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Sustainability Spotlight Statement

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Brewer's spent grain (BSG) is the most abundant side-stream in the brewing industry. Since BSG is rich in protein, several research has been conducted in extracting and characterising the protein fraction. This research article investigates the potential of BSG-derived protein to be added in different beverage systems resulting in significant insights for the development of high protein soft drinks and in further consideration of sensory attributes. Applications of upcycled proteins scares, but if thoroughly tested, can contribute to transforming our food system towards more sustainable food systems by reducing food loss during food production process. This work aligns with SDG2 (enhancing food security), SDG3 (food fortification), SDG12 (upcycling of by-products), and SDG13 (reducing food losses that cause global warming).



Benefits and challenges of fortified beverage systems with brewer's spent grain-derived protein isolates.

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Abstract

Plant protein soft drinks align with current consumer demand for hydration, nutrition and sustainability yet faces challenges due to protein aggregation and precipitation in an acidic environment. This study evaluated the effects of two different barley and rice protein isolates, EverPro[®] Dark Fraction (EDF) and a decolourised version, EverPro[®] Light Fraction (ELF), at ingredient inclusion levels of 0%, 2.5%, 5% and 10% in diverse beverage systems including water as a control, apple juice, a full sugar and sugar free carbonated drink, a sweetened ice tea, an unsweetened black



22 tea and a non-alcoholic beer. Physicochemical properties were comprehensively assessed. Protein
23 inclusion, particularly at 10%, elevated pH to a more neutral range (from 3.1 and 4.88 to 5.0 and 7.8).
24 Increasing the addition of both ingredients also elevated viscosity (from 1.04 and 1.46 to 2.54 and 5.43
25 mPa·s), density (0.999 and 1.045 to 1.030 and 1.074 g/cm³) as well as lubrication properties of all
26 beverages with ELF exhibiting higher values compared to EDF. Dispersion stability improved
27 dramatically including turbidity and particle size at higher inclusion levels (10%), influenced by changes
28 in pH further from the proteins isoelectric point. Low inclusion levels (2.5%) caused maximum turbidity
29 and separation rate. Principal component analysis revealed distinct clustering by beverage matrix and
30 protein type, with apple juice most affected. These findings demonstrate a step towards soft drinks
31 fortified with upcycled protein from brewers spent grain with added nutritional benefits for future
32 commercially potential.

33 1. Introduction

34 Conventional soft drinks offer little to no nutritional value, primarily composed of water, sugar or
35 sweeteners, acidulants and flavourings¹. Other soft drinks, such as juices, teas and non-alcoholic
36 beers, also offer limited nutritional value but can provide essential micronutrients including vitamins,
37 minerals, antioxidants and polyphenols. Protein-based beverages are continuing to grow as popular
38 functional or sports product. Most research has been focused on plant-based milk alternatives and
39 the ongoing challenges they face, such as nutrition, stability and flavour². These plant-based beverages
40 or drinks are made from a variety of plant sources including cereals, nuts, and legumes, whereas
41 typical commercial plant-based milk substitutes include the use of almonds, cashews, hazelnuts, rice,
42 quinoa, oat, hemp and soy³⁻⁵. Consumers are seeking a shift away from traditional protein smoothies
43 or shakes to clear beverage options that can address thirst and provide hydration⁶. Beverages such as
44 these can be a convenient option to supply a high dose of protein. The functional drinks market is one
45 of the fastest growing industries, with functional waters emerging as one of the most popular
46 beverages in this category⁶. However, most soft drinks have an acidic pH range, which can cause



47 proteins to precipitate⁷. Furthermore, these beverages are commonly thermally processed to extend
48 their shelf life by reducing harmful microbes and inactivating enzymes. This is typically achieved
49 through methods like pasteurisation or ultra-high temperature (UHT) sterilisation, which may alter
50 their flavour, aroma and colour^{4,5}. As a result, stabilizing protein in both acidic conditions and high
51 temperatures presents a challenge, as turbidity, phase separation, viscosity and gelation can be
52 affected⁸. Additionally, the incorporation of plant-based proteins into beverages is often associated
53 with challenges such as sensorial, imbalanced amino acid profiles, low bioavailability, and the
54 presence of antinutrients. These sensory challenges can include undesirable beany flavours,
55 astringency, bitterness and mouthcoating mainly attributed from the polyphenols⁹⁻¹¹. These hurdles
56 can limit consumer acceptance. Specifically for carbonated beverages, including high levels of protein
57 could also lead to processing issues during production such as excessive foaming^{1,12,13}. Protein
58 hydrolysates can be an excellent ingredient for fortifying these beverages due to their high solubility
59 and greater thermal and acidic stability when compared to larger molecular size proteins. Methods
60 used for hydrolysing proteins can include biological processes (including fermentation and enzyme
61 treatment), physical, or chemical processes, while each method has its own advantages and
62 disadvantages¹⁴. Brewing is an example of a process that causes protein degradation leading to
63 partially hydrolysed proteins and small peptides in brewers spent grain¹⁵.

64 The demand for high protein food and beverage products has risen significantly in recent years with a
65 particular emphasis on plant-based protein options as consumers increasingly identify as vegetarian,
66 vegan or even flexitarian, and due to environmental and ethical considerations¹⁶. As a result, majority
67 of the leading dairy companies are incorporating plant-based alternatives into their product portfolio.
68 Numerous studies have focused on animal-based protein beverages, particularly fortifying various
69 flavoured soft drinks with whey protein¹⁷⁻²⁰. Many of these whey-based flavoured soft drinks are
70 formulated with low to medium protein contents (ranging from 0.8% to 6% per 100 ml) and are limited
71 to certain beverage types such as juices, while tea and non-alcoholic beers have not been explored.
72 Moreover, there has been limited research on the inclusion of plant-based proteins in soft drinks.



73 The valorisation of protein ingredients recovered from food processing side streams is critical to
74 reduce food waste and improve resource efficiency for producing alternative proteins to address
75 future protein demands. EverPro™ Dark Fraction (EDF) and Everpro™ Light Fraction (ELF) produced by
76 the company EverGrain Ingredients, are commercially available barley and rice protein isolates
77 derived from upcycled brewers spent grain^{21,22}. ELF (beige/sandy colour) is a decolourised version of
78 EDF (dark brown colour), with higher L* brightness values and distinctive physiochemical properties²³.
79 This study tested two hypotheses 1) Does EDF and ELF affect techno-functionality of the beverages?
80 And if so, is the impact similar or significantly different? and 2) Does the ingredient addition level
81 change the techno-functionality of the beverages? If yes, are these changes beneficial or do they cause
82 challenges?

83 2. Materials and Methods

84 2.1. Materials

85 The barley and rice protein isolates, EDF and ELF were obtained from EverGrain Ingredients (St. Louis,
86 MO, USA). The apple juice from concentrate (AJ), carbonated lemon and lime soft drink (LL),
87 carbonated lemon and lime soft drink sugar free (LL Free), sweetened peach flavoured ice tea (IT),
88 unsweetened black tea bags (BT) and non-alcoholic lager beer (NAB) were all purchased from local
89 supermarkets in Cork, Ireland. The list of ingredients and nutritional composition of each beverage
90 used in the study are shown in Table 1. Chemicals for the analyses were purchased from Sigma-Aldrich
91 (St. Louis, MO, USA) unless stated otherwise.

92 2.2. Preparation of protein beverages

93 EDF and ELF were added singly to a variety of beverage systems (water, AJ, LL, LL Free, IT, BT, and NAB)
94 at four different concentrations, 0%, 2.5%, 5% and 10%. These concentrations were chosen to match
95 the protein content of protein soft drinks that were available on the global market from a previous
96 study performed (i.e. for commercial relevance)²⁴. According to the protein content of the ingredients
97 each beverage had a final protein content of approximately 2%, 4% and 8% with the addition of 2.5%,



98 5% and 10% (w/w) of ingredient, respectively²³. Protein solubility was done on ingredients exceeding
99 80% across pH 2 – 9, relevant to soft drinks acidic value. A range of different drinks were chosen to
100 cover a variety of beverage matrices. Carbonated beverages (LL, LL Free, NAB) were decarbonated
101 before protein addition using ultrasonication as previously described²⁴. The BT was prepared using
102 package instructions by brewing 2 teabags in 1 L of boiled water for 4 min, followed by the removal of
103 the teabags and cooling until it reached room temperature (~20 °C). The beverages were mixed using
104 a vortex, then left for 2 hrs to allow further hydration, followed by a 30 s mixing stage using an Ultra-
105 Turrax equipped with a S10N-10G dispersing element at speed 3 (IKA Labortechnik, Janke and Kunkel
106 GmbH, Staufen, Germany). All beverages were freshly prepared prior to analysis.

107 2.3. pH

108 The pH of each beverage was measured using a calibrated pH meter (Mettler Toledo, Ohio, United
109 States) at 20 °C.

110 2.4. Apparent Viscosity

111 Rheological behaviour was determined using a rheometer (MCR301, Anton Paar GmbH, Graz, Austria)
112 equipped with a concentric cylinder measuring attachment (Anton Paar GmbH, Graz, Austria) as
113 previously described²⁴. A shear sweep was performed, measured as a function of shear rate ranging
114 from 0.5 to 100 (1/s) at 20 °C. The apparent viscosity at 80.7 (1/s) was evaluated and used to compare
115 the beverages.

116 2.5. Liquid Density

117 The liquid density (g/cm³) of the soft drinks was determined using the Anton Paar density meter DMA
118 4500 M (Anton Paar GmbH, Austria). The sample was loaded and measured based on the oscillating
119 U-shaped-tube principle where the sample is excited and oscillated at an oscillation period specific for
120 the sample mass and volume at 20°C.

121 2.6. Turbidity



122 Turbidity was measured using a turbidity meter (Thermo Scientific Eutech TN-100 Waterproof
 123 Turbidimeter, Singapore, Singapore). Samples were measured at a wavelength of 850 nm at 20 °C. A
 124 sample volume of 10 ml was used to determine turbidity ranging from 0 – 2000 nephelometric
 125 turbidity unit (NTU).

126 2.7. Dispersion Stability

127 Dispersion stability was determined using an analytical centrifuge (LUMiSizer, LUM GmbH, Berlin,
 128 Germany), through phase separation based on light transmission during centrifugation. The
 129 separation rate, an indication of stability was reported. The cycle configuration involved taking 100
 130 profiles in 10 s intervals during centrifugation at 1000 rcf for 17 min, at a wavelength of 865 nm and a
 131 temperature of 20 °C. The percentage of integrated light transmission increased over time and the
 132 separation rate in is expressed as the % transmission per minute, as previously reported²⁵.

133 2.8. Particle Size Distribution

134 The average particle size (nm) and polydispersity index (PDI) of each soft drink was measured using
 135 dynamic light scattering technology with the Zetasizer Nano Z (Malvern Instruments Ltd.,
 136 Worcestershire, UK), equipped with a 633 nm laser and set size range of 0.3 nm – 10 µm. A refractive
 137 index of 1.45 and absorbance at 0.001 specific for proteins was used at 20 °C.

138 2.9. Foam Capacity and Foam Stability

139 Foaming properties of the beverages were determined by frothing 20 ml of samples using an Ultra-
 140 Turrax equipped with a S10N-10G dispersing element (IKA Labortechnik, Janke and Kunkel GmbH,
 141 Staufen, Germany), at the maximum speed of 6 for 30 s. Sample expansion was calculated using the
 142 following equations ²⁶:

$$143 \text{ Foaming capacity (\%)} = \left(\frac{\text{Foam height immediately after foaming}}{\text{Initial sample height}} \right) \times 100$$

$$144 \text{ Foam stability (\%)} = \left(\frac{\text{Foam height after 1 hour}}{\text{Foam height immediately after foaming}} \right) \times 100$$



145 2.10. Tribology

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146 Tribological measurements were conducted on a rheometer MCR301 using the BC-12.7 ball-on-3-pins
147 tribology attachment (Anton Paar GmbH, Graz, Austria) at 37 °C as described²⁷. The attachment
148 contains changeable tribopairs (a glass ball and polydimethylsiloxane (PDMS) pins), which were
149 supplied by Anton Paar (Graz, Austria). New pins were used for each test consisting of one run-in
150 period and two repeated measurements of Stribeck curves. Before starting a test, the pin-holder and
151 ball-holder were washed gently using a dilute detergent solution and rinsed thoroughly as well as
152 being rinsed in acetone and dried with lab-wipes with the tribopairs. The test sequence was as
153 followed: first the glass ball was lowered until a target force of 3 N was reached and held at that height
154 for an equilibration period of 1 min. Furthermore, the following settings of the measurement system
155 were selected: a logarithmic increase in sliding speed from 10⁻⁸ to 1 m/s was performed recording 80
156 data points with point duration from 1 to 10 s logarithmic, followed by a resting period of 1 min. Only
157 the 0% and the 10% (w/w) beverages of EDF and ELF were measured using 1 ml aliquots. Three
158 consecutive Stribeck curve measurements were performed for each sample. The first measurement
159 served as a run-in period and was excluded from the data set. Each sample was performed in triplicate,
160 resulting in six evaluated datasets per sample.

161 2.11. Statistics

162 All analyses were performed in triplicate. Statistical analysis was performed using Origin lab pro2025b
163 (Northampton, MA, USA). A one-way ANOVA with post hoc Tukey test ($p < 0.05$), principal component
164 analysis, as well as a correlation analysis were performed.

165 3. Results

166 The results are separated into two parts. First, the changes in techno-functionality due to protein
167 fortification with EDF and ELF at different concentrations are reported. The second part reveals the
168 degree of changes of the beverages due to either EDF or ELF, illustrated in a principal component
169 analysis (PCA) biplot.



170 **3.1. Impact of protein fortification on techno-functionality of beverages**

171 **3.1.1. pH**

172 The pH values of the beverages are displayed in Table 2. All beverages were quite different in their pH
173 values, with the lowest pH values observed for IT (3.10) followed by LL (3.30), LL Free (3.30) and AJ
174 (3.51). The pH of NAB (4.32) and BT (4.88) are slightly higher, while the highest pH value was observed
175 for water (6.71).

176 The addition of EDF and ELF caused an increase in pH, with ELF elevating the value to a higher extend
177 compared to ELF. Increased inclusion of either EP™ ingredient consistently elevated the pH of all
178 beverages significantly. However, in water, the addition of 2.5% of either EDF or ELF caused an
179 increase in pH from 6.71 (0% addition) to 8.15 and 7.45, respectively, but higher addition levels did
180 not further raise the pH. The highest pH values (>7) in beverages fortified with 10% of EDF were
181 observed in water (8.25) followed by BT (7.8) and NAB (7.3). The addition of 10% EDF to acidic
182 beverages elevated their pH to 6.7 in IT, to 6.6 in LL, to 6.5 in LL Free, and to 5.5 in AJ. Addition of ELF
183 followed the same trend in these beverages with slightly lower final pH values. A significant strong
184 positive correlation between the addition level of either EP ingredient in IT and the resulting pH was
185 observed ($r = 0.97$, $p \leq 0.05$). Interestingly, the addition of ELF in AJ showed a strong correlation
186 between addition level and pH ($r = 0.97$, $p \leq 0.05$), but fortifying with EDF did not show significance.

187 **3.1.2. Apparent Viscosity**

188 The formulation of a beverage can impact viscosity, which is an important functional property capable
189 of influencing the mouthfeel/texture. The apparent viscosity of the beverages at 80.7 1/s are
190 presented in Table 2. Among the controls, LL Free exhibited the lowest viscosity (1.04 mPa·s) followed
191 by water (1.07 mPa·s), IT (1.09 mPa·s), and BT (1.10 mPa·s). The highest viscosity among the controls
192 were found in NAB (1.46 mPa·s) followed by LL (1.25 mPa·s) and AJ (1.23 mPa·s).



193 Fortification with both EDF and ELF increased apparent viscosity across all beverages, with ELF addition
194 resulting in higher viscosity values than EDF. Water showed a clear increase in viscosity with EP
195 addition, reaching significantly higher values of 3.19 mPa·s (EDF) and 4.82 mPa·s (ELF) at 10% inclusion.
196 AJ with 10% inclusion was shown to be the most viscous beverage, particularly with ELF (5.43 mPa·s),
197 followed by EDF (4.07 mPa·s) with statistical significance. LL showed moderate increases, with 10%
198 addition of EDF and ELF resulting in viscosity values of 2.81 mPa·s and 3.98 mPa·s, respectively. LL Free
199 increased to 2.54 mPa·s (EDF) and 3.41 mPa·s (ELF) at 10% inclusion levels. NAB displayed apparent
200 viscosities of 3.19 mPa·s (EDF) and 4.82 mPa·s (ELF) at 10% ingredient addition, with ELF inclusion
201 resulting in a significantly higher value than EDF. Additionally, the inclusion of either EP ingredient
202 showed a higher impact on IT than on BT.

203 All beverages demonstrated a significantly strong positive correlation ($r = 0.96 - 1.00$, $p \leq 0.05$)
204 between protein inclusion level and apparent viscosity, with the exception of EDF addition in IT where
205 the correlation was not significant.

206 3.1.3. Liquid Density

207 Density is an important physical parameter reflecting the content of soluble solids of a soft drink,
208 meaning the density of a solution is dependent on the concentration of dissolved substances within
209 the product. The impact of EDF and ELF fortification on beverage density is illustrated in Table 2. As a
210 reference value, water showed an average density value of 1 g/cm³. Regarding the controls, beverages
211 containing sugars exhibited higher densities compared to sugar free beverages; AJ (1.0448 g/cm³), LL
212 (1.0175 g/cm³), IT (1.0177 g/cm³) and NAB (1.0156 g/cm³), whereas LL Free (0.9997 g/cm³) and BT
213 (0.9995 g/cm³) displayed lower densities.

214 Fortification with both EP ingredients led to elevated density values in all beverages, while ELF showed
215 significantly higher density values compared to EDF. Water with 10% addition of EP ingredients
216 resulted in the lowest density values at 1.0295 g/cm³ and 1.0304 g/cm³, similar to BT at 1.0303 g/cm³
217 and 1.0309 g/cm³ for EDF and ELF inclusion, respectively. LL and LL Free reached similar densities at



218 10% inclusion with both EPs (1.05 g/cm³ (EDF) and 1.03 g/cm³(ELF)). NAB and IT increased to 1.046
219 g/cm³ and 1.048 g/cm³ at 10% EP inclusion. AJ showed the highest densities at 10% addition level,
220 with a value of 1.0728 g/cm³ determined for EDF addition, and 1.0743 g/cm³ for ELF. addition
221 All beverages displayed a strong positive correlation between protein inclusion and density ($r = 0.99$,
222 $p \leq 0.05$), revealing higher amounts of soluble solids with increasing EP fortification.

223 3.1.4. Turbidity

224 Turbidity reflects the clarity and transparency of a beverage. The turbidity results are displayed in
225 Table 3. Control samples (0% inclusion) exhibited low turbidity, ranging from 0.2 – 27.5 NTU, with
226 water being the lowest and BT the highest.

227 Addition of both EP ingredients significantly increased turbidity in all beverages. Water samples with
228 EP inclusion consistently exhibited low turbidity across all concentrations, with ELF causing slightly
229 higher values than EDF. The turbidity of most beverages was dependent on concentration levels. The
230 most substantial increases in turbidity were observed in the LL beverages fortified with ELF at 2.5%,
231 giving values out of range (≥ 2000 NTU). An addition of 2.5% EDF to LL showed higher turbidity (1768
232 ± 14 NTU) compared to LL Free (927 ± 53 NTU). IT and BT also showed high turbidity levels, especially
233 at 2.5% inclusion of both EP ingredients. However, ELF caused higher turbidity in IT and BT than EDF.
234 In AJ, the fortification with EDF caused the highest turbidity value at an inclusion level of 2.5%, while
235 fortification with ELF reached the highest turbidity at 10% addition level. The fortification of NAB with
236 EP ingredients displayed moderate turbidity increases. The addition of ELF to NAB revealed a
237 significantly strong positive correlation with turbidity ($r = 0.95$, $p \leq 0.05$). Overall lower protein
238 inclusions (2.5%) generally resulted in the most turbid samples (excluding water), while the higher
239 inclusion level of 10% often reduced turbidity, observed in LL (EDF and ELF), LL Free (EDF and ELF), IT
240 (EDF and ELF), BT (EDF) and NAB (EDF).

241 3.1.5. Dispersion Stability



242 An indication of dispersion stability is a low separation rate. The separation rate of each beverage is
243 presented in Table 3. According to the light transmission profile (shown in Figure 1), all beverage
244 controls (0% EP inclusion) showed low separation rates (≤ 0.08 %/min).

245 Water samples containing either EDF or ELF at all inclusion levels also showed similarly low values (\leq
246 0.2 %/min). Both EDF and ELF addition increased separation rates across all beverages with no
247 consistent difference between the two ingredients. However, inclusion level showed a strong
248 influence on separation behaviour. AJ showed sedimentation and a high separation rate at every EP
249 concentration (highest in AJ ELF 5% = 6.18 ± 0.02 %/min). Both LL and LL Free followed a pattern similar
250 to IT, where separation rates were the highest at low inclusion levels (2.5%), and significantly lower at
251 10%, indicating improved stability at higher protein levels. Dispersion stability was also influenced by
252 2.5% inclusion of both EPs in NAB. BT demonstrated a clear protein concentration-dependent effect
253 for EDF, with a significant positive correlation observed between addition level and separation rate (r
254 = 0.98, $p \leq 0.05$).

255 Across all beverages (excluding water), more than half of the samples displayed their highest
256 separation rates at 2.5% protein inclusion, whereas the lowest rates were consistently observed at
257 10% addition, indicating greater stability at higher protein levels.

258 3.1.6. Particle size Distribution

259 The average particle size (nm) and polydispersity index (PDI) values are presented in Table 3. Among
260 the control beverages, water showed no detectable particle size. AJ exhibited the largest particle size
261 (2560 ± 48.29 nm), followed by LL Free (1035 ± 110.4 nm) and LL (917 ± 218.67 nm). IT showed the
262 smallest particle size (187 ± 30.64 nm), and BT (309 ± 27.64 nm) and NAB (465 ± 47.33 nm) also
263 displayed comparatively low values. Across beverages, the addition of EP ingredients increased
264 particle size, with no consistent differences observed between EDF or ELF. Increasing EP concentration
265 did not lead to a uniform trend across the beverages. Water and BT were the only samples where
266 increasing EP addition levels produced a clear trend. AJ with 2.5% and 5% fortification with ELF



267 resulted in the highest values observed (8050 ± 1247 nm and 11180 ± 529 nm, respectively). LL Soft
268 drinks displayed a peak in particle sizes at 2.5% with both EPs (2700 – 4000 nm), followed by a marked
269 decrease at 10% inclusion level (320 – 360 nm). IT showed a similar pattern with significant
270 differences. NAB showed no clear relationship between inclusion level and particle size, with values
271 varying independently of EP concentration.

272 Regarding PDI, the majority of the samples obtained a PDI > 0.5 (90% of all samples) indicating
273 polydisperse systems with a wide particle size distribution.

274 3.1.7. *Foam Capacity and Stability*

275 The foam capacity and stability were analysed, and the results are displayed in Table 2. Among the
276 control samples, water, LL, and LL Free produced no measurable foam, while AJ, IT, and BT exhibited
277 low foam capacities ($26.64\% \pm 5.17$, $24.45\% \pm 2.37$, and $27.95\% \pm 2.75$, respectively). NAB displayed
278 the highest foaming capacity ($121.75\% \pm 0.47$) among the controls. Foam stability at 60 min was
279 generally low, with values of $84.85\% \pm 5.25$ (AJ), $47.22\% \pm 4.81$ (IT), $63.10\% \pm 4.29$ (BT), and $39.91\% \pm$
280 3.27 (NAB) determined for the control beverages.

281 Inclusion of either EDF or ELF significantly increased the foam capacity and stability in all beverages
282 compared to controls, except for NAB, which showed minimal changes. No consistent differences
283 were observed between EDF and ELF across the beverages. Foam capacity ranged from 113.57% to
284 142.64% with protein inclusion, showing no clear correlation with EP addition level in most beverages.
285 Overall, lower and higher protein concentrations led to a similar foam capacity, indicating that foam
286 formation was largely dependent on the presence of EP protein rather than the specific addition level.
287 Despite the increases in foam capacity with EP fortification, foam stability remained below 50% in
288 most samples.

289 3.1.8. *Tribology*



290 Soft tribology is a technique used to measure the frictional properties of food and beverages which
291 can be linked with the friction between tongue and pallet leading to mouthfeel. Figure 2 displays the
292 Stribeck curves of beverages with EDF and ELF at 10% addition level. The presented curves for the
293 beverages can be divided into static ($< 10^{-8} - 10^{-5}$ m/s), boundary ($10^{-5} - 10^{-4}$ m/s) and beginning of
294 mixed ($> 10^{-4}$ m/s) regimes.

295 Water had the highest frictional factor compared to all other beverages, followed by the remaining
296 beverage controls including LL Free, LL, NAB, AJ, IT and BT, showing frictional factors between 10^{-5} to
297 10^{-2} . although NAB and AJ showed higher frictional factors between sliding speeds 10^{-5} and 10^{-4} .

298 The inclusion of EP ingredients increased lubricating properties. ELF showed higher lubricating
299 properties in the beverages compared to EDF. AJ exhibited the highest frictional properties when
300 either of both ingredients were included. LL soft drinks showed similar Stribeck curves across static
301 and boundary regimes. The addition of ELF, followed by EDF, to BT gave the highest lubricating
302 properties. NAB showed the second highest lubricating properties followed by IT with EP inclusion.

303 3.2. Principal Component Analysis biplot

304 Two principal component analysis (PCA) biplots were performed to visualise the changes in beverage
305 characteristics following fortification with EDF (Figure 3A) or ELF (Figure 3B). The biplots captured a
306 total variance of 62.93% for EDF fortification and 58.27% for ELF fortification.

307 The biplot illustrating the influence of EDF (Figure 3A) revealed five distinct groups including group 1:
308 AJ 0%; group 2: water 0%; group 3: AJ EDF 10%; group 4: the remaining control (0%) beverages; group
309 5: the remaining 10% EDF beverages. Lower inclusion levels (2.5 and 5%) show the gradual
310 modification of beverage techno functionality, with majority of samples clustering in the region
311 associated with high dispersion instability properties. As EDF increased, a clear progression in sample
312 distribution was observed. The distinct separation of AJ (0%) and water (0%) from the other beverages
313 suggests notable differences in their techno-functional profiles. AJ with 10% EDF stands out distinctly



314 due to its high density, separation rate, particle size, PDI and viscosity, of all which are positively
315 correlating. The remaining 10% EDF beverages cluster together, sharing similar measurements for pH,
316 foam capacity and turbidity, also positively correlating.

317 Similarly, the biplot in Figure **3B** (influence of ELF) also showed five groups. Group 1: AJ 0%; group 2:
318 water 0%; group 3: AJ ELF 10%; group 4: the remaining control (0%) beverages; group 5: the remaining
319 10% ELF beverages. Increasing ELF concentration produced clear directional shifts in sample
320 clustering, with higher inclusion levels (5% and 10%) associated with lower dispersion instability
321 properties.

322 In both biplots, controls (indicated as "0%") clustered distinctly apart from samples with 10% EP
323 ingredient addition, highlighting the impact of inclusion level on techno-functionality of the beverages.
324 Hence, ingredients both show individual changes in beverage characteristics.

325 **4. Discussion**

326 The protein beverage market continues to expand, fuelled by health-conscious consumers seeking
327 clear beverage alternatives that satisfy both hydration and nutritional requirements without the
328 heaviness of protein smoothies or shakes. This trend highlights importance of developing protein
329 ingredients that perform well in a variety of beverage systems. Numerous studies have explored
330 fortifying fruit juices with protein to enhance nutritional value using primarily whey protein
331 ingredients (i.e. concentrates, isolates and hydrolysates) at levels between ~ 0.8 to 6%, as well as
332 collagen hydrolysate at a protein content of ~2.5%^{17-20,28,29}. The nutritional quality of EDF and ELF have
333 been previously characterised with both ingredients containing a high protein content (>80%). All
334 essential amino acids meet the FAO requirements for individuals above >3 years old with the
335 exception of lysine, with notable levels of branch chain amino acids. Dynamic *in vitro* digestibility of
336 EDF also demonstrated favourable amino acid digestibility with a DIAAS of 67%, exceeding values of
337 other plant-based proteins studied^{15,24}. It was further demonstrated that these ingredients possess
338 techno-functional properties that make them promising candidates for beverage applications²⁴.



339 However, those findings were limited to testing in water. The present study addresses this gap by
340 investigating both EP ingredients at various inclusion levels (2.5%, 5% and 10%) across a range of
341 commonly consumed beverages, determining whether certain beverage properties pose challenges
342 for incorporation. As these ingredients are upcycled from low cost BSG, they offer sustainable and
343 economic advantages over proteins from dedicated crops like pea, faba or soy. However, ELF's extra
344 processing increases its cost relative to EDF. Higher inclusion levels (2.5 – 10% w/w; 2-8g
345 protein/100ml) cover cost effective moderate fortification to premium formulations consistent with
346 market data showing higher protein contents and carbonated types were more expensive²⁴. Pictures
347 of each beverage are displayed in Figure 4, where clear differences can be seen between the inclusion
348 of EDF and ELF. Fortifying beverages with EDF resulted in solutions with brown shade colours,
349 increasing in darkness with increased addition level. The addition of ELF, on the other hand, caused a
350 yellow shade colour at low concentrations, turning to dark orange and even brown colours at higher
351 inclusion levels. This is, putatively, due to the lighter colour of ELF powder, where the decolourisation
352 process likely removed many of these Maillard reaction compounds (e.g. melanoidin) originating from
353 the brewing process²³ However, residual pigments and their concentration showed to proportionally
354 darken the beverages.

355 The majority of soft drinks have low pH values due to the addition of acidulants and, in the case of
356 carbonated beverages, the presence of dissolved carbonic acid¹. EverPro[®] ingredients possess the
357 unique functional property of being highly soluble over a range of pH values. At pH 4, the pH closest
358 to their isoelectric points, both EDF and ELF exhibited high protein solubility values of 82% and 80%
359 respectively²³. The pH values of the chosen beverages (excluding water) fell within the acidic range of
360 3.1 – 4.9, as expected due to the presence of acidulants such as malic and citric acid¹. The beverages
361 ranked by acidity from highest to lowest pH were as follows: IT > LL > LL Free > AJ > NAB > BT. The BT
362 and NAB showed the lowest acidity amongst the beverages (excluding water). BT includes organic
363 acids and polyphenols, such as tannins, developed from the oxidation/fermentation of tea leaves
364 which can possess weakly acidic properties³⁰. Additionally, NAB contains low amounts of organic acids



365 synthesised during the brewing process^{27,31}. IT exhibited the highest acidity, attributed to a
366 combination of organic acids, polyphenols from the black tea extract, and added acidulants^{1,30}.
367 Increased inclusion of both EP ingredients substantially increased the pH value closer to the neutral
368 range. When both EP ingredients were gradually added to water, there was no change in average pH
369 for all inclusion levels of EDF and ELF. Since proteins can act as a buffer, the addition of EP ingredients
370 to a soft drink can interact with its acidic components. Specifically acidic amino acids with negatively
371 charged side chains, such as aspartic and glutamic acid (~30g/100g of EP protein), can bind to the
372 hydrogen ions in an acidic environment³². This interaction can reduce free hydrogen concentration,
373 leading to an increase in pH within the beverage demonstrating their buffering capacity. Organic acids
374 and ash can also contribute to a higher buffering capacity, where the slightly higher ash content
375 observed in EDF compared to ELF may have influenced its elevated pH^{23,32}.

376 The liquid density of a beverage can be influenced by the amount of total soluble solids present³³.
377 Additionally, soluble solids in a beverage system can elevate viscosity. Both density and viscosity
378 showed similar trends. Beverage controls containing higher sugar contents (LL, AJ) exhibited higher
379 density and viscosity results compared to those that used artificial sweeteners (IT, LL Free), as reported
380 previously^{34,35}. When sugar is replaced with artificial sweeteners, the reduction in bulk or density of
381 the beverages leads to a significant decrease in viscosity, whereas if added at equivalent
382 concentrations, these parameters remain comparable³⁶. As artificial sweeteners can be 200 to 500
383 times sweeter than sucrose, only very minimal quantities are required³⁷ evident in the reduction of
384 sugar from 4.7g in LL to 0g in LL Free, replaced by with aspartame and acesulfame K. Although the
385 NAB is low in sugar, it showed the highest apparent viscosity, which is likely due to the presence of
386 complex carbohydrates, such as beta glucan and dextrin from the malted barley, which contribute to
387 viscosity²⁷. Higher concentrations of both EP ingredients resulted in increased density and viscosity
388 values with significant positive correlations (with the exception of IT EDF for viscosity), as seen
389 previously in fruit flavoured beverages fortified with whey protein ingredients¹⁹. Specifically, the
390 addition of ELF led to higher density and viscosity values compared to EDF. The PCA biplots clearly



391 demonstrates this by the distinct separate clusters of 0% and 10% inclusions. ELF went through further
392 processing compared to EDF, particularly a decolourisation step. Decolourisation has been shown to
393 alter the functional properties of proteins, particularly increasing water holding capacity, thereby
394 increasing the viscosity³⁸. This occurs due to an oxidation process expanding the protein structure and
395 allowing for the more efficient binding of water³⁸. Similar results have been observed for decolorised
396 soy protein isolate, which also demonstrated increased water holding capacity³⁹. EP originates from
397 brewers spent grain and has been shown to consist of low molecular weight proteins and peptides²³.
398 This lack of large and folded structure of intact proteins can cause the lower water holding capacity
399 leading to a lower change in viscosity. Additionally, ELF resulted in larger particle sizes in the water
400 solution compared to EDF, which could be attributed to the different native pH values of the
401 ingredients in water affecting protein charge. EDF has a higher pH than ELF, thus being further away
402 from the isoelectric point (pI ~3.4), leading to higher repulsion forces and improved dispersion and/or
403 hydration²³. This was shown by Dissanayake et al. (2013) where whey protein dispersions exhibited
404 increased viscosity with decreasing pH values, due to increased repulsive forces at higher pHs and
405 enhanced aggregation of protein at lower pHs.

406 Stability in soft drinks is essential for maintaining consistent quality, appearance, and flavour
407 throughout the products shelf life, ensuring consumer satisfaction. Many ready-to-drink protein
408 beverages with both animal- and plant-based protein sources on the market tend to have neutral pHs
409 with few being acidic^{24,41}. Stabilizing plant-based protein ingredients in an acidic environment can be
410 a challenge as these proteins tend to precipitate around their isoelectric point, typically within this pH
411 range. The pH of these beverages is important as it has been found that pH values far from their
412 isoelectric point play a key role in the clearness of beverages because of the electrostatically repulsive
413 forces amongst unfolded proteins or aggregated proteins/peptides⁴². This was seen in the current
414 study where EP inclusion level at 2.5% in soft drinks resulted in the most acidic pH values and exhibited
415 the highest turbidity and separation rate. On the other hand, 10% addition resulted in the highest pH
416 values, with the clearest appearance and the lowest separation rates with little to no sedimentation.



417 Particle size trends indicated that the largest size was observed at the 2.5% addition, with significant
418 correlations not observed with increased inclusion levels. As seen in the PCA biplots (Figure 3), AJ is
419 distinctly separated at both 0% and 10% inclusions due to its high particle size and separation rate,
420 likely attributed to its natural composition including residual pulp which increases particle size, thus
421 affecting stability as highlighted⁴³. One study demonstrated that adjusting the pH to an optimum value
422 resulted in a reduction in the mean particle size for both whey protein isolate/hydrolysate, with
423 beverages with smaller particle sizes showing the clearest appearance¹⁸. One method of trying to
424 stabilize an acidic protein beverage can be the use of different polysaccharides such as beta glucan,
425 low methoxyl pectin and chitosan that can alter the charge characteristics, potentially increasing
426 stability⁴⁴. The presence of beta glucan in the NAB beverages may explain the low separation rates
427 observed across the varying EP concentrations. Another study investigated the use of six different
428 hydrocolloids (as stabilizing agents) to prevent phase separation, where particle size decreased with
429 increasing concentrations of some stabilizers⁴⁵.

430 While raising the pH can improve solubility and stability of proteins, it may also impact the expected
431 tangy, acidic flavour as well as colour of a soft drink. Additionally, a higher pH can increase
432 susceptibility to microbial spoilage as well as decrease the effectiveness of preservatives (i.e. sorbic
433 and benzoic acid) and therefore measures such as pasteurisation or UHT need to be considered¹.

434 Soft tribology analysis provides insight into the mouthfeel of oral processing of foods and beverages.
435 Higher frictional coefficients are generally associated with increased surface roughness and sensations
436 such as astringency or dryness, while lower friction reflects smoother lubrication behaviour⁴⁶. This
437 method has been widely applied to beverages such as beer, wine, tea and soft drinks^{27,47-49}. In this
438 study, ELF showed higher lubricating properties than EDF. These results are consistent with a previous
439 study²³ that reports that the presence of free amino acids and organic acids can influence frictional
440 properties, and ELF showed reduced metabolite^{50,51}. Compared with their controls, NAB and AJ
441 showed elevated frictional factors at the boundary regime believed to be associated with

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442 astringency⁵². Comparable findings have been reported for other NABs, where high friction
443 corresponds with sensory descriptors of thin or watery mouthfeel relative to alcoholic ones described
444 as creamy and smooth²⁷. LL Free had higher frictional properties compared to LL with added sugar.
445 The addition of EDF or ELF decreased frictional factors, therefore may sensorially decrease astringency
446 and increase the viscosity of the mouthfeel. Contrary to expectations, BT and IT, typically perceived
447 as astringent, exhibited the opposite tribological behaviour with the highest lubricating properties,
448 even in control samples with no EP addition. One explanation is that the addition of protein may
449 facilitate the formation of a thin, lubricating interfacial layer, improving sliding between the ball and
450 pin, as previously proposed⁵³. Although polyphenols generally increase oral friction by forming
451 precipitating complexes with proteins, the behaviour of BT and IT suggests that the nature of protein–
452 polyphenol interactions depend on factors such as molecular size, charge, tannin flexibility and protein
453 conformation⁵⁴. For example, large protein–polyphenol aggregates can form lubricating films⁵⁵, while
454 tea types also differ in polyphenol composition, with green tea typically containing higher polyphenol
455 levels than black tea⁵⁶. However, while this study shows IT exhibiting smaller particle sizes (~350 nm)
456 and BT showing larger particle sizes (~2000 nm), the literature shows conflicting findings, with studies
457 reporting that mainly smaller particle sizes have reduced frictional parameters^{46,53}. However, the tests
458 in the current study were conducted without the addition of saliva which would further influence the
459 lubrication behaviour under real oral conditions. Therefore, this discrepancy needs to be considered
460 and highlights how a combination of factors can be involved in tribological behaviour.

461 5. Conclusion

462 Several beverage systems were evaluated following the addition of EDF and ELF at various
463 concentrations. Clear differences were found depending on both the concentration of protein added,
464 and the type of beverage system studied. Increased protein concentration elevated the pH across all
465 beverage systems, leading to a trend whereby beverages became more stable and clearer at higher
466 protein levels due to changes in solubility as a result of shifts in their isoelectric points. Moreover,



467 protein inclusion increased both viscosity and liquid density accordingly. Lubricating properties
 468 increased with the addition of EP ingredients in all beverages, with ELF showing more effective
 469 lubrication in all beverage systems. Beverages with polyphenols, such as BT and IT, demonstrated the
 470 lowest frictional parameters regardless of protein inclusion level. Incorporating both EP ingredients
 471 into these beverages enhanced their protein content by offering a convenient ready-to-drink protein
 472 soft drink, while also promoting sustainability benefits through the use of upcycled proteins.
 473 Nevertheless, the current study highlights the need for further research to address the impact of
 474 protein addition, particularly plant-based proteins, on the stability and quality of these beverages. No
 475 single beverage system demonstrated a clear advantage over another and it should be noted that
 476 protein inclusion can potentially compromise sensory properties, which should be carefully
 477 considered during formulation. Future work should investigate the antioxidant properties of these
 478 ingredients and their interactions within the beverage systems including polyphenols, particularly in
 479 tea based beverages. Furthermore, trials to ensure microbial stability are essential for safe
 480 consumption, especially in ready-to-drink soft drinks. Continued research is key for advancing the
 481 development of nutritious, stable, safe, consumer accepted protein fortified beverages.

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6. Abbreviations

EP	EverPro™
EDF	EverPro™ Dark Fraction
ELF	EverPro™ Light Fraction
AJ	Apple juice
LL	Lemon and lime
LL Free	Lemon and lime sugar free
IT	Ice tea (sweetened)
BT	Black tea (unsweetened)
NAB	Non-alcoholic beer

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492 **Niamh Ahern**.; Resources, Aylin W. Sahin and **Elke K. Arendt**.; Data Curation, **Niamh Ahern**; Writing –
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517**7. References**518
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620 **8. Tables**

621 **8.1. Table 1 – Ingredients list and nutritional composition in grams per 100ml of beverages from**
 622 **labels. An * - indicates carbonated soft drink.**

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Soft Drink	Calories (kcal)	Carbohydrates (g)	Of which sugar (g)	Ingredients List
Water	0	0	0	Water
Apple Juice	46	11.1	11.1	Apple juice from concentrate
Lemon & Lime*	19	4.7	4.7	Carbonated Water, Sugar, Acids (Citric Acid, Malic Acid), Natural Lemon and Lime Flavouring with other Natural Flavourings, Acidity Regulator (Sodium Citrate), Sweeteners (Acesulfame K, Sucralose)
Lemon & Lime Free*	1	0	0	Carbonated Water, Acids (Citric Acid, Malic Acid), Natural Lemon and Lime Flavouring, Acidity Regulator (Sodium Citrate), Sweeteners (Aspartame, Acesulfame K), Preservative (Sodium Benzoate)
Ice Tea Peach	13	3.1	3	Water, Sugars (Sucrose, Fructose), Acids (Malic Acid, Citric Acid), Black Tea Extract (0.12%), Peach Juice from Concentrate (0.1%), Acidity Regulator (Trisodium Citrate), Flavourings, Antioxidant (Ascorbic Acid), Sweetener (Steviol Glycosides from Stevia)
Black Tea	0	0	0	100% Black Tea
Non – Alcoholic Beer*	14	3.1	0.2	Water, Malted Barley, Hops, Yeast



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8.2. **Table 2** – Physicochemical properties of soft drinks including EverPro Dark Fraction (D) or EverPro Light Fraction (L). Statistical analysis was performed within the same drink identifying the impact of EverPro on the soft drinks' characteristics. Different lower-case letters within the same beverage type indicates significant differences ($p < 0.05$). n.a – non applicable.

	Soft Drink and EverPro inclusion level	pH	Apparent Viscosity at 80.7 1/s (mPa.s)	Density (g/cm ³)	Foaming capacity (%)	Foaming stability (%)
Control	Water control (0%)	6.71 ± 0.00 ^a	1.07 ± 0.13 ^a	0.9985 ± 0.0000 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
	Water D 2.5%	8.15 ± 0.02 ^b	1.27 ± 0.13 ^{ab}	1.0061 ± 0.0001 ^b	120.03 ± 3.53 ^b	36.9 ± 0.57 ^f
	Water D 5%	8.25 ± 0.05 ^b	1.57 ± 0.08 ^{bc}	1.0138 ± 0.0000 ^c	128.99 ± 1.26 ^{bc}	37.07 ± 1.38 ^f
	Water D 10%	8.24 ± 0.05 ^b	2.56 ± 0.17 ^d	1.0295 ± 0.0000 ^d	121.92 ± 2.44 ^b	28.14 ± 1.94 ^d
	Water L 2.5%	7.45 ± 0.01 ^c	1.36 ± 0.06 ^{ab}	1.0064 ± 0.0001 ^e	134.12 ± 3.22 ^c	19.89 ± 0.19 ^b
	Water L 5%	7.45 ± 0.01 ^c	1.81 ± 0.09 ^c	1.0143 ± 0.0001 ^f	128.57 ± 1.10 ^{bc}	23.9 ± 1.33 ^c
	Water L 10%	7.45 ± 0.01 ^c	3.29 ± 0.10 ^e	1.0304 ± 0.0001 ^g	125.92 ± 7.91 ^{bc}	33.12 ± 0.65 ^e
Juice	Apple Juice control (0%)	3.51 ± 0.01 ^a	1.23 ± 0.05 ^f	1.0448 ± 0.0000 ^e	26.64 ± 5.17 ^c	84.85 ± 5.25 ^a
	Apple Juice D 2.5%	4.26 ± 0.08 ^b	1.89 ± 0.01 ^e	1.0517 ± 0.0001 ^d	124.83 ± 2.50 ^{ab}	34.64 ± 1.17 ^{bc}
	Apple Juice D 5%	4.74 ± 0.02 ^c	2.68 ± 0.47 ^{cd}	1.0586 ± 0.0001 ^c	130.68 ± 2.54 ^{ab}	36.87 ± 1.70 ^{bc}
	Apple Juice D 10%	5.49 ± 0.06 ^d	4.07 ± 0.25 ^b	1.0728 ± 0.0010 ^b	133.82 ± 2.37 ^a	43.99 ± 1.93 ^b
	Apple Juice L 2.5%	4.12 ± 0.01 ^e	2.04 ± 0.06 ^{de}	1.052 ± 0.0001 ^d	120.23 ± 7.04 ^b	37.99 ± 6.19 ^{bc}
	Apple Juice L 5%	4.49 ± 0.01 ^f	2.88 ± 0.28 ^c	1.0594 ± 0.0002 ^c	122.91 ± 3.64 ^b	32.01 ± 5.59 ^{cd}
	Apple Juice L 10%	4.98 ± 0.02 ^g	5.43 ± 0.12 ^a	1.0743 ± 0.0001 ^a	122.61 ± 4.90 ^b	21.22 ± 1.83 ^d
Soft drink (full sugar)	Lemon Lime control (0%)	3.30 ± 0.02 ^f	1.25 ± 0.06 ^d	1.0175 ± 0.0000 ^e	0.00 ± 0.00 ^e	0.00 ± 0.00 ^f
	Lemon Lime D 2.5%	5.03 ± 0.06 ^d	1.59 ± 0.05 ^{de}	1.0249 ± 0.0001 ^d	118.05 ± 3.77 ^{cd}	38.41 ± 1.49 ^b
	Lemon Lime D 5%	5.68 ± 0.02 ^b	1.86 ± 0.17 ^{cd}	1.0325 ± 0.0001 ^c	113.57 ± 1.16 ^d	35.22 ± 0.62 ^{bc}
	Lemon Lime D 10%	6.57 ± 0.02 ^a	2.81 ± 0.27 ^b	1.0473 ± 0.0005 ^b	142.64 ± 2.34 ^a	45.36 ± 2.03 ^a
	Lemon Lime L 2.5%	4.65 ± 0.0 ^e	1.80 ± 0.04 ^d	1.0249 ± 0.0001 ^d	124.68 ± 2.96 ^{bc}	29.64 ± 1.49 ^d
	Lemon Lime L 5%	5.07 ± 0.01 ^d	2.26 ± 0.03 ^c	1.0325 ± 0.0003 ^c	124.44 ± 2.22 ^b	23.23 ± 2.05 ^e
	Lemon Lime L 10%	5.56 ± 0.01 ^c	3.98 ± 0.27 ^a	1.0482 ± 0.0002 ^a	123.74 ± 2.06 ^{bc}	34.31 ± 0.36 ^c
Soft drink (no sugar)	Free Lemon Lime control (0%)	3.30 ± 0.02 ^f	1.04 ± 0.03 ^e	0.9997 ± 0.0000 ^e	0.00 ± 0.00 ^b	0.00 ± 0.00 ^c
	Free Lemon Lime D 2.5%	4.95 ± 0.02 ^d	1.39 ± 0.03 ^d	1.0072 ± 0.0000 ^d	122.17 ± 3.55 ^a	36.83 ± 3.21 ^a
	Free Lemon Lime D 5%	5.63 ± 0.01 ^b	1.72 ± 0.06 ^c	1.0149 ± 0.0001 ^c	121.74 ± 2.17 ^a	36.31 ± 2.59 ^a
	Free Lemon Lime D 10%	6.48 ± 0.04 ^a	2.54 ± 0.18 ^b	1.0303 ± 0.0009 ^b	127.25 ± 9.12 ^a	38.61 ± 3.13 ^a
	Free Lemon Lime L 2.5%	4.65 ± 0.00 ^e	1.64 ± 0.08 ^{cd}	1.0073 ± 0.0001 ^d	122.46 ± 1.26 ^a	17.75 ± 1.79 ^b
	Free Lemon Lime L 5%	5.08 ± 0.01 ^c	1.82 ± 0.12 ^c	1.0153 ± 0.0001 ^c	120 ± 6.14 ^a	18.95 ± 1.27 ^b
	Free Lemon Lime L 10%	5.60 ± 0.01 ^b	3.41 ± 0.05 ^a	1.0314 ± 0.0003 ^a	120.29 ± 6.28 ^a	35.04 ± 2.96 ^a
Sweetened tea-based beverage	Ice Tea control (0%)	3.10 ± 0.00 ^a	1.09 ± 0.13 ^f	1.0177 ± 0.0000 ^a	24.45 ± 2.37 ^e	47.22 ± 4.81 ^a
	Ice Tea D 2.5%	4.71 ± 0.01 ^b	1.54 ± 0.12 ^e	1.025 ± 0.0001 ^b	117.3 ± 2.56 ^d	36.81 ± 1.89 ^{bc}
	Ice Tea D 5%	5.44 ± 0.02 ^c	1.93 ± 0.14 ^{cd}	1.0326 ± 0.0001 ^c	118.3 ± 3.69 ^{cd}	30.25 ± 1.20 ^{cd}
	Ice Tea D 10%	6.68 ± 0.02 ^d	3.09 ± 0.06 ^b	1.0479 ± 0.0000 ^d	122.46 ± 1.26 ^{bcd}	34.92 ± 2.25 ^{bcd}
	Ice Tea L 2.5%	4.53 ± 0.01 ^e	1.73 ± 0.10 ^{de}	1.0252 ± 0.0000 ^e	125.02 ± 1.63 ^{abc}	29.75 ± 3.05 ^d



	Ice Tea L 5%	4.99 ± 0.01 ^f	2.25 ± 0.03 ^c	1.0328 ± 0.0001 ^f	128.6 ± 3.63 ^{ab}	19.44 ± 0.71 ^g
	Ice Tea L 10%	5.56 ± 0.03 ^g	4.21 ± 0.19 ^a	1.0485 ± 0.0002 ^g	131.16 ± 1.26 ^a	39.23 ± 0.84 ^b
Tea (unsweetened)	Black Tea control (0%)	4.88 ± 0.02 ^a	1.10 ± 0.06 ^e	0.9995 ± 0.0000 ^a	27.95 ± 2.75 ^d	63.1 ± 4.29 ^a
	Black Tea D 2.5%	7.56 ± 0.01 ^b	1.33 ± 0.06 ^{de}	1.0071 ± 0.0000 ^b	129.63 ± 3.39 ^{ab}	40.55 ± 1.70 ^b
	Black Tea D 5%	7.72 ± 0.04 ^c	1.57 ± 0.08 ^{cd}	1.0144 ± 0.0005 ^d	131.2 ± 3.09 ^a	39.23 ± 0.84 ^b
	Black Tea D 10%	7.79 ± 0.02 ^d	2.30 ± 0.04 ^b	1.0303 ± 0.0001 ^e	121.25 ± 4.01 ^{bc}	35.69 ± 0.68 ^{bc}
	Black Tea L 2.5%	6.83 ± 0.02 ^e	1.36 ± 0.07 ^{de}	1.0073 ± 0.0001 ^b	130.94 ± 1.48 ^a	18.14 ± 1.81 ^d
	Black Tea L 5%	7.20 ± 0.01 ^f	1.80 ± 0.14 ^c	1.0152 ± 0.0000 ^c	122.65 ± 3.54 ^{abc}	30.93 ± 2.08 ^c
	Black Tea L 10%	7.32 ± 0.03 ^g	3.42 ± 0.27 ^a	1.0309 ± 0.0003 ^f	118.72 ± 2.62 ^c	30.89 ± 1.26 ^c
Malt-based beverage	Non Alcoholic Beer control (0%)	4.32 ± 0.00 ^a	1.46 ± 0.05 ^c	1.0156 ± 0.0000 ^d	121.75 ± 0.47 ^{bc}	39.91 ± 3.27 ^a
	Non Alcoholic Beer D 2.5%	6.01 ± 0.02 ^b	1.77 ± 0.06 ^c	1.0227 ± 0.0002 ^c	124.09 ± 2.20 ^{bc}	35.32 ± 2.39 ^a
	Non Alcoholic Beer D 5%	6.64 ± 0.03 ^c	2.06 ± 0.18 ^c	1.0306 ± 0.0004 ^b	117.39 ± 2.17 ^c	26.59 ± 4.31 ^b
	Non Alcoholic Beer D 10%	7.27 ± 0.01 ^d	3.19 ± 0.32 ^b	1.0457 ± 0.0006 ^a	133.36 ± 3.58 ^a	41.3 ± 1.31 ^a
	Non Alcoholic Beer L 2.5%	5.26 ± 0.01 ^e	1.89 ± 0.06 ^c	1.0231 ± 0.0001 ^c	126.35 ± 4.39 ^{ab}	11.55 ± 0.89 ^c
	Non Alcoholic Beer L 5%	5.62 ± 0.02 ^f	2.30 ± 0.14 ^c	1.0309 ± 0.0000 ^b	118.15 ± 2.83 ^c	5.52 ± 0.06 ^c
	Non Alcoholic Beer L 10%	6.08 ± 0.01 ^g	4.82 ± 0.33 ^a	1.0464 ± 0.0003 ^a	125.89 ± 1.88 ^{ab}	25.11 ± 2.04 ^b

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632 **8.1. Table 3 – Dispersion properties of soft drinks including EverPro Dark Fraction (D) or EverPro**
 633 **Light Fraction (L). Statistical analysis was performed within the same drink identifying the**
 634 **impact of EverPro on the soft drinks' characteristics. Different lower-case letters within the**
 635 **same beverage type indicates significant differences ($p < 0.05$). n.a – non applicable.**

	Soft Drink and EverPro inclusion level	Turbidity (NTU)	Particle size (nm)	Polydispersity index	Separation rate (%/min)
Control	Water control (0%)	0.16 ± 0.04 ^a	n.a	n.a	0.00 ± 0.00 ^a
	Water D 2.5%	25.7 ± 0.53 ^b	298 ± 16 ^d	0.59 ± 0.04 ^a	0.09 ± 0.00 ^b
	Water D 5%	42.73 ± 0.06 ^c	306 ± 21 ^{cd}	0.65 ± 0.04 ^a	0.14 ± 0.00 ^c
	Water D 10%	58.53 ± 0.58 ^d	417 ± 7 ^b	0.57 ± 0.02 ^a	0.20 ± 0.00 ^d
	Water L 2.5%	52.5 ± 0.17 ^e	256 ± 9 ^e	0.64 ± 0.19 ^a	0.06 ± 0.00 ^e
	Water L 5%	96.93 ± 0.78 ^f	338 ± 9 ^c	0.55 ± 0.05 ^a	0.11 ± 0.00 ^f
	Water L 10%	184.33 ± 0.58 ^g	562 ± 7 ^a	0.56 ± 0.08 ^a	0.17 ± 0.01 ^g
Juice	Apple Juice control (0%)	4.26 ± 0.05 ^c	2560 ± 48 ^d	1.00 ± 0.00 ^a	0.04 ± 0.00 ^d
	Apple Juice D 2.5%	625 ± 22.07 ^b	4607 ± 1228 ^c	0.85 ± 0.26 ^a	5.26 ± 0.46 ^b
	Apple Juice D 5%	360.67 ± 9.45 ^d	2560 ± 491 ^d	0.22 ± 0.08 ^b	6.09 ± 0.19 ^{ab}
	Apple Juice D 10%	466.67 ± 6.51 ^e	6509 ± 420 ^{bc}	0.83 ± 0.29 ^a	5.4 ± 0.13 ^{ab}
	Apple Juice L 2.5%	670.33 ± 13.32 ^b	8050 ± 1247 ^b	1.00 ± 0.00 ^a	5.82 ± 0.28 ^{ab}
	Apple Juice L 5%	548.67 ± 12.22 ^f	11180 ± 528 ^a	1.00 ± 0.00 ^a	6.18 ± 0.02 ^a
	Apple Juice L 10%	956.67 ± 41.31 ^a	834 ± 46 ^d	0.85 ± 0.13 ^a	1.21 ± 0.54 ^c
Soft drink (full sugar)	Lemon Lime control (0%)	0.24 ± 0.11 ^a	917 ± 219 ^b	0.45 ± 0.13 ^c	0.02 ± 0.00 ^c
	Lemon Lime D 2.5%	1768.67 ± 13.61 ^b	3354 ± 324 ^a	1.00 ± 0.00 ^a	2.84 ± 0.30 ^a
	Lemon Lime D 5%	617.67 ± 14.01 ^d	943 ± 80 ^b	0.22 ± 0.03 ^d	0.49 ± 0.04 ^b
	Lemon Lime D 10%	770.33 ± 62.31 ^c	338 ± 14 ^b	0.72 ± 0.06 ^b	0.19 ± 0.02 ^{bc}
	Lemon Lime L 2.5%	> 2000	3977 ± 1342 ^a	1.00 ± 0.00 ^a	2.68 ± 0.11 ^a
	Lemon Lime L 5%	807.00 ± 37.24 ^c	253 ± 11 ^b	0.50 ± 0.04 ^c	0.13 ± 0.00 ^c



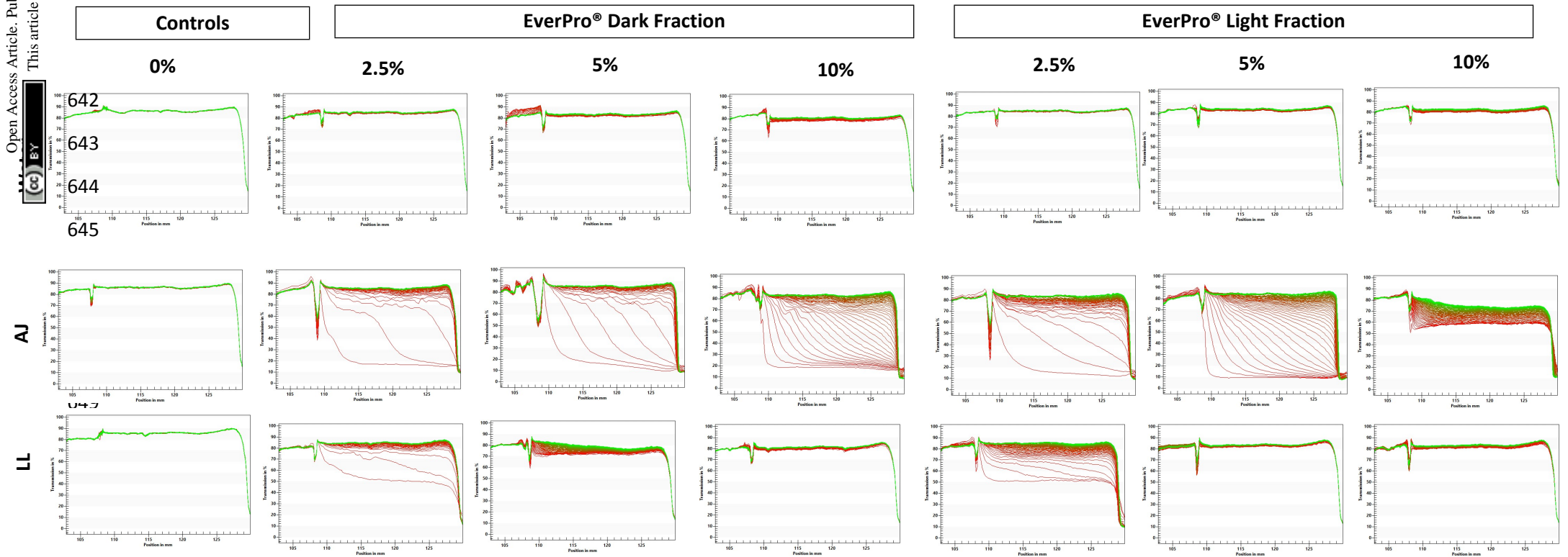
	Lemon Lime L 10%	165.00 ± 37.36 ^e	406 ± 20 ^b	0.67 ± 0.03 ^b	0.20 ± 0.01 ^e
Soft drink (no sugar)	Free Lemon Lime control (0%)	0.38 ± 0.25 ^a	1035 ± 110 ^{cd}	0.47 ± 0.13 ^c	0.03 ± 0.00 ^c
	Free Lemon Lime D 2.5%	927.00 ± 52.85 ^b	3981 ± 742 ^a	1.00 ± 0.00 ^a	3.31 ± 0.05 ^a
	Free Lemon Lime D 5%	984.00 ± 70.00 ^b	1571 ± 38 ^c	0.80 ± 0.04 ^{ab}	1.82 ± 0.10 ^b
	Free Lemon Lime D 10%	564.67 ± 39.80 ^c	319 ± 18 ^d	0.65 ± 0.07 ^{bc}	0.21 ± 0.01 ^c
	Free Lemon Lime L 2.5%	> 2000	2668 ± 671 ^b	1.00 ± 0.13 ^a	3.23 ± 0.14 ^a
	Free Lemon Lime L 5%	267.33 ± 13.61 ^d	277.07 ± 27 ^d	0.54 ± 0.01 ^c	0.21 ± 0.02 ^c
	Free Lemon Lime L 10%	189.67 ± 32.52 ^d	360 ± 46 ^d	0.67 ± 0.01 ^{bc}	0.19 ± 0.01 ^c
Sweetened tea-based beverage	Ice Tea control (0%)	23.67 ± 0.42 ^d	187 ± 31 ^c	0.30 ± 0.07 ^{bc}	0.04 ± 0.01 ^e
	Ice Tea D 2.5%	980.00 ± 3.61 ^a	3543 ± 365 ^a	0.58 ± 0.43 ^{abc}	4.16 ± 0.27 ^b
	Ice Tea D 5%	953.33 ± 16.86 ^a	3621 ± 540 ^a	0.94 ± 0.10 ^a	2.76 ± 0.23 ^c
	Ice Tea D 10%	82.03 ± 2.80 ^d	366 ± 47 ^c	0.71 ± 0.01 ^{ab}	0.19 ± 0.01 ^e
	Ice Tea L 2.5%	1006.33 ± 54.86 ^a	2427 ± 515 ^b	0.15 ± 0.05 ^c	5.16 ± 0.07 ^a
	Ice Tea L 5%	781.00 ± 12.12 ^b	1736 ± 182 ^b	0.89 ± 0.20 ^a	0.98 ± 0.10 ^d
	Ice Tea L 10%	186.00 ± 4.36 ^c	341 ± 4 ^c	0.54 ± 0.00 ^{abc}	0.20 ± 0.01 ^e
Tea (unsweetened)	Black Tea control (0%)	27.47 ± 1.25 ^f	309 ± 28 ^d	0.68 ± 0.07 ^a	0.08 ± 0.01 ^e
	Black Tea D 2.5%	789.00 ± 12.29 ^c	145 ± 7 ^d	0.14 ± 0.01 ^b	0.25 ± 0.02 ^e
	Black Tea D 5%	737.67 ± 12.06 ^{de}	191 ± 5 ^d	0.15 ± 0.02 ^b	0.35 ± 0.01 ^{de}
	Black Tea D 10%	699.67 ± 13.01 ^e	2337 ± 76 ^a	0.14 ± 0.01 ^b	0.90 ± 0.09 ^{cd}
	Black Tea L 2.5%	1641.33 ± 13.8 ^a	197 ± 6 ^d	0.15 ± 0.01 ^b	1.31 ± 0.00 ^c
	Black Tea L 5%	765.67 ± 17.62 ^{cd}	607 ± 120 ^c	0.26 ± 0.02 ^b	5.13 ± 0.29 ^a
Malt-based beverage	Black Tea L 10%	1151.33 ± 20.50 ^b	1809 ± 215 ^b	0.51 ± 0.19 ^a	3.53 ± 0.43 ^b
	Non Alcoholic Beer control (0%)	5.05 ± 0.15 ^e	465 ± 47 ^c	0.55 ± 0.03 ^{ab}	0.03 ± 0.00 ^e
	Non Alcoholic Beer D 2.5%	766.33 ± 9.29 ^a	2472 ± 305 ^a	0.16 ± 0.09 ^c	1.03 ± 0.02 ^a
	Non Alcoholic Beer D 5%	258.33 ± 23.16 ^b	380 ± 40 ^c	0.57 ± 0.04 ^{ab}	0.15 ± 0.01 ^c
	Non Alcoholic Beer D 10%	112.10 ± 33.05 ^d	614 ± 88 ^{bc}	0.51 ± 0.12 ^b	0.19 ± 0.01 ^b
	Non Alcoholic Beer L 2.5%	88.90 ± 1.73 ^d	336 ± 27 ^c	0.71 ± 0.05 ^a	0.12 ± 0.0 ^d
	Non Alcoholic Beer L 5%	92.97 ± 1.96 ^d	355 ± 56 ^c	0.6 ± 0.12 ^{ab}	0.16 ± 0.01 ^c
Non Alcoholic Beer L 10%	163.33 ± 1.53 ^c	973 ± 177 ^b	0.44 ± 0.02 ^b	0.21 ± 0.00 ^b	

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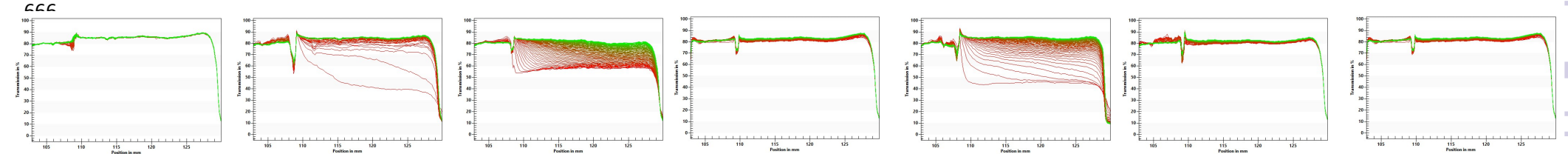
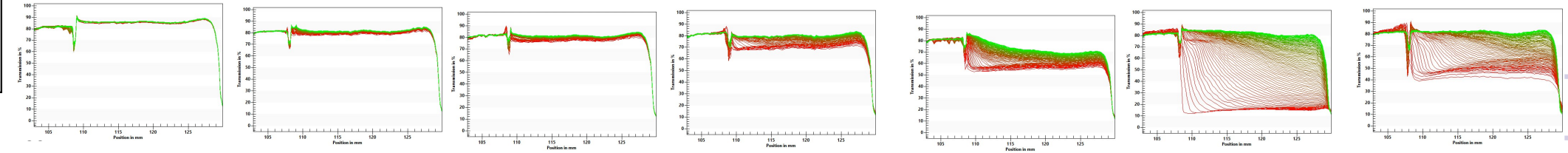
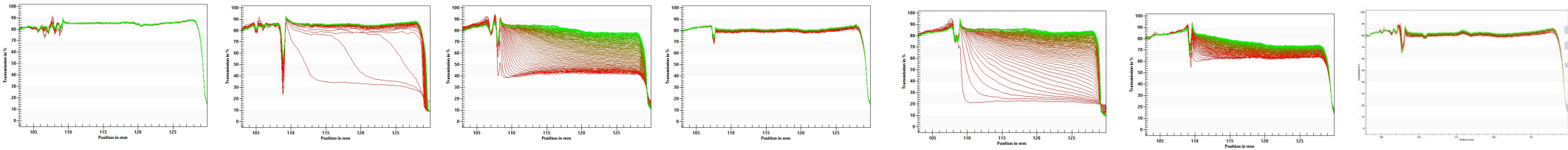
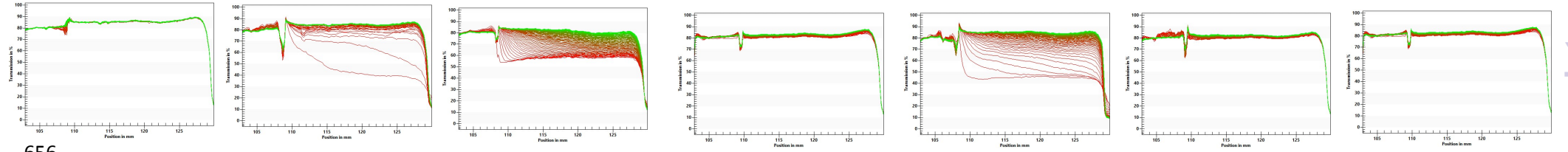
638 **9. Figures**

639 **9.1. Figure 1** - Lumisizer light transmission profiles of soft drinks containing 0%, 2.5%, 5%, 10% of EverPro Dark Fraction (EDF) or EverPro Light Fraction
 640 (ELF) as a function of time. (AJ) apple juice, (LL) lemon and lime, (LL Free) lemon and lime sugar free, (IT) ice tea, (BT) black tea, (NAB) non-alcoholic
 641 beer.





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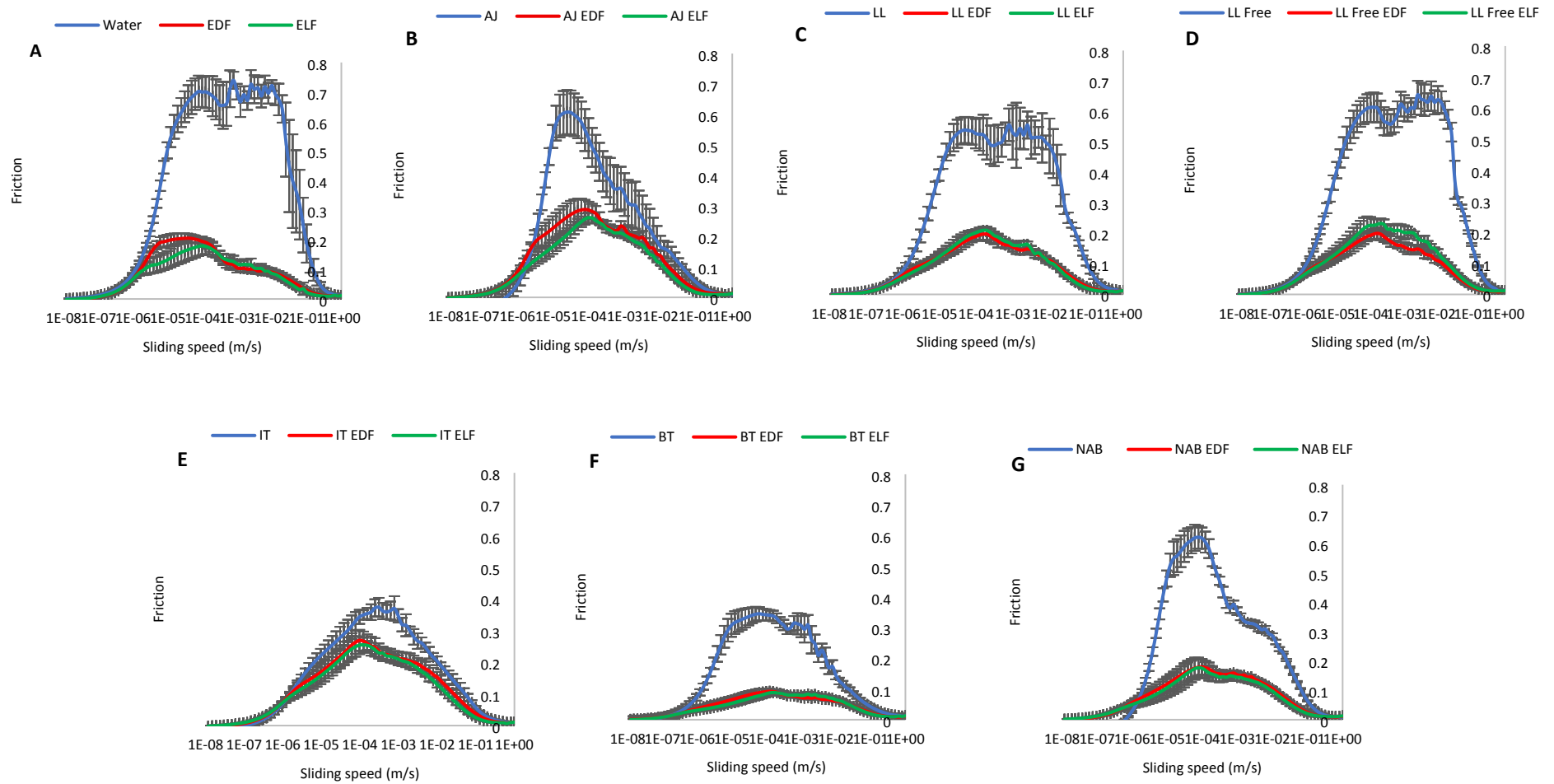


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9.1. **Figure 2** – Stribeck curves of soft drinks containing EDF and ELF at 10%. Error bars indicate standard deviation. (A) water ²³, (B) apple juice, (C) lemon and lime, (D) lemon and lime sugar free, (E) ice tea, (F) black tea, (G) non-alcoholic beer.



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677 9.2. **Figure 3** – Principal component analysis biplot. (A) EverNAVIPro Dark (EDF) and (B) Light

678 Fraction (ELF) at 0% (●), 2.5%(●), 5%(●) and 10% (●) ingredient inclusion level of beverages

679 analysed including water, apple juice (AJ), lemon and lime (LL), lemon and lime sugar free

680 (LL free), ice tea (IT) black tea (BT) and non-alcoholic beer (NAB) .

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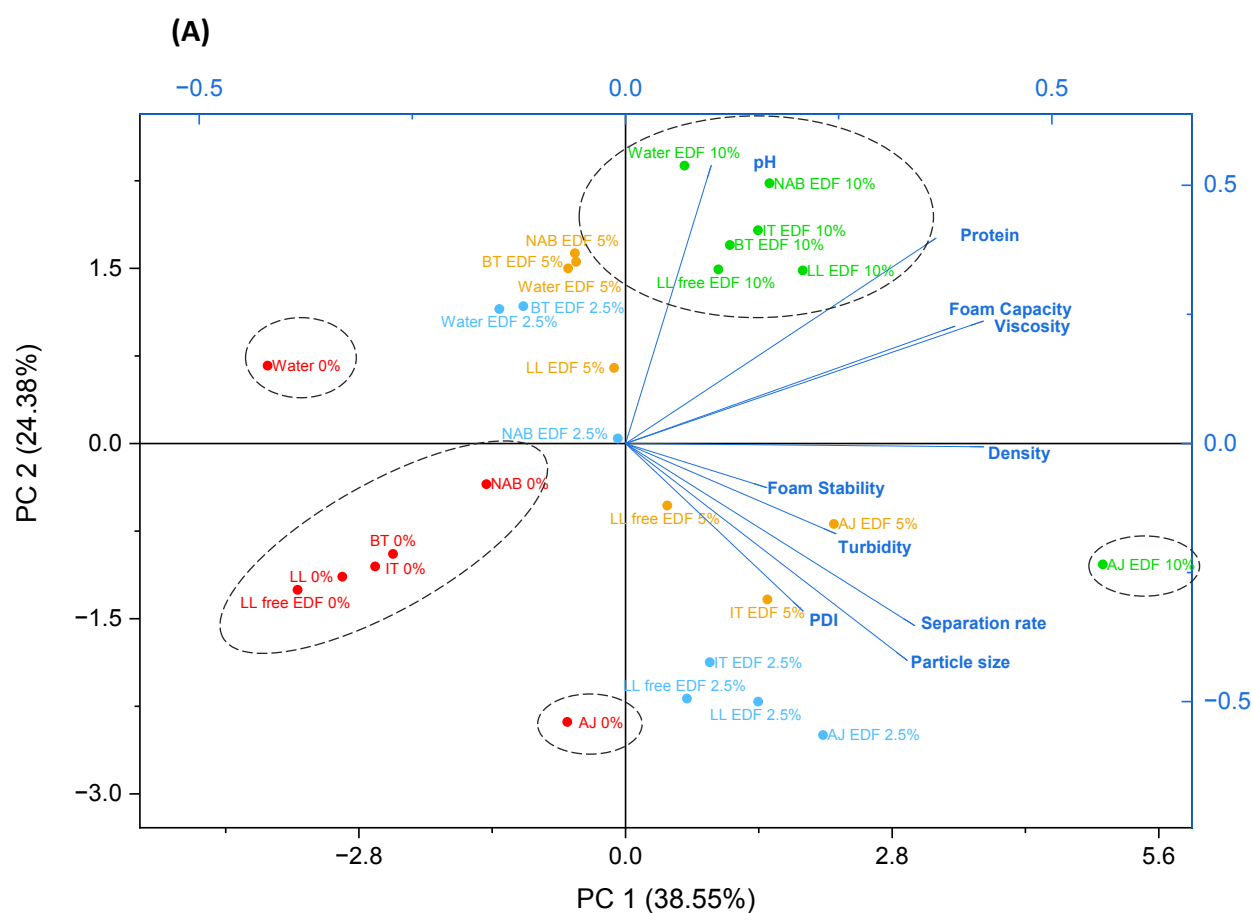
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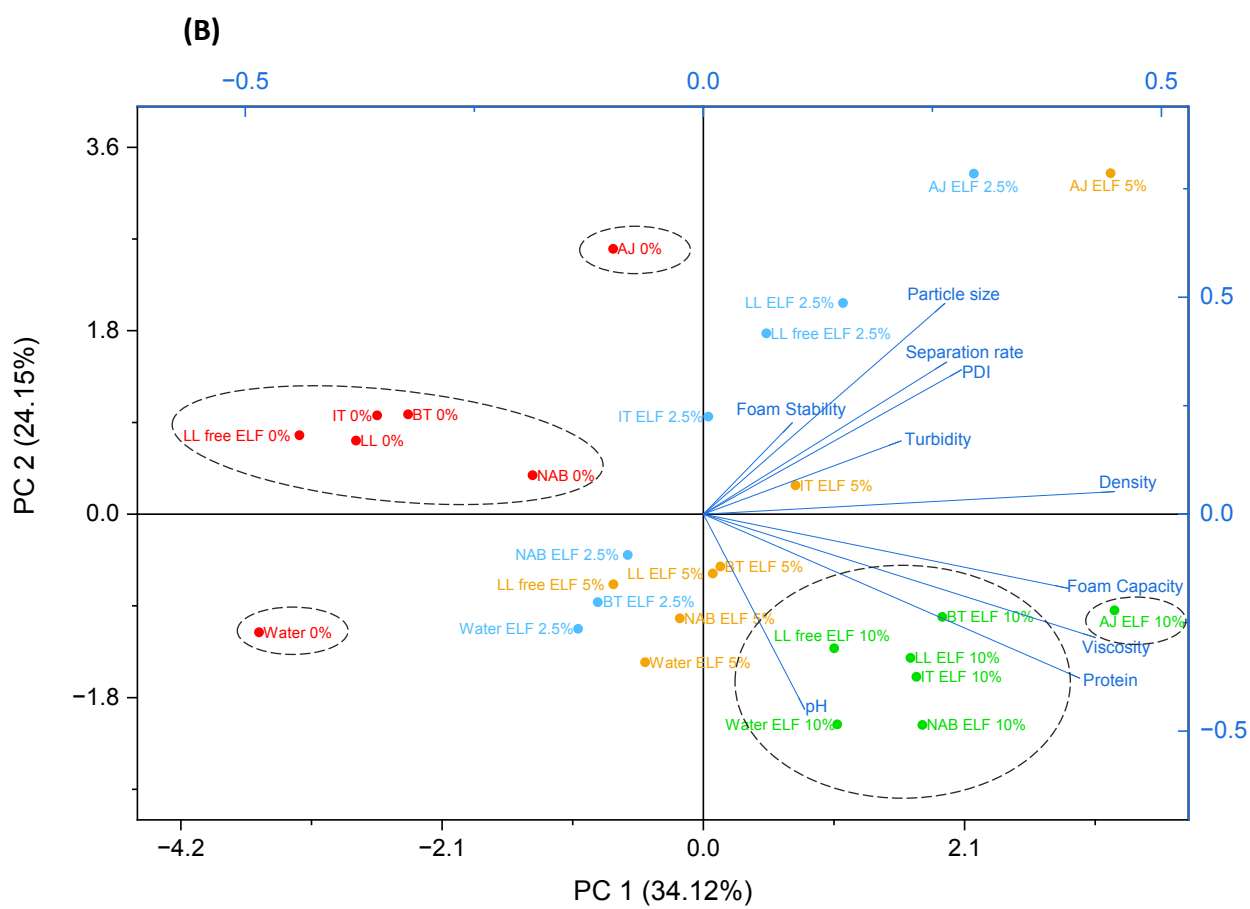
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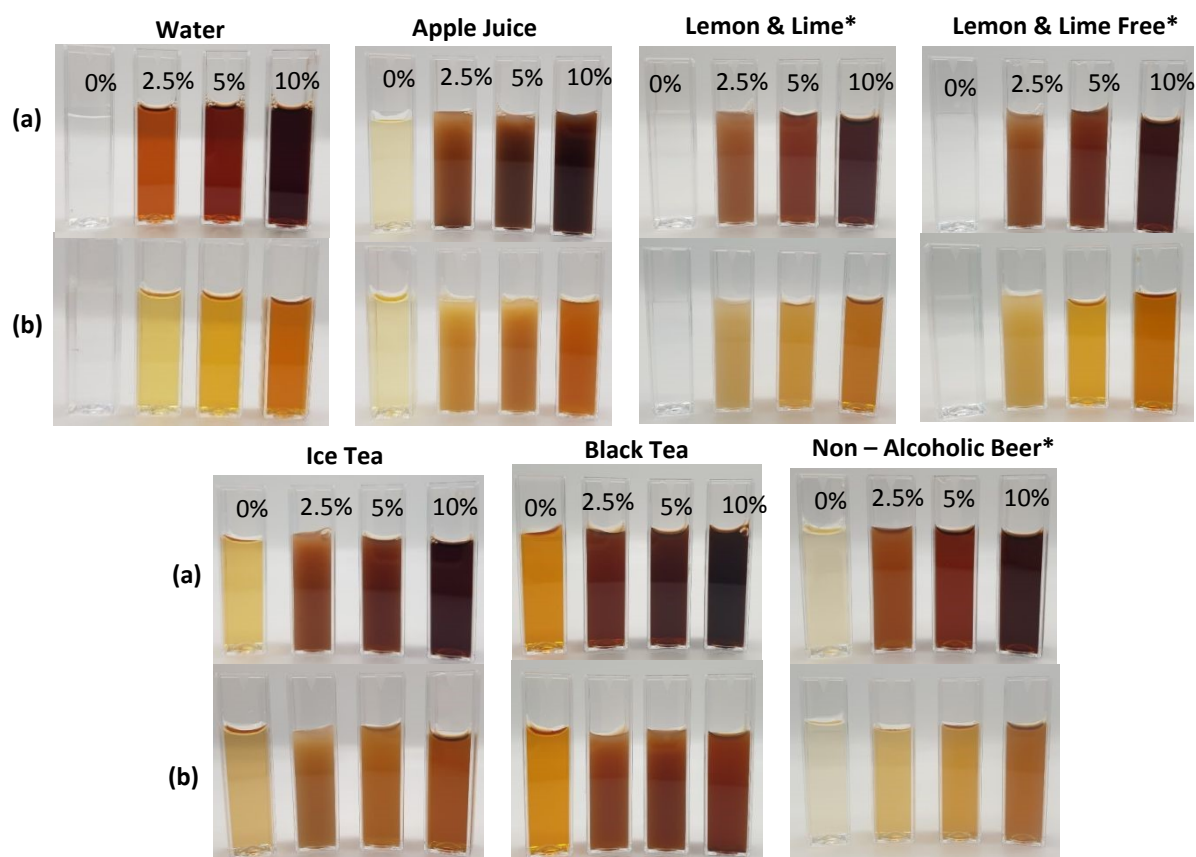
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696 **9.1. Figure 4** – Images of beverages at all inclusion levels where row (a) EverPro Dark Fraction

697 (EDF) and row (b) EverPro Light Fraction (ELF). An asterisk (*) indicates beverages that were

698 decarbonated before analysis.



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Benefits and challenges of fortified beverage systems with brewer's spent grain-derived protein isolates.

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Data Availability Statement

Data will be made available on request.

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