In vitro digestion of fish oils rich in n-3 polyunsaturated fatty acids studied in emulsion and at the oil–water interface

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1 # In vitro digestion of fish oils rich in n-3 polyunsaturated fatty acids
2 studied in emulsion and at the oil/water interface

3

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6

7

8 ## Abstract

9 The *in vitro* digestion of β-lactoglobulin stabilized emulsions rich in the n-3 polyunsaturated
10 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were studied using
11 several physicochemical techniques. Artificial media for mouth, stomach and small intestine
12 were used in a sequential static *in vitro* digestion method. Different sizing techniques were
13 compared to follow the droplet size during the digestion steps, including diffusing wave
14 spectroscopy (DWS) which allowed direct measurements on undiluted emulsions. Titration of
15 fatty acids confirmed that the digestion of such emulsified fish oils is partial. The study of the
16 digestion at the oil/water interface using tensiometry revealed specific affinities between
17 lipids and proteins. These could explain the emulsion and the single droplet lipolysis.
18 Nevertheless, by comparing our results to a previous study of fish oil lipolysis, we identified
19 two other important factors. Those were the aqueous solubility and the rate of hydrolysis of
20 the individual fatty acids, the emulsion with the most soluble and hydrolysable ones being
21 digested more quickly.

22

23 ## 1. Introduction

24 Polyunsaturated fatty acids are essential nutrients which have recognized positive health
25 effects, among which the very long chain n-3 eicosapentaenoic acid (EPA, 20:5) and

docosahexaenoic acid (DHA, 22:6) are especially beneficial.[1] Nevertheless, contrary to the saturated or monounsaturated fatty acids, there are still some open questions concerning their bioavailability, from the digestion to the absorption and uptake.[2,3] In particular, there are only few studies concerning the digestion of these fatty acids. *In vivo*, there are clear effects of the lipid composition and of the food matrix, although the design of the study (especially dose and duration but also the outcome measure) makes it difficult to compare the results quantitatively.[1] There are only a few studies where an estimation of the absorption percentage was performed. After 24 hours collection of the lymph of rats, the absorption from fish oils was in the range 47-57 % relative to corn oil,[4] and the absorption from trieicosapentaenoyl glycerol and tridocosahexaenoyl glycerol was about 80 % and 85 % respectively.[5] After 8-12 hours collection of blood of men, the plasma absorption from fish oil was 69 % for EPA and 61 % for DHA relative to linseed oil,[6] increased to 90 % and 68 % respectively in case of co-ingestion of a high-fat meal.[7] *In vitro*, for the lipolysis of EPA and DHA in non-emulsified oil form, Bottino et al.[8] reported a resistance to pancreatic lipase, which was confirmed by Yang et al.[9] and Martin et al.[10]. The latter compared the lipolysis of fish oils to that of an oil containing partial glycerides, resulting in a change in the interfacial lipolysis but not in the bulk micellar solubilization, concluding this was the limiting step. This is reminiscent of Ostwald ripening, for which the solubilization process can occur at the interface and in the bulk.[11]

Recently, there was an intensification of *in vitro* digestion of emulsions, as a model system for food which can be incrementally complexified.[12-16] One goal is to understand the role of the microstructures on the digestion of nutrients. Many authors focused on emulsified vegetable oils stabilized by milk proteins,[17-24] yet not focusing on the lipids but rather on the change of droplet microstructure (destructuration) during digestion.

1 In this article, we report the investigation of the relation between the destructuration and the
2 *in vitro* lipid digestion of emulsions in order to identify the bulk and interfacial mechanisms.
3 Because very long chain polyunsaturated fatty acids have special digestion features, we
4 expected to gain insight from their digestion in emulsion. As the main sources for these acids
5 are marine, notably from oily fishes and algae, we focused on emulsions made of two marine
6 fish oils with distinct compositions. We used different techniques to study the impact of this
7 composition on the structural and physicochemical changes during digestion. Especially,
8 diffusing wave spectroscopy (DWS) and pH-stat were used to investigate the bulk behavior of
9 droplets in undiluted emulsions whereas drop tensiometry was used to resolve the molecular
10 interactions at the oil/water interface of a single oil droplet.
11
12 2. Materials and methods
13 2.a. Materials
14 The marine fish oils EPAX1050TG (abbr. f-1050) or OMEGAVIE1812TG (abbr. f-1812)
15 were provided by Polaris (Pleuven, France). EPAX1050TG contains a minimum of 10 wt%
16 EPA and 50 wt% DHA, respectively 18 wt% EPA and 12 wt% DHA for
17 OMEGAVIE1812TG (see table 1 for the oils composition as measured by GC-FID).
18 Tricaprylin was provided by Sigma-Aldrich France (T9126). β-lactoglobulin (β-LG) was
19 purified from whey protein isolate in our laboratory. In all preparations, Milli-Q water having
20 an electrical resistivity of 18.2 *MΩ.cm* was used. All chemicals were from Sigma-Aldrich
21 France except when mentioned.
22 2.b. Emulsion preparation
23 Oil/water emulsions of dispersed oil volume fraction $\phi = 0.2$ were prepared using a 0.5 wt%
24 solution of β-lactoglobulin in 10 *mM* NaH_2PO_4 buffer at pH 7.0 and one marine fish oil or
25 tricaprylin (a medium chain triglyceride). A total volume of 10 *mL* was placed in a 50 *mL*

1 plastic vial, pre-emulsified for 1 *min* at 15000 *rpm* using a rotor-stator homogenizer
2 (SilentCrusher M equipped with the 12F generator, from Heidolph Instruments, Germany).
3 Immediately after, the pre-emulsion was sonicated using a Misonix Sonicator 4000 equipped
4 with a microtip probe 419 (Qsonica, Connecticut, USA) for 2 *min*, alternating 15 *s* sonication
5 and 15 *s* pause. A total of three cycles were performed with a 2 *min* pause between them to let
6 the emulsion cool. The total energy brought to the emulsions was always about 1.6 *kJ*.
7 2.c. Emulsion digestion
8 The protocol we followed was adapted from Versantvoort et al.[25,18] with some concentration
9 changes to account for some *in vivo* data[26] and the specific activity of the enzymes we used
10 (see table 2). Briefly, three stock salts solutions were prepared to mimic the ionic
11 environments and pH of three parts of the digestive tube: 1) the mouth, 2) the stomach and 3)
12 the small intestine.
13 The day of the *in vitro* digestion, enzymes and proteins were added to these solutions:
14 pancreatic α-amylase (10080) and gastric mucin type II (M2378) in 1), gastric pepsin
15 (P7012), gastric mucin type II and bovine serum albumin (103703 from MP Biomedicals,
16 Illkirch, France) in 2), bovine serum albumin, pancreatic lipase type II (L3126), pancreatin
17 8xUSP (P7545) and bile extract (B8631) in 3).
18 A 5 *mL* emulsion (ϕ = 0.2) was brought to 37 °C in a 30 *mL* amber glass vial on a hotplate
19 with constant magnetic stirring of 300 *rpm*. To work at a constant volume, some emulsion
20 was removed before the artificial medium was added. The mouth step lasted 5 *min* with a
21 dilution such that ϕ = 0.091. The stomach step lasted 120 *min* with ϕ = 0.0435 and the
22 intestinal step lasted 240 *min* with ϕ = 0.0235.
23 To measure the fatty acids release using a pH-stat setup, separate digestions were performed
24 with no salts in the emulsion nor in the digestion media, keeping all other conditions the
25 same. After the mouth and gastric steps with no measurement, the intestinal step was followed

1 using 0.02 *M* NaOH to titrate the fatty acids production by maintaining a pH of 8.0, which
2 was maximized to give high pancreatic lipase activity[27] and ionization of the long fatty
3 acids,[28] while approaching the higher pH value reported for the small intestine *in vivo*.[26] The
4 result was calculated as the percentage of fatty acids molecules released deduced from the
5 volume of NaOH added, considering that 1 triglyceride produces 2 fatty acids.[20] This volume
6 was recorded every 3 *s* (1200 points / hour). Note that when salts were included (results not
7 shown), this volume was systematically much lower, what we interpreted as an effect of the
8 complex salts mixture, neutralizing a part of the fatty acids produced.
9 2.d. Laser diffraction
10 Volume-based particle size distribution was measured at 37 °C using a MasterSizer S
11 equipped with a 2 *mW* He-Ne laser of λ = 633 *nm* and the 300RF lens (Malvern Instruments
12 Ltd., Worcestershire, UK). The detection limits are 0.05 and 900 μm. The refractive index n_0
13 of the aqueous phase was 1.33 and the properties of the dispersed phase (fish oil) were 1.46
14 for the refractive index and 0.001 for the absorption. Samples were pre-diluted with the
15 desired salts solution, then diluted with distilled water at 37 °C in the dispersion unit to reach
16 an oil volume concentration near 0.01% for the circulation in the measurement cell.
17 2.e. Dynamic light scattering
18 Back-scattering measurements of the mean droplet diameter were obtained at 37 °C using a
19 Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 4 *mW*
20 He-Ne laser of λ = 633 *nm*. The exact angle between the laser beam and the detector
21 (avalanche photodiode) is 173°. The laser power is automatically attenuated to collect an
22 optimal scattered intensity. The measurement position was set to the maximum of 4.65 *mm*,
23 that is 3.65 *mm* inside the sample as we used disposable 12 *mm* square polystyrene cuvettes
24 with 1 *mm* thick walls (Brand, Wertheim, Germany). The optical properties and dilution
25 factor were the same as previously for laser diffraction. A 30 *s* acquisition was generally

1 enough to obtain a stable measurement. The intensity-based particle size distribution was
2 used.
3 2.f. Zeta potential
4 The Zetasizer Nano ZS apparatus was also used to measure the zeta potential, with the same
5 parameters and dilution factor than for particle sizing, but in a special capillary cell
6 (DTS1060). The zeta potential is measured by laser Doppler electrophoresis using a phase
7 analysis light scattering technique.
8 2.g. Diffusing wave spectroscopy
9 To obtain the mean droplet diameter in undiluted samples, we used a homemade DWS
10 equipment. We go into the details of this method in a separate article.[29] It is based on the
11 cross-correlation of two independent PhotoMultiplierTubes signals, obtained by splitting the
12 beam collected by a single optical fiber. These materials and the electronic correlator were
13 supplied by Correlator.com (Bridgewater, NJ, US). The light source is produced by a 100 *mW*
14 DPSS laser of λ = 532 *nm* provided by Laserglow Technologies (Toronto, Canada). Both
15 back- and forward- scattering geometries can be set. The sample consists in 3 *mL* emulsion in
16 a closed disposable 12 *mm* square polystyrene cuvette, maintained to 37 °C on a hotplate.
17 2.h. Drop tensiometry
18 The Tracker drop tensiometer (I.T.Concept-Teclis, Longessaigne, France) was used to
19 perform model digestions on a single rising oil droplet at the end of a vertical curved needle.
20 The droplet characteristics are controlled by a motor acting on the syringe according to
21 droplet shape analysis feedbacks. The needle was placed in an optical glass cuvette of 8 *mL*
22 (Hellma GmbH, Müllheim, Germany) filled with the desired solution. Just before setting up,
23 the oil was purified in a Sep-Pak Silica cartridge (WAT051900, Waters SAS, France).
24 The interfacial tension between oil/buffer or oil/β-LG solution at constant interfacial area was
25 allowed to reach equilibrium at 25 °C, then the temperature was set to 37 °C. At equilibrium,

1 a fraction of the aqueous phase was removed and replaced by the same fraction of the first
2 digestion medium using a micropipette, then successively by the other media, using the same
3 compositions, dilution factors and durations described earlier.
4
5 At least 2, usually 3 independent emulsions, digestions and associated measurements were
6 done on different days in order to check the overall repeatability of the study. The results are
7 presented as averages and standard deviations, except if stated otherwise.
8
9 3. Results
10 Figure 1 shows a comparison of the release of fatty acids for the two fish oils and the
11 tricaprylin emulsions during the intestinal step with no salts (previously incubated in the
12 mouth and the gastric media with no salts). Although the initial slope is quite similar for both
13 fish oils, there is statistically more fatty acids released for f-1812 than for f-1050 after 5
14 hours. Overall, the quantity released after 5 hours of intestinal incubation only represents 45-
15 49 mol% of the producible fatty acids for f-1050 and 49-53% for f-1812, whereas it reaches
16 90-95 mol% for tricaprylin.
17 Figure 2 sums up the results for the evolution of the mean droplet size during the *in vitro*
18 digestion, as measured by the different techniques. Those are in fair agreement except for
19 dynamic light scattering (DLS) during the intestinal step. The deviation of the measurements
20 is also higher for DLS, which is due to a multimodal distribution. Nevertheless, all techniques
21 reveal similar trends: a small increase in size during the mouth step, a large increase during
22 the gastric step, and a decrease during the intestinal step. The initial emulsions are quite
23 similar in diameter, with a mean of 775 *nm* for f-1812 and 825 *nm* for f-1050. During the
24 digestion, the size for emulsion droplets of f-1812 is always slightly smaller than that for
25 emulsion droplets of f-1050.

1 Figure 3 shows the evolution of the average zeta potential during the *in vitro* digestion. There
2 is an overall correlation with the droplet size trends, because the smaller the zeta potential
3 absolute value is, the less stable against flocculation the droplets are, hence the higher the
4 apparent droplet size is, as seen e.g. during the gastric step. As a function of the oil type, the
5 interface of the f-1050 emulsion is always more charged than the f-1812 one.
6 Figure 4 shows representative interfacial tension measurements for the f-1050 oil against a 10
7 *mM* NaH_2PO_4 buffer in the presence or in the absence of 0.5 wt% β-LG. When β-LG is
8 present, there is a sharp initial interfacial tension decrease followed by a gradual one, due to
9 its adsorption and reorganization respectively. The start of each digestion step is located on
10 the curves. The pH was measured at the end of each digestion step, of 6.5, 4 and 8
11 respectively. During the mouth step, there is a sharp interfacial tension decrease in the
12 absence of β-LG, or a small increase in the presence of β-LG. During the gastric step, there is
13 a gradual decrease in the absence of β-LG, or a sharp initial increase followed by a small
14 gradual one in the presence of β-LG. During the intestinal step, there is a sharp initial
15 decrease followed by a gradual decrease. Experiments were also done only with salts in the
16 digestion media, which allowed to check that those trends are not due to the salts themselves
17 or to the pH changes.
18 In order to compare those trends quantitatively for the two fish oils, we plot the interfacial
19 tension variation (difference) for each digestion step separately. The origin of time is set at the
20 beginning of each digestion step (a few seconds after the addition of the medium) and the
21 interfacial tension is lowered by the value at the end of the previous step (a few seconds
22 before the addition of the medium). Figures 5-6-7 show the results for the mouth, gastric and
23 intestinal steps respectively. Overall, those graphs show the influence of the initial interfacial
24 layer of β-LG, as the main differences are seen by comparing its presence or absence. During
25 the mouth step, the oil type does not play a significant role. In the contrary, f-1050 is more

affected by the gastric step than f-1812. The oil type also plays a role during the intestinal step where the results for the two fish oils are distinct.

4. Discussion

Concerning the titration of fatty acids during digestion of emulsions, the difference between the two fish oils seems to reflect their composition, as the release is higher for f-1812 than for f-1050, in agreement with their average molar mass, which can be calculated to be of 890 and 1000 $g.mol^{-1}$ respectively, representing the influence of the distribution of the carbon chain lengths. For the tricaprylin, with a short carbon length and a molar mass of 470 $g.mol^{-1}$, the release is almost complete, which was expected. Using β-LG emulsions, Li and McClements[20] indeed found that the *in vitro* release is much higher for tricaprylin/tricaprin as medium-chain triglycerides (97 mol%) compared to corn oil as long-chain triglycerides (45.5 mol%) after 30 minutes. Although our final values agree with those, all of our emulsions show much slower digestion kinetics. This can be due to several factors, but the main ones might be the absence of calcium and the relatively high lipid concentration with respect to the lipase and bile salts concentrations in our experiments.[20] For two non-emulsified fish oils (composition similar to f-1812) in the presence of phosphatidyl choline, Martin et al.[10] found a total release of fatty acids around 20 wt%, which can be calculated to represent about 36 mol% release. We find a significantly higher total release, which is likely due to a higher efficiency of the emulsified form, mostly because the droplets are then much smaller and more stable. The release is nevertheless low, which could also be explained by the specific resistance of EPA and DHA to pancreatic lipase, demonstrated *in vitro*.[8-9] In our results, there is indeed less release for the oil containing the higher cumulated amount of EPA+DHA (f-1050).

1 The measurement of the droplet size gives information about the structure of the emulsion in
2 term of droplet interactions for each digestion step. The different techniques are in fair
3 agreement, so DWS is able to measure an accurate size in undiluted complex systems,
4 extending our previous results with undiluted model systems.[29] The sizes are in qualitative
5 agreement with previous studies using β-LG or whey protein isolate (WPI) emulsions. For the
6 mouth step, Silletti et al.[17] found almost no change in the particle size distribution of an
7 emulsion mixed with human saliva close to neutral pH, indicating no flocculation. Hur et al.[18]
8 found the same result for emulsions stabilized by WPI with the *in vitro* protocol of
9 Versantvoort et al.[25]. For the gastric step, Hur et al.[18] found a small decrease in size when
10 expressed as the surface-based mean D3,2 whereas the volume-based distributions were
11 constant. We found a significant increase, up to one order of magnitude for the volume-based
12 size, as many other authors reported,[19,21,22,24,30] and attributed to flocculation/coalescence
13 caused by pepsin. For the intestinal step, a more or less pronounced decrease in size was
14 reported, attributed to deflocculation and digestion processes.[16,18,19,21,23] This is also what we
15 found, with a quick deflocculation as most of the decrease took place at the very beginning of
16 the intestinal step, and only a small gradual decrease followed (results not shown).
17 The variations of the zeta potential during *in vitro* digestion are in good agreement with the
18 ones reported for similar emulsions stabilized by β-LG,[19,21,22] WPI,[18,24] or for milk.[23] In the
19 details, a discrepancy is seen for the gastric step, as we found slightly negative zeta potentials
20 after 2 hours in this medium (as Macierzanka et al.[19]), whereas other authors reported values
21 between 10-20 mV.[18,21,22,24] This is not surprising, as the absolute values are influenced by
22 many other parameters than the interfacial layer, as the composition of the digestion
23 media,[19,21,23] the ionic strength,[22] the pH,[24] and as we found, the oil type.[23] Our result can be
24 explained by the interfacial competition at pH 4 between β-LG, being positively charged
25 below its isoelectric point (pI ≈ 5, Hur et al.[18]), and pepsin or mucin, being negatively charged

1 above their isolectric point (pI ≈ 3, Righetti et al.[31], Bansil and Turner[32]). Comparing the two
2 oils, the initial emulsion shows a higher zeta potential absolute value indicating more β-LG
3 interfacial interactions in the f-1050 case, and this is conserved throughout the digestion.
4 Although enzymologists pioneered the study of the mechanisms of digestion at the interface,[33]
5 their systems were too simplified to extrapolate to digestion of complex foods. Interfacial
6 studies were recently relived for that matter.[15,34] However, to our knowledge, there is only one
7 other oil/water interface study with successive digestion steps, but with no mouth step and
8 using no lipase.[19] During the mouth step, we interpret the variations of interfacial tension as
9 mucin and α-amylase adsorption or competition with β-LG at the interface, in the absence or
10 presence of β-LG respectively. It was indeed reported that mucin and α-amylase can adsorb at
11 interfaces.[35-37] During the gastric step in the absence of β-LG, the interfacial tension decreases
12 as more mucin and some pepsin adsorb. In the presence of β-LG, it increases, what could be
13 due to a competition at the interface in favor of mucin and pepsin, in agreement with the
14 negative zeta potential value. This increase could also be partly due to the production of
15 peptides by proteolysis, as shown by Macierzanka et al.[19]. During the intestinal step, the
16 initial decrease is due to the adsorption of bile salts, which are known to desorb various
17 amphiphiles.[34] All trends and interpretations agree well with Macierzanka et al.[19]. However,
18 contrary to them, our intestinal step includes a pancreatic lipase, responsible for the gradual
19 decrease of the interfacial tension as lipolysis releases tensioactive fatty acids and 2-
20 monoglycerides.[38]
21 We interpret the interfacial differences between the two fish oils by considering the gastric
22 and intestinal steps. In the absence of β-LG, more adsorption of digestive proteins for f-1050
23 during the gastric step likely induces less adsorption of bile salts and pancreatic lipase activity
24 during the intestinal step compared to f-1812. In the presence of β-LG, more competition
25 and/or proteolysis for f-1050 during the gastric step likely induces less adsorption of bile salts

1 and pancreatic lipase activity during the intestinal step compared to f-1812. The same trends
2 are true if the comparison is set after the initial sharp decrease due to bile salts in order to
3 isolate the lipolysis contribution.[39] So, the presence of β-LG always induces less adsorption of
4 bile salts and pancreatic lipase activity, what could be due to a resistant interfacial network of
5 residual β-LG[40-41] and/or of peptides[42] reported at the end of the gastric step, in agreement
6 with our hypotheses. These results also agree qualitatively with the zeta potential
7 measurements. Overall, we found more interactions between proteins and lipids in the f-1050
8 case. Different interactions were also reported for pepsin and β-LG at the olive oil or
9 tetradecane interfaces.[41] In our case, this result is likely due to the difference in the lipid
10 composition. It is indeed known that certain physiological proteins have different affinities for
11 saturated and unsaturated fatty acids.[43] Another probable explanation of the interfacial tension
12 variations during the intestinal step is a higher resistance of EPA and DHA to pancreatic
13 lipase for f-1050, as it contains more EPA+DHA. The interfacial tension and the bulk pH-stat
14 measurements are indeed in agreement, showing less release for f-1050. All of these
15 hypotheses suggest that the release is controlled by the interfacial interactions.
16 Nevertheless, the effect of the molar mass could also explain the release trends. In other
17 words, the digestion might also be controlled by the bulk solubilization, lower for the oil with
18 longer carbon chains. To go beyond the molar mass interpretation and compare quantitatively,
19 figure 8 shows the simulated aqueous solubility of the main fatty acids of the two fish oils
20 (accounting for about 80 wt% of their fatty acids), together with the results of Yang et al.[9] for
21 their rates of hydrolysis in a menhaden oil. For this oil, the proportions of fatty acids in the sn-
22 2 position on the triglycerides were reported by the same group (Myher et al.[44]). We used the
23 highest solubility estimate of ALOGPS 2.1 software by Tetko et al.[45] as it was close to the
24 experimental value around 40 °C for saturated fatty acids.[46] The aqueous solubility can be
25 seen as an indicator of the solubility in bile salt micelles, as these two solubilities are

1 correlated by a power law (usual R between 0.6-0.8) for the data reported by Wiedmann and
2 Kamel.[47] Figure 8 illustrates the interplay between the interfacial lipolysis and the bulk
3 solubilization processes. We see that the longer the chain length, the lower the aqueous
4 solubility and rate of hydrolysis. The sn-2 position proportion is usually not far from the
5 theoretical random distribution 33.3 mol%, except for C18:1 and C22:6, much lower and
6 higher respectively. When considering the main fatty acids in the compositions, we clearly
7 discriminate the two fish oils, f-1812 having more fatty acids of high aqueous solubility and
8 hydrolysis rate, f-1050 having only more DHA, of low aqueous solubility and hydrolysis rate.
9 This means that the f1812 emulsion should release fatty acids faster than the f-1050 one,
10 which is the case. Even more obviously, with a simulated aqueous solubility of 1200 $mg.L^{-1}$,
11 caprylic acid is indeed quickly released from the tricaprylin emulsion. The preferential sn-2
12 position of DHA could play a role but it should not influence the interfacial tension decrease
13 during the intestinal step because such characterization measures the release of fatty acids as
14 well as of 2-monoglycerides. The difference is thus due to the other factors. Both the bulk
15 solubilization and the interfacial hydrolysis could explain why the release for fish oils is so
16 low compared to most oils, having no highly polyunsaturated nor very long chain fatty acids.
17 In another study comparing tricaprylin and triolein emulsions, the limiting process was found
18 to be the bulk solubilization.[39] This can be seen in figure 8 for C18:1 which is relatively much
19 more hydrolysable than soluble. In real oils, these properties can be coupled, requiring
20 independent measurements to identify the limiting process for each lipid. Bile salts are likely
21 responsible, as they are known to control both the bulk solubilization[48,49] and the interfacial
22 lipolysis[27,49,50] as a non-monotonous function of their concentration.
23
24 5. Conclusion

1 Concerning the different sizing techniques, all of them were able to measure the changes in
2 the droplet size during the digestion steps. Nevertheless, the size indicators should be chosen
3 adequately to take multimodal distributions into account. DWS was found to be accurate to
4 work directly with undiluted complex emulsions.
5 All bulk methods, namely titration, droplet sizing, and zeta potential were not able to evidence
6 major differences between the digestion of the two emulsified fish oils. Nevertheless, the two
7 latter allowed to follow the structural changes during the different digestion steps. Globally,
8 we recovered the result that the *in vitro* lipolysis of emulsified fish oils is only partial, what
9 raises questions about the bioaccessibility of very long chain polyunsaturated fatty acids.
10 The interfacial technique was able to show the details of the interactions between lipids and
11 proteins at the interface during the digestion steps. Digestive proteins and peptides
12 adsorption/competition with β-LG could explain the subsequent trends of the interfacial
13 lipolysis. Further work is in progress with simpler oils and digestion media in order to fully
14 interpret the interactions.
15 By comparing our results to a previous study of the *in vitro* lipolysis of a fish oil, we
16 concluded that there is an interplay between the bulk solubilization and the rate of hydrolysis
17 of the individual fatty acids, the emulsion with the most soluble and hydrolysable ones being
18 digested more quickly. Our experiments did not allow to distinguish between these factors.
19 Again, a work with simpler oils and digestion media is under progress to understand this
20 aspect.
21 Although it did not seem to play a major role in this study, the position of the fatty acids on
22 the triglycerides will also be investigated to determine the chain lengths of the retained and
23 released fatty acids.
24

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	f-1812	f-1050
C14:0	7.11 ± 0.08	0.25 ± 0.03
C15:0	0.54 ± 0.02	0.08 ± 0.01
C16:0	16.46 ± 0.04	2.97 ± 0.09
C18:0	3.51 ± 0.08	3.07 ± 0.14
C20:0	0.40 ± 0.02	0.70 ± 0.03
C16:1 n-9	0.04	0.00
C16:1 n-7	7.81 ± 0.09	0.78 ± 0.01
C17:1	2.36 ± 0.03	0.00
C18:1 n-9	9.75 ± 0.06	6.94 ± 0.08
C18:1 n-7	3.02 ± 0.06	1.15 ± 0.14
C20:1	0.98 ± 0.05	2.62 ± 0.07
C16:2 n-4	1.27 ± 0.01	0.00
C18:2 n-6	1.73 ± 0.01	1.26 ± 0.04
C20:2 n-6	0.00	0.41 ± 0.05
C18:3 n-6	0.69 ± 0.03	0.00
C18:3 n-3	0.99 ± 0.08	0.00
C18:4 n-3	3.69 ± 0.05	0.80 ± 0.01
C20:4 n-6	1.15 ± 0.09	1.94 ± 0.09
C20:4 n-3	0.81 ± 0.03	0.82 ± 0.04
C20:5 n-3	22.03 ± 0.09	15.36 ± 0.09
C22:5 n-3	2.00 ± 0.22	3.13 ± 0.19
C22:6 n-3	13.66 ± 0.04	57.71 ± 0.23

Table 1: Fatty acids composition of fish oils (wt%).

Mouth	Salts solution	Proximal small intestine	Salts solution
Saliva: 1 g/L α-amylase (50 U/mg) 0.2 g/L urea 0.03 g/L uric acid 0.05 g/L mucin	0.896 g/L KCl 0.2 g/L KSCN 0.888 g/L NaH_2PO_4 0.570 g/L Na_2SO_4 0.3 g/L NaCl 1.694 g/L $NaHCO_3$ pH 6.8	*Duodenal juice (60%):* 9 g/L pancreatin (16 U/mg) 1.5 g/L porcine pancreatic lipase (100-400 U/mg) 0.1 g/L urea 0.1 g/L BSA	7.012 g/L NaCl 3.388 g/L $NaHCO_3$ 0.564 g/L KCl 0.215 g/L HCl 37% 0.2 g/L $CaCl_2.2H_2O$ 0.080 g/L KH_2PO_4 0.050 g/L $MgCl_2$ pH 8.1
Stomach	Salts solution		
Gastric juice: 1 g/L pepsin (2500-3500 U/mg) 3 g/L mucin 0.02 g/L urea 0.02 g/L uric acid 0.2 g/L BSA	2.752 g/L NaCl 0.266 g/L NaH_2PO_4 0.824 g/L KCl 0.4 g/L $CaCl_2.2H_2O$ 0.306 g/L NH_4Cl 7.75 g/L HCl 37% pH 2	*Bile juice (30%):* 60 g/L bile extract 0.250 g/L urea 0.1 g/L BSA	5.259 g/L NaCl 5.785 g/L $NaHCO_3$ 0.376 g/L KCl 0.180 g/L HCl 37% 0.222 g/L $CaCl_2.2H_2O$ pH 8.2
		Water (10%)	Water pH 6.5-7

Table 2: Compositions of the artificial digestion media before dilution of the emulsion. Each medium is made of a stock salts solution to which enzymes and organic compounds are added the day of the digestion.

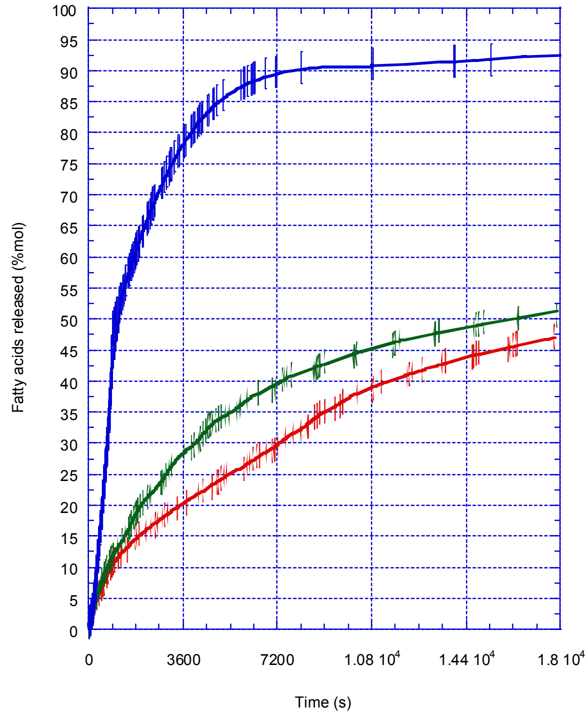

Figure 1: (Color online) Titration of fatty acids released during the intestinal step (no salts) for tricaprylin emulsions, f-1812 emulsions, and f-1050 emulsions (from top to bottom). The deviations are shown as error arcs.

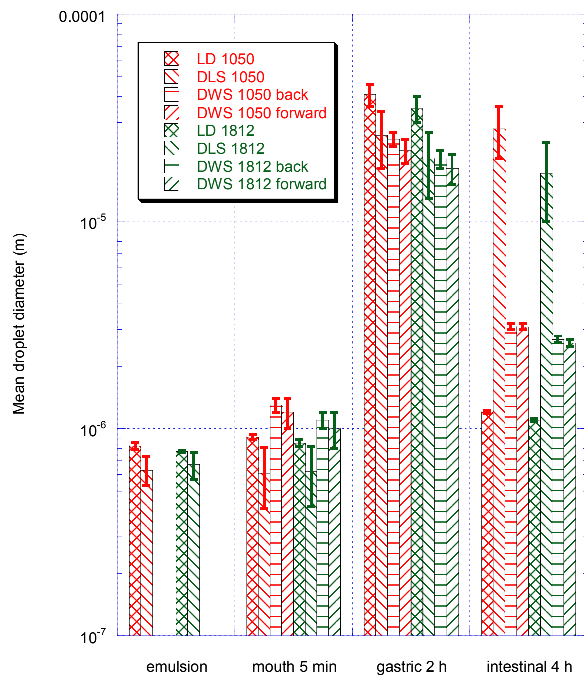

Figure 2: (Color online) Mean droplet diameter measured by different techniques during each digestion step for f-1050 emulsions (left bars) and f-1812 emulsions (right bars). See legend.

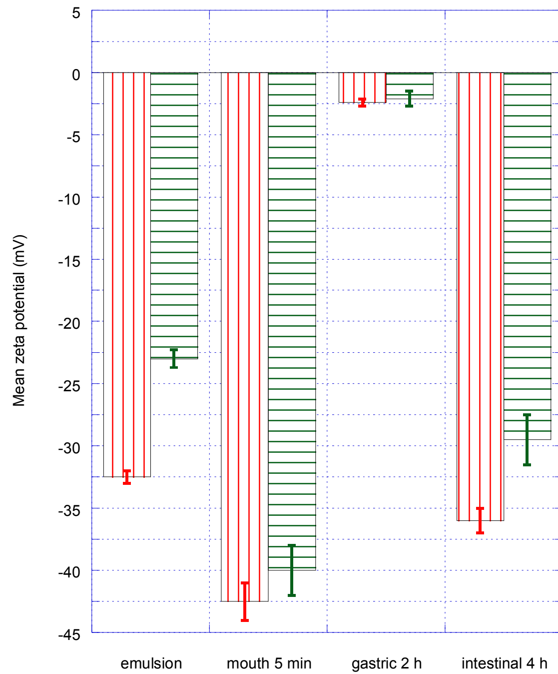

Figure 3: (Color online) Mean zeta potential during each digestion step for f-1050 emulsions (vertical filling) and f-1812 emulsions (horizontal filling).

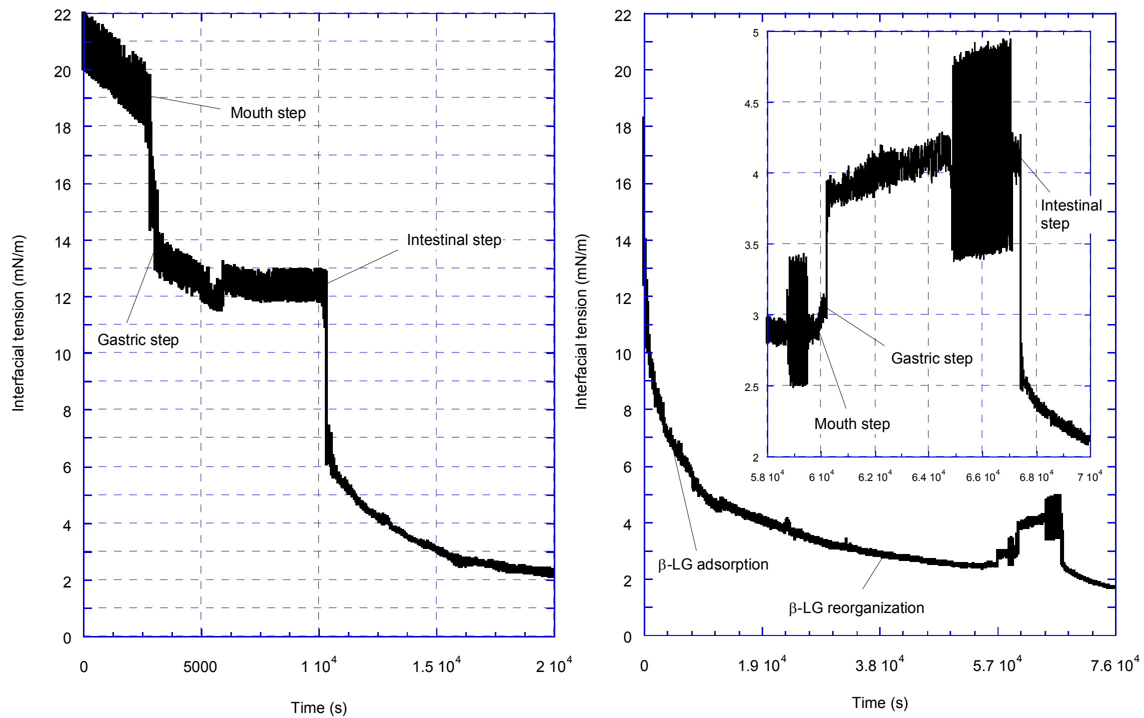

Figure 4: Representative digestion profiles for the f-1050/buffer interfacial tension in the absence (left) or in the presence (right) of 0.5 wt% β-LG in the initial 10 *mM* NaH_2PO_4 buffer.

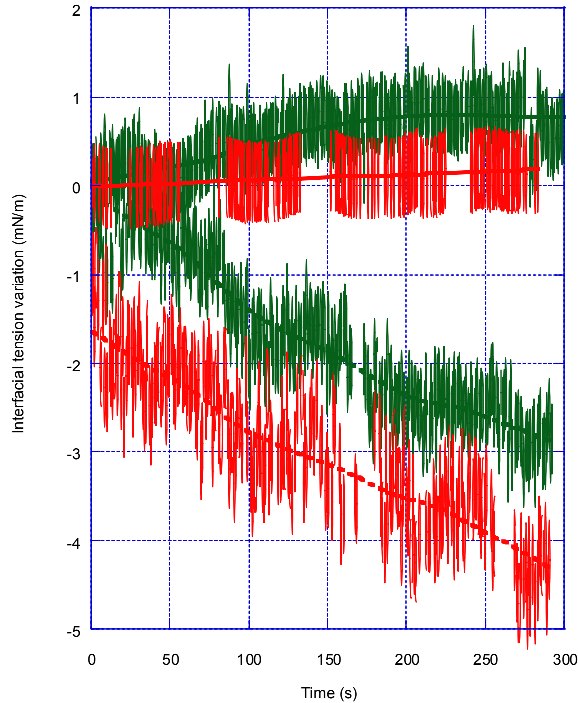

Figure 5: (Color online) Average interfacial tension variation during the mouth step for f-1050 in the absence (lowest red dotted line) or in the presence (lowest red full line) of 0.5 wt% β-LG. Same for f-1812 (green highest lines). The deviations are shown as error arcs.

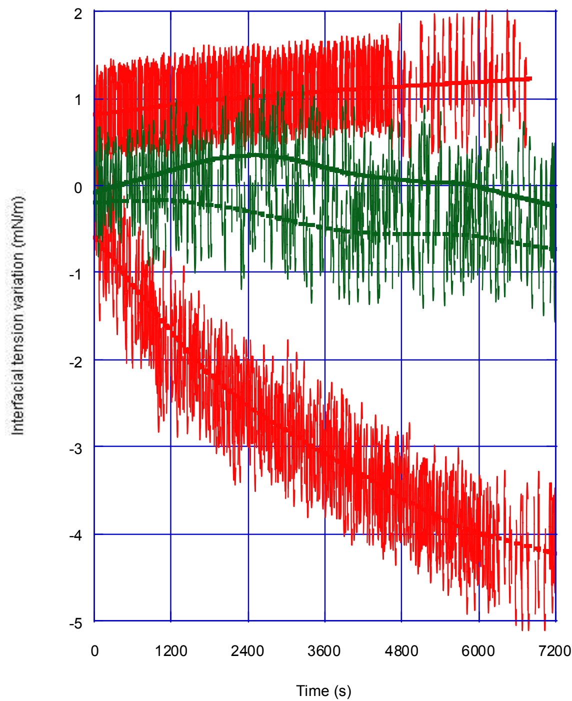

Figure 6: (Color online) Average interfacial tension variation during the gastric step for f-1050 in the absence (lowest dotted line) or in the presence (highest full line) of 0.5 wt% β-LG. Same for f-1812 (highest dotted line and lowest full line respectively). The deviations are shown as error arcs.

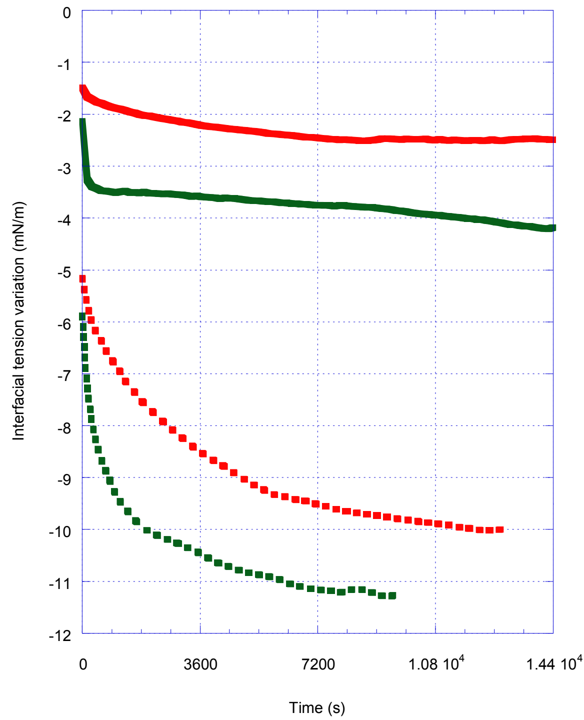

Figure 7: (Color online) Average interfacial tension variation during the intestinal step for f-1050 in the absence (highest dotted line) or in the presence (highest full line) of 0.5 wt% β-LG. Same for f-1812 (lowest lines). The deviations are within the line thickness.

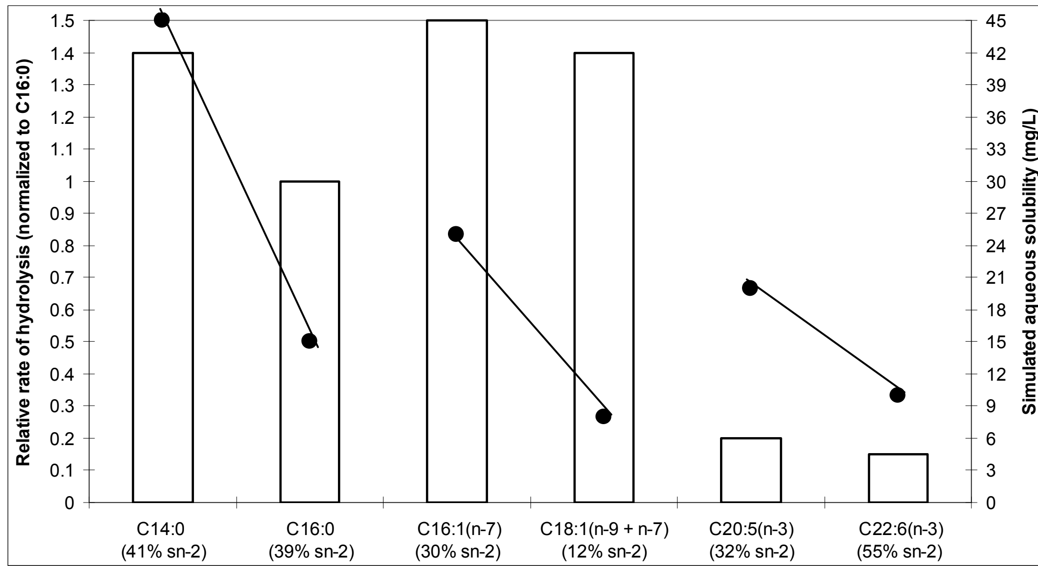

Figure 8: For the main individual fatty acids: relative rate of hydrolysis from Yang et al.[9] in a menhaden oil (bars) and simulated aqueous solubility (points). The proportions of fatty acids in the sn-2 position on the triglycerides are estimated from Myher et al.[44]. Lines are guides for the eyes.