Impacts of organic matter digestion protocols on synthetic, artificial and natural raw fibers

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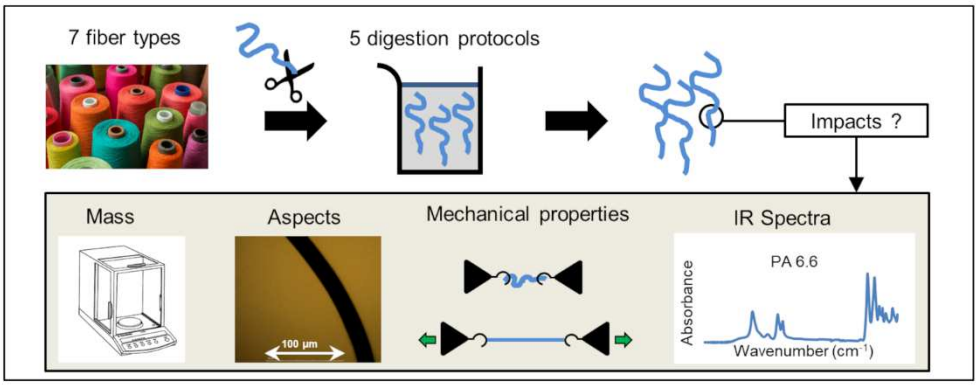
1 Abstract

2 As microplastic (MPs) studies grow, environmental concerns of all kinds of fibers are currently
3 investigated. However, there is a gap in data regarding the impacts of digestion protocols on fibers
4 integrity. This work focuses on the impact of five commonly used digestion protocols on the seven
5 most produced fibers in traditional textile: three synthetics (polyamide 6.6 (PA 6.6), polyethylene
6 terephthalate (PET) and polyacrylonitrile (acrylic)), one artificial (viscose), two vegetal natural
7 (cotton and flax) and one animal natural (wool). The protocols to be tested were selected based on the
8 literature: 10 % KOH at 40 °C for 24 h; 10 % KOH at 60 °C for 24 h; diluted NaClO at room
9 temperature (~ 20 °C) for 15 h; 30 % H_2O_2 at 40 °C for 48 h; Fenton's reagent with 30% H_2O_2 for 2 h
10 at room temperature (~20 °C). The fibers were characterized before and after digestion. The effects of
11 those protocols on fibers integrity have been assessed using several of their performance parameters.
12 High degradations were observed for PET with 10 % KOH 60 °C whereas almost no impact was
13 observed at 40 °C. H_2O_2 digestion affects mechanical properties of different fibers, particularly
14 PA 6.6. Both protocols should be avoided for synthetic fibers analyses. NaClO digestion mainly
15 affected flax and viscose. Diluted NaClO at room temperature for 15 h, 10 % KOH at 40 °C for 24 h
16 and Fenton's reagent are more appropriate to maintain fibers integrity.

17 KEYWORDS: fibers, digestion protocols, impact assessment, microplastic

18 Graphical abstract



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1. Introduction

Recent research on plastic pollution has raised environmental concerns about synthetic fibers pollution. However, fibers pollution is not limited to synthetics as all types of fibers are found in the environment. According to the International Organization for Standardization (in ISO/TR 11827:2012 Textiles - Composition testing - Identification of fibers), fibers are classified into two main categories: (i) man-made and (ii) natural fibers. Natural fibers are produced from crops or farming whereas man-made fibers are manufactured from chemical manufacturing processes. Man-made fibers are divided into artificial fibers, such as viscose (which come from cellulose, a natural polymer) and synthetic fibers which mainly come from petroleum resources. Biobased synthetic fibers, which constitute less than 1 % of the production rate (TextileExchange, 2018), will not be considered here.

From 1950 to 2015, ~600 Mt of polyester, polyamide, and acrylic fibers were dumped and accumulated in the environment (Geyer et al., 2017). In 2017, the fiber production exceeded 100 Mt for the first time and reached 105 Mt of which 68 % (71.5 Mt) was man-made *i.e.* artificial and synthetic fibers. Polyester, polyamide, and acrylic fibers are the most produced synthetic fibers, accounting for 62 % of the global fiber production (TextileExchange, 2018). Significant leaks to the environment occur owing to wear and tear of textiles and synthetic products. Besides synthetic fibers, artificial and natural fibers have been reported to significantly pollute the environment as microscopic litter, particularly in biota matrices (Lusher et al., 2013; Remy et al., 2015; Rochman et al., 2015; Zhao et al., 2016). Artificial and natural fibers can behave as pollutant vectors (Zhao et al., 2016) and further research in needed to study their impact on the environment.

In microplastic studies, fibers < 5 mm are generally categorized as microfibers even though fibers of this size limit is not always rigorously used (Arthur et al., 2009; Frias and Nash, 2019). Quantitatively, fibers were reported as the major component of microplastics or plastic-suspected particles (compared with various shapes such as fragments), and have been found in oceans (Law et al., 2010), rivers (Rillig, 2012), fish stomachs (Collard et al., 2015), atmospheric outfall (Dris et al., 2016), sediments (Ng and Obbard, 2006) and recently, in bottled water (Welle and Franz, 2018). The

ubiquity of microfibers may have negative effects on aquatic and terrestrial biota particularly, by reducing the energy budgets available in food as well as exposing species to pathogens or contaminants and oxidative stress (Foley et al., 2018; Song et al., 2019; Watts et al., 2015).

Fibers have been found in every compartment of urban water *i.e.*, drinking water (Mintenig et al., 2019; Pivokonsky et al., 2018), wastewater treatment plants (Magnusson and Norén, 2014; Talvitie et al., 2015), sewage sludge (Lv et al., 2019; Mahon et al., 2017), and stormwater (Dris et al., 2018; Piñon-Colin and al., 2020). Boucher and Friot, 2017 estimated that 35 % of microplastics released into oceans comes from the laundry of synthetic textiles. Several studies have attributed significant fiber releases to washing (De Falco et al., 2019; Hernandez et al., 2017; Napper and Thompson, 2016). However, fiber pollution is not limited to only washing as apparel and furnishing textiles contribute significantly to fiber pollution throughout their life (Henry et al., 2019). Owing to the various environmental matrices (biota, water, sediments, etc.,) fiber extraction and quantification in the environment have proved to be scientific challenges. This variety of matrices include different organic matter (OM) and mineral matter. Moreover, fiber quantification requires the removal of OM and mineral matter without fiber degradation. Therefore, various protocols have been developed to this end. Mineral matter is commonly removed by density separations whereas OM is commonly removed by digestion protocols. Digestion protocols commonly used in various studies are tested on different types of matrices and different polymers. In most cases, polymers in the form of fragments are tested. In all studies that have tested digestion protocols for microplastic extraction, only a few have considered fibers (Cai et al., 2019; Hurley et al., 2018; Piarulli et al., 2019; Thiele et al., 2019). Moreover, the considered fibers are mostly synthetics, even though significant amounts of natural fibers have been reported from environmental samples (Zhao et al., 2016). Several reagents have been assessed, *i.e.* acids like HCl or HNO_3 (Avio et al., 2015; Catarino et al., 2017; Cole et al., 2014), bases like NaOH or KOH (Dehaut et al., 2016; Karami et al., 2017; Mintenig et al., 2017; Piarulli et al., 2019; Thiele et al., 2019), oxidizing agents such as H_2O_2 (Dris et al., 2018; Hurley et al., 2018; Mintenig et al., 2014; Nuelle et al., 2014), enzymes (collagenase, proteinase, etc.,) (Cole et al., 2015; Courtene-Jones et al., 2017; Löder et al., 2017; Mintenig et al., 2017; Piarulli et al., 2019), a

73 combination of some reagents presented above (Cai et al., 2019; Collard et al., 2015), and more
74 recently, Fenton's reagent (Hurley et al., 2018; Tagg et al., 2017) which is produced from an
75 oxidation reaction catalyzed with Fe^{2+} ions commonly used in wastewater treatments (Kuo, 1992).

76 Acids seemed too aggressive for fragments (Avio et al., 2015), particularly for polyamide (PA),
77 polyethylene terephthalate (PET) and high-density polyethylene (HDPE) (Catarino et al., 2017).
78 Bases such as KOH seemed suitable (Dehaut et al., 2016; Karami et al., 2017) especially before 4 d at
79 room temperature (Piarulli et al., 2019). Damage was observed for polyester fibers exposed to 10 %
80 KOH after 4 d at room temperature (Piarulli et al., 2019). Impacts of 10 % KOH at 60 °C and 40 °C
81 after 48 h on fibers such as PET, polypropylene (PP), PA, acrylic, and rayon (viscose filaments) have
82 been recently assessed (Thiele et al., 2019). Rayon turned out to be dissolved at 60 °C but not at 40 °C
83 (Thiele et al., 2019). H_2O_2 is a common oxidizer used in digestion protocols but different microplastic
84 fragments turned out to be impacted such as PA 6.6, which is dissolved when exposed to H_2O_2 at
85 high temperatures (> 60 °C), PP, and polyethylene (PE) (Hurley et al., 2018; Nuelle et al., 2014;
86 Sujathan et al., 2017). Fenton's reagent has recently been proposed as a highly effective in digestion
87 protocols with no degradation reported on tested polymers (mostly fragments) (Hurley et al., 2018;
88 Tagg et al., 2017). Only PET fibers were tested. Enzymatic protocols have also been reported to be
89 highly effective with no damage to polyester and polypropylene fibers (Piarulli et al., 2019).
90 However, these protocols are generally expensive. Less expensive enzymatic digestion protocols exist
91 but are generally time consuming (at least 6 d) (Mintenig et al., 2017). A combination of NaClO and
92 HNO_3 has proved to be aggressive for polyvinyl chloride (Collard et al., 2015). A combination of
93 H_2O_2 and KOH has proved to have a strong impact on natural and semi-synthetic fibers (Cai et al.,
94 2019).

95 The impact of digestion protocols on synthetic fibers is not the only problem; blends of synthetic and
96 nonsynthetic fibers have been found in samples. Owing to the growing concerns about their adverse
97 effects on ecosystems, reliable and robust protocols that preserve the integrity of all kinds of fibers, or
98 at least, a knowledge of which protocol(s) may have an impact on the various fibers is needed.

Almost no data is available on mass changes, mechanical degradations, and/or microscopic modifications of fibers for the various digestion protocols. To fill this data gap, five different digestion protocols were tested on seven different industrial raw fibers *i.e.*, three synthetic fibers, one artificial fiber, and three natural fibers. To fill the knowledge gap highlighted above, changes in mass, diameter, mechanical properties, and chemical dissolution after digestion protocols were evaluated using five performance parameters: (i.) mass, (ii.) morphology, (iii.) tenacity, (iv.) elongation at break and (v.) infrared spectra.

For reliable results, we tested fibers with a similar diameter to fibers commonly found in samples collected from the environment. Because OM can be particularly diverse and our study only focused on the impacts of digestion protocols on fibers and did not consider the digestion efficiency of OM, we selected protocols previously tested on organic matter.

Currently available data on the impact of digestion protocols on fibers in their original state is inadequate. To avoid parasitic chemical reactions, the most crucial chemical parameters were precisely measured and controlled (volume, fiber type, temperature, and the composition of the solution). Our samples were free of OM. In this manner, potential influence of OM on reactions was avoided. As such, we observed impacts on fibers from only digestion protocols.

Various databases are commonly referred to when predicting reactions between polymers and solvents, such as the Hildebrandt or the Hansen solubility parameters (Wypych, 2016). However, parameters such as temperature, concentration, and reaction time are important factors that could affect those predictions and necessitate the need for imposing an experimental validation procedure.

2. Material and methods

2.1. Prevention of microplastic contamination

Contamination mitigation was ensured using the following precautions:

- All the solutions used were preliminarily filtered on GF/D Whatman filters (Sigma Aldrich, 2.7 µm).

- All the glass vessels and filters were heated at 500 °C for 2 h before use. Vessels were then rinsed with filtered water and filtered 50 % ethanol (96 %, technical). Plastic materials were avoided and only 100 % cotton laboratory coats were worn.
- Fibers were stored in glass beakers covered by aluminum foils. Moreover, beakers were all covered by aluminum foils during experiments.

2.2. Tested protocols

To be fit for operation, digestion protocols must be (i) easy to apply (in only one step), (ii) fast (less than 48 h), and (iii) inexpensive. For the latter two, enzymes were not considered. Acids were also avoided because important degradations were observed after acidic digestions (Avio et al., 2015; Catarino et al., 2017). Five protocols were selected:

- Protocol 1: 50 ml of 10 % KOH (v/w) for 24 h at 40 °C adapted from Karami et al., 2017;
- Protocol 2: 50 ml of 10 % KOH (v/w) for 24 h at 60 °C adapted from Dehaut et al., 2016;
- Protocol 3: 50 ml of 1:4 diluted NaClO (La Croix ®, 3.6 w% of active chloride) for 1 night (15 h) at room temperature (~20 °C) adapted from Collard et al., 2015;
- Protocol 4: 50 ml of 30 % H_2O_2 (v/v) for 48 h at 40 °C adapted from Mintenig et al., 2014;
- Protocol 5: 50 ml of Fenton's reagent for 2 h, using 33.3 ml of 30 % H_2O_2 (v/v) with 16.7 ml of an iron(II) sulfate heptahydrate catalyst solution (20 g of $FeSO_47H_2O$ dissolved in 1 L of distilled water) acidified with sulfuric acid (H_2SO_4, pH = 3) adapted from Hurley et al., 2018.

Only temperature differed for Protocols 1 and 2. Fibers and particularly synthetic fibers, are known to be sensitive to heat (Wypych, 2016). The tentative glass transition temperatures (Tg) for PET and PA 6.6 are close to 60 °C (tentative glass transition temperature for PET: 60–85 °C and for PA 6.6: 56–70 °C (Wypych, 2016)), which means high temperatures (> 60 °C) can impact their structure. Moreover, Thiele et al., 2019 observed a total dissolution of viscose when exposed to 10 % KOH at 60 °C for 48 h. Other works found polymer degradations owing to high temperatures (>70 °C) (Munno et al., 2017). For these reasons, the impact of temperature was investigated. For Protocol 3, contrary to the original protocol from Collard et al., 2015, digestion was reduced to a one-step process

150 to test NaClO potential impacts on fibers. Reagent impacts on fiber integrity for a combination of
151 reagents is complex. Moreover, a slightly lower concentration was tested in this study to reduce the
152 potential impacts on fibers. In this paper, Protocols 1 to 5 are denoted as follows: KOH 40 °C, KOH
153 60 °C, NaClO, H_2O_2, and Fenton.

154 2.3. Tested fibers

155 We tested widely available commercial raw fibers used for textile manufacture with worldwide
156 circulation (TextileExchange, 2018): three synthetics (polyethylene terephthalate (PET), polyamide
157 6.6 (PA 6.6), and polyacrylonitrile (acrylic)), one vegetal artificial (viscose), two vegetal natural
158 (cotton and flax), and one animal natural (wool). We selected raw fibers in our tests for three main
159 reasons:

160 • Studies on mass variations require large amounts of fibers (at least 800 mg per sample).
161 However, collecting large amounts of environmental fibers would have been complicated
162 because the extraction of environmental fibers is time consuming. Practically, only a few mg
163 would have been extracted.
164 • Artificial weathering would have been appropriate but it raises two issues: (i) which method
165 should be considered as the most representative for environmental fibers analyses (UV
166 exposure, mechanical or biological degradation, hydrolysis or a combination of these
167 methods?); (ii) no current standards exist concerning acceptable thresholds for parameters
168 used during artificial weathering.
169 • Raw fibers are supposed to be more resistant than environmental fibers. Thus, if a digestion
170 protocol affects raw fibers, it need not be tested on environmental fibers.

171 For these reasons, we selected raw fibers as reference materials as they provided a basis for a better
172 assessment and better reproducibility. We acquired the raw fibers from GEMTEX (textile materials
173 engineering laboratory, Roubaix, France), in the form of filaments with an initial length of ~10 cm.
174 Those fibers correspond to commonly used materials before industrial spinning processes. We
175 characterized these fibers before and after subjecting them to digestion protocols. As the fibers are

commercial raw fibers, they include additives. We were not able to obtain specific information about the additives from the supplier. Because fibers from environmental samples also include additives (which are generally unknown) and as the final objective of the study is to obtain a method suitable for environmental samples, it is appropriate to test this methodology on commercial raw fibers with unknown additives and not on laboratory-prepared ones, to achieve a higher representativity.

2.4. Performance parameters and experimental design

Digestion protocols were carried out following the steps below: for a given digestion protocol, 200 ± 20 mg of each fiber (800 ± 20 mg for mass variation assessment, to ensure a higher representativity) were cut using cleaned scissors (fiber length from 1 mm to 5 cm) and plunged into 50 ml of reagent (KOH, H_2O_2, NaClO, or Fenton's reagent) in a beaker using metallic clamps. 200 ml of reagent was used for mass variation assessment as a higher fiber mass was used to test this parameter (except for Fenton's reagent, for which 100 ml was used to avoid violent reaction). Scissors and clamps were rinsed thoroughly between samples with filtered water followed by filtered 50 % ethanol (96 %, technical) to avoid contamination. Reaction occurred in beakers and on a heating plate IKA® RT, 15-15 position (when heating was needed) at the required temperature (temperature precision: ± 2 °C), without stirring. Temperatures were regularly checked using a checktemp 1 HI 98509 Hanna Instruments® precision thermometer. After the allotted time, the heating was stopped and the solutions containing the fibers were filtered on GF/D Whatman filters (Sigma Aldrich, porosity: 2.7 µm and Ø 90 mm). Filters that contained digested fibers were stored in glass Petri dishes, put in a desiccator for 48 h, and then dried in an oven at 40 °C for 48 h. Finally, fibers were put in a controlled atmosphere (20 °C, 65 % relative humidity) for at least 24 h. The parameters of the fibers before and after digestion are presented in Results and discussion.

2.4.1. Mass variation

For mass variation, each digestion protocol was tested three times, *i.e.*, for each digestion protocol, three different fiber batches of 800 mg of each fiber type underwent the same digestion protocol. The

fibers were weighed before being used in the digestion protocols. When dry, the fibers were weighed using a Sartorius® CPA225D precision balance (± 0.01 mg).

2.4.2. Morphological aspects

The fibers were longitudinally and cross-sectionally observed using an optical microscope MOTIC B1-223, at three magnifications (x10, x40, x60). For longitudinal observations, it was necessary to isolate a few fibers and put them into two glass slides. After the longitudinal observations, a small number of fibers were parallelized and cut using a microtome (Hills Inc.), for cross-sectional observations. When damages were detected or suspected, fibers were pasted on an aluminum stub with a double face adhesive then coated with a uniform platinum film by vacuum evaporation (Jeol JFC-1100E) and observed using a Scanning Electron Microscope (SEM) (Jeol 6301F) fitted with an X-ray Energy Dispersive Spectrometer (Silicon Drift X-Max 80 mm^2 detector and Aztec Advanced-INCA350 analyzer, Oxford Instruments). To observe damages and characterize deposits on fibers, secondary electron analysis and X-ray analysis were performed, respectively.

2.4.3. Mechanical properties

The tenacity and elongation of the fibers at break were assessed. Tenacity, as shown in Equation 1, corresponds to the ratio between the maximum longitudinal strength that a fiber can endure, and its linear density:

Equation 1. Definition of tenacity (cN/Tex)

$$\text{Tenacity}\left(\frac{\text{cN}}{\text{Tex}}\right) = \frac{\text{Maximum tensile strength (cN)}}{\text{Linear density (Tex)}} \qquad (1)$$

The maximum tensile strength of fibers was assessed following the NF EN ISO 5079 standard on a tensile testing machine (Zwick (1456)); the cell force used was 10 N, with a preload of 0.2 N/m². All the tests were carried out at standard atmosphere conditions (20 ± 2 °C; HR 65 ± 5 %). The lengths of the samples were 20 mm and the deformation rate were 20 mm/min, with the preload.

Linear density also called fiber count usually expressed in Tex or dTex (10^{-7} kg. m^{-1}), is the ratio between mass and length. Two different methods were used for linear density measurement: (i) measurement of radius of the fibers and (ii) vibroscope measurements (only for synthetic fibers). The first method is a direct measurement of fiber radius using a microscope. Linear density is shown in Equation 2.

Equation 2. Linear density depending on fiber radius

$$\text{Linear density} = 10^{-7}\rho\pi r^2 \qquad (2)$$

with linear density in dTex; ρ = density in kg. m^{-3}; and r = fiber radius in m

30 radius measurements were taken for each fiber (digested and nondigested) except for PET and acrylic as they were too dark for direct observation of their fiber radius. To solve this problem, PET and acrylic fibers were mixed with light-colored fibers (wool) and their radii compared. 10 radius measurements were taken for the dark-colored fibers. The second method was only applied to synthetic fibers (PET, PA 6.6, and acrylic). It required the use of a vibroscope (Lenzing instrument). For this method, 10 measurements were taken.

Elongation at break is the ratio between the initial length and the maximum length before the rupture of the fiber. Elongation at break and tenacity were measured at the same time using the tensile test bench at the same conditions outlined above.

For synthetic fibers (PET, PA 6.6, and acrylic), at least 5 and up to 30 tensile tests were performed. Owing to handling difficulties, elongation at break and tenacity measurements were adapted for artificial and natural fibers (samples length was 10 mm) and up to six tensile tests were performed.

2.4.4. Infrared spectra

To study the spectral modifications of the different fibers after digestion, fibers were chemically characterized using a Fourier-Transform Infrared (FTIR) spectroscope (Thermo Scientific® Nicolet iN10 MX) in transmission mode. A few fibers were put on Whatman® anodisc inorganic filter

membrane (porosity: 0.2 µm, Ø 25 mm) and analyzed using the same parameters for each fiber type. The detector used was a Thermo Scientific® MCT/A Cooled 25 mm detector, (spectral range of 4000 to 1200 cm^{-1} to avoid interferences with the anodisc filter, detection time of 12 seconds (64 scans) in absorbance mode). Spectroscope aperture used was 20 µm in width and 20 µm in height. For each experiment, six infrared spectra on six different fibers were collected. To compare nondigested and digested fibers, an auto-baseline correction was applied to all infrared spectra. Then, for a given fiber type, an average of the six spectra was applied. Digested fibers spectra were compared to nondigested ones using the average spectra and the correlation comparison software "Q-check" (Thermo Scientific®), which gives a percentage of correlation between two spectra by comparing all the different peaks of both infrared spectra.

2.5. Evaluation of digestion protocols impacts

To evaluate the impact of digestion protocols, impact indicators were defined for variations of morphological aspects, mass, tenacity, elongation at break, and infrared spectra correlations. To ensure a better comparison and to avoid statistical bias owing to extreme values, median values were used instead of mean values. For a given parameter value, a score was given depending on the impact indicators previously defined: 0 for "no data", 1 for "not affected", 2 for "slightly affected", 3 for "affected", and 4 for "highly affected". All impact indicators and scores for each parameter are reported in Table 1. For example in Table 1, for mass variation, fibers were observed to be slightly affected (score = 2) when mass variation ranged between 5-10 %. They were observed to be highly affected (score = 4) when mass variation reached beyond 25 %. The same indicator values were used for tenacity and elongation at break measurements. It is important to note that when fibers were too brittle to be handled, the maximum score for tenacity and elongation at break was attributed. For morphological impacts, different key indices were considered for structural modifications such as radius reduction, fiber tear, modification of rugosity, flattening, and color variation.

Parameters	Score	Indicator
Mass variation	0	No data
	1	Ranges from 5 to -5 %
	2	Ranges from 10 to -10 %
	3	Ranges from 25 to -25 %
	4	> 25 or < -25 %
Morphological aspects	0	No data
	1	No visible modification
	2	Slight modifications are visible
	3	Degradations and/or structural changes are visible
	4	The fiber structure is highly degraded
Tenacity	0	No data
	1	10 % decrease
	2	20 % decrease
	3	50 % decrease
	4	> 50 % decrease
Elongation at break	0	No data
	1	10 % decrease
	2	20 % decrease
	3	50 % decrease
	4	> 50 % decrease
FTIR Spectral comparison	0	No data
	1	Correlation between 100 and 90 %
	2	Correlation between 90 and 80 %
	3	Correlation between 80 and 60 %
	4	Correlation below 60 %

Table 1. Impact indicators and scores for each parameter

3. Results and discussion

3.1. Mass variation

Median values for mass variations of all the fibers after the different digestion protocols are reported in supplementary data (Table S5). Considering the balance precision (0.01 mg) and the initial fiber mass (800 ± 20 mg), the significance of the results is confirmed when mass variations are higher than 0.5 %. However, after KOH 40 °C, KOH 60 °C, and Fenton, mass gains of 5–10 % are observed for almost all the fibers. This is most likely attributed to potassium or iron deposits on fibers which were

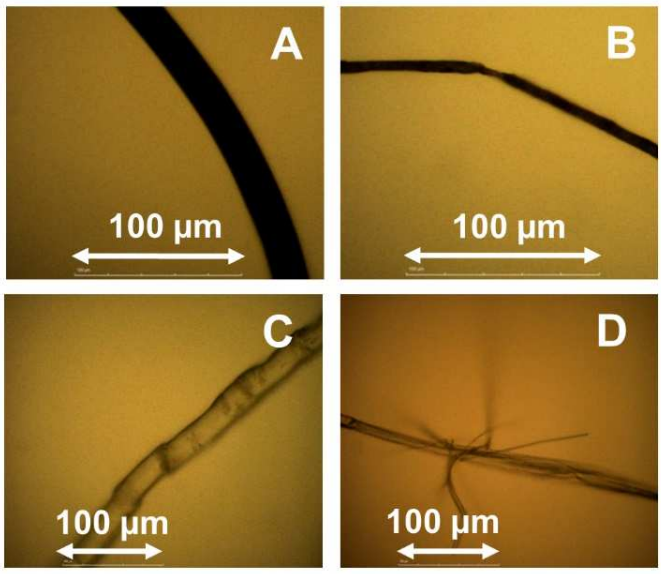
observed on fibers via scanning electron microscopy (see supplementary data, Figure S1). Owing to the deposits observed, it is important to be vigilant on mass variations. For this reason, the interpretation of results focused on important mass variations (> 20 %, in absolute value).

Wool is dissolved after KOH 40 °C, KOH 60 °C, and strongly affected after NaClO. As such it was not considered in the other tests except in H_2O_2 and Fenton's reagent). Wool is composed of proteins (mostly keratin) known to be easily dissolved by various solvents (Lewin, 2006).

PET is strongly affected by KOH 60 °C, 65.6 % of mass loss (median value) is observed for PET fibers, owing to fiber degradation. PET is known to have poor resistance to alkalis (Wypych, 2016). Nevertheless, this observation shows the importance of temperature as KOH 40 °C does not affect PET mass. For PET, temperatures close to T_g facilitate solvent migration in amorphous zones, which eventually provokes fiber dissolution. Viscose mass decreases after H_2O_2 (~20 % mass loss).

3.2. Morphological aspects

Except for PET and flax after specific digestion protocols, no other morphological alterations are revealed by the optical microscope. PET is highly altered after KOH 60 °C (see Figure 1) whereas no degradation is observed for KOH 40 °C. After KOH 60 °C PET diameter decreases from 20 to approximately 12 µm, but not consistently. Moreover, the shape of the fiber changes, from smooth to coarse (Figure 1). From SEM images, Figure 2 confirms that PET is highly degraded after KOH 60 °C. The fibers are observed to reduce in radius from 20 µm before KOH 60 °C to less than 12 µm after KOH 60 °C. Buckling zones are observed (see the red circle in Figure 2D). Buckling zones are attributed to a decrease in the global mechanical properties of the fibers from frequent observations. This hypothesis is confirmed by tenacity and elongation at break variations (see "mechanical properties"). SEM images also confirm an increase of the surface roughness for the digested fibers. KOH 60 °C on PET was repeated and another SEM analysis was performed on those fibers. We observed similar degradation. This observation confirms our first hypothesis that PET could be degraded with temperatures close to or above 60 °C.



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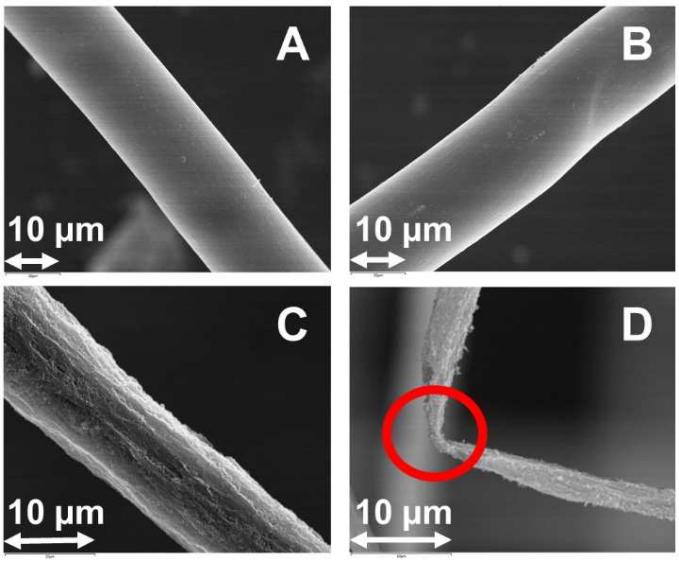
307 Figure 1. Optical microscope images (x40); A: PET before digestion; B: PET after KOH 60

308 °C; C: Flax hull before digestion; D: Flax hull after NaClO.



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310 Figure 2. SEM images obtained by secondary electron analysis of PET fibers before (A and

311 B) and after (C and D) KOH 60 °C. The red circle shows a buckling zone.

312 The flax cell wall is shattered by NaClO as shown in Figure 1D. The NaClO probably dissolved a part

313 of this cell wall during the digestion. This observation is confirmed by SEM (see supplementary data

314 Figure S2).

Finally, iron and potassium depositions are observed and confirmed after Fenton, KOH 40 °C, and KOH 60 °C respectively, using X-ray analysis (Figure S1).

3.3. Mechanical properties

3.3.1. Synthetic fibers

Boxplots of tenacity and elongation at break for synthetic fibers before and after the different digestion protocols are presented in Figure 3.

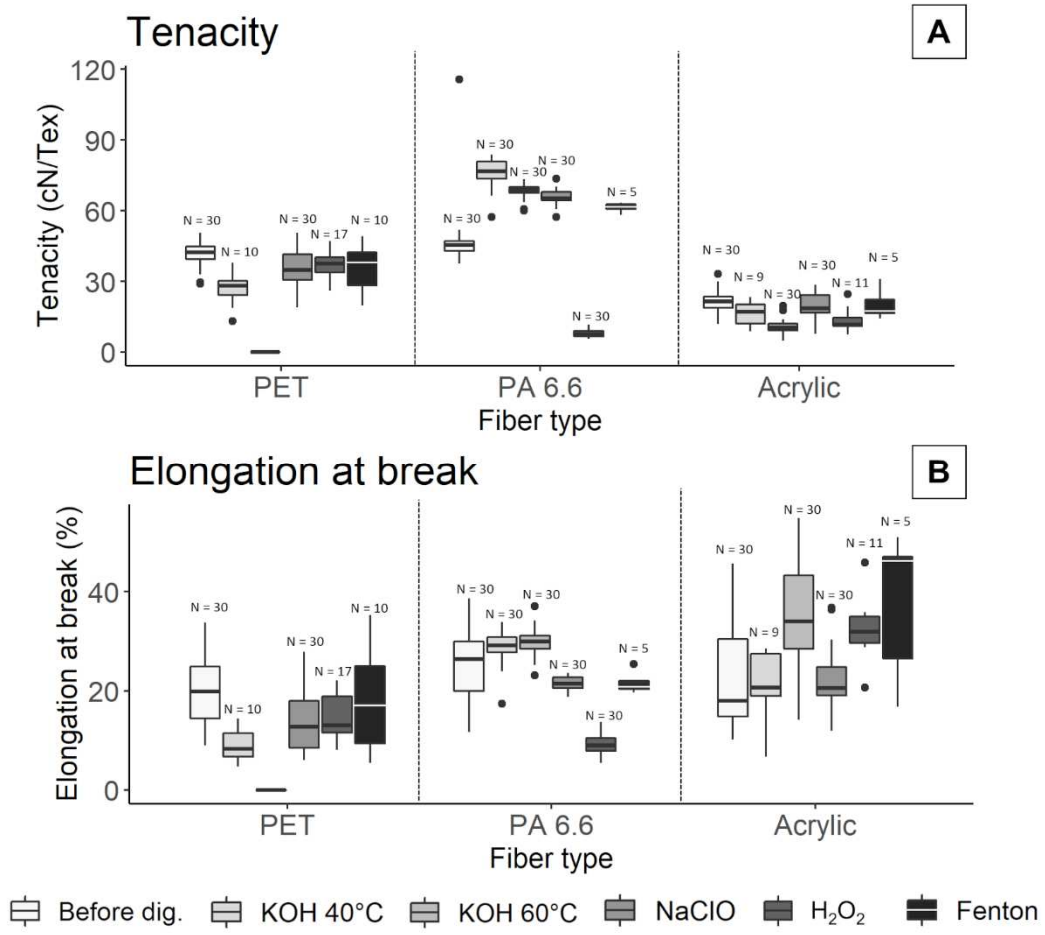

Figure 3. Boxplots of tenacity (A) and elongation at break (B) for synthetic fibers before digestion (noted "Before dig.") and after the different digestion protocols. Black dots correspond to extreme values.

Statistical significance between the results before and after each digestion was tested using a Mann-Whitney-Wilcoxon test. P-values for tenacity and elongation at break are presented in Table 2. Significance is not confirmed for grayed-out values.

		PET	PA 6.6	Acrylic
KOH 40 °C	Tenacity	3.10E-05	5.60E-10	1.70E-02
	Elongation	6.80E-05	2.00E-02	9.60E-01
KOH 60 °C	Tenacity	-	5.60E-10	7.40E-10
	Elongation	-	3.70E-03	3.00E-04
NaClO	Tenacity	8.10E-04	5.60E-10	1.70E-01
	Elongation	1.50E-03	5.70E-02	5.20E-01
H_2O_2	Tenacity	5.50E-03	3.00E-11	3.90E-04
	Elongation	1.40E-02	4.10E-11	1.10E-02
Fenton	Tenacity	6.70E-02	1.00E-03	5.60E-01
	Elongation	4.60E-01	4.40E-04	3.20E-02

Table 2. P-values for tenacity and elongation at break values after Mann-Whitney-Wilcoxon test for each fiber and protocol (significance is not confirmed for grayed-out values)

For PET, tenacity, and elongation at break median values decrease after each digestion protocol. This trend is more pronounced for PET which underwent KOH 40 °C. As for tenacity, PET after KOH 60 °C was too brittle to be tested.

PA 6.6 presents unexpected behavior. For this fiber, increases of tenacity (which are significant) are observed for all protocols, to the exception of H_2O_2. Those increases are difficult to interpret. PA 6.6 after H_2O_2 presents a significant decrease for both tenacity and elongation at break, showing a mechanical degradation.

For acrylic, tenacity slightly decreases after KOH 60 °C and H_2O_2. However, elongation at break is not affected the same way. For this parameter, values are globally stable or slightly increase.

3.3.2. Artificial and natural fibers

Tenacity and elongation at break for artificial and natural fibers before and after the different digestion protocols are presented in Figure 4.

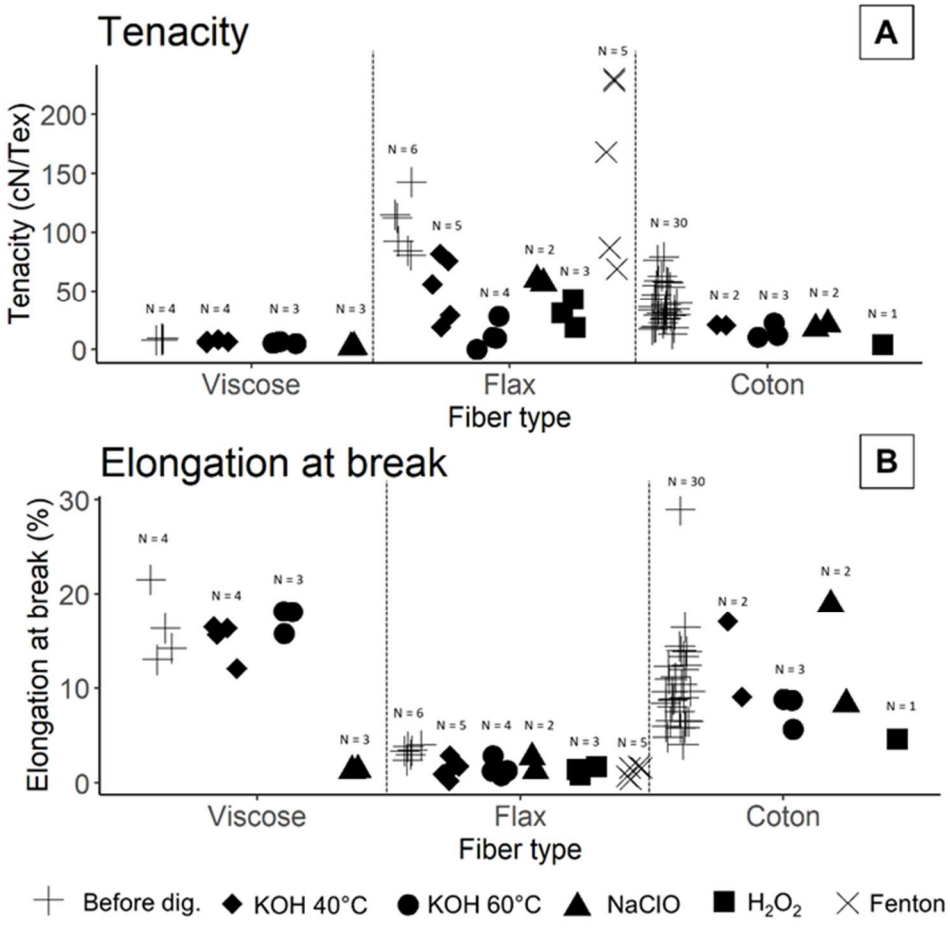

Figure 4. Tenacity (A) and elongation at break (B) for natural and artificial fibers before digestion (noted "Before dig.") and after the different digestion protocols

Owing to the number of tests (generally below 5 tests), statistical significance cannot be confirmed for artificial and natural fibers. However, different trends can be observed.

Viscose tenacity before digestion is particularly low (median value = 8.46 cN/Tex) compared to other fibers. Its tenacity decreases after KOH 40 °C (6.96 cN/Tex) and KOH 60 °C (5.42 cN/Tex). Even though this decrease might not be significant, it is more marked for NaClO (2.00 cN/Tex, a decrease of 76.3 %). For this fiber, elongation at break is stable after KOH 40 °C and KOH 60 °C. Elongation

at break seems however affected after NaClO, for which an important decrease is observed after these tests (median value = 1.35 cN/Tex, a decrease of 91.2 %). After H_2O_2 and Fenton, viscose was too brittle to be tested and broke at each manipulation with metallic clamps (even though, no degradation is visible neither by optical microscope nor SEM).

For flax, KOH 40 °C, KOH 60 °C, NaClO, and H_2O_2 have an impact on tenacity. Elongation at break generally decreases (~50 % decrease for median values of all digestions).

For cotton, 30 tests were performed before digestion. It was however not possible to reproduce as many tests after digestions. For tenacity, values after digestions generally correspond to the lowest values found before digestion, which could indicate a global decrease for all digestions. For elongation at break, no trend can be observed. Only one test was performed after H_2O_2, owing to the brittleness of cotton after this digestion.

3.3.3. Discussion regarding the changes in tenacity and elongation at break

Changes in tenacity and elongation at break after digestion protocols are attributed to chemical resistance. According to Wypych (2016), PET has a poor resistance to alkalis which explains why its tenacity and elongation at break decreased after KOH 40 °C and KOH 60 °C. A slight PET dissolution may start at 40 °C, causing slight mechanical degradations. This dissolution is much greater for KOH at 60 °C. Other digestion protocols have a lower impact.

PA 6.6 is known for its mechanical strength and its good chemical resistance to most common solvents (Wypych, 2016). Increases in tenacity would imply a higher mechanical resistance to traction. Because elongation at break is globally stable for nearly all protocols, the elasticity of PA 6.6 did not change. Even though results from PA 6.6 tests were unclear, there could be sorption mechanisms between ions (K^+, Na^+, Cl^-) and the amorphous zone of PA 6.6 as observed between water molecules and polyamide fibers (Lewin, 2006; Puffr and Šebenda, 1967). These interactions may have changed the mechanical properties of PA 6.6. For Fenton, more tests should be performed to confirm these trends. On the contrary, mechanical degradation was observed after the H_2O_2. 30 % H_2O_2 oxidized PA 6.6, this concentration is too high to maintain PA 6.6 integrity.

For acrylic, its tenacity and elongation at break are globally stable. However, its tenacity slightly decreases after KOH and H_2O_2 resulting in a small impact. Its elongation at break varies within the same range of values before and after all digestions. Acrylic is known to be resistant to many solvents, which was confirmed by our test results (Wypych, 2016).

For viscose, its tenacity and elongation at break were impacted by NaClO. Moreover, H_2O_2 and Fenton were too aggressive for this fiber. Viscose is known as a more brittle fiber owing to its structure and manufacturing process (regenerated cellulose) (Wypych, 2016).

As opposed to cotton and viscose, flax is composed of hemicellulose and lignin in addition to cellulose which could explain the observed decreases following different protocols. Lignin dissolution may explain the degradations (confirmed on SEM for flax exposed to NaClO, see supplementary data Figure S2).

Cotton has chemical resistance to many solvents which could explain why no particular impacts were observed after digestion protocols (Lewin, 2006; Wypych, 2016).

3.4. Infrared spectra

Using Q-check software (see Material and methods), the correlation of infrared spectra of digested and nondigested fibers were compared (Figure 5).

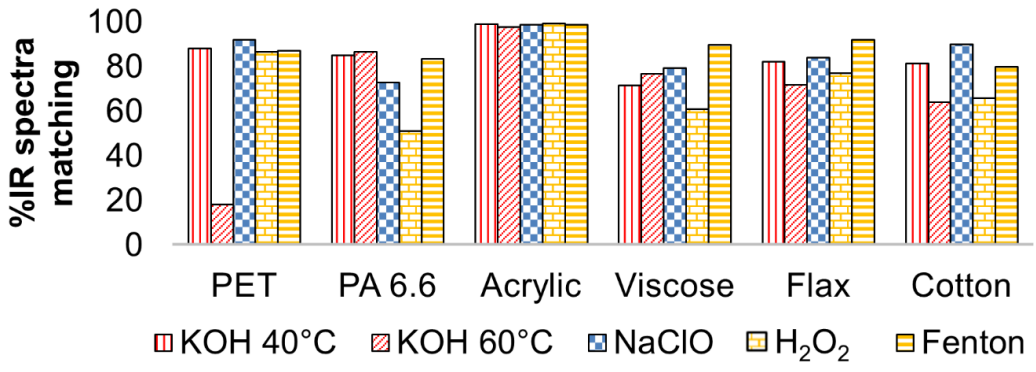

Figure 5. Matching percentages between nondigested and digested fibers infrared signals for each fiber type and each digestion protocol. The comparison was performed with Q-check from Thermo Scientific® analysis software.

PET after KOH 60 °C and PA 6.6 after H_2O_2 show the lowest correlation values of all studied fibers (17.8 % and 50.7 % respectively). The low value of PET after KOH 60 °C is most likely attributable to the reduction in diameter (from 20 µm to ~12 µm). Additionally, 10 µm generally corresponds to the smallest sample size that can be analyzed by FTIR spectroscopy.

For these fibers, infrared spectra are available in supplementary data (Figure S3 and Figure S4). Most of the digestions have an impact on the infrared signals of viscose, flax, and cotton, with most of the correlation percentages lying between 60 and 80 %. Acrylic has almost no signal modifications for any digestion protocol (all values were higher than 90 %). In terms of signal modification, KOH 40 °C, NaClO, and Fenton are the most suitable digestion protocols for reducing the impacts on infrared spectra.

3.5. Evaluation of digestion protocols impacts

By applying the different impact indicators previously defined, a score for each parameter value for each tested fiber was obtained. Radar charts in Figure 6 present an overview of all the impacts observed. Detailed variations of all parameters of each fiber after each digestion are reported in supplementary data (Table S5).

According to Figure 6, acrylic is the most resistant fiber; its performance parameters are not significantly impacted. Figure 6 shows that digestion impacts depend on fiber nature.

KOH 60 °C and H_2O_2 have the strongest impacts which, however, differ significantly. KOH 60 °C has a targeted impact on PET Additionally, all performance parameters highly impacted by KOH 60 °C (see Figure 6). Mass variation in PET owing to degradation after KOH 60 °C (65.6 % of mass loss) could lead to underestimations of weight if KOH 60 °C, for example, is followed by Pyr-GC-MS. To a lesser extent, KOH 60 °C also impacts the mechanical properties of flax and cotton as well as viscose mass. This is attributable to temperature as KOH 40 °C reduced those impacts, even though tenacity and elongation at break are still affected (Figure 6). Surprisingly, viscose was not dissolved, contrary to what Thiele et al., 2019 observed after KOH 60 °C.

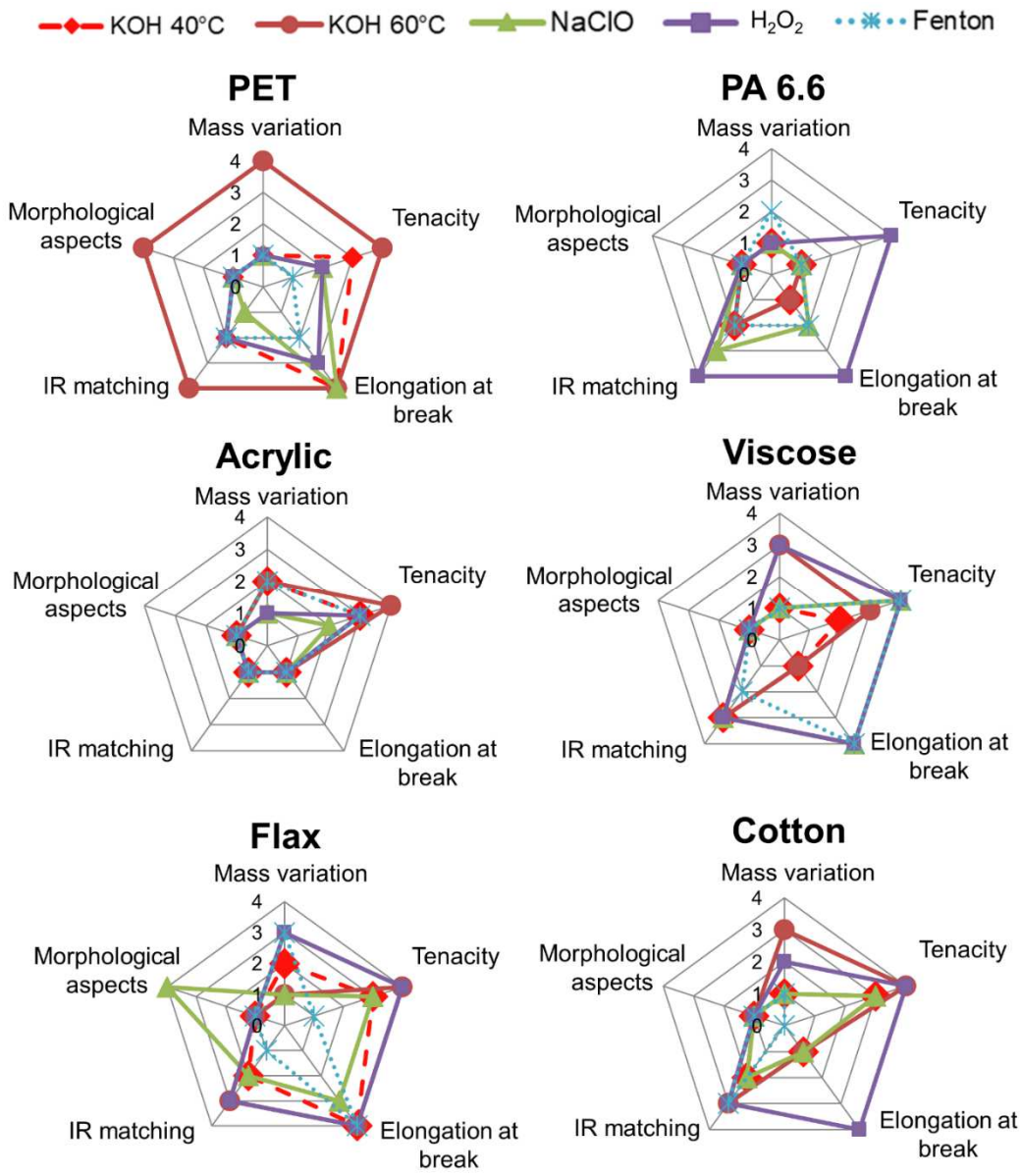

Figure 6. Radar diagrams of the impacts of digestion protocols for each fiber type

H_2O_2 affects the mechanical properties (tenacity and elongation at break) and IR spectra of PA 6.6 and potentially viscose, flax, and cotton. Brittleness of these fibers after H_2O_2 has been observed during handling. This brittleness could potentially lead to fiber fragmentation which could result in counting errors and false estimations. H_2O_2 impacts on PA 6.6 support observations made by previous works (Hurley et al., 2018; Nuelle et al., 2014; Sujathan et al., 2017) even though we did not observe dissolution of PA 6.6. This could be attributed to the length of the used fibers (>1 mm and up to 5 cm). The observed impacts of this digestion could mainly be attributed to two parameters: (i)

concentration, (ii) the reaction time (48 h), or probably a combination of both. Previous works used more diluted H_2O_2 solutions (Sujathan et al., 2017).

It is worth noting that our results do not prove fibers fragmentation during protocols. Rather, they only compare the impact of digestion protocols on different fiber parameters. Our results form a basis for developing a more suitable digestion protocol for fiber extraction.

KOH 40 °C, NaClO, and Fenton have the smallest impacts on fibers. Karami et al., 2017 showed an increase in the recovery rate of microplastics and digestion efficiency when KOH 40 °C was used instead of KOH 60 °C. Piarulli et al., 2019 showed PES fibers degraded after 4 d of exposure in KOH 1M at room temperature. More tests are needed to find out the optimum time and temperature for KOH digestion, but 10 % KOH at 40 °C for 24 h is promising to be effective in maintaining fiber integrity.

Apart from flax and viscose, NaClO does not impact most of the parameters of the fibers. However, the efficiency of this protocol on OM should be assessed as it is commonly combined with HNO_3 (Collard et al., 2015).

Finally , Fenton shows high efficiency for OM removal (Hurley et al., 2018; Tagg et al., 2017) with small impacts on all tested fibers to the exception of viscose mechanical properties. This protocol tends to be a suitable digestion protocol for fragments and fibers analysis. This could be explained by the short reaction time (reaction ended in 15 minutes, although we let the reaction occur for 2 h) without additional heating. However, an iron deposition was observed on GF/D filters, which may complicate the analysis of microplastics, particularly counting. Iron concentration seems too high and could be reconsidered to avoid those deposits. However, iron deposits have no influence on infrared spectra (Figure 5).

To the exception of wool, results showed the relatively high resistance of natural fibers. This observation supports the need of fiber characterization after digestion protocols. More techniques should be developed to distinguish synthetic, semi synthetic and natural fibers.

Distinction between man-made cellulosic (e.g. viscose) and natural cellulosic fibers (e. g. cotton, flax) in environmental samples is still a scientific challenge since degraded cellulosic fibers are generally difficult to differentiate (Cai et al., 2019; Remy et al., 2015; Zhu et al., 2019). Thus, fiber classification may be reconsidered for environmental fibers by distinguishing: (i) synthetic fibers, (ii) cellulosic fibers and (iii) proteinaceous fibers which mainly come from animals. This new classification could help for a better comparison between samples.

4. Conclusion

10 % KOH 60 °C for 24 h and 30 % H_2O_2 40 °C for 48 h have the strongest impacts on raw fibers particularly synthetics and should be avoided for microfibers analysis. 10 % KOH 40 °C for 24 h, 1:4 diluted NaClO for one night (15 h) at room temperature (~ 20 °C) and Fenton's reagent are more appropriate to maintain fibers integrity.

The tests within this study are performed on industrial raw fibers with no weathering. More tests should be applied on weathered or artificially weathered fibers to assess digestion impacts on fibers.

Moreover, parameters could probably modify fiber stability towards digestion protocols, such as: (i) formulation parameters (molar mass, additives…) and (ii) proceeding parameters (crystallinity, specific surface area…). In the future, more data should be collected on the impacts of those parameters.

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