difference could be related to the very high
317 molecular weight that could hinder the penetration phenomena. When the pH is
318 increased up to 6, greater k_{ads} values are observed for all samples except for LM123
319 where no statistical change is found. This evidence could be related to the discussed
320 influence of pH on intermolecular interactions: weaker interactions in the bulk phase
321 could promote the macromolecule adsorption at the interface and this effect becomes
322 more evident for HM145 that is characterised by a marked dependence on pH, as
323 already discussed.
324 As far as the specific rearrangement rates (k_r) are concerned (see Table 2), it can be seen
325 that at pH=6 values are higher for LM pectins with respect to HM samples and they do
326 not exhibit a dependence on molecular weight. When pH is reduced to 4 they decrease
327 significantly for all samples; the lower change is observed for HM104 that exhibits a
328 behaviour less dependent on pH conditions in agreement with the data previously
329 discussed.

330 At lower pH, where intermolecular interactions are more probable, it seems that
331 molecular weight becomes a more important factor and the lowest k_r is shown by the
332 sample with highest MW (see table 2).

333 Kinetic data on adsorption phenomena can be useful in investigating foaming processes,
334 in fact, in previous studies on protein behaviour, they have been shown to be related to
335 the easiness of foam formation (Langevin, 2000; Martin et al., 2002; Murray, 2007).
336 Foaming involves, generally, prolonged and repeated shearing of bubbles, until desired
337 size is obtained, and is less rapid than, for instance, emulsification (Murray, 2007).
338 Therefore, diffusion and adsorption times become very important (probably more
339 important than in emulsification) because the bubble formation is determined by
340 monolayer building: the faster is the surface agent diffusion towards the interface, the
341 more probable is the bubble formation. According to these considerations, it seems that
342 sample LM96 could be the most effective agent during foaming because it exhibits the
343 highest diffusion rate, at both pH values, (approximately 110% greater than that shown
344 by HM104, that is, the sample with a similar MW, see Table 2) and adsorption rates
345 equal to (or greater than) the values of the other pectins (differences to HM104 are not
346 statistically significant). This is also in agreement with surface pressure data (Fig. 2)
347 that evidence, for LM96, an almost absent lag period: this is considered an important
348 condition to obtain optimal foaming capacity (Maldonado-Valderrama & Patino, 2010).
349 In addition, the highest pH value seems more convenient for the process, yielding
350 higher rates.

351

352 *3.3 Dilatational rheological properties*

353 Dynamic dilatational moduli were obtained for all pectins, at pH 4 (Fig. 3) and 6 (Fig.
354 4), at saturation concentration.

355 It is worth noticing that for all samples, at both pH values, the phase angle ranges
356 between 20° and 5° (Fig. 3B and 4B) suggesting a prevalent solid-like behaviour of the
357 interface; moreover, the complex modulus (Fig. 3A and 4A), in log-log scale is almost
358 linear with the frequency, suggesting that interfacial layers behave as a 2D critical gel
359 (Bouriat et al., 2004; Dicharry et al., 2006). According to this approach a power law
360 model can be used to fit E^* as function of frequency (Seta et al., 2012)

361

$$E^* = k\omega^n \quad (5)$$

362 Two parameters are obtained: n , considered an indirect measure of the structuring
363 degree, and k , value of E^* extrapolated at 1 Hz, which give a measure of the interfacial
364 strength (Seta et al., 2012). Obtained parameters are reported in Table 3. It can be seen
365 that a different pH dependence is observed for LM and HM samples. When HM pectins
366 are considered, an increase in both k and n is observed, even if the increase in k is
367 important (approximately 94%, see Table 3) only for sample HM145 that exhibited,
368 also in other tests, a marked dependence on pH (see data in Table 2). It can be
369 speculated that, at low pH, intermolecular interactions in the bulk hinder diffusion and
370 adsorption phenomena at the interface, as already discussed, yielding a weaker and less
371 structured interfacial layer. At higher pH, according also to diffusion and adsorption
372 results, interfacial coverage is probably larger and therefore, a stronger and more
373 structured layer is obtained.

374 Low methoxyl pectins, on the contrary, evidence a slight increase of k from pH 6 to pH
375 4 (Table 3), this behaviour is unexpected when compared to HM samples. Anyway, it
376 could be attributed to the combined effects of intermolecular interactions in the bulk and
377 at the interface. It could be speculated that even if at pH 4 intermolecular interactions in
378 the bulk hinder diffusion and adsorption phenomena, molecules at the interface can
379 strongly interact yielding a strong interfacial layer. On the other hand, increasing pH,
380 interactions among molecules decreases both in the bulk, promoting molecules
381 migration to the interface, and at the interface causing the formation of a weaker layer.
382 Differences among pH dependence of LM and HM samples could be attributed,
383 therefore, to differences in the amount and distribution of carboxylic groups.

384 Dynamic data, describing the film viscoelasticity, are important, mainly, for the
385 investigation of stability issues because destabilisation phenomena, such as Ostwald
386 ripening, coalescence, etc. seem to be hindered by an increase in surface viscoelasticity
387 (Martin et al., 2002; Murray, 2007).

388 Relevant differences among tested samples are not evident at pH 6, whereas they are
389 present at pH 4 where a more complex situation can be observed; LM pectins, with
390 respect to HM ones, are characterised by both higher k (i.e. a stronger layer) and n (i.e.
391 less structured layers) values. According to the literature (Martin et al., 2002; Murray,
392 2007) it seems that consistent and elastic layers should be more resistant to phenomena
393 such as coalescence and drainage and this would suggest that LM samples could yield

394 more stable foams. It is worth noticing that the effects of structuring degree of layers are
395 not known and they could affect stability under high shear where weaker layers could be
396 damaged by shear action.

397

398 **4. CONCLUSIONS**

399 Four pectins at two different DM and MW were investigated at the air/water interface
400 with transient and dynamic tests in dilatational kinematic conditions.

401 The transient interfacial tests have shown that the velocity of diffusion increases with
402 the pH value and decreases with MW (data shown in Table 2). The situation is different
403 for the adsorption velocity, which shows, generally, an increment with pH, but not a
404 clear trend with MW. Finally, the rearrangement values, obtained at long times, show
405 higher values at pH 6 and a trend that seems dependent on DM, whereas no apparent
406 dependence on MW is observed.

407 Additional information was obtained with dynamic tests in interfacial saturation
408 conditions: from the experimental results (Fig.4A) it is evident that the LM pectins
409 show the higher complex modulus at lower pH whereas at higher values differences are
410 smaller. Even in this case a clear effect of molecular weight was not observed.

411 Experimental results evidenced that, for HM samples, improved surface properties are
412 obtained at higher pH (i.e. pH=6), as suggested by the higher values of all determined
413 parameters (reported in Table 3); on the other hand, a more complex behaviour was
414 observed for LM samples: in this case it seems that the higher pH value promotes all
415 adsorption steps (diffusion, adsorption and rearrangement) but the obtained layer is
416 weaker than that observed at lower pH.

417 Among the tested samples, LM pectins seem particularly interesting because they yield
418 stronger interfacial layers that could improve foam stability; in addition, LM sample
419 with low MW exhibits fast diffusion phenomena that could improve foam production.

420 It was observed that commercial citrus pectins are characterised by an appreciable
421 interfacial activity even if measured values of interfacial pressure are lower than those
422 observed for common emulsifiers and are comparable to values measured for some
423 proteins at lower concentrations (see for instance data obtained by (Seta et al., 2012)).
424 Moreover, it seems that intermolecular interactions, affected by DM and carboxylic

425 group distribution, seem to affect observed properties significantly whereas no clear
426 dependence on molecular weight was found.
427

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- 546

547 **Table captions**

548 Table 1 Sample identification, degree of methylation (DM), molecular weight (MW),
549 protein content, equilibrium surface pressure (π_{eq}) at pH 4 and pH 6 of pectins used. The
550 same letters, for each parameter, identify the absence of statistically significant
551 differences

552 Table 2 Velocity of diffusion (k_{dif}), adsorption (k_{ads}) and rearrangement (k_r) for HM and
553 LM pectins at pH 4 and 6. The same letters, for each parameter, identify the absence of
554 statistically significant differences

555 Table 3 Rheological parameters of interfacial “gel model” (k and n) at the A/W
556 interface at pH 4 and 6. The same letters, for each parameter, identify the absence of
557 statistically significant differences

558

559

560 **Figure captions**

561 Figure 1 Surface pressure equilibrium data, π_{eq} , at pH 4 (A) and pH6 (B) for samples
562 HM 104 (blue diamond), HM145 (red square), LM96 (green triangle), LM123 (orange
563 circle). Concentration, c , is measured as g/100g. Standard error was computed over
564 three repetitions.

565 Figure 2 Temporal evolution of surface pressure, π , of biopolymer adsorbed films at the
566 air/water interface at saturation concentration, at pH 4 (A) and pH6 (B) for samples HM
567 104 (blue diamond), HM145 (red square), LM96 (green triangle), LM123 (orange
568 circle). Data are reported as a function of time, t , square root according to Eq. 1. Error
569 bars are not reported to make the figure more readable; standard error was computed
570 over three repetitions and is lower than 10%.

571 Figure 3 Surface dilatational complex modulus, E^* (A), and phase angle, δ , (B), as
572 frequency function, ω , at air/water interface at pH=4, for samples HM 104 (blue
573 diamond), HM145 (red square), LM96 (green triangle), LM123 (orange circle).
574 Standard error was computed over three repetitions.

575 Figure 4 Surface dilatational complex modulus, E^* (A), and phase angle, δ , (B), as
576 frequency function, ω , at air/water interface at pH=6, for samples HM 104 (blue
577 diamond), HM145 (red square), LM96 (green triangle), LM123 (orange circle).
578 Standard error was computed over three repetitions.

Table 1 Sample identification, degree of methylation (DM), molecular weight (MW), protein content, equilibrium surface pressure (π_{eq}) at pH 4 and pH 6 of pectins used. The same letters, for each parameter, identify the absence of statistically significant differences

Pectin ID	DM (%)	MW (kDa)	Protein (g/100g)	π_{eq} at pH 4 (mN·m)	π_{eq} at pH 6 (mN·m)
HM104	65.3	104	1.70±0.1	23.4±0.3 ^a	24.7±0.5 ^d
HM145	65.1	145	1.50±0.1	19.4±0.5 ^b	25.1±0.7 ^d ^f
LM96	41	96	1.80±0.1	28.0±0.2 ^c	29.6±0.1 ^e
LM123	42.9	123	1.90±0.1	23.0±0.8 ^a	26.2±0.3 ^f

Table 2 Velocity of diffusion (k_{dif}), adsorption (k_{ads}) and rearrangement (k_r) for HM and LM pectins at pH 4 and 6. The same letters, for each parameter, identify the absence of statistically significant differences

	pH 4			pH 6		
	k_{dif} ($\text{mN}\cdot\text{m}^{-1}\text{ s}^{-0.5}$)	$k_{\text{ads}}\cdot 10^{-4}$ (s^{-1})	$k_r\cdot 10^{-4}$ (s^{-1})	k_{dif} ($\text{mN}\cdot\text{m}^{-1}\text{ s}^{-0.5}$)	$k_{\text{ads}}\cdot 10^{-4}$ (s^{-1})	$k_r\cdot 10^{-4}$ (s^{-1})
HM104	$0.7 \pm 0.1^{\text{a}}$	$3.5 \pm 0.1^{\text{a}}$	$21.3 \pm 0.3^{\text{a}}$	$0.75 \pm 0.01^{\text{a}}$	$3.895 \pm 0.006^{\text{c}}$	$24.7 \pm 0.2^{\text{e}}$
HM145	$0.41 \pm 0.04^{\text{b}}$	$2.7 \pm 0.3^{\text{b}}$	$8.8 \pm 0.9^{\text{b}}$	$0.56 \pm 0.04^{\text{d}}$	$3.8 \pm 0.2^{\text{c}}$	$26 \pm 1^{\text{e}}$
LM96	$1.5 \pm 0.1^{\text{c}}$	$3.3 \pm 0.1^{\text{a}}$	$16 \pm 1^{\text{c}}$	$1.55 \pm 0.02^{\text{c}}$	$3.8 \pm 0.1^{\text{c}}$	$33.0 \pm 0.6^{\text{f}}$
LM123	$0.45 \pm 0.03^{\text{b}}$	$3.38 \pm 0.09^{\text{a}}$	$19.63 \pm 0.08^{\text{d}}$	$0.75 \pm 0.02^{\text{a}}$	$3.39 \pm 0.07^{\text{a}}$	$34.6 \pm 0.3^{\text{e}}$

Table 3 Rheological parameters of interfacial “gel model” (k and n) at the A/W interface at pH 4 and 6. The same letters, for each parameter, identify the absence of statistically significant differences

	pH 4		pH 6	
	k (mN·m ⁻¹ ·s ⁻¹)	n (-)	k (mN·m ⁻¹ ·s ⁻¹)	n (-)
HM104	32 ± 3 ^a	0.07 ± 0.02 ^a	39 ± 2 ^c	0.15 ± 0.04 ^{ef}
HM145	31 ± 1 ^a	0.105 ± 0.003 ^b	60 ± 4 ^d	0.19 ± 0.01 ^f
LM96	72 ± 2 ^b	0.22 ± 0.01 ^c	51 ± 6 ^d	0.17 ± 0.04 ^{cef}
LM123	75 ± 7 ^b	0.15 ± 0.02 ^d	59.3 ± 0.3 ^d	0.14 ± 0.01 ^{de}

Figure 1A
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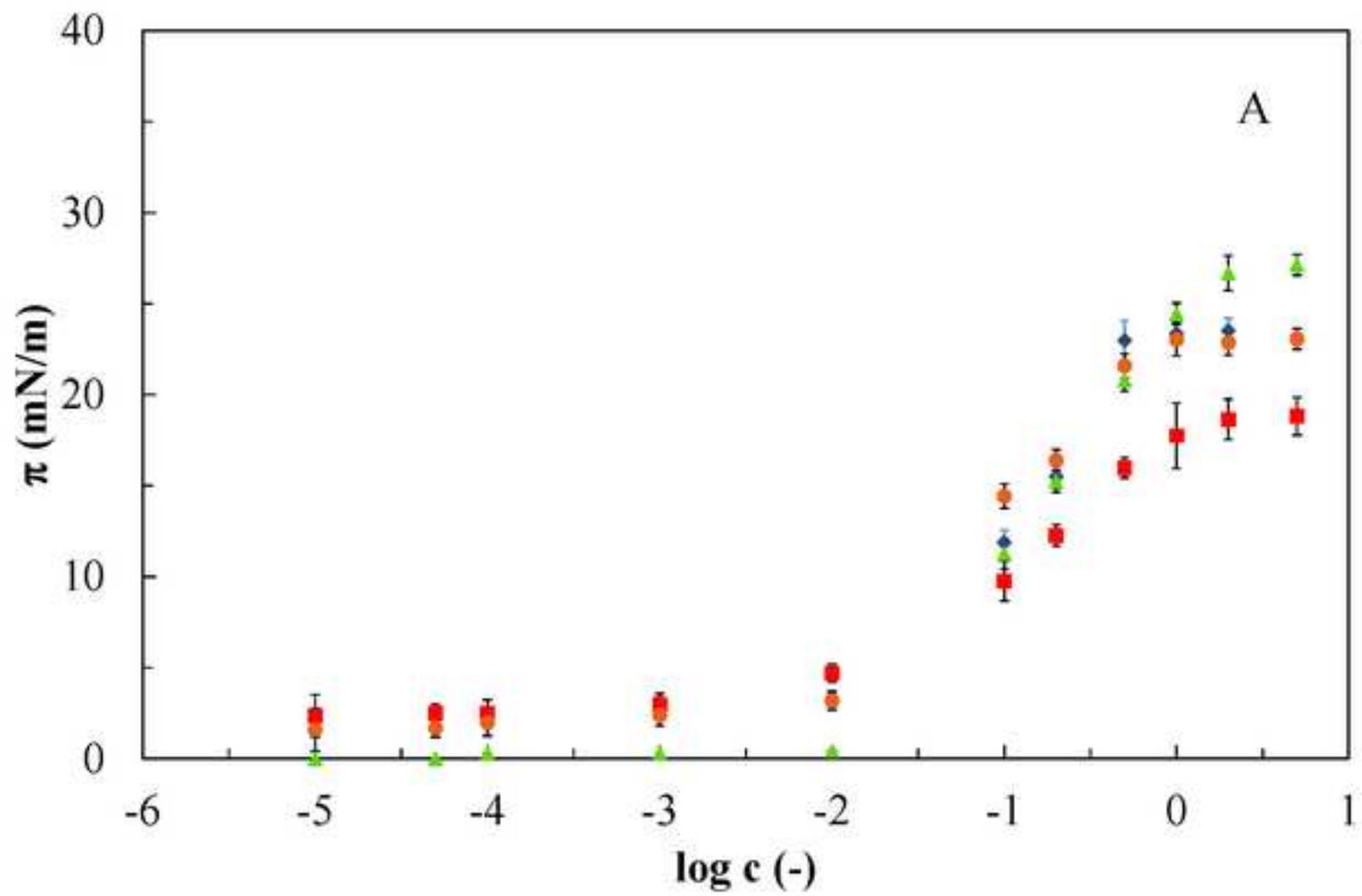


Figure 1A

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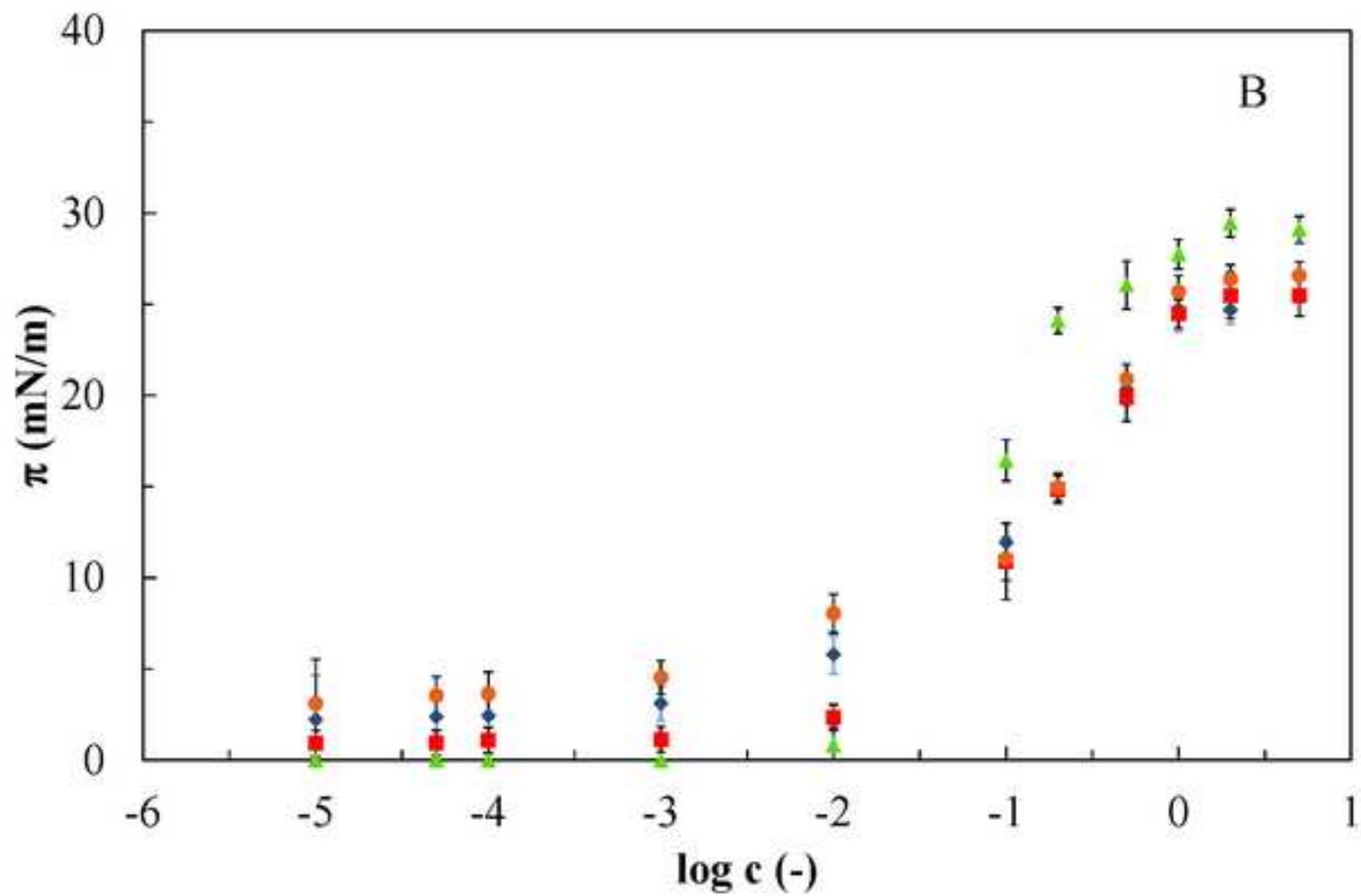


Figure 1B

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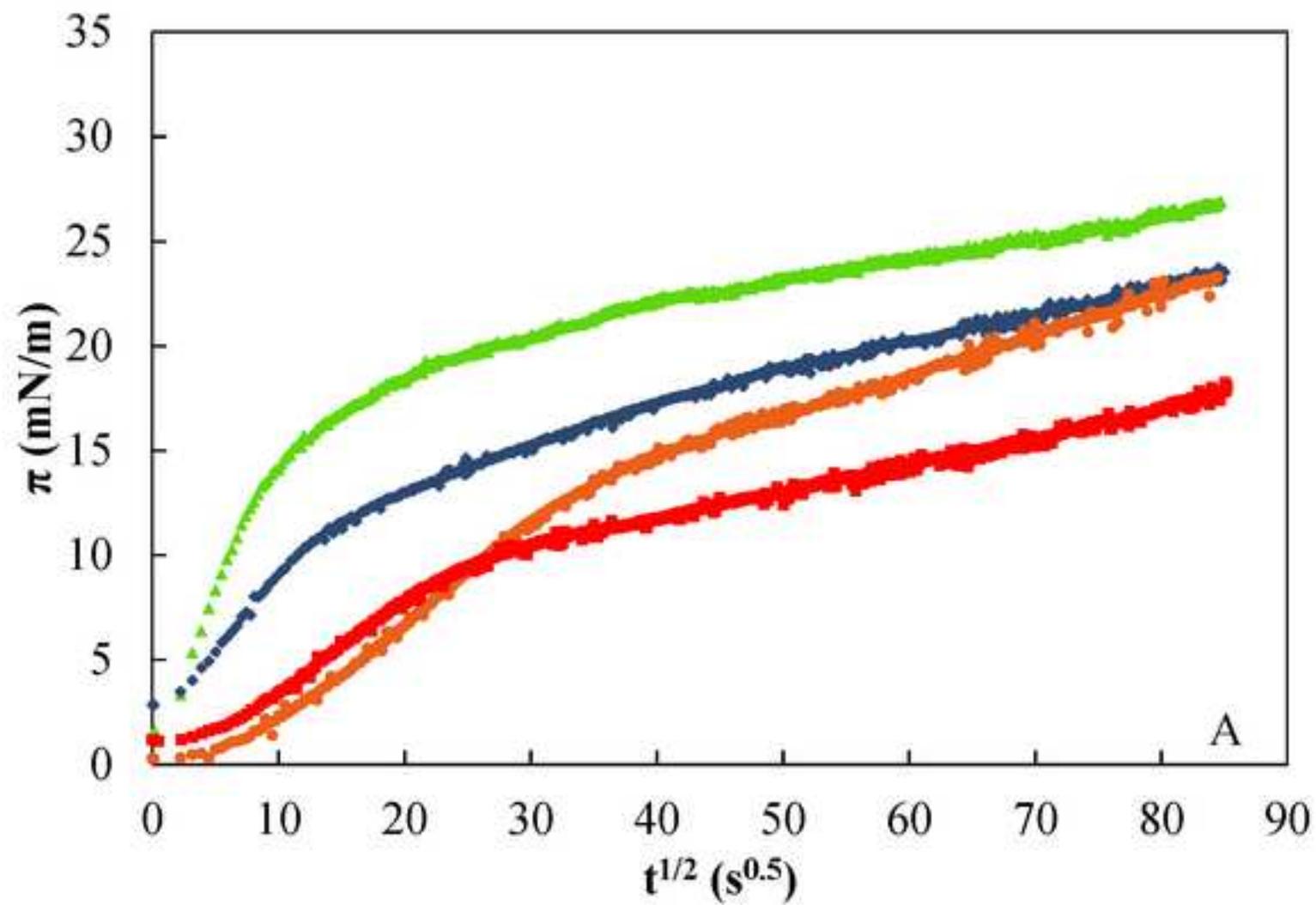


Figure 2A

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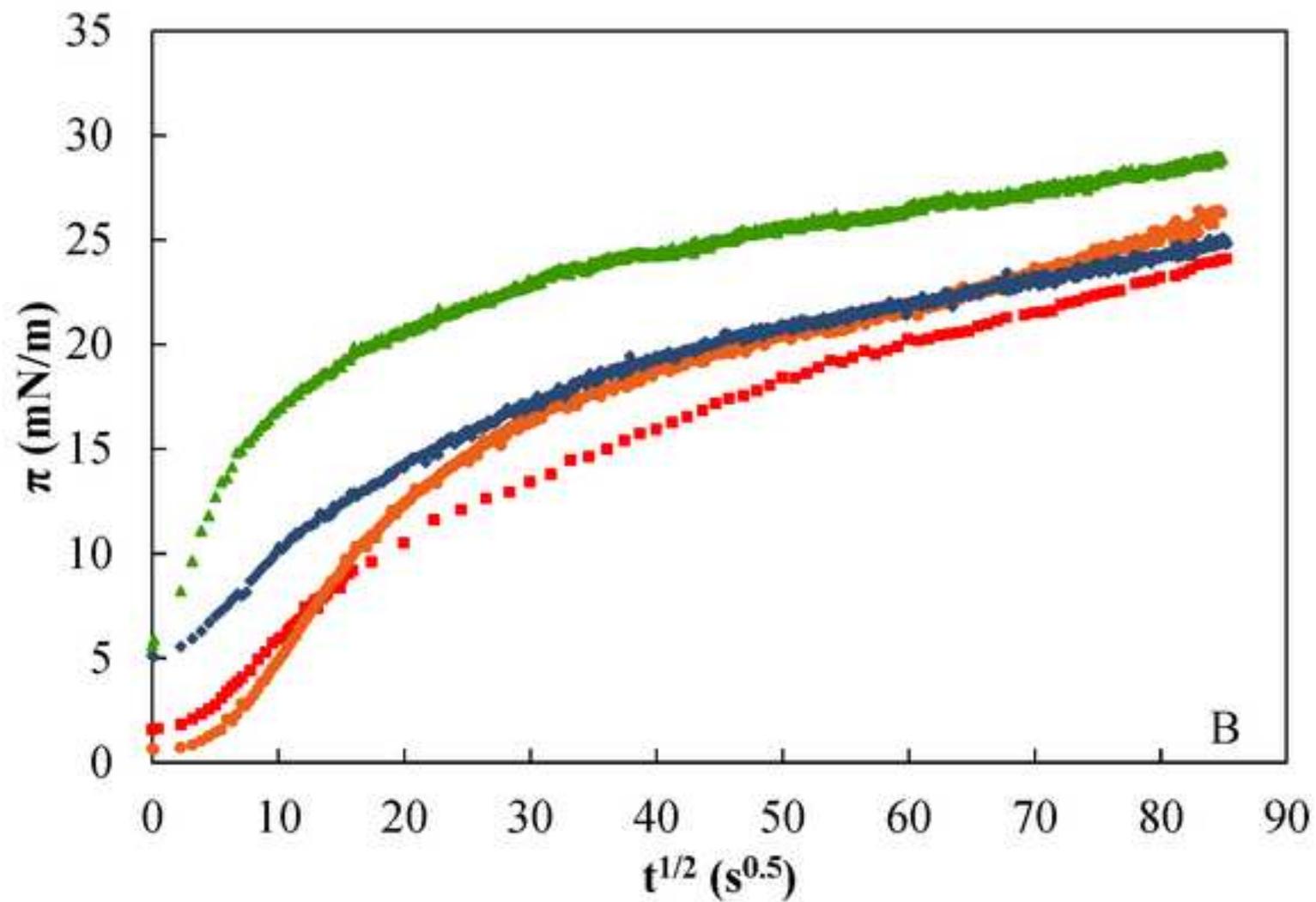


Figure 2B

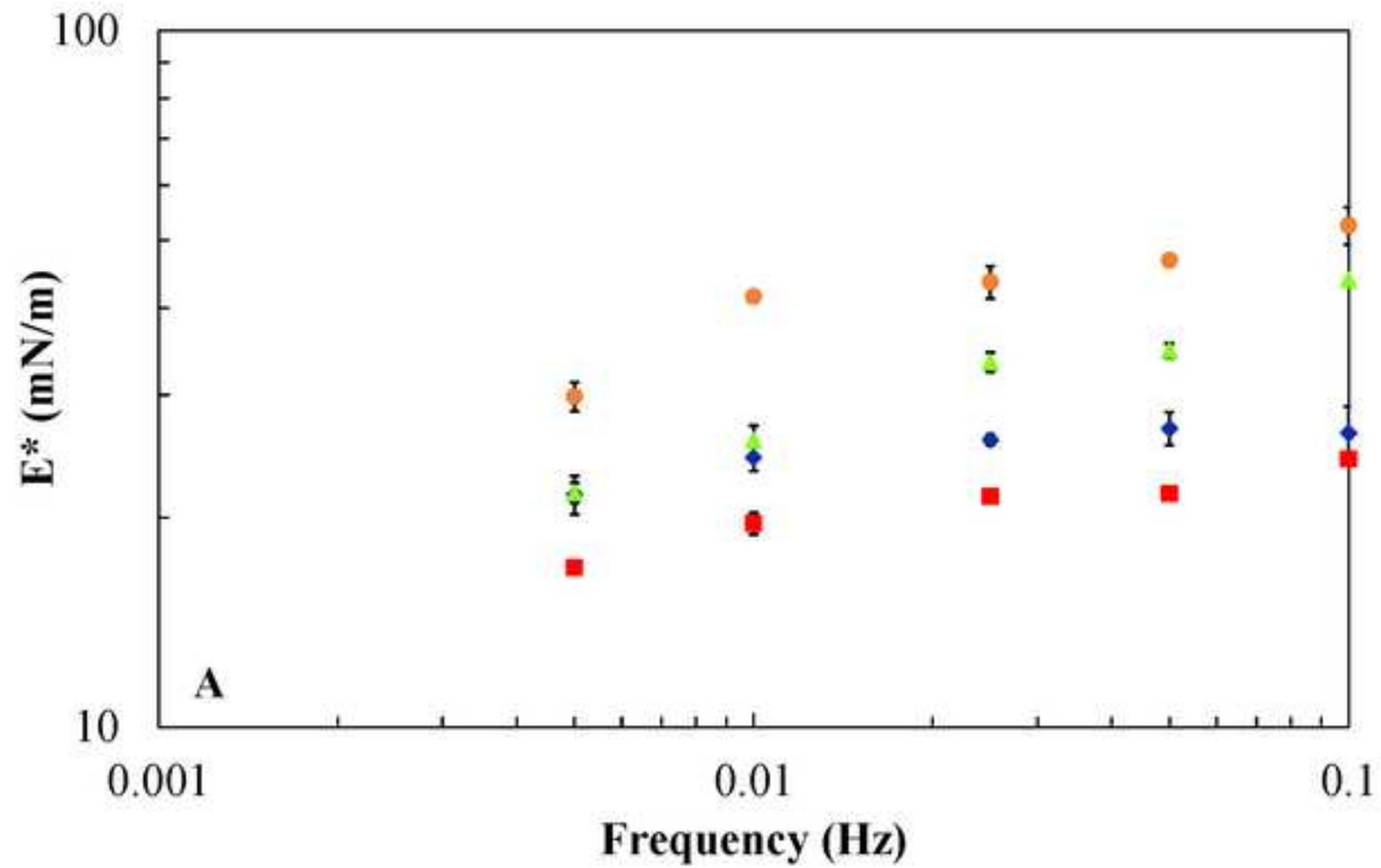


Figure 3A

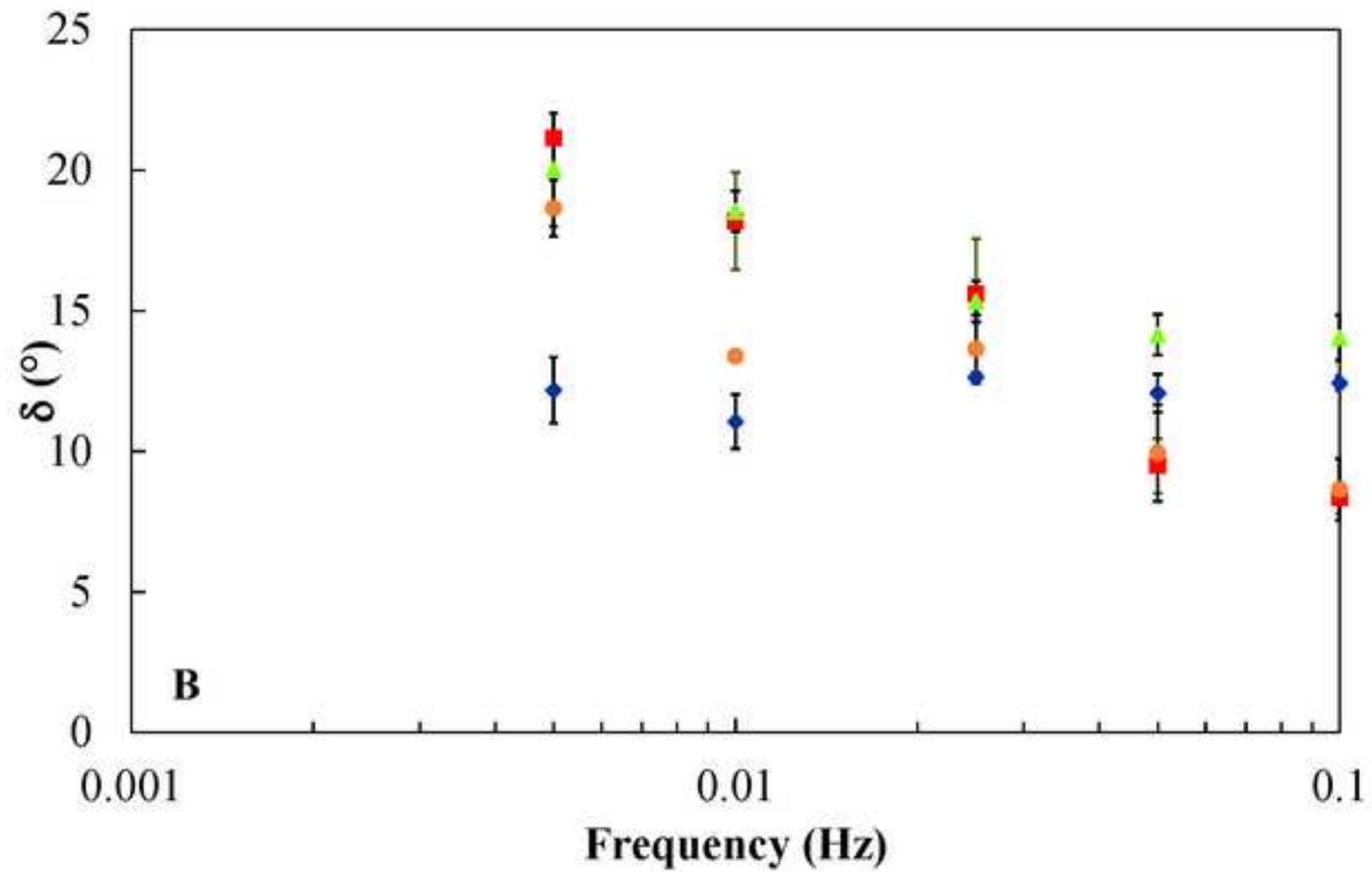


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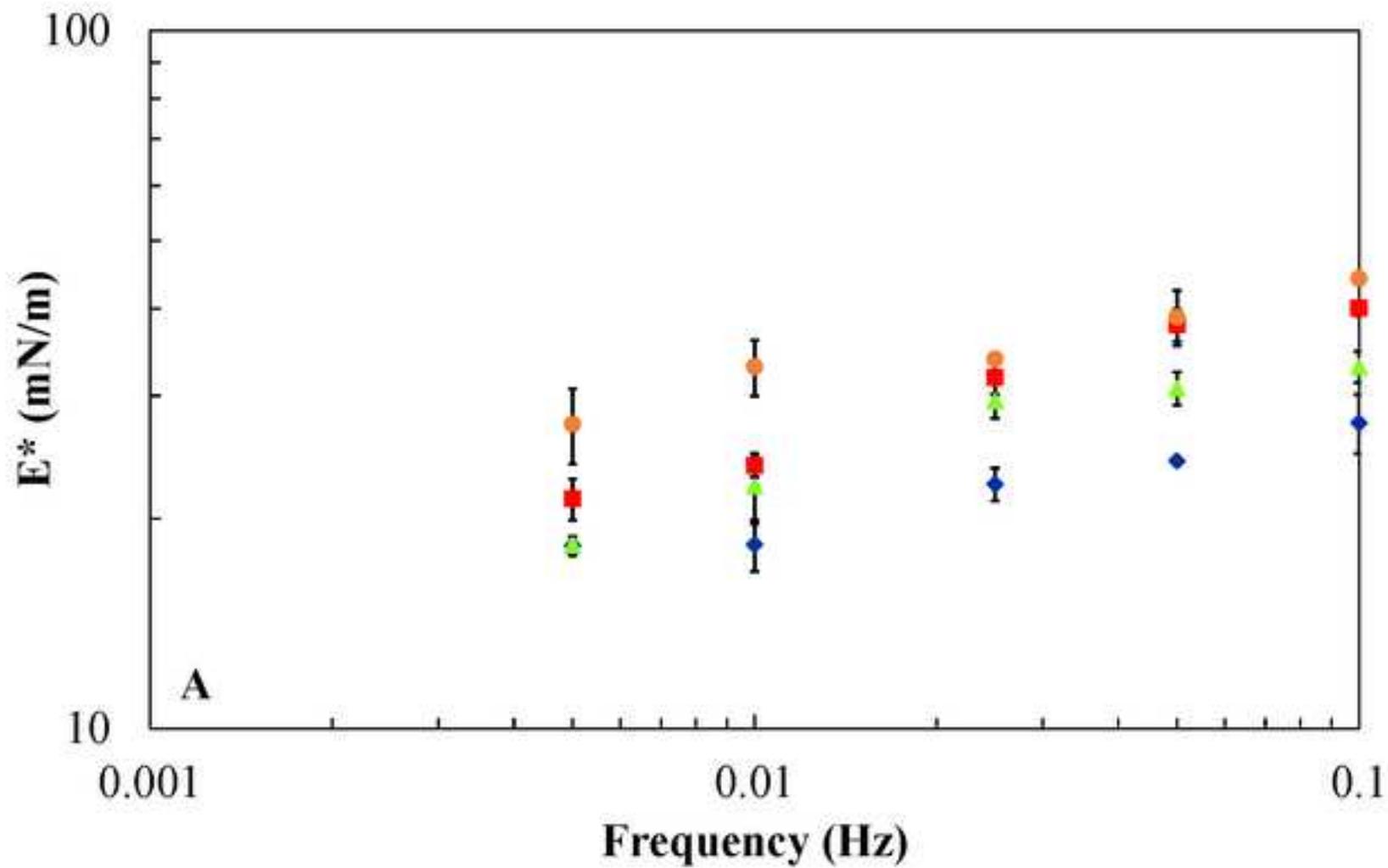


Figure 4A

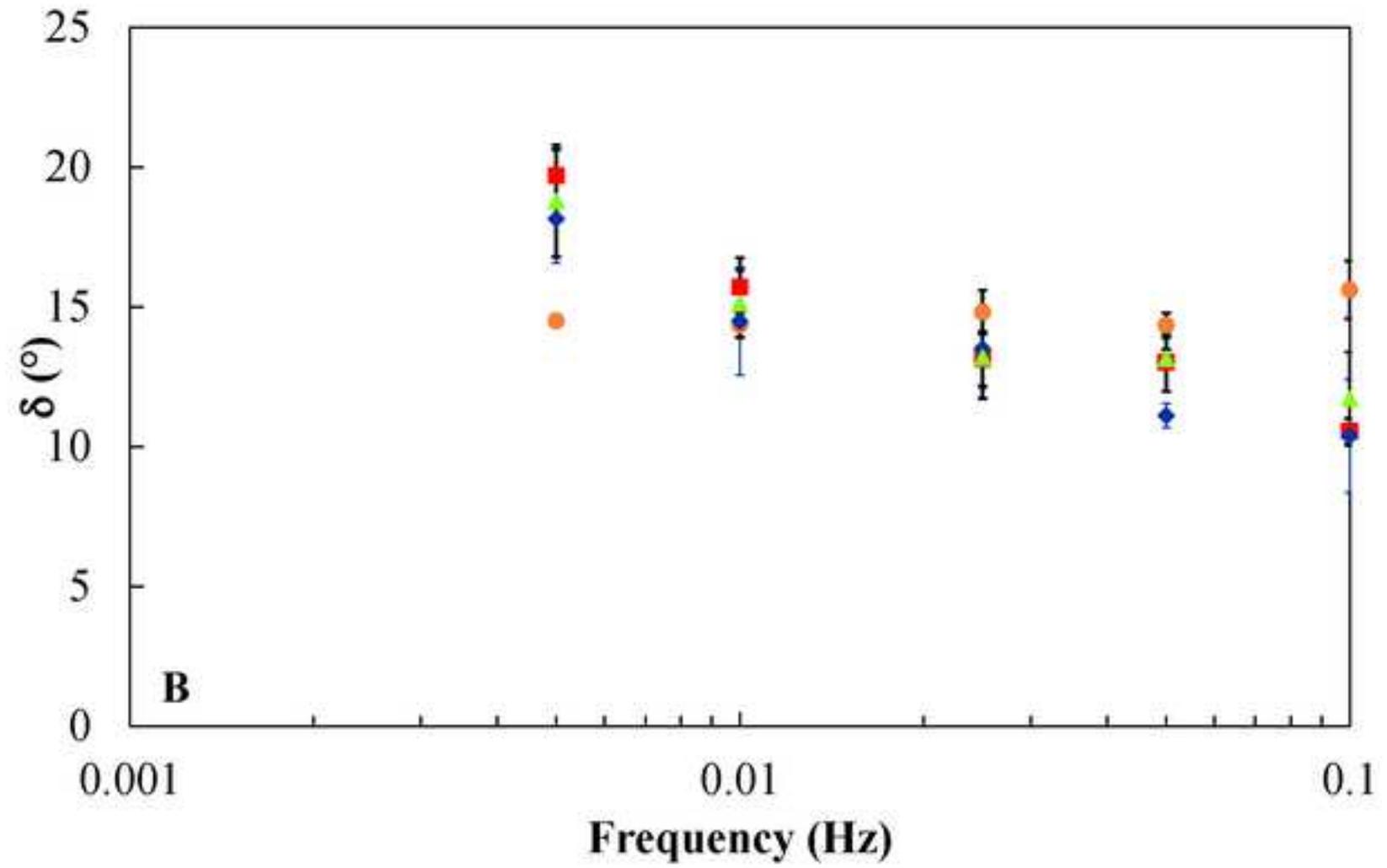


Figure 4B