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Abstract: By-products of tomato processing are rich in bioactive compounds and their recovery might bring significant economic and environmental benefits. High-pressure homogenization (HPH) (1-10 passes at 100 MPa) was used as a disruption method to recover valuable compounds from tomato peels, using solely water as process medium. Micronization of tomato peels suspensions by HPH reduced their size distribution below the visual detection limit, because of the complete disruption of individual plant cells. With respect to high-shear mixing (5 min at 20000 rpm), HPH processing (10 passes) caused an increased release of intracellular compounds, such as proteins (+70.5%), and polyphenols (+32.2%) with a corresponding increase in antioxidant activity (+23.3%) and reduction in oil-water interfacial tension (-15.0%). Remarkably, also the release of water-insoluble lycopene in the aqueous supernatant increased, enabling the recovery of up to 56.1% of the initial peel content, well above what reported when using organic solvents or supercritical CO2.

Università di Salerno
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Manuscript title: "High-Pressure Homogenization treatment to recover bioactive compounds from tomato peels"

Dear Editor,

On behalf of my coauthors, I would like to submit the present article for consideration for possible publication in the *Journal of Food Engineering*.

The manuscript concerns the work that the research groups involved (from the University of Salerno and ProdAl Scarl, from the University of Zagreb and from Unilever R&D Vlaardingen) have carried out in the last years for the valorization of agro-food industrial residues, through the recovery of bioactive compounds.

In particular, this contribution is the continuation and the expansion of the initial work reported in the article "Novel approaches to oil structuring via the addition of high-pressure homogenized agri-food residues and water forming capillary bridges", recently published in the *Journal of Food Engineering* (Journal of Food Engineering 236 (2018) 9-18).

The main contribution of this work, in terms of novelty and originality, is the development of the concept of unlocking the intracellular compounds using a physical disruption, yet mild, technology, such as the high-pressure homogenization. In particular, the results have shown that the recovery of lycopene positively compares with previous literature data, even though only water was used as extraction solvent, rather than mixtures of organic solvents or supercritical CO_2. This is quite remarkable, considering that lycopene is not soluble in water; a possible explanation, discussed in the manuscript, is the formation of complexes of lycopene with hydrocolloids, which could be exploited as natural delivery systems.

In terms of impact, the results of our study pave the way towards the application in the food industry of a green, sustainable and purely physical process for the valorization of agro-food by-products and residues.

On behalf of the authors, I declare that we have consulted the Guide for Authors in preparing the submitted manuscript, and confirm that we have prepared the manuscript in compliance with the Ethics in Publishing Policy as described in the Guide for Authors. In addition, I also declare no conflict of interest.

Independently on the final evaluation, I would like to thank you for the opportunity of being considered for this Journal, as well as of receiving important suggestions for the improvement of our work.

Best regards,

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Highlights

- Tomato peel suspensions were treated in water by high-pressure homogenization (HPH)
- Water was used as a process medium, without the need for organic solvents
- HPH caused the size reduction of tomato peels below the visual detection limit
- HPH increased the release of intracellular compounds (protein, sugars, bioactives)
- 10 HPH passes extracted in water 56.1% of the total lycopene content of the peels

High-Pressure Homogenization treatment to recover bioactive compounds from tomato peels

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Declarations of interest: none

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Abstract

By-products of tomato processing are rich in bioactive compounds and their recovery might bring significant economic and environmental benefits. High-pressure homogenization (HPH) (1-10 passes at 100 MPa) was used as a disruption method to recover valuable compounds from tomato peels, using solely water as process medium. Micronization of tomato peels suspensions by HPH reduced their size distribution below the visual detection limit, because of the complete disruption of individual plant cells. With respect to high-shear mixing (5 min at 20000 rpm), HPH processing (10 passes) caused an increased release of intracellular compounds, such as proteins (+70.5%), and polyphenols (+32.2%) with a corresponding increase in antioxidant activity (+23.3%) and reduction in oil-water interfacial tension (-15.0%). Remarkably, also the release of water-insoluble lycopene in the aqueous supernatant increased, enabling the recovery of up to 56.1% of the initial peel content, well above what reported when using organic solvents or supercritical CO_2.

Keywords: *High-pressure homogenization*; *Tomato peels*; *Lycopene*; *Bioactive compounds; Agro-food by-products; Natural functional ingredients*

1. Introduction

Tomato processing by-products are generally used as animal feed or compost, despite they are still rich in high value-added compounds, hence projecting significant economic and environmental benefits from their exploitation as functional food ingredients.

On a dry basis, the tomato processing by-products (peels and seeds) are rich in polyphenols (> 1000 mg GAE/kg (Nour et al., 2018)), fibers (about 50 wt % (Nour et al., 2018)), proteins (between 10 (Elbadrawy and Sello, 2016) and 18 wt % (Nour et al., 2018)), and carotenoids, such as β-carotene (about 95 mg/kg (Nour et al., 2018)) and lycopene (about 500-800 mg/kg (Nobre et al., 2009; Nour et al., 2018)).

Lycopene which is the main component of tomato-residues carotenoids is found to have significant beneficial effects on human health (Story et al., 2010). It is accumulated in higher concentrations in tomato peels (33.2 % - 72.3 %) than in the flesh (Zuorro et al., 2011), which is motivating the interest in the valorization of the tomato processing by-products, which consists mainly of tomato peels (Viuda-Martos et al., 2014). However, it is important to remark that lycopene is found predominantly in the chromoplast of plant tissues. During the ripening process of tomatoes, chloroplasts undergo transformation to chromoplasts and lycopene biosynthesis increases dramatically (Kirk and Tilney-Bassett, 1978). Lycopene, as a highly hydrophobic molecule, is located inside the vesicles generated from the inner membrane of the plastid and is arranged exclusively within the inner part of the lipid bilayer (Fiedor and Burda, 2014). In addition, within the vegetable cells, lycopene is present in a complexed form with proteins (Agarwal et al., 2001a; Erdman et al., 1988). Because of all these reasons, lycopene recovery from tomato peels requires intensive thermal or mechanical treatments and organic solvents.

In general, also the other active ingredients of tomato peels are tightly locked inside the plant cells, with consequent significant resistances to mass transfer during conventional extraction processes, such as solvent, enzymatic or thermal extraction (Chan et al., 2014; Franco et al., 2007). Because of that, novel methods are under investigation, for the partial or total disintegration of the vegetable cells. Polysaccharides are predominantly located between the primary and secondary cell walls, whereas proteins (with a prevalent amino acid content of glutamic acid, aspartic acid, arginine, leucine, lysine (Elbadrawy and Sello, 2016; Nour et al., 2018)), phenols (in particular caffeic, protocatechuic, vanillic, and gallic acid and catechin (Elbadrawy and Sello, 2016)), and other antioxidants (in particular flavonoids, β-carotene and

lycopene (Nour et al., 2018)), along with lipids (in particular, fatty acids (Elbadrawy and Sello, 2016; Nour et al., 2018)), are found in the inner bodies of the cells, i.e. vacuoles and lipid vesicles. Therefore, the permeabilization of the primary cell membranes, such as those induced by Pulsed Electric Fields (PEF) (Pataro et al., 2018a), might not be sufficient, and more intensive or selective processes are needed to open up the secondary membranes (Donsì et al., 2013, 2010).

For example, high-shear mixing (HSM), routinely used to prepare foams, emulsions, and suspensions, is able to mill coarse particles suspended in a fluid, under the strong shear forces generated by the high rotation speed of 10,000-20,000 rpm (Chen et al., 2014). However, literature data have shown that this process is able to disaggregate cell lumps, but not to efficiently disrupt vegetable cells (Mustafa et al., 2018). High-pressure homogenization (HPH) has been reported to be a fast and effective method to micronize plant tissue in suspension and to unlock the bioactive compounds entrapped in cells, with high extraction yields (Mustafa et al., 2018; Pataro et al., 2018b; Shouqin et al., 2004). Moreover, HPH is able to produce a homogeneous size distribution of the vegetable particles suspended in a liquid, by forcing the liquid under the effect of pressure through a specifically designed homogenization valve (Patrignani and Lanciotti, 2016). High-pressure homogenization (HPH) is a technique specifically suitable for industrial applications, because of the ease of operation, scalability, reproducibility, and high throughput (Liedtke et al., 2000; Schultz et al., 2004).

The main objective of this work is to investigate the potential of HPH processing of suspensions of tomato peels in water to induce high levels of cell disruption and high yields of recovery of intracellular compounds, such as lycopene, total polyphenols, proteins, and polysaccharides. The final goal of this research is, hence, to investigate the possibility of full exploitation of the by-products of the tomato processing industry, achieving the concept of zero residual waste, and developing a green, sustainable process, which uses water as recovery medium.

2. Materials and Methods

HPLC grade methanol, ethanol, acetonitrile, and 2,4,6-tripyridyl-S-triazine (TPTZ) were supplied from Sigma-Aldrich (Steinheim, Germany). Sulphuric acid was purchased from Sigma Aldrich (St. Louis, USA). Analytical grade formic acid was purchased from Riedel-deHaën (Seelze, Germany). The chemicals for total polyphenols (Folin-Ciocalteau reagent), lycopene standard, bovine serum albumin (A7030) standard, and D-Glucose (G8270) standard were purchased from Sigma-Aldrich (Milan, Italy). Peanut oil (Sagra, Italy) was bought from a local supermarket. Piccadilly tomatoes were purchased from a local market and were immediately (fresh) used for this research. Figure 1. depicts the experimental workflow, including sample preparation, treatments carried out and analysis of samples.

[Figure 1 here]

2.1. Pretreatment of the samples

Tomatoes were blanched according to conventional methods adopted in a processing factory (FDP s.r.l., Fisciano, Italy) at 95 °C in a steam oven for 3 min (Pataro et al., 2018b). Blanched tomatoes were then immediately ice-cooled and manually peeled with a laboratory blade. The pulp was completely removed and fresh tomato peels were ready for further processing. The moisture content of the tomato peels after preparation was 80 wt %. The tomato peels were mechanically milled in a laboratory blender to the size of 1-2 mm and then distilled water was added to a final concentration of 10 wt % of tomato peels. The suspension was immediately used for the HSM and HPH treatments.

2.2. High-shear mixing and high-pressure homogenization

Tomato peels suspensions (300 mL) were subjected to HSM at 20000 rpm for 5 min with a T-25 Ultra Turrax device (IKA, Germany) equipped with an S25 N18 G rotor. In order to avoid any temperature rises, the treatment was carried out in an ice bath. Additionally, before HPH processing, to prevent the blockage of the homogenization valve, the HSM suspensions were sieved with a mesh size of 600 μm as a precaution. Sieving removed only a small fraction of the solids from the suspension (the final concentration always remained > 9.75 wt %). HPH

was carried out using an orifice valve assembly (orifice diameter of 150 μm) at 100 MPa for up to 10 passes. A tube-in-tube heat exchanger was used immediately upstream and downstream of the orifice valve, in order to ensure that the product temperature was always kept below 24 °C. 15 mL of extracts were taken after 1, 3, 5, 7 and 10 passes for further analyses. HSM samples were used as the controls for the corresponding HPH samples. The adopted sample labels are as follows: HSM for high-shear mixing, HPH, followed by a number, for high-pressure homogenization for different passes; for example, HPH 1 indicates one pass, and HPH 10 indicates 10 passes of high-pressure homogenization.

2.3. Macro and microscope imaging and particle size measurement

After HSM and HPH treatments, the suspensions (2 mL) were poured in small Petri dishes to acquire photographs with a Nikon Coolpix S7000 camera. Microscopic images were acquired with an inverted optical microscope (Nikon Eclipse TE2000-S) at 100× magnification. The particle size distribution of the obtained suspensions was characterized by light diffraction (Malvern Mastersizer 2000, Malvern Instruments Ltd., UK), and expressed in terms of the characteristic diameters d(0.1), d(0.5), d(0.9) as well as of the volume weighted mean diameter D[4,3] and surface weighted mean diameter D[3,2], as previously discussed (Mustafa et al., 2018).

2.4. Recovery of aqueous supernatant and pellet fractions from tomato peels suspensions

The tomato peels suspensions, treated by HSM and HPH, were subjected to centrifugation for 10 min at 5 °C and 6500 rpm (PK121R model, ALC International, Cologno Monzese, IT), in order to separate the aqueous supernatant, containing the intracellular compounds released during the treatment, from the cell debris. After centrifugation, the supernatant was filtered through Whatman no. 4 filters under vacuum, to remove residual particles. The obtained aqueous supernatant was then used directly for the analysis of total polyphenols, antioxidant activity, total sugars, total proteins, lycopene and surface activity.

Furthermore, the aqueous supernatant was evaporated in a Büchi Rotavapor R-300 Evaporator System until dry and was resuspended in the same volume of ethyl lactate for spectrophotometric analysis, or in acetone for HPLC analysis.

In contrast, the pellet, recovered from centrifugation, was subjected to solvent (acetone or ethyl lactate) extraction for the quantification of residual lycopene. In particular, 1 g of the pellet was extracted with acetone or ethyl lactate (60 mL) under agitation in a thermostated orbital shaker at 180 rpm and 20 °C in the dark. After 1 h extraction, the solvent pellet extracts were subjected to centrifugation for 10 min at 5 °C and 6500 rpm and then filtered through Whatman no. 4 filter paper. Ethyl lactate extracts were used for UV-Vis analyses. Acetone extracts were used for the determination of lycopene by HPLC.

2.5. Total polyphenols

The total polyphenols content in the aqueous supernatant was determined by adopting a previously proposed method (Slinkard and Singleton, 1977) with slight modifications. The supernatant (1 mL) was added to a test tube along with 5 mL of Folin Ciocalteu reagent (diluted 1:10 with distilled water). 4 mL of 7 % Na_2CO_3 was then added, vortexed and left in a dark chamber for 1 h at room temperature. Distilled water was used as a blank. Absorbance was then measured at 765 nm. Gallic acid was used as a calibration standard. Results were expressed as mg Gallic Acid Equivalents (GAE) per volume (L) of the aqueous supernatant.

2.6. Antioxidant activity - FRAP

The antioxidant activity of the aqueous supernatant was evaluated by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), modified as described by Bobinaite et al. (Bobinaitė et al., 2015). A standard calibration curve was obtained for ascorbic acid so that the FRAP values were expressed as μmol of ascorbic acid equivalents (μmol AA) per volume (L) of the aqueous supernatant.

2.7. Total proteins

The total water-soluble protein concentration in the aqueous supernatant was determined using the Lowry method (Lowry et al., 1951) with some changes. Briefly, The Folin-Ciocalteau reagent was initially diluted in distilled water (1:2, v/v) then 0.5 mL of the diluted reagent was added to 1 mL of supernatant. Previously, supernatant was mixed with 5 mL of the reactive C – 50 volumes of reagent A – 2 % (w/v) Na_2CO_3 + 0.1 mol dm^{-3} NaOH and with

1 mL of reactive B – ½ volume of 0.5 % (w/v) $CuSO_4 \cdot 5H_2O$ + ½ volume of 1 % $KNaC_4H_4O_6 \cdot 4H_2O$). 35 minutes after the start of the chemical reaction, absorbance was measured at 750 nm against a blank (5 mL reactive C + 1 mL of distilled water + 0.5 mL Folin-Ciocalteau reagent) using a V-650 Spectrophotometer (Jasco Inc. Easton, MD, USA). Bovine serum albumin (BSA) standard was used for the calibration curve so that the results were expressed as mg equivalent of BSA per volume (L) of the aqueous supernatant.

2.8. Total sugars

The total sugars concentration in the aqueous supernatants were determined by the DuBois method (Dubois et al., 1956). Briefly, 0.2 mL of 5 wt % phenol and 1 mL of concentrated sulfuric acid was added to 0.2 mL of previously diluted supernatant in the test tube. Samples were incubated at 25 °C for 30 minutes in the dark before reading the absorbance at 490 nm against a blank (distilled water). D-Glucose was used as a calibration standard, and the results were expressed as mg of equivalent to D-Glucose per L of the aqueous supernatant.

2.9. Oil-water interfacial tension measurements

The pendant drop method was used to measure the interfacial tension of the aqueous fraction of the tomato peels suspensions treated by HSM or HPH against peanut oil. The supernatant was recovered from the samples subjected to centrifugation for 10 min at 5 °C and 6500 rpm and then filtered through a Whatman no. 4 filter paper. Details are given elsewhere (Donsì et al., 2012). The interfacial tension was measured in dynamic mode, during 1000 s, over 200 frames, using a CAM200 apparatus (KSV Instruments, Finland) equipped with an image analyzer software (CAM 101, KSV Instruments).

2.10. UV-Vis spectrometry and HPLC lycopene analyses

UV-Vis spectra analysis was performed for a wavelength range between 200 and 700 nm for tomato peel water aqueous supernatant, and the pellet extracts in ethyl lactate, as obtained from the centrifugation of the tomato peels aqueous suspensions treated by HSM (control) and HPH (1, 3, 5, 7 and 10 passes). Lycopene in ethyl lactate was quantified from UV-Vis spectrophotometer (Jasco Inc., Easton, USA) reading at 472 nm, upon obtaining a calibration

curve for lycopene standard in ethyl lactate and is expressed as mg of lycopene per L of extract. Lycopene in the aqueous supernatant was determined from UV-Vis spectrophotometer (Jasco Inc., Easton, USA) reading at 350 nm, in correspondence of the absorbance maxima observed for complexes of lycopene with bovine serum albumin (Galdón et al., 2013).

The lycopene content in the acetone extracts of the dried aqueous supernatant or of the pellet obtained from the centrifugation of the tomato peel suspensions was measured by reversed-phase high-performance liquid chromatography using isocratic elution and UV detection at 472 nm (Waters, Belgium). A carotenoid C30 reversed-phase column (250 × 4.6 ID, 3 μm) from YMC Corporation (Waters, Belgium) was used with MeOH/isopropyl alcohol/THF (30:30:35) containing 250 ppm BHT and 0.05% TEA as a mobile phase. The flow rate was 1 mL/min, column temperature was 35 °C and the injection volume of 20 μL, according to a previously described method (Cucu et al., 2012).

2.13. Statistical analysis

All the experiments were carried out in triplicate. The obtained dataset was analyzed with XLSTAT add-on for Microsoft Office 2016. The data are represented as means with standard deviations. One-way analysis of variance (ANOVA) was used for determination of whether the means between samples differ significantly from each other. The significance (p<0.05) was established using the posthoc t-tests with Bonferroni adjustment. All of the data are expressed as mean ± SD of the values. Correlation analysis was also performed using the same statistical package. Percentage of bioactive compounds and antioxidant activity change relative to the control (HSM) was calculated with $relative\ change\% = \frac{HPHX'-HSM}{HSM} \times 100$ formula, where x' is the number of passes. Agglomerative hierarchical clustering (AHC) with Euclidean distance Dissimilarity and Agglomeration Ward's method was performed.

3. Results and Discussion

3.1. Physical characteristics of tomato peel suspensions

The HSM and HPH treatments induce a measurable disruption of the peels, which became more evident at increasing the number of HPH passes. Preliminary tests, carried out to characterize the particle size distribution of tomato peels suspensions treated at different HPH passes, showed that after 10 passes no significant changes occurred. Therefore, 10 passes were set as the limiting number of passes of HPH treatments.

Visually, as shown in Figure 2, the tomato peels suspension became progressively more homogeneous in appearance, as the number of passes is increased. These observations can be correlated with the microscopical observations, reported in Figure 3, which shows that HSM treatment caused only the fragmentation of the peel tissue into smaller cell aggregates, with negligible effects on cell integrity. As HPH treatment was applied, the tomato peels were further fragmented, and, at the same time, the individual cells were progressively disrupted, as suggested by the large fraction of filamentous debris appearing in the suspension, likely as the results of cell wall breakage.

[Figure 2 here]

[Figure 3 here]

The particle size of the tomato peels suspension is described in Table 1, through the characteristic diameters d(0.1), d(0.5), d(0.9), D[4,3], and D[3,2] as a function of treatment intensity. The results confirm that, when increasing the number of passes, a decrease of the characteristic diameters of the tomato peels suspension is observed. However, after 5 passes, only modest changes in size occurred.

Remarkably, the results of Table 1 and Figure 3 clearly show that HSM treatment is not able to destroy individual plant cells, whereas HPH treatment does. In particular, after 10 passes, the plant cells are completely disrupted, with most of the intracellular content being released in the suspension.

Table 1. Characteristic diameters (μm) of the particle size distribution of the tomato peels aqueous suspensions treated by HSM (control) and HPH (1, 3, 5, 7 and 10 passes).

HPH passes	d(0.1)	d(0.5)	d(0.9)	D[4,3]	D[3,2]
0 (HSM)	101.2 ± 3.2	330.9 ± 24.4	1006.5 ± 42.4	443.6 ± 11.3	150.8 ± 11.6
1	57.4 ± 1.6	298.1 ± 6.6	967.3 ± 18.0	429.9 ± 16.6	110.0 ± 8.3
3	19.2 ± 1.8	165.0 ± 52.2	679.4 ± 53.1	272.9 ± 27.2	38.9 ± 4.3
5	12.5 ± 0.1	57.4 ± 0.6	491.7 ± 6.7	165.0 ± 1.1	23.8 ± 0.5
7	10.5 ± 0.2	42.1 ± 3.3	297.6 ± 38.4	104.1 ± 9.3	18.8 ± 0.8
10	9.3 ± 0.6	30.9 ± 1.8	111.2 ± 2.4	49.7 ± 0.8	16.1 ± 0.8

A recent study about the consumer perception of tomato purees enriched with dietary fibers and polyphenols revealed that most of the preferences went to the puree containing particles in the size range 250 μm - 500 μm, which was associated to sensations of fresh tomato, as well as crispiness, granularity and vegetable notes (Torri et al., 2015), which are, instead, not perceived in a lower size range. Therefore, in the perspective of using the tomato peels suspension as a food ingredient in juices/sauces/purees, a minimum number of passes of 5 is recommended to obtain a particle distribution within the suggested range (d(0.9) of HPH 5 is 491.7 μm), which, in addition to providing a grainy texture are also visible by naked eye, whereas more intense conditions (HPH 7 and HPH 10) exhibit a size distribution below such range. In the case of addition to products with a smoother texture, a smaller size distribution (> 5 passes) would, instead, be preferable.

3.2. Total polyphenols and antioxidant activity

After centrifugation, the water supernatant was subjected to further analyses of total polyphenols, antioxidant activity, total proteins, and total sugars. These results are presented in Table 2, where it can be observed that, when the number of HPH passes increased, the concentration of total polyphenols recovered in the supernatant increased. In particular, the suspensions treated by HPH exhibited an increasing antioxidant activity in comparison with

HSM suspensions. For example, after 10 HPH passes, the antioxidant activity, as measured by the FRAP method, increased of 23.3% with respect to HSM suspension, from 220.7 ± 0.6 µmol AA/L to 272.2 ± 0.8 µmol AA/L, which corresponds to an increase of total polyphenols of 32.2%. Moreover, total polyphenol concentration could be well correlated with the antioxidant activity in the supernatant ($r = 0{,}997$), as determined by FRAP assay. Furthermore, both polyphenol concentration and antioxidant activity could be correlated with the concentration of total proteins in the supernatant ($r_{TPC} = 0{,}993$, $r_{FRAP} = 0{,}989$), because the HPH technique is non-selective, and the release of intracellular compounds depend mainly on the extent of cell disruption. Previous studies about the microfluidization of aqueous suspensions of corn and wheat bran exhibited similar trends for the antioxidant activity (Wang et al., 2014, 2013). The authors related this observation with the increased release of bioactive compounds in water upon micronization treatment, which also induced an enhanced bioaccessibility of the antioxidant compounds, which are generally contained inside the cells. It is likely that the HPH process substantially loosened the tightly packed architecture of the plant tissue, disrupted and opened the plant cells, thus making the antioxidant compounds, bound to the cell structure, to become accessible to the molecules present in the surrounding liquid phase.

Table 2. The concentration of total polyphenols (TPC), antioxidant activity (FRAP), and concentration of total proteins and sugars in the supernatant obtained from the centrifugation of the tomato peel aqueous suspensions treated by HSM (control) and HPH (1, 3, 5, 7 and 10 passes). In addition, also the percentage variation (%) with respect to the HSM treatment (control) is reported.

HPH passes	**TPC (mg GAE/L)**		**FRAP (µmol AA/L)**		**Total proteins (mg BSA/L)**		**Total sugars (mg D-Glu/L)**	
	Absolute value	*Relative change**	*Absolute value*	*Relative change**	*Absolute value*	*Relative change**	*Absolute value*	*Relative change**
0 (HSM)	$38.9 \pm 3.1^{a-c}$	-	$220.7 \pm 0.6^{a-e}$	-	$386.4 \pm 22.9^{a-e}$	-	2.4 ± 0.1	-
1	$44.2 \pm 0.5^{d,e,f}$	+13.6%	$242.3+2.0^{a,f-h}$	+9.8%	$548.3 \pm 13.6^{a,f,g}$	+41.9%	2.4 ± 0.3	-0.8%
3	47.7 ± 1.4	+22.7%	261.1 ± 4.2^{b}	+18.3%	$585.9 \pm 07.6^{b,h}$	+51.6%	2.6 ± 0.1	+7.0%
5	$50.1 \pm 0.5^{a,d}$	+28.7%	$267.8 \pm 3.5^{c,f}$	+21.4%	$643.8 \pm 05.5^{c,h}$	+66.6%	2.5 ± 0.2	+4.1%
7	$50.8 \pm 1.1^{b,e}$	+30.5%	$270.1 \pm 1.1^{d,g}$	+22.4%	$651.7 \pm 18.5^{d,f}$	+68.7%	2.5 ± 0.1	+1.2%
10	$51.4 \pm 1.0^{c,f}$	+32.2%	$272.2 \pm 0.8^{e,h}$	+23.3%	$658.8 \pm 31.7^{e,g}$	+70.5%	2.7 ± 0.2	+11.5%

*relative change (%) with respect to HSM treatment (control).

Values superscripted with the same letter within a column are significantly different according to the posthoc t-test with Bonferroni adjustment ($p<0.05$)

3.3. Proteins and sugars

The release of proteins in the supernatant was found to be in correlation with the total polyphenols content and antioxidant activity, likely due to the ability of polyphenols to associate with proteins (Siebert et al., 1996). The lowest total protein concentration in the HSM suspension (386.4 mg BSA/L) was significantly different from all of the HPH suspensions (1 to 10 passes). Furthermore, compared to the control, the total released proteins after only 1 pass increased of 41.9% with respect to control, whereas after 10 passes they increased by 70.5%. However, in comparison to the total amount of proteins typically contained in the peels (10-18 wt % of the dry weight (Elbadrawy and Sello, 2016; Nour et al., 2018)), the maximum amount recovered in the supernatant is less than 3 wt % of the dry weight of the used peels.

In the case of total sugars, HPH caused only a moderate, and non-statistically significant, increase (about +11%) with respect to the control (HSM sample), with the highest concentration measured in the supernatant after 10 HPH passes (2.7 mg D-Glu/L).

3.4. Interfacial activity

The release of intracellular compounds, such as proteins and polysaccharides, affected also the interfacial tension of the aqueous phase of tomato peel suspensions, recovered as supernatant after centrifugation. The results of oil-water interfacial tension measurements on the supernatant of suspensions treated by HSM (control) and HPH (1-10 passes), measured by the pendant-drop method, are presented in Figure 4.

When increasing the number of passes, it is clearly visible a drop in the interfacial tension of the supernatant. The HSM suspension exhibited the highest interfacial tension values, while as the number of HPH passes increased, the interfacial tension of the treated suspension gradually decreased: the lowest value of the asymptotic interfacial tension, of 14.1 mN/m, was observed after HPH 10 passes, in comparison with a value of 15.9 mN/m for HSM. The drop in the interfacial tension can be ascribed to the release of surface active molecules, such as proteins, from the intracellular space to the aqueous extract.

[Figure 4 here]

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353 *3.5. Lycopene*

354 The release of the intracellular compounds, especially carotenoids, from tomato peel cells in
355 the aqueous phase, by HSM and HPH treatments has been further investigated by UV-Vis
356 spectra analysis of the supernatant, in a wavelength range between 200 and 700 nm. Figure 5a
357 shows the UV-Vis spectra of the aqueous supernatants obtained from the tomato peel
358 suspensions. The aqueous supernatant spectra clearly reveal that the highest peaks are
359 achieved after 5 to 10 HPH passes, while the lowest peaks are observed for the sample treated
360 only by HSM, suggesting an increased release of intracellular materials, as the treatment
361 intensity is increased. In addition, the UV-Vis spectroscopy analyses in Figure 5b show the
362 visible absorption spectrum of the ethyl lactate-extracted lycopene from the pellet. Ethyl
363 lactate was used because of its efficiency in solubilizing lycopene (Silva et al., 2018; Strati
364 and Oreopoulou, 2011). The data of Figure 5b clearly show that a significant fraction of
365 carotenoids (and in particular lycopene) is still trapped inside the tomato peel cells, but this
366 fraction is reduced as the HPH passes are increased, in accordance with what observed from
367 Figure 5a.

368

369 [Figure 5 here]

370

371 The residual content of lycopene in the pellet was quantified by extraction of the pellet with
372 ethyl lactate, using a calibration curve made with different working standard solutions of
373 lycopene (Figure 6).

374

375 [Figure 6 here]

376

377 Interestingly, the residual content of lycopene in the pellets decreased from an initial value of
378 4.1 mg/g (wet basis), as characterized for the peels prior to any treatment (balance curve, at 0
379 HPH passes), to 3.3 mg/g (wet basis) after HSM, and to 1.9 mg/g (wet basis) after 3 passes.
380 Additional processing after 3 passes caused only a marginal additional release of lycopene, as

shown by the residual value of 1.6 mg/g (wet basis) in the pellet after 10 passes, which corresponds to a residual content of lycopene in the pellet of 39.2%.

The evaluation of lycopene in the supernatant was more difficult, because lycopene, which is insoluble in the aqueous phase, is likely to be complexed/associated with hydrocolloids present in tomato (e.g. proteins, pectin) that help to stabilize the lycopene suspension (Jazaeri et al., 2018). The solubility of these stabilizing hydrocolloids, however, is affected by organic solvents and they precipitate in the presence of ethyl lactate, which in turn may significantly decrease the measured concentration of lycopene if strongly trapped in the precipitated hydrocolloid. Previous studies reported similar absorption spectra, with absorbance maxima at 275 nm and 350 nm (this last wavelength was used in the present work) of complexes of lycopene with bovine serum albumin (Galdón et al., 2013). Therefore, the concentration of lycopene in the supernatant was determined directly from the UV-Vis spectra of the aqueous supernatant, calibrating the concentration in the supernatant of the HSH sample with the initial measured content of lycopene in the peels. As shown in Figure 6, this approach is validated by the closing mass balance on lycopene for all the remaining samples. In addition, the HPLC spectra, reported in section 3.6, qualitatively confirm these results.

Based on this assumption, the maximum amount of lycopene recovered in the supernatant corresponded to 56.1% of total initial lycopene (2.3 mg/g of peels on a wet basis).

This is in agreement with the observation that the size-reduction induced by high-pressure microfluidization of tomato ketchup increased the detectable lycopene levels of the ketchup samples (Mert, 2012).

3.6. Carotenoids in the supernatant

The HPLC analysis of the pellet and of the dried supernatant extracted by acetone, reported in Figure 7, shows that lycopene is the main carotenoid, both in the supernatant and the pellets after centrifugation of the treated tomato peel suspensions. In addition, Figure 7 also confirms the results from the UV-Vis analysis: (a) the pellet from HSM suspensions contains significantly higher amounts of lycopene than the pellet from the suspensions treated by 5 HPH passes; coherently, (b) the supernatant from the suspensions treated by 5 HPH passes contain a significantly higher amount of lycopene than the supernatant from the HSM suspensions.

The HPLC analysis also confirmed the quantitative results obtained by UV-Vis analysis. This is particularly relevant, considering that the lycopene content of the analyzed tomato peels (3.86 mg/g on a wet basis, corresponding to 19.3 mg/g on a dry basis) is significantly higher than the values typically reported in literature (about 0.5-0.8 mg/g on a dry basis (Nobre et al., 2009; Nour et al., 2018)).

When taking into account the dietary factors and food properties, the bioavailability of lycopene is the lowest from raw sources, whereas mild processed foods show slightly better bioavailability, and thermally processed foods the highest bioavailability (Honest et al., 2011). Therefore, the lycopene uptake in the human body is more favorable when consuming variously processed tomato products (Böhm and Bitsch, 1999; Gärtner et al., 1997; Goñi et al., 2006; Granado-Lorencio et al., 2007; Porrini et al., 1998; Rao and Agarwal, 1998; Richelle et al., 2002). Thermal and mechanical food processing improves lycopene bioavailability by disrupting the cell walls and weakening the chemical bonds between lycopene and the raw tissue matrix, hence making lycopene more accessible (Agarwal et al., 2001b; Shi and Le Maguer, 2000). According to these studies, our results show that a significant release of lycopene from the rigid tomato peel structure into the aqueous phase can be achieved by intense high-pressure processing, with a potential increase of its bioavailability.

[Figure 7 here]

3.7. Dendrogram of sample groupings

The dendrogram analysis of the influence of the different treatments performed on the dissimilarities between the samples is represented in Figure 8. The dendrogram analysis is based on the results of particle size distribution d(0.1), d(0.5), d(0.9), D[4,3], D[3,2], total polyphenols, total proteins, total sugars, antioxidant activity and interfacial tension of the supernatant, as well as lycopene content in the pellet and supernatant from tomato peels suspensions treated by HSM and HPH. The analysis reveals the formation of three main clusters. In the first cluster, HSM (control) and HPH 1 suspensions are grouped together, because they are more statistically similar than the other treatments. HPH 5 and HPH 7 suspensions were also found to be grouped together with significant similarities, alongside the

443 HPH 3, which is slightly more dissimilar, forming the third cluster. HPH 10 is isolated as a
444 single separate cluster, because it is significantly different from HSM and HPH 1 samples,
445 and only slightly more similar to the other samples in terms of the measured parameters.

446

447 [Figure 8 here]

448

449 **4. Discussion**

450 The reported results clearly show that not only water-soluble compounds were released in
451 significant concentrations in the aqueous phase, but also hydrophobic molecules, among
452 which the most abundant in tomato peels is lycopene. In particular, lycopene concentration in
453 the aqueous supernatant reached the value of about 8.5 mg/L, which is remarkable because
454 lycopene is insoluble in water. Therefore, it can be hypothesized that HPH is able to
455 completely open the vegetable cells and release hydrophobic lycopene and stabilizing it in the
456 aqueous phase by complexation with the extracted proteins in colloidal particles. This is
457 clearly visible from Figure 9, where the different experimental stages followed for the
458 isolation of lycopene are shown. Initially, the aqueous extract is obtained as supernatant after
459 the centrifugation of the HPH-processed peel suspension. At this stage, a fraction of lycopene
460 remains in the pellet, bound to the centrifuged cell debris, while another fraction (up to 56%)
461 is suspended in the aqueous phase. Subsequently, the supernatant is dried in a rotavapor, and
462 the residual solids redispersed in acetone. The addition of acetone causes the precipitation of
463 proteins and polysaccharides, while lycopene is completely dissolved. At this stage, it can be
464 supposed that the complexes are broken, and, consequently, pure lycopene can be recovered
465 upon filtration of the acetone solution. This is confirmed by the typical lycopene color
466 appearing in the acetone solution, as well as by its identification by HPLC (Figure 7).
467 Ongoing work in our laboratory is addressed to verify this hypothesis, to identify the type of
468 complex and investigate the bioaccessibility of lycopene in complexed form, which could be
469 exploited as an all-natural delivery system in aqueous products for lycopene.

470

471 [Figure 9 here]

472

From the processing point of view, it is interesting to compare the yield of extraction of the current work with the literature data, using different solvents and different technologies. Figure 10 reports, comparatively, the amount (in mg) of lycopene extracted per gram of dry weight of peels, considering a moisture content of 75%, when the value was not explicitly reported.

[Figure 10 here]

Interestingly, the data of Figure 10 show that the yields of extraction of lycopene observed in this work are comparable with the results obtained through solvent extraction assisted by pectinase and cellulase enzymes (Choudhari and Ananthanarayan, 2007) or ultrasounds (US) (Eh and Teoh, 2012; Kumcuoglu et al., 2014; Silva et al., 2018). The lycopene yields achieved in this work were also significantly higher than what obtained by PEF-assisted solvent extraction (Luengo et al., 2014), supercritical CO_2 extraction (Hatami et al., 2019; Rubashvili et al., 2018), and conventional solvent extraction from dried peels using different combinations of hexane, acetone and ethanol or methanol (Kaur et al., 2008; Luengo et al., 2014; Rao et al., 1998).

However, it must be highlighted that, as mentioned in section 3.7, the initial content of lycopene in the peels used in this work is significantly higher than the typical value reported in the literature, and differences in lycopene yields of Figure 10 are also likely to depend on the biological variability of the tomatoes.

In addition to the advantages in terms of lycopene recovery, the use of water as an extraction solvent is intrinsically environmentally benign especially because it prevents the need for organic solvents, which are generally toxic and require their complete removal from the exhaust material before its disposal or further use (i.e. animal feed or compost).

Remarkably, in the case of the proposed technology, it is possible to obtain by means of only physical processing in water a tomato peel suspension, which is very rich in lycopene (up to 56.1% of the initial content of tomato peels). This suspension can be used as it is, as a food ingredient or the aqueous phase can be separated by centrifugation, still carrying a significant concentration of lycopene (up to 39.2% of the initial content, at the optimal processing conditions). The pellet (exhaust material) can be disposed of exactly as the initial residue, as

504 no chemicals were involved in its processing, which adds a significant benefit to the proposed
505 technology (Naviglio et al., 2008). Therefore, even in the case where pure lycopene is the
506 desired product, rather than a total extract rich in lycopene, the solvent extraction can be
507 carried out directly on the aqueous supernatant, drastically reducing the exhaust material to be
508 disposed of.

5. Conclusions

The use of high-pressure homogenization (HPH) for the treatment of agro-industrial by-products is able to cause the complete disruption of the plant cells, and the release of intracellular material. In this work, we applied the HPH treatment to tomato peels, preliminary dispersed as aqueous suspensions. The HPH suspensions of tomato peels represent a by-product with high added value because the cell aggregates are reduced in size below the perception of the naked eye, with better properties than the simply highly sheared suspensions, because richer in polyphenols, proteins and lycopene. In particular, at increasing the number of HPH passes, a higher amount of total proteins, total polyphenols are released as well as the greater antioxidant power of the suspensions can be observed. In particular, the HPH treatment enables the release of lycopene from the rigid structure of tomato peel cells to the aqueous phase, without the need of any organic solvent, significantly improving its recovery through a green, sustainable and purely physical process, to make a product ready for human consumption. The obtained suspensions could be exploited as functional foods or can be added back to the transformed peeled tomato products, to enrich their bioactive potential.

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707

Figure Captions

Figure 1. Experimental workflow for sample preparation, treatment, and analysis.

Figure 2. Images of tomato peel aqueous suspensions after HSM (control) and HPH (1, 3, 5, 7 and 10 passes) treatments.

Figure 3. Micrographs of tomato peel aqueous suspensions after HSM (control) and HPH (1, 3, 5, 7 and 10 passes) treatments. Scale bars correspond to 100 μm.

Figure 4. Interfacial tension of the supernatant of the suspensions treated by HSM (control) and HPH (1, 3, 5, 7 and 10 passes), as a function of the measurement time.

Figure 5. UV-Vis spectra of (a) the aqueous supernatant, and of (b) ethyl lactate extracts from the pellet, as obtained from the centrifugation of the tomato peels aqueous suspensions treated by HSM and HPH 1-10.

Figure 6. Lycopene content (reported per wet mass of peels) evaluated by ethyl lactate extraction from the supernatant and the pellet obtained from the centrifugation of the tomato peels aqueous suspensions treated by HSM (control, 0 HPH passes) and HPH (1-10 passes). Values superscripted with the same letter are significantly different according to the posthoc t-test with Bonferroni adjustment ($p<0.05$).

Figure 7. HPLC spectra of (a, b) the pellet resuspended in acetone and of (c, d) the dried supernatant resuspended in acetone, obtained from the centrifugation of the tomato peels aqueous suspensions treated by HSM (a, c) and 5 HPH passes (b, d).

Figure 8. Dendrogram of treatment influence on dissimilarities between the samples based on obtained results of particle size distribution d(0.1), d(0.5), d(0.9), D[4,3], D[3,2], total polyphenols, total proteins, total sugars, antioxidant activity, interfacial tension of the supernatant and lycopene content in the pellet and supernatant from tomato peels suspensions treated by HSM and HPH.

Figure 9. Pictures of (a) the tomato peel aqueous suspension processed by HPH after centrifugation, and of (b) the supernatant after removal of water and resuspension in the same volume of acetone, and (c) after filtration to remove suspended solids.

Figure 10. Comparison of the amount of lycopene recovered from tomato peels by HPH treatment in water (this work) with literature data, where different technologies and solvents were used.

Figure 1

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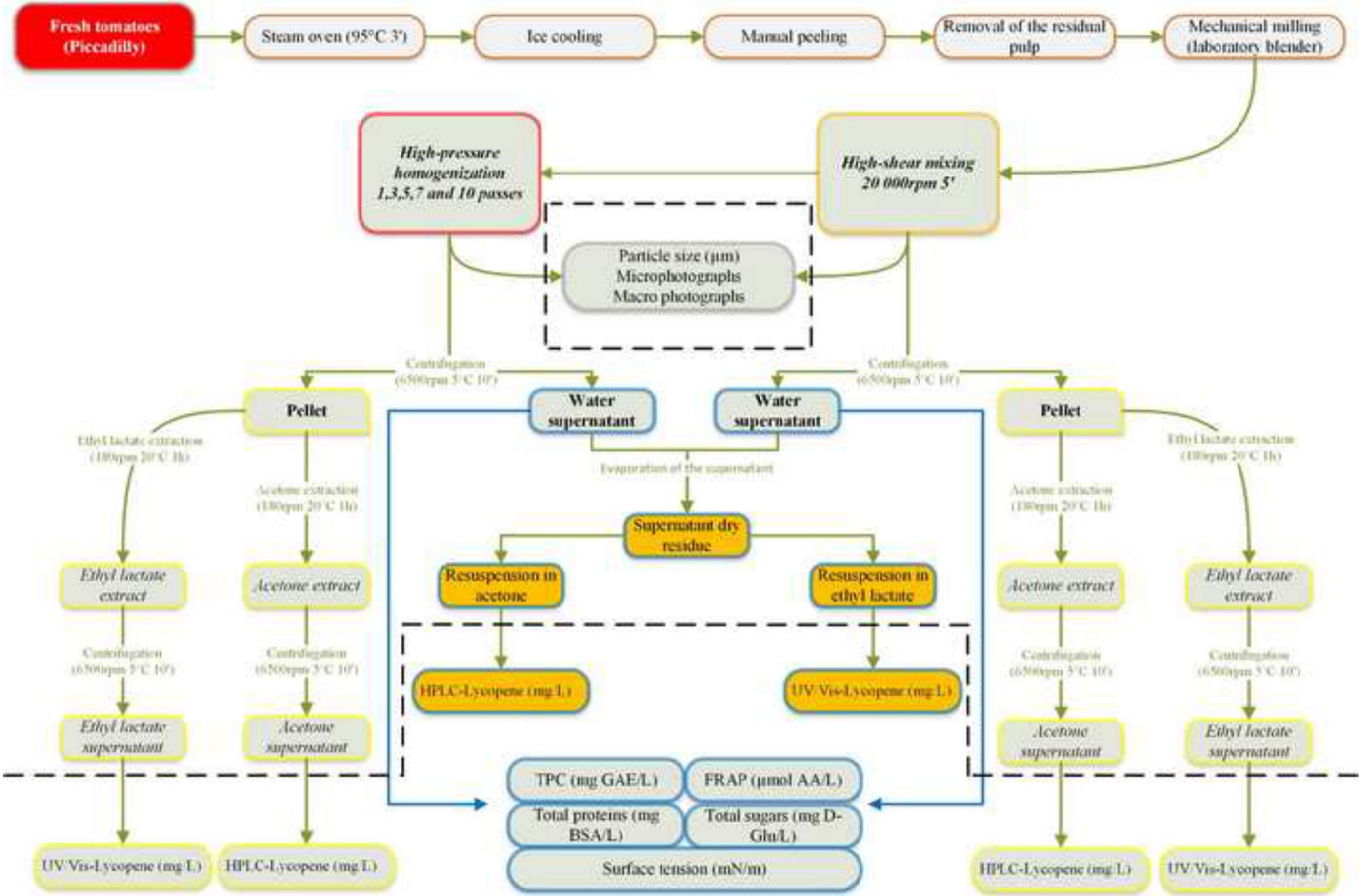

Figure 2

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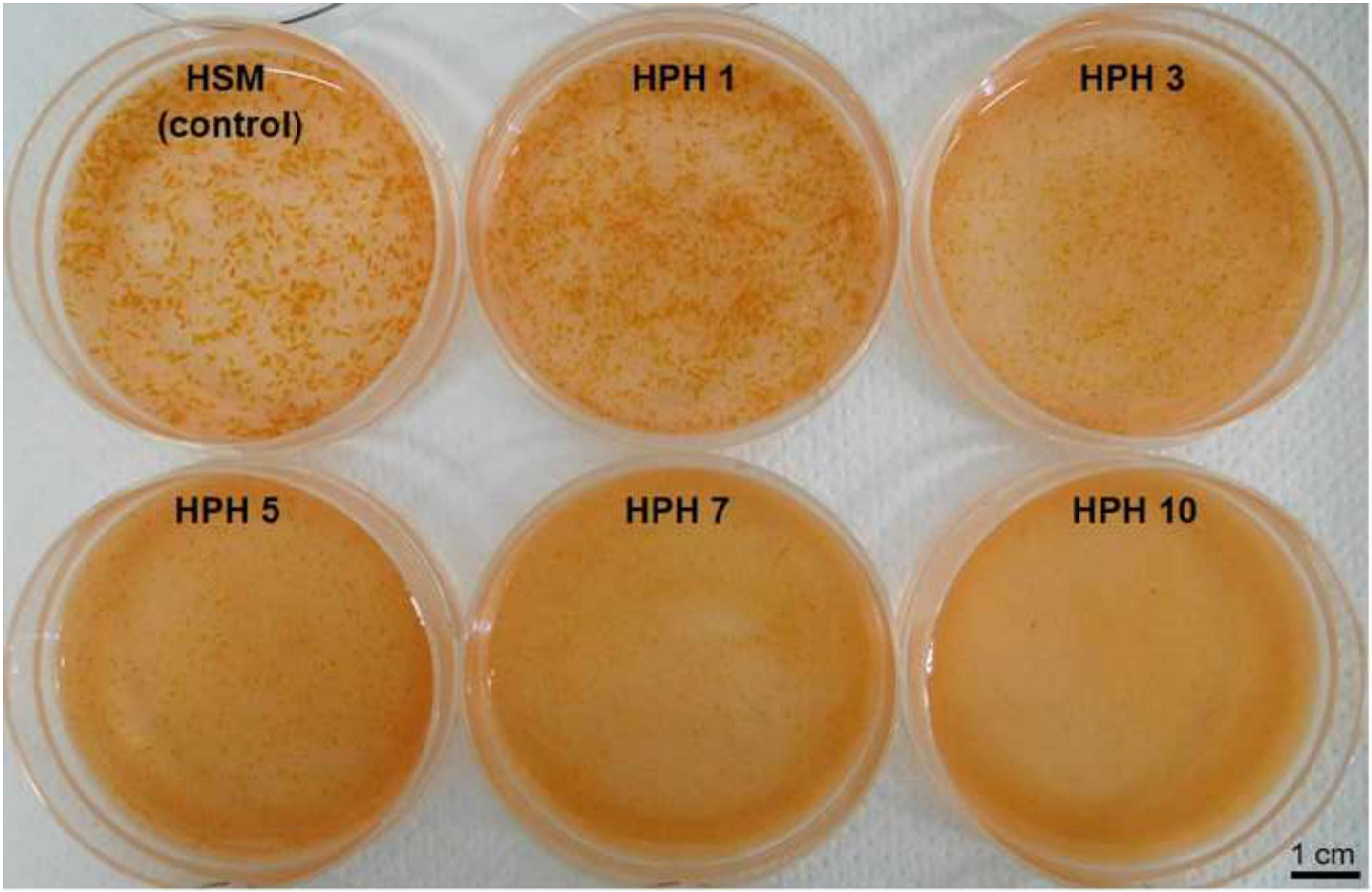

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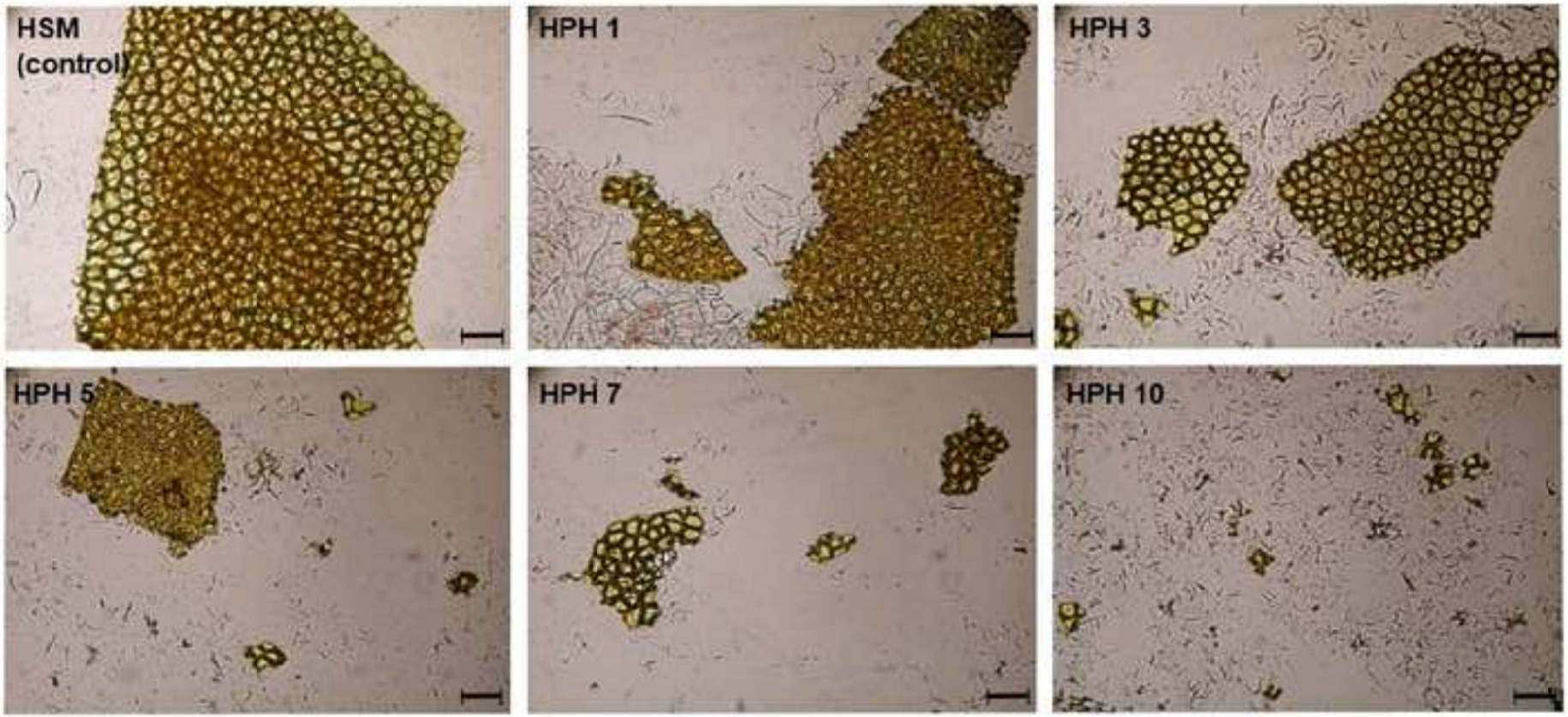

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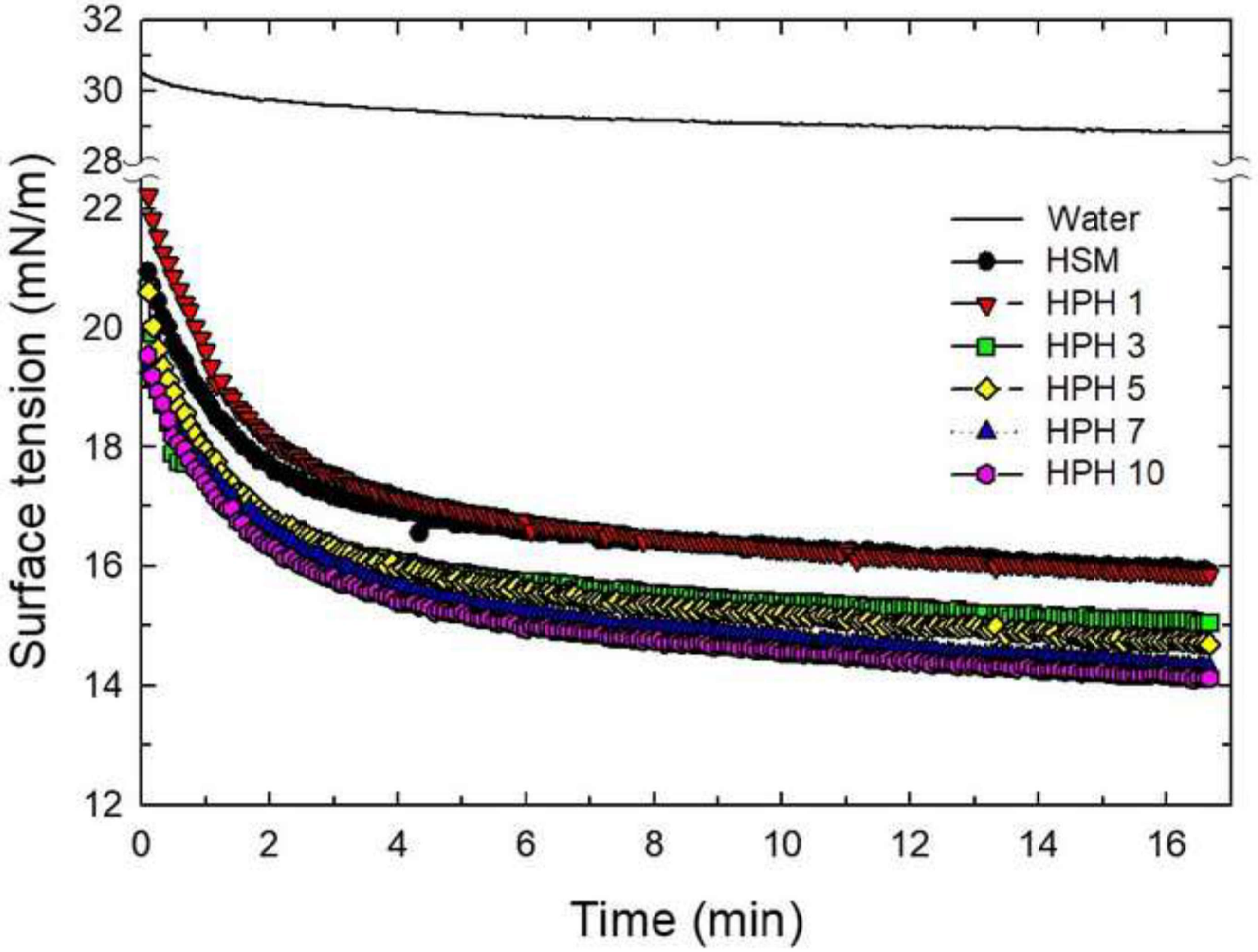

Figure 5

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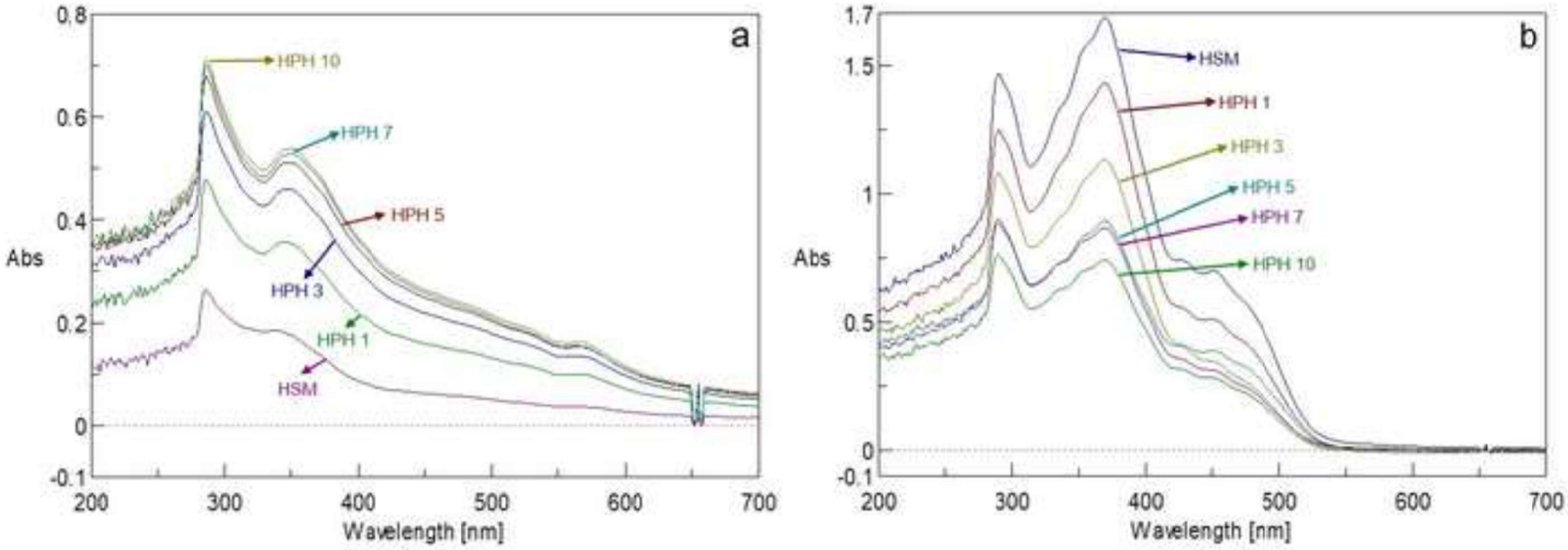

Figure 6
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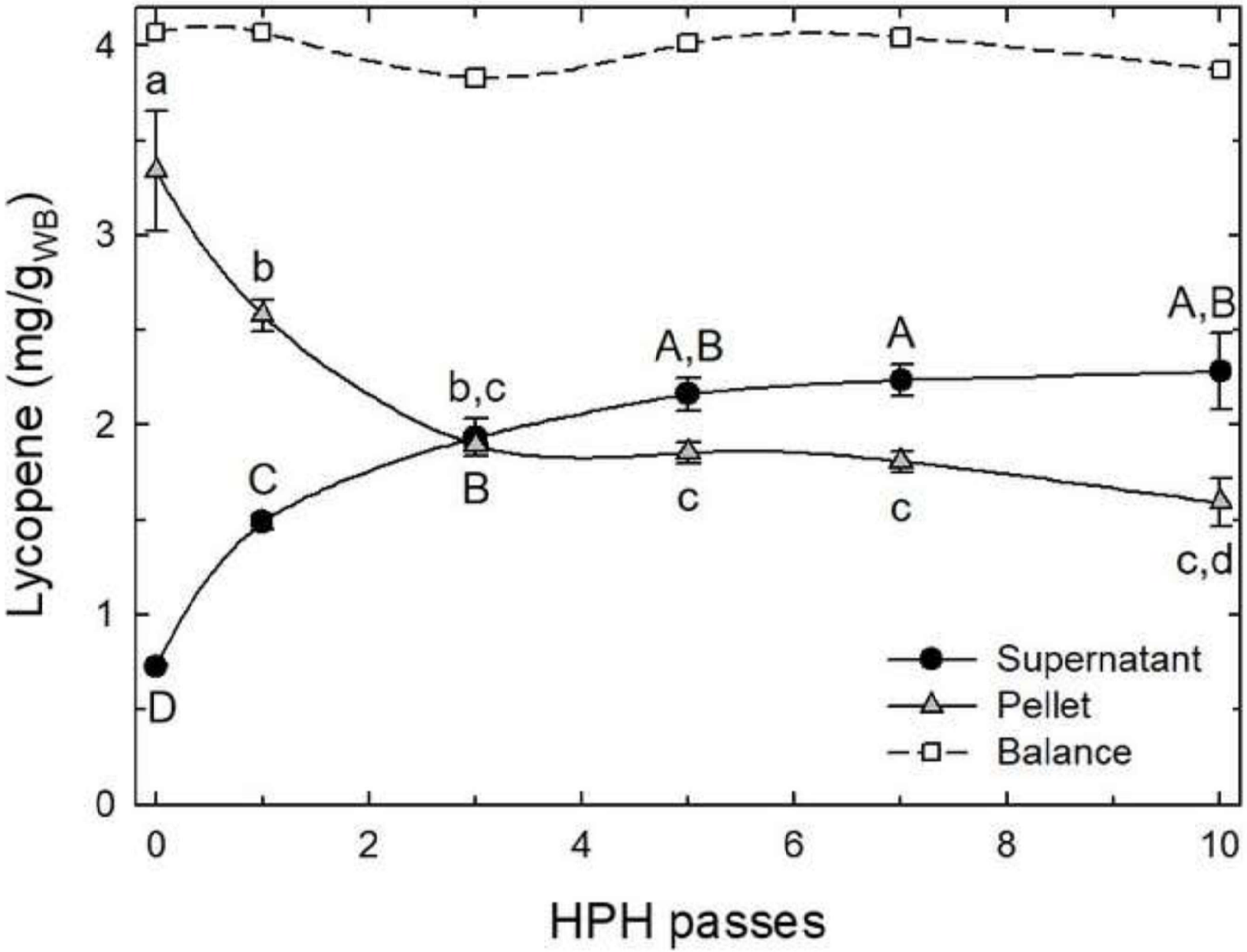

Figure 7

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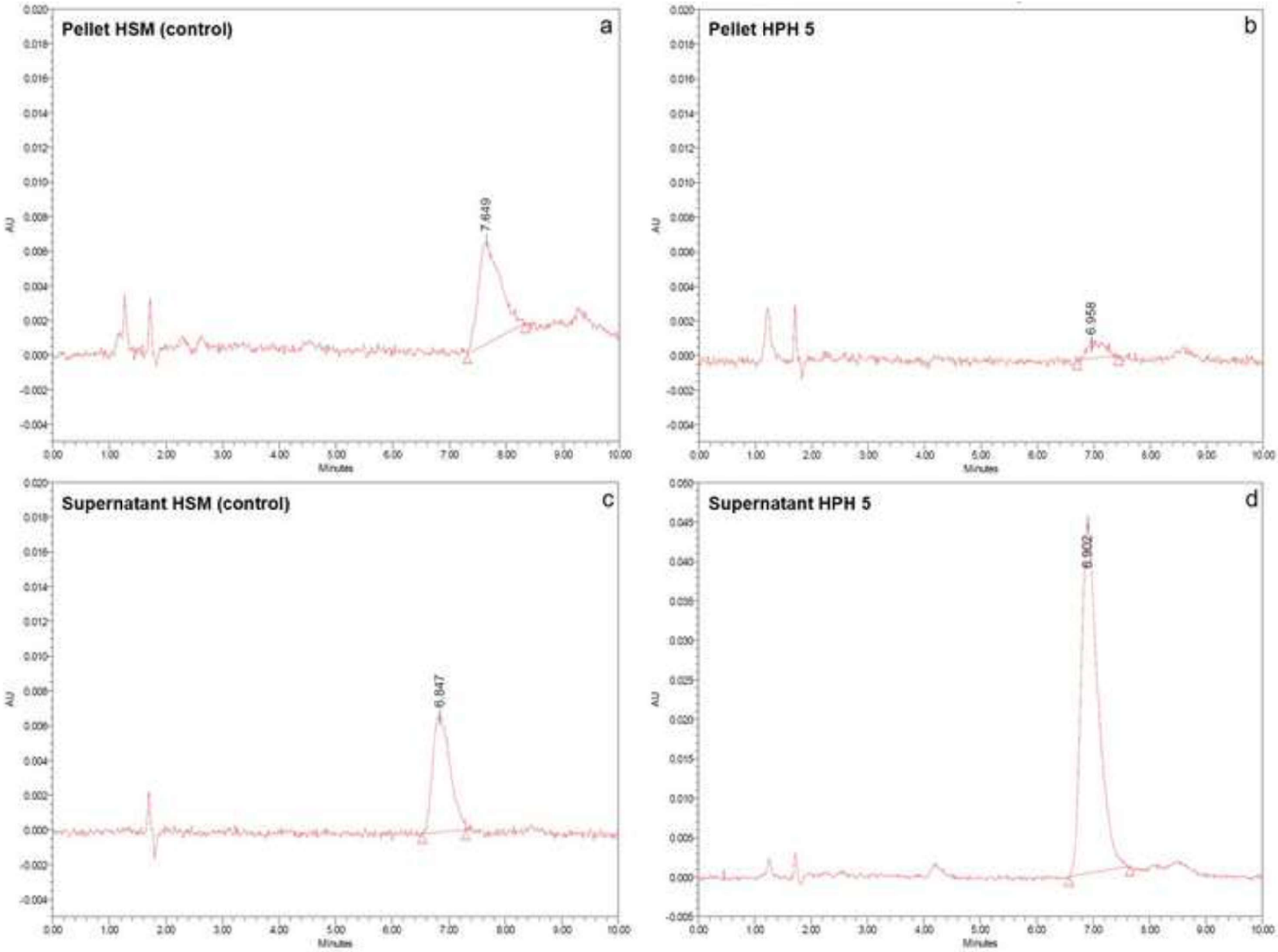

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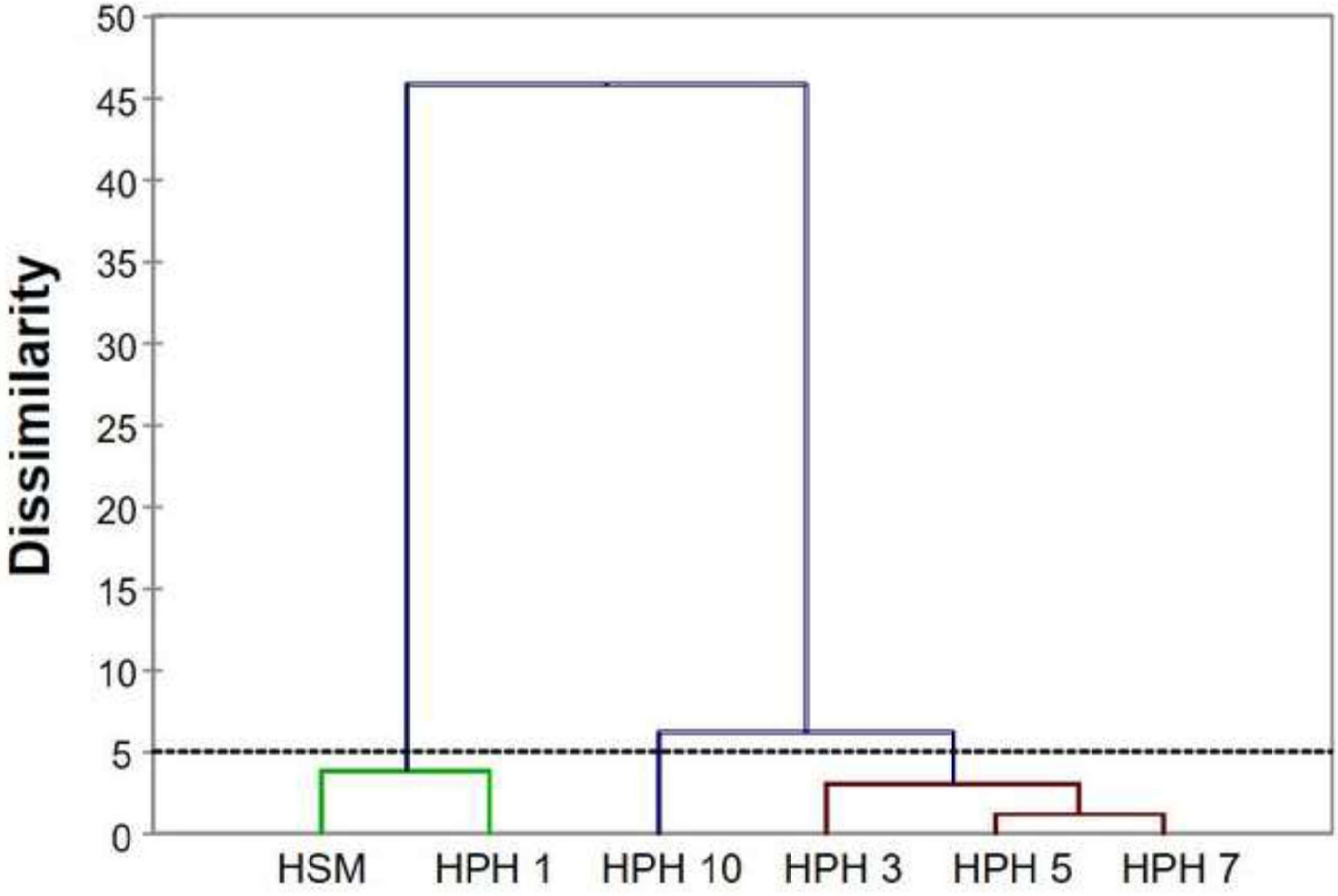

Figure 9
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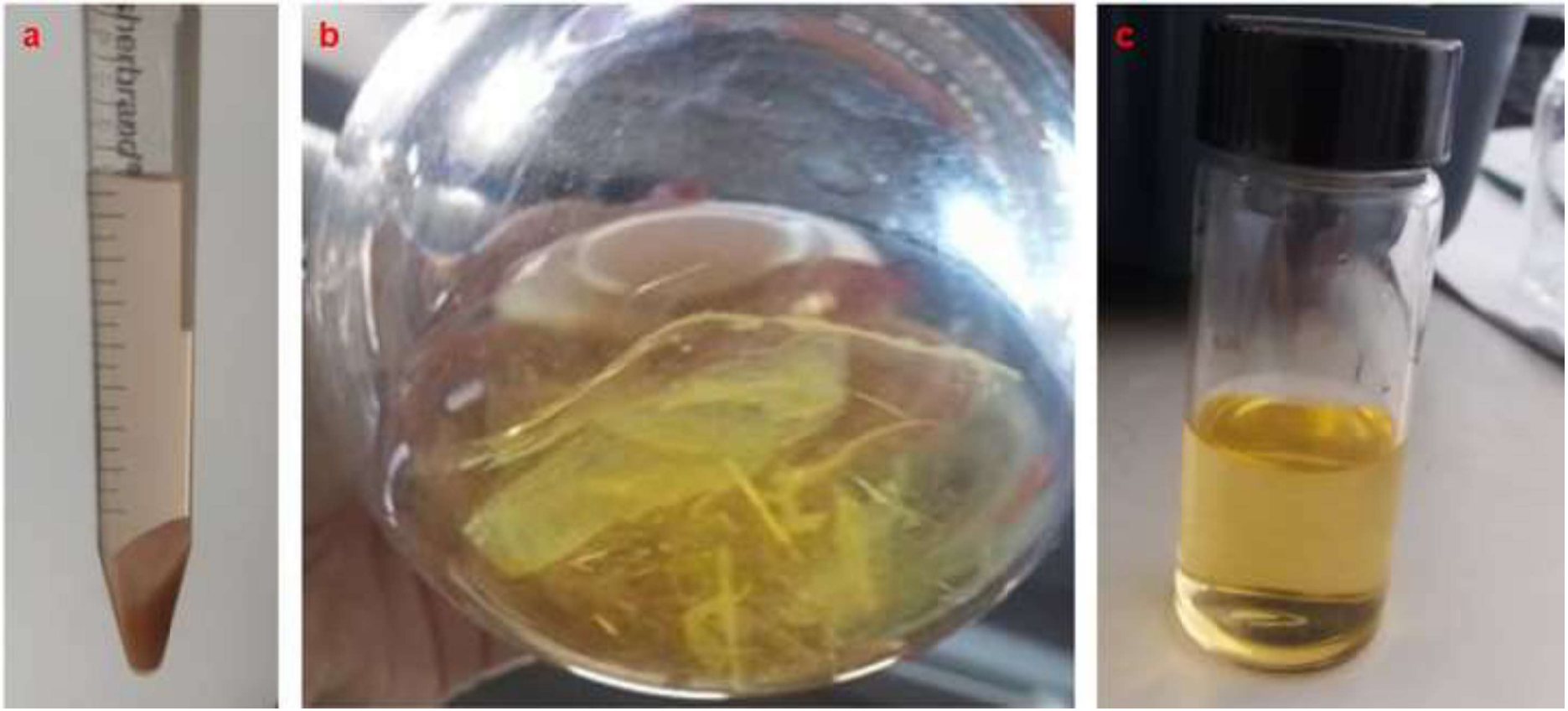

Figure 10

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