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ABSTRACT

The effect of the presence of surfactant micelles and of the mode of incorporation (pre-homogenization or post-homogenization) on the antioxidant efficiency of a homologous series of n-alkyl gallates phenolipids (G0, G3, G8, G12 or G16) was investigated in oil-in-water nanoemulsions. In both absence and presence of surfactant micelles, G12 and G16 were the best antioxidants. The effect of the mode of incorporation was modulated by the presence of surfactant micelles. In absence of surfactant micelles, G8 and G16 had higher efficiency when incorporated pre-homogenization, suggesting that the mode of incorporation promoted a distinct initial distribution of these compounds. In contrast, in presence of surfactant micelles, the antioxidants could be incorporated in any phases without efficiency loss. These results demonstrate the important role of surfactant micelles in modulating the antioxidant efficiency and could be used by the food industry to optimize emulsion formulations.

1. Introduction

Lipids are commonly found dispersed in nature and in manufactured products. They form diverse oil-in-water (O/W) emulsions in a large range of foods and beverages such as mayonnaise and dairy products (Chen, McClements, & Decker, 2013; Vieira, McClements, & Decker, 2015). Unsaturated lipids are prone to lose hydrogen atoms, a chemical process triggering a sequence of reaction known as lipid oxidation. This process may lead to sensorial, nutritional, and health benefit losses, along with the generation of potentially toxic compounds, thus limiting the shelf life of products in which they appear (Chen et al., 2013; Vieira et al., 2015; Vieira, Zhang, & Decker, 2017). The addition of antioxidants is a strategy currently used for preventing lipid oxidation, but selecting the optimal antioxidant is not a simple task because of conflicting anti- and pro-oxidant mechanisms which remain poorly understood and mastered in most complex lipid systems (Barden, Barouh, Villeneuve, & Decker, 2015; Decker et al., 2017).

The polar paradox (Porter, Black, & Drolet, 1989) was the first attempt to predict antioxidants' behavior in lipid systems by stating that polar antioxidants are more effective in bulk oils, whereas nonpolar antioxidants are more efficient in O/W emulsions. Frankel, Huang, Kanner, and German (1994) extended this concept by proposing that nonpolar antioxidants concentrate at the oil-water interface of O/W emulsions, where lipid oxidation is supposed to initiate. Therefore, in addition to their chemical reactivity, physical location of antioxidants in lipid systems is also critical for their efficiency.

However, one main hurdle for predicting antioxidant efficiency in emulsion-based foods using the polar paradox is that emulsions are complex multicomponent systems, composed of oil, water, surfactants, and other food components. Among these, surfactants in excess in the aqueous phase of O/W emulsions, above their critical micelle concentrations (CMC), form surfactant micelles that have been suggested to influence antioxidant reactions. For example, the physical location of antioxidants in O/W emulsions can be influenced by micelles, modifying their antioxidant efficiency (Kiralan, Doğu-Baykut, Kittipongpittaya, McClements, & Decker, 2014; Panya et al., 2012).

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The mode of incorporation of antioxidants in dispersed lipids can also play a role in the antioxidant efficiency (Barden et al., 2015; Durand et al., 2019). The distribution of an antioxidant is influenced by its solubility and partitioning behavior in the different regions of the emulsion (oil core, aqueous phase, interface), and by the type of transport of antioxidant species between oil droplets (diffusion, collision-exchange-separation, and or micelle-assisted transfer) (Laguerre, Bily, Roller, & Birtić, 2017). Moreover, both partitioning and transport can be affected by the antioxidant interactions with other components in the system, including the surfactant interfacial layer and the surfactant micelles present in the aqueous phase. Therefore, depending on the phase in which the antioxidants are initially incorporated (oil or aqueous phases), their ability to reach the interface and inhibit free radicals formation (initiation) or to reach a dynamic equilibrium within the system may significantly differ between antioxidants, thus affecting their efficiency (Barden et al., 2015; Durand et al., 2019).

Thus, in the present study, we investigated the effect of the mode of incorporation, in absence and presence of surfactant sodium dodecyl sulfate (SDS) micelles, on the antioxidant efficiency of a homologous series of gallate alkyl esters applied to rapeseed O/W nanoemulsions (emulsions with droplet size «1 μm). Here, SDS was chosen as an anionic small sized emulsifier. Similarly, gallic acid and its esters were used as model antioxidants due to the fact that such antioxidants are widely used in food and cosmetic sectors. To our knowledge, there is a lack of studies examining the effect of surfactant micelles and of the mode of incorporation of antioxidants on their antioxidant efficiency. Improving our understanding of the impact of these parameters on antioxidant behavior is essential to successfully identify effective compounds, capable of extending the shelf life of new food formulations enriched with bioactive lipids, such as omega-3 fatty acids.

2. Material and methods

2.1. Chemicals and samples

Isopropanol, isooctane, methanol and 1-butanol were acquired from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), iron sulfate, ammonium thiocyanate, 1,1,3,3-tetramethoxypropane (TEM), cumene hydroperoxide, trichloroacetic acid, thiobarbituric acid, mono and dibasic sodium phosphate, and propyl gallate (98% purity) were obtained from Sigma-Aldrich (St. Louis, MI, USA). Gallic acid (G0) (99.7%), propyl gallate (G3) (>98%), octyl gallate (G8) (>99%), and dodecyl gallate (G12) (>99%) were purchased from Sigma-Aldrich, and hexadecyl gallate (G16) (>95%) were from Tokyo Chemical Industry (TCI, Portland, OR, USA).

Rapeseed and sunflower oils were obtained from local markets in Montpellier, France, and they were used without further purification. Concerning rapeseed oil, its characteristics were as follows: peroxide value 1.47 mmol/kg oil, α-tocopherol: 326 mg/kg oil (756 μM) and γ-tocopherol: 433 mg/kg oil (1039 μM). Fatty acid composition (mg/g of total fatty acid) was as follows: 16:0, 44 mg/g; 16:1 n-7, 2 mg/g; 18:0, 18 mg/g; 18:1 n-9, 623 mg/g; 18:2 n-6, 197 mg/g; 18:3 n-3, 99 mg/g; 20:0, 5 mg/g; 20:1 n-9, 12 mg/g. The total percentage of polyunsaturated fatty acids in the rapeseed oil was 296 mg/g.

2.2. Experimental design

O/W nanoemulsions were prepared by homogenization of 1 g of rapeseed oil with 99 g of aqueous phase. The aqueous phase consisted of phosphate buffer solution (10 mmol/L, pH 7.0) and SDS at two concentration levels: 5 mmol/L or 20 mmol/l in the final emulsion. The lower concentration corresponds to a SDS concentration below its critical micelle concentration (bCMC), which is around 8 mmol/L (Fuguet, Ràfols, Rosés, & Bosch, 2005), and it was selected based on preliminary tests that determined the lowest necessary content of surfactant for the nanoemulsion physical stabilization over 15 days. The higher SDS concentration was selected to be above the critical micelle concentration (aCMC). Calculations (Decker et al., 2017) indicated that nanoemulsions needed 1.17 mmol/L of SDS to cover the entire surface, and the remaining SDS (approximately 18 mmol/L, thus, much higher than the CMC of 8 mmol/L) would be available in the aqueous phase to form micelles.

For nanoemulsion preparation, oil and aqueous phases were initially mixed using an Ultra-Turrax IKA (Janke & Kunkel IKA-Labortechnik, Staufen, Germany) for 2 min at 5.65 m/s speed (da Silveira et al., 2020). The resulting coarse emulsions were then homogenized on a Microfluidizer (9 K, Microfluidics, Newton, MA, USA) at an operating pressure of 40 MPa for 3 cycles.

Gallic acid and its alkyl esters were diluted in pure ethanol and incorporated in the oil phase before the homogenization step (i.e. pre-homogenization) to give a concentration of 50 μmol/L in the final nanoemulsion, followed by sonication (Branson Ultrasonic Cleaner B-12, 50 Hz, Brookfield, CT, USA) for 15 min to ensure antioxidant dispersion. Alternatively, the antioxidant solutions were incorporated in the aqueous phase of the nanoemulsions after the homogenization step (i.e. post-homogenization). For incorporation, emulsions from each treatment (60 mL) were placed in 100 mL flasks and an appropriate volume of the antioxidant solution was added to give a final concentration of 50 μmol/L, followed by 15 min of sonication. Ethanol was added to control (no antioxidants) treatments. Ethanol concentration in the final nanoemulsions was negligible (<1 mg/g). For the oxidation experiments, 20 mL of nanoemulsions were transferred into 25-mL screw capped amber vials, and incubated in the dark at 40 °C for 15 days under gentle orbital stirring. Samples were collected every two days for analysis. Three replicates of oxidation for each antioxidant treatment were carried out.

2.3. Droplet size distribution

The nanoemulsion droplet size distribution was determined by laser light scattering using a Mastersizer 3000 laser diffractometer (Malvern Instruments, Malvern, UK). Measurements were performed at 25 °C in fresh nanoemulsions (day 0) and at the end of the storage period (day 15), to evaluate oil droplet stability. Nanoemulsion samples were suitably diluted in distilled water to reach 6–7% laser obscuration value and the refractive index of rapeseed oil and water at 25 °C was taken as 1.47 and 1.33, respectively. Each sample was measured five times in succession to obtain a mean-size distribution curve according to the distribution-volume and each distribution was characterized by the mean volume-weighted mean ($D_{3,2}$).

2.4. Determination of lipid oxidation

The evaluation of lipid oxidation in the nanoemulsions was carried out by monitoring the formation of both primary (lipid hydroperoxides) and secondary (2-thiobarbituric acid reactive substances, TBARS) oxidation products.

Peroxide value (PV) was determined according to Shantha and Decker (1994) adapted to microplate dimensions. Briefly, 300 mg of samples were mixed with 1.5 mL of isooctane (3 mL)/isopropanol solution (1 mL) and vortexed three times for 10 s. An aliquot of this mixture was then added to methanol (3 mL)/butanol (7 mL) to give a final volume of 260 μL in the microplate well. Then, 2.5 μL of aqueous ammonium thiocyanate (300 mg/mL) and 2.5 μL of ferrous solution (0.144 mol/L) were added, and the mixture was incubated at room temperature for 10 min. Following the incubation period, sample absorbance was measured at 510 nm using an Infinite M1000 microplate reader (Tecan, Gröedig, Austria) equipped with the Magellan software. A standard curve was prepared with cumene hydroperoxide. Analysis were performed in triplicate and results were expressed as mmol/kg oil.

TBARS were determined according to Yi et al. (2019), with a few modifications. Nanoemulsions (300 mg) were mixed with 0.6 mL of the

TBARS solution (150 mg/mL) trichloroacetic acid + 3.75 mg/mL thiobarbituric acid and 0.25 mol/L HCl) and heated at 95 °C for 15 min. Following the heating step, samples were cooled on an ice bath for 5 min and centrifuged (6720×*g*) for 10 min. The absorbance of the supernatant was read on the Infinite M1000 microplate reader described earlier at 532 nm. The TBARS content (mg malondialdehyde per kilogram oil (MDA/kg oil)), was determined according to a calibration curve obtained from 1,1,3,3-tetramethoxypropane as external standard. Analyses were performed in triplicate.

2.5. Partition coefficient (log P) and solubility measurement

In a 10-mL glass tube, 2 mg of gallic acid or its alkyl esters were added to 4 mL 75 mmol/L KH_2PO_4 buffer solution (aqueous phase) and 4 mL of octan-1-ol or sunflower oil. The mixture was then vortexed for 1 h, at room temperature, and left to equilibrate for 30 min. Both phases were collected with a Pasteur pipette and extemporaneously analyzed by high performance liquid chromatography (HPLC) using an Agilent Zorbax eclipse C18 1.8 μm, 30 × 4.6 mm analytical column. Injection volume was 2 μL. HPLC flow rate was 1 mL/min with the following mobile phases: 1 mL/L of TFA in water (A) and acetonitrile (B). The gradient was as follow (% volume ratio): 0 min, A at 90% and B at 10%; 15 min, A at 0% and B at 100%; 16 min, A at 90% and B at 10%; and 20 min, A at 90% and B at 10%. The column temperature was set at 25 °C and the detection was performed at 280 nm. Calibration curves were previously prepared in each phase (buffer, octan-1-ol and sunflower oil) for each antioxidant until octyl gallate. Longer chain esters such as dodecyl and hexadecyl gallate esters were not soluble in the buffer solution, therefore, their partition coefficient was not experimentally measurable.

For the solubility measurements, 10 mL of the above-mentioned buffer solution or sunflower oil were added to an unknown amount of gallic acid and its alkyl esters. The suspension was magnetically stirred for 16 h at room temperature and a particular attention was brought to keep the solutes at saturation. After filtration through a 0.45 μm filter, the supernatant was analyzed by HPLC.

2.6. Statistical analysis

Results are presented as means ± standard deviations of analyses conducted in triplicate. Treatments were compared using one-way analysis of variance (ANOVA) using Statistica v. 13.4 (TIBCO Software Inc., Round Rode, Texas, USA).

3. Results and discussion

3.1. Droplet size distribution and physical stability of nanoemulsions

Overall, and as targeted, all nanoemulsions showed similar droplet size distribution (Table 1 and Fig. 1S, *supplementary material*). Fresh bCMC and aCMC nanoemulsions had MODE mean droplet size ($D_{3,2}$) of 100 ± 5 nm and 80 ± 1 nm, respectively (Table 1). This slightly lower droplet size of aCMC compared to bCMC (approximately 23%) was expected and could be attributed to a higher emulsifier concentration in droplet loaded with more SDS being able to enhance droplet disruption during homogenization and further prevent droplet coalescence (Schröder, Laguerre, Sprakel, Schroën, & Berton-Carabin, 2017). The effect of droplet size on lipid oxidation rate is still controversial. However, Costa, Freiría-Gándara, Losada-Barreiro, Paiva-Martins, and Bravo-Díaz (2020) and Costa, Losada-Barreiro, et al. (2020) demonstrate that, in O/W nanoemulsions, some small variations in droplet sizes between 80 and 130 nm, thus similar to the droplet size range reported in our study, do not alter the oxidative stability of O/W fish and olive oil nanoemulsions. Such variations did not either modify the partition of gallate alkyl esters (G3-G12) nor their antioxidant efficiency. Therefore, it was assumed that the reduced variation in droplet size between the bCMC and aCMC emulsions did not interfere with the oxidation rate in our study.

The mode of incorporation of antioxidants did not affect the droplet diameter of nanoemulsions. Indeed, for fresh bCMC samples, the mean droplet size was 110 ± 2 nm and 100 ± 1 nm (addition of antioxidants pre-homogenization or post-homogenization, respectively), while for fresh aCMC nanoemulsions, these values were 80 ± 3 nm for both modes of incorporation (Table 1). This suggests that the pre-homogenization addition of amphiphilic gallate alkyl esters did not contribute to oil droplet disruption during the emulsification process. Phenolipids are designed to hold enhanced surface activity properties (Figueroa-Espinoza, Laguerre, Villeneuve, & Lecomte, 2013) and adsorb to oil droplet surfaces. However, they usually do not facilitate emulsion formation or physical stability due to their much lower concentration compared to surfactants and lack of electrically charged groups or steric effects in the molecule (McClements & Decker, 2018). Moreover, literature data suggest that gallate alkyl esters, specifically short (G4) and long chain compounds (G16), show lower surface tension effectiveness

Table 1
Droplet size ($D_{3,2}$, nm) of fresh and oxidized nanoemulsions.

Below CMC (bCMC)[a]										
Mode of incorporation	Time (days)	Control	Gallic acid	Propyl gallate	Octyl gallate	Dodecyl gallate	Hexadecyl gallate	Mean[b]	Mean Day 0[c]	Mean day 15[d]
Pre-homogenization	0	100 ± 4	110 ± 9	110 ± 2	110 ± 1	110 ± 0.3	110 ± 1	110 ± 2	100 ± 5	100 ± 1
	15	100 ± 1	100 ± 4	100 ± 1	100 ± 2	100 ± 3	100 ± 2			
Post-homogenization	**0**	100 ± 4	100 ± 4	100 ± 4	100 ± 4	100 ± 4	100 ± 4	100 ± 1		
	15	100 ± 1	100 ± 0.4	90 ± 3	100 ± 3	100 ± 0.4	100 ± 4			
Above CMC (aCMC)[a]										
Pre-homogenization	**0**	80 ± 2	80 ± 2	80 ± 2	80 ± 1	90 ± 2	80 ± 2	80 ± 3	80 ± 1	80 ± 2
	15	80 ± 1	80 ± 1	80 ± 0.2	80 ± 1	80 ± 2	80 ± 1			
Post-homogenization	**0**	80 ± 2	80 ± 2	80 ± 2	80 ± 2	80 ± 2	80 ± 2	80 ± 3		
	15	80 ± 1	70 ± 1	80 ± 2	80 ± 0.2	80 ± 1	80 ± 1			

[a] Mean droplet size ± Standard deviation.
[b] Mean droplet size of fresh nanoemulsions (day 0) containing antioxidants incorporated pre-homogenization or post-homogenization.
[c] Mean droplet size of fresh nanoemulsions (day 0) containing antioxidants for both modes of incorporation.
[d] Mean droplet size of oxidized nanoemulsions (day 15) containing antioxidants for both modes of incorporation.

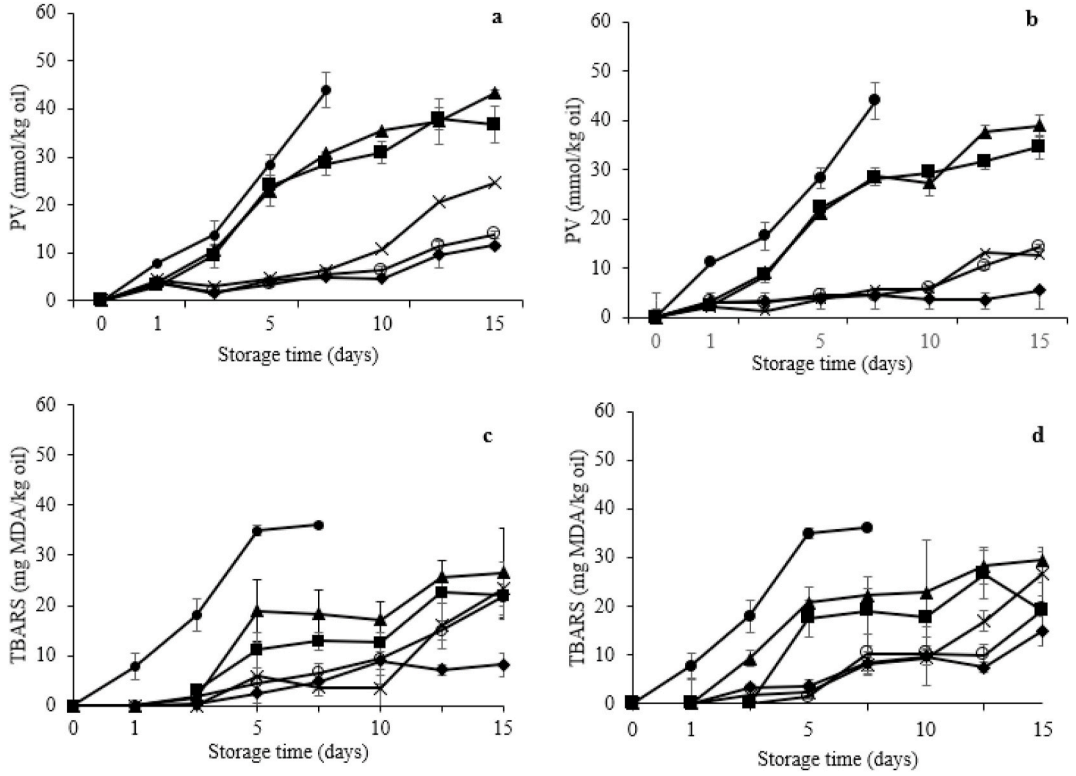

Fig. 1. Oxidative stability of below CMC (bCMC) nanoemulsions. a) Peroxide Value (PV) and b) 2-thiobarbituric acid reactive substances (TBARS) for nanoemulsions with antioxidants added pre-homogenization; c) PV and d) TBARS for nanoemulsions with antioxidants added post-homogenization —●— Control; —▲— Gallic acid (G0) —■— propyl gallate (G3); —×— octyl gallate (G8); —○— dodecyl gallate (G12); —◆— hexadecyl gallate (G16).

compared to SDS (Maldonado et al., 2011). This could explain the low impact of the antioxidants on the droplet formation and size.

Nanoemulsions were physically stable over the 15 days (Table 1 and Fig. 1S, *supplementary material*). bCMC nanoemulsions containing antioxidants showed a slightly lower stability compared to bCMC control and aCMC samples (Fig. 1S, *supplementary material)*, with an increased span in droplet size distribution (on average 50 nm higher in bCMC oxidized samples than in bCMC fresh ones, data not shown). This suggests that, at low surfactant concentrations, alkyl gallates could have favored the occurrence of some coalescence during storage. However, the differences in droplet size between fresh and oxidized bCMC nanoemulsions were in the order of 10 nm or less (Table 1). Thus, the system was considered stable.

3.2. Oxidative stability of nanoemulsions

As evidenced by PV and TBARS results, gallic acid and its alkyl esters showed antioxidant activity compared to the control, for all tested modes of incorporation and surfactant concentrations (Figs. 1 and 2). As already reported in earlier studies, gallate alkyl esters generally show higher antioxidant activity in O/W emulsions than their unesterified parent molecule, which was attributed to a higher partitioning of esters into the interfacial phase where antioxidants can more effectively prevent oxidation (Freiría-Gándara, Losada-Barreira, Paiva-Martins, & Bravo-Díaz et al., 2018a; Phonsatta et al., 2017). Moreover, at pH 7.0, gallic acid is negatively charged (pKa COOH = 4.33, ACD/Labs) and will be electrostatically repelled by the anionic SDS-coated interface, which could also explain the lower antioxidant efficiency of this compound compared to electrostatically neutral ester derivatives. pH is indeed another environmental factor that can directly influence lipid oxidation in emulsions and antioxidants efficiency. In the present study, we fixed pH at 7 but one must keep in mind that pH variations could impact oxidation rates in emulsions as indicated by Kiokias, Gordon, & Oreopolou (2017) who studied sunflower o/w emulsions prepared with Tween 20 as emulsifier and showed that oxidation wase faster as pH increased from 3.0 to 7.0.

Among esters, G3 had a similar efficiency to that of G0 in all cases (Figs. 1 and 2) except for TBARS values obtained after post-homogenization of antioxidant in aCMC nanoemulsion (Fig. 2d). This is unexpected if we consider partitioning data between water and octan-1-ol (Table 2). Indeed, with an experimental log P_W^{oct} (pH 7.0) of 1.92 ± 0.02 (theoretical: 1.72), only 1.2% of the population of G3 molecules partition into the aqueous phase, while it is 99.5% for G0 (experimental log P_W^{oct} pH 7.0 = −2.28 ± 0.16, theoretical: 2.27) (Table 2). To verify that this trend was also found in a binary mixture of oil and water, we then measured the log P_W^{oil} for G0 and G3 (Table 2). Partitioning measurements were done with sunflower because published data are available on other antioxidants using these phases/solvents (Schröder, Laguerre, Sprakel, Schroën, & Berton-Carabin, 2020). In addition, it has been demonstrated that the nature of the oily phase does not significantly change the log P (Freiría-Gándara et al., 2018a).

Compared with log Pw^{oct}, the log Pw^{oil} at pH 7.0 changed only slightly for G0 (−2.28 vs. −2.03 ± 0.01), with a partitioning in the aqueous phase of 99.08%. However, by replacing the octan-1-ol phase of the binary system by sunflower oil - while keeping all other parameters unchanged - this parameter changed remarkably for G3 (log P_W^{oil} = −0.14 vs. log Pw^{oct} = 1.92) (Table 2), and the proportion of the G3 population partitioning in the aqueous phase changed from 1.2 to 58.0%. These data agree with Freiría-Gándara, Losada-Barreira, Paiva-Martins, and Bravo-Díaz (2018), who also report a notable difference between log P_W^{oct}

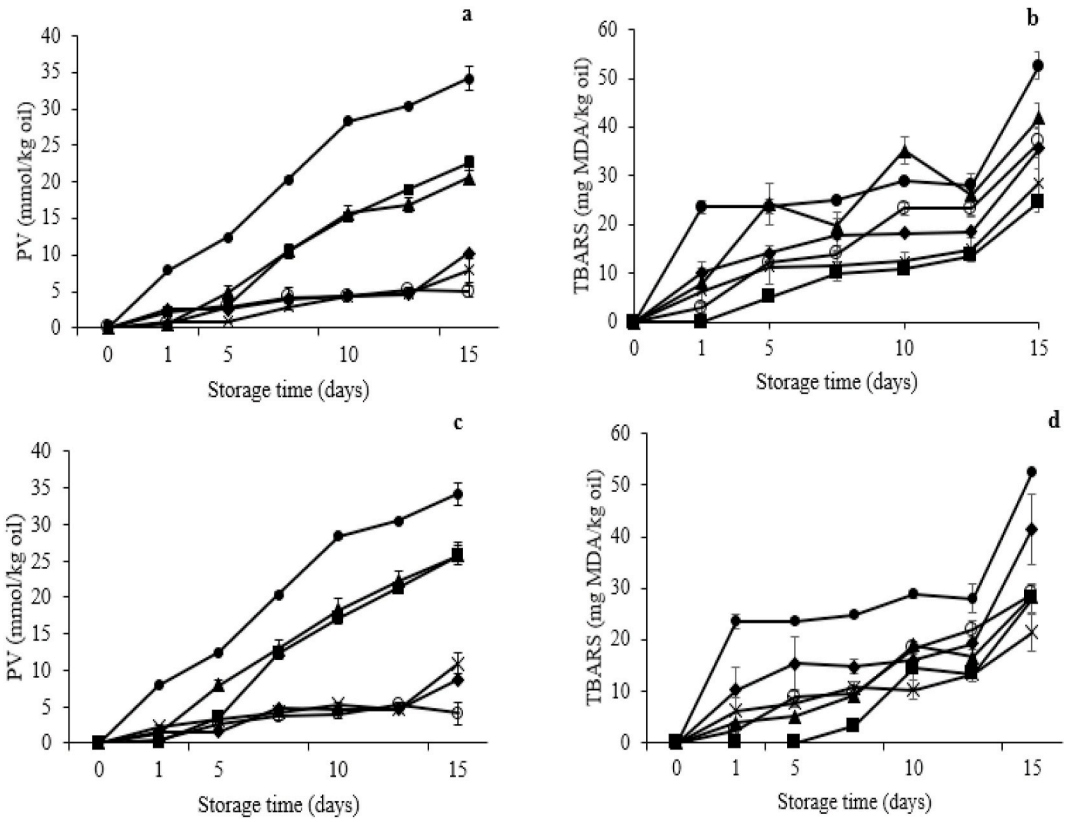

Fig. 2. Oxidative stability of above CMC (aCMC) nanoemulsions. a) Peroxide Value (PV) and b) 2-thiobarbituric acid reactive substances (TBARS) for nanoemulsions with antioxidants added pre-homogenization; c) PV and d) TBARS for nanoemulsions with antioxidants added post-homogenization. Control; Gallic acid (G0) propyl gallate (G3); octyl gallate (G8); dodecyl gallate (G12); hexadecyl gallate (G16).

Table 2
Solubility and Partition data for gallate alkyl esters.

Compounds	Solubility in water at pH 7.0 (mmol/L)	Partition in octan-1-ol:water mixture				Partition in sunflower oil:water mixture		
		Partition in water (wt. %)	Partition in octanol (wt. %)	Experimental Log $P_{W(7.0)}^{oct}$ [a]	Theoretical Log P_W^{oct} [b]	Partition in water (wt. %)	Partition in oil (wt. %)	Experimental Log $P_{W(7.0)}^{oil}$ [c]
Gallic acid (G0)	21.16 ± 0.03	99.46 ± 0.19	0.54 ± 0.19	−2.28 ± 0.16	−2.27	99.08 ± 0.03	0.92 ± 0.03	−2.03 ± 0.01
Propyl gallate (G3)	15.79 ± 0.19	1.20 ± 0.06	98.80 ± 0.06	1.92 ± 0.02	1.72	58.02	41.98	−0.14
Octyl gallate (G8)	0.0035 ± 0.0018	0.22 ± 0.27	99.78 ± 0.27	>2.6	4.27	0	100	Non measurable
Dodecyl gallate (G12)	0	0	100	Non measurable	6.30	0	100	Non measurable
Hexadecyl gallate (G16)	0	0	100	Non measurable	8.34	0	100	Non measurable

[a] Flask method based on six independent measurements in a biphasic octan-1-ol:10 mM phosphate buffer, pH 7.0.
[b] Log $P_{W(7.0)}^{oct}$ calculated using Advanced Chemistry Development (ACD/Labs) software V11.02 (© 1994–2019 ACD/Labs).
[c] Flask method measurements in a biphasic sunflower oil:10 mM phosphate buffer, pH 7.0.

and log Pw^{oil} for G3: while for log P_W^{oct} the value was 1.73, for olive and soybean oils, log $P_W^{oil} = -0.07$, and for corn oil log $P_W^{Oil} = 0.10$. Such partition values result from intermolecular interactions between the solute and the two phases of possible partition. These interactions can be electrostatic or from hydrogen bonding between the solute and the two phases. In the case of G3, such interactions in oils/water seems to differ from the ones existing in octanol/water whereas for G0, the difference of interactions depending on the two systems are only minor. This suggests that distributional data measured between oil and water are more accurate to explain the similar antioxidant efficiency between G0 and G3 (Figs. 1 and 2). Both antioxidants were indeed significantly partitioned in the aqueous phase where their efficiency to scavenge radicals formed at the interface by lipid hydroperoxide decomposition is not optimal. This is further supported by previous studies that observe a higher concentration of G3 and G0 in the aqueous phase accompanied with a lower antioxidant efficiency of these compounds compared to longer chain gallates found in higher concentration at the interface (Ferreira, Costa, Losada-Barreiro, Paiva-Martins, & Bravo-Díaz, 2018; Freiría-Gándara, Losada-Barreiro, Paiva-Martins, & Bravo-Díaz, 2018). For example, Freiría-Gándara, Losada-Barreiro, Paiva-Martins, and

Bravo-Díaz (2018) report that up to 75% of G0 and 50% of G3 partition into the aqueous phase of olive oil emulsions, and that these compounds present lower antioxidant efficiency than G4 (maximum of 40% partitioned into the aqueous phase) and G8, which shows higher partition at the interface.

Interestingly, the water solubility at pH 7.0 also showed similar values for G0 and G3: 3.6 g/L (21.16 mmol/L) vs. 3.4 g/L (15.79 mmol/L) (Table 2). These solubility limits were 424 and 316 times higher, respectively, than the tested antioxidant concentrations (50 μmol/L), thus indicating that the diffusion through the aqueous phase was allowed for them. By contrast, 0.001 g/L (1.8 μmol/L) of G8 could be solubilized in the aqueous phase, which was 28 times lower than the tested concentration.

In bCMC nanoemulsions (Figs. 1 and 3), for PV, the antioxidant activity increased with increasing the antioxidant chain length. G16 showed the highest ($p < 0.05$) efficiency in both modes of incorporation after 15 days, accompanied by G12 ($p > 0.05$) in post-homogenization (Fig. 1a and c, Fig. 3a and c). For TBARS, although this behavior was less obvious, it was possible to observe a similar tendency (Fig. 1b and d, Fig. 3b and d), which is in agreement with the polar paradox (Porter et al., 1989).

In aCMC nanoemulsions (Figs. 2 and 4), when considering PV alone, a cut-off effect seemed to appear after 11 days for the dodecyl chain ($p < 0.05$) (Fig. 4a and c), suggesting that the presence of micelles could have induced this effect. Variations in the chain length of occurrence of the cut-off effect, as well as its non-occurrence, have already been observed by other authors (Alemán et al., 2015; Ferreira et al., 2018; Sørensen, Villeneuve, & Jacobsen, 2017), but the mechanisms involved are still poorly understood. Among the possible environmental factors influencing the efficiency of antioxidants of different polarities, the nature and concentration of surfactant is among be the most important (McClements & Decker, 2018). For example, different types of molecular interactions between surfactant and antioxidants can alter their local concentration (Panya et al., 2012) or their transport mode (Laguerre et al., 2017) in multicompartmentalized systems. Surfactant micelles may thus be able to solubilize the antioxidants, reducing their concentration or favoring their action at the interface (Kiralan et al., 2014; McClements & Decker, 2018).

As in bCMC nanoemulsions, micelles were absent, it could be assumed that gallic acid and its alkyl esters will distribute in the aqueous phase, the oil droplet interior, or at the droplet surface according to their inherent partitioning characteristics. Therefore, from Table 2 and literature (Freiría-Gándara, Losada-Barreira, et al., 2018; Ferreira et al., 2018), exogenous gallates alkyl esters will predominantly locate at the interface or into the oil droplet core, where endogenous tocopherols also predominate (Kiralan et al., 2014). Synergistic interactions between tocopherols and phenolic compounds, such as gallic acid, caffeic acid and rosmarinic acid, are reported in literature (Pazos, Andersen, Medina, & Skibsted, 2007; Panya et al., 2012). For example, Wang et al. (2019) observe that gallate alkyl esters G3, G8 and G12 showed synergistic effect for regeneration of α-tocopheroxyl radicals into α-tocopherol in Tween 20-stabilized O/W emulsions. As our nanoemulsions were prepared with unstripped rapeseed oil, hydrophobic gallates (G8, G12, G16) more associated with the interface and oil droplet may have exerted a synergistic interaction with endogenous tocopherols in bCMC nanoemulsions, which could partially explain the antioxidant efficiency order observed (Figs. 1 and 3). In aCMC nanoemulsions, the presence of micelles may have altered the interaction between the highly hydrophobic antioxidants and tocopherols (Sørensen et al., 2017), solubilizing them and taking them away from the oil droplet, which could account for the cut-off effect observed after 11 days in aCMC samples (Fig. 4).

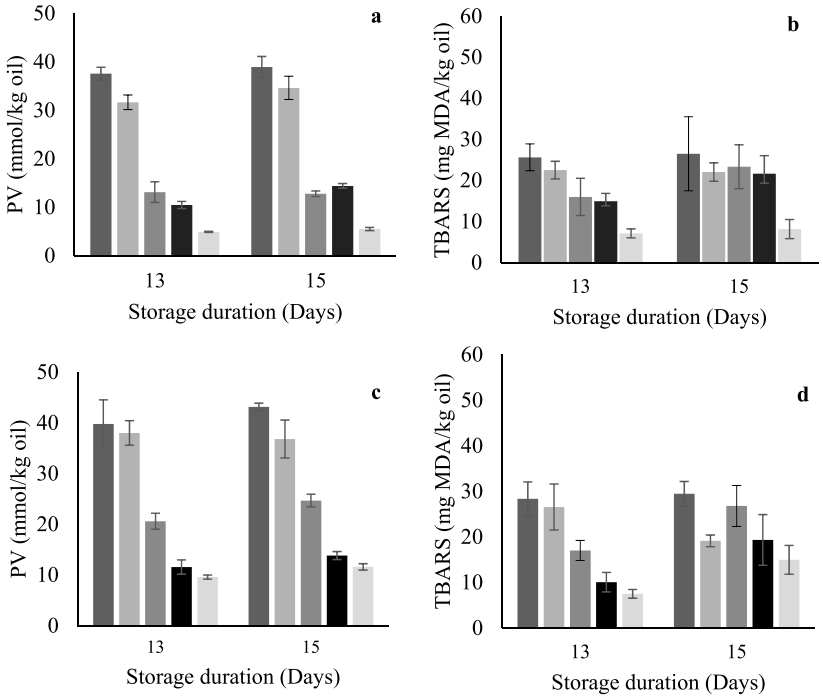

Fig. 3. Oxidative stability of below CMC (bCMC) nanomemulsions at days 13 and 15. a) Peroxide Value (PV and b) 2-thiobarbituric acid reactive substances (TBARS) for nanoemulsions with antioxidants added pre-homogenization; c) PV and d) TBARS for nanoemulsions with antioxidants added post-homogenization. Bars show mean ± standard deviation. Gallic acid (G0); propyl gallate (G3); octyl gallate (G8); dodecyl gallate (G12); hexadecyl gallate (G16).

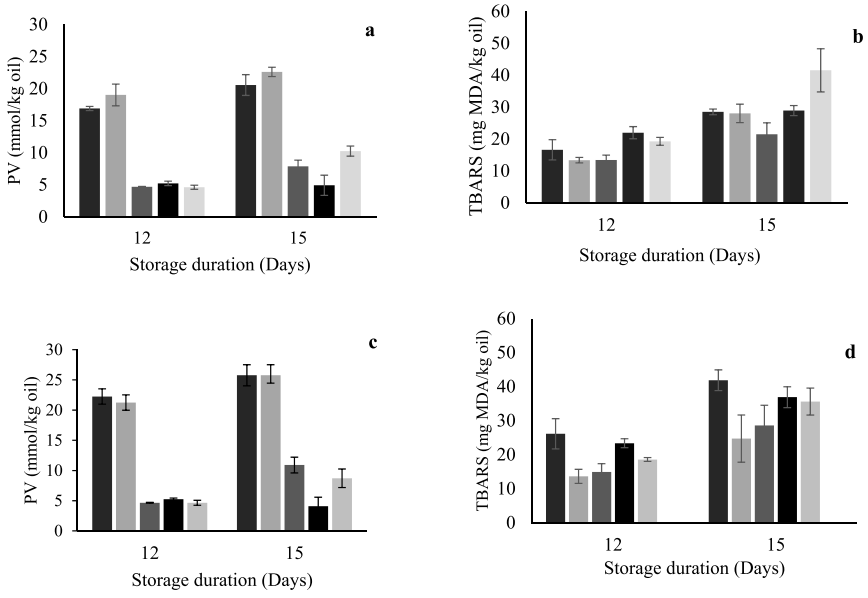

Fig. 4. Oxidative stability of above CMC (aCMC) nanomemulsions at days 12 and 15. a) Peroxide Value (PV) and b) 2-thiobarbituric acid reactive substances (TBARS) for nanoemulsions with antioxidants added pre-homogenization; c) PV and d) TBARS for nanoemulsions with antioxidants added post-homogenization. Bars show mean ± standard deviation. . Gallic acid (G0); propyl gallate (G3); octyl gallate (G8); dodecyl gallate (G12); hexadecyl gallate (G16).

3.3. *Effect of the mode of incorporation and of the surfactant concentration*

Recent studies highlight hurdles in predicting the efficiency of antioxidants in dispersed lipid systems based only on their partitioning and physical location (Decker et al., 2017). These difficulties originate mainly from the fact that many additional factors influencing antioxidant efficiency are poorly understood and need further investigations. Among these factors, we hypothesized that the mode of incorporation of antioxidants (pre-homogenization or post-homogenization) could affect their partition in the emulsion system, modifying their efficiency. Based on earlier reports on the ability of surfactant micelles to modulate the antioxidant activity (Berton-Carabin, Ropers, & Genot, 2014; Kiralan et al., 2014; Laguerre et al., 2017; Panya et al., 2012), we also investigated these different incorporation systems at two surfactant concentrations corresponding to absence (bCMC) or presence (aCMC) of micelles.

For bCMC (Figs. 1 and 2S, *supplementary material*), our results indicated that when antioxidants were incorporated during pre-homogenization, the following efficiency ranking could be observed for PV: G16 > G12~G8>G3~G0 (Fig. 1a). Considering the inhibition of primary oxidation compounds (PV value), the addition of antioxidants at post-homogenization lead to a decrease ($p < 0.05$) of G8 and G16 efficiency. The antioxidant ranking was: G16~G12 > G8>G3~G0 (Fig. 1c). However, for secondary oxidation compounds (TBARS) (Fig. 1b and d), no difference was observed between the distinct modes of incorporation ($p > 0.05$), except for G16 at day 15, with addition pre-homogenization showing lower TBARS value ($p < 0.05$) than post-homogenization.

Adding lipophilic antioxidants in oil before it is dispersed as a multitude of droplets enables these antioxidants to be already in a dynamic equilibrium (i.e. distributed in all droplets) at day 0. On the contrary, when they are incorporated post-homogenization, they must diffuse to all droplets to reach the dynamic equilibrium, which may be a long process in the absence of surfactant micelles due to the null water-solubility of these antioxidants (Table 2). Moreover, as was previously observed with long chain esters of chlorogenic acids (Laguerre et al., 2009) one can also envisage that such long chain phenolipids when incorporated after homogenization would form aggregates in the aqueous phase, limiting therefore their antioxidant efficiency. This could explain the differences in PV (G8 and G16) induced by a different mode of incorporation.

These results agree with those reported by Durand et al. (2019) for a liposome system, who observe a higher antioxidant efficiency of medium and long chain gallate alkyl esters (C10–C16) when blended with phospholipids before vesicles formation (here pre-homogenization) compared to when added to the liposome suspension (here post-homogenization). The significant effect of mode of incorporation on antioxidant efficiency is also demonstrated in low-moisture foods (Barden et al., 2015). Despite the huge compositional and structural differences between the above-mentioned and the present systems, Barden et al. (2015) also conclude that incorporation of highly hydrophobic rosmarinate esters (C12 and C20) in the oil phase during dough preparation is more effective to prevent lipid oxidation.

Unexpectedly, G12, which can also be considered as a low polarity compound (Table 2), did not show the same behavior as G8 and G16, as the mode of incorporation did not affect its antioxidant activity in bCMC nanoemulsions (Fig. 1). Since no micelle-assisted transport of G12 can take place, it suggested that G12 added post-homogenization could only reach a dynamic equilibrium in droplets if (i) it is water soluble enough to diffuse through the aqueous phase or (ii) it is exchanged between two droplets when they collide with each other (collision-fission transport) (Laguerre et al., 2017). To verify this point, we incubated G12 overnight at saturation in water under magnetic stirring at room temperature. After filtration, the supernatant was analyzed by HPLC and, as expected, no peak could be observed (Table 2). The insolubility of G12 in water suggests that this antioxidant is probably exchanged (if exchanged) by collision-fission transport. Thus, given the fact that the surfactant also contained a dodecyl hydrocarbon chain positioned at the droplet surface, it could be hypothesized that G12 is co-localized with SDS at the

interface.

This could explain the distinct behavior of G12 compared to the other lipophilic gallate alkyl esters in two ways. First, an antioxidant located at the interface can inhibit the initiation of oxidation instead of the propagation of this process, which is a much more effective way to counteract oxidation (Schröder et al., 2020). Second, if reaching a dynamic equilibrium is important for G12 to express its antioxidant activity, then being at the interface in bCMC nanoemulsions will certainly increase the inter-droplet transfer efficiency during collision-fission events. Indeed, if the antioxidant is in the oily core of the droplets as for G16, this exchange is disfavored since the antioxidant will have to migrate from the interior to the interface, and then to cross the molecular layer formed by the surfactant at this interface. By contrast, if G12 is located at the interface, this exchange is facilitated.

This putative interfacial G12/SDS co-location may be favored by the effect of the chain length compatibility which has already been demonstrated in many surfactant systems such as monomolecular films, micelles, microemulsions, macroemulsions, among others (James--Smith, Alford, & Shah, 2007). When surfactant such as SDS as well as other hydrocarbon surface-active molecules are aligned at interfaces, the properties of the interface are impacted, to a great extent, by the matching or mismatching of the alkyl chain lengths. For example, among different alcohols (C8, C10, C12, C14, C16, and C18) added pre-homogenization in a SDS-stabilized O/W emulsion, only dodecanol is able to increase the adsorption of SDS at the oil/water interface (James-Smith et al., 2007). By analogy with our gallate alkyl esters, we can speculate that a chain length compatibility between SDS and G12 can synergistically co-partition them at the interface. Whether this chain length compatibility effect would affect the droplet size is unclear for us, but our results (Table 1) suggest it does not, although further studies are necessary to confirm this.

In the presence of surfactant micelles (aCMC) (Figs. 2 and 3S, *supplementary material*), no distributional differences of antioxidants in the nanoemulsion was observed, whatever their modes of incorporation ($p > 0.05$). Surfactant micelles can rapidly exchange lipid components between micelles and emulsion droplets (Laguerre et al., 2017). Thus, formation of co-micelles between the surfactant and antioxidants could have assisted transport of hydrophobic (hence non-water-diffusible) gallate alkyl esters through the aqueous phase between droplets. This hypothesis agrees with a recent finding showing by flow-cytometry that surfactant micelles are able to transfer lipid oxidation products between emulsion droplets faster than in emulsions lacking these structures (Li, McClements, & Decker, 2020). Although it needs further confirmation, it is possible that such phenomena are also occurring for the inter-droplet transfer of hydrophobic antioxidants.

Another important observation is that the efficiency order among antioxidants was unchanged when considering both primary oxidation compounds (PV) or secondary ones (TBARS) in bCMC nanoemulsions, while it strongly differed in aCMC nanoemulsions. Indeed, in aCMC nanoemulsions, when considering secondary oxidation compounds only, no significant differences between antioxidants were observed whatever their mode of incorporation (Fig. 2b and d). On the contrary, for primary oxidation compounds, antioxidants can be clustered in two groups: (i) G0-G3 with a high water-solubility and (ii) G8-G12-G16 with a low water-solubility (Table 2 and Fig. 2a and c). The first group was much less efficient than the second one when considering their ability to counteract the formation of primary oxidation compounds. As the antioxidant activity of G0 and G3 regarding TBARS was much less impacted by the presence of surfactant micelles than that of G12 and G16, this suggests that surfactant micelles somehow hindered the antioxidant action of hydrophobic phenolipids but not that of hydrophilic ones to counteract the formation of secondary oxidation products. This can be noted in the generally higher values of TBARS for G12 and G16 in both modes of incorporation (Fig. 2b and d and Fig. 4b and d), although for G12 this trend seemed to disappear in the last days of oxidation (Fig. 4b and d).

Furthermore, the rate of oxidation in aCMC nanoemulsions was lower than in bCMC, as evidenced by the lower PV in aCMC samples (control and added of antioxidants) and TBARS (control samples) at the end of the storage period (Figs. 1 and 2). These results suggest that the presence of surfactant micelles somehow played an antioxidant role. One possible influence of micelles to reduce oxidation could be their interaction with tocopherols (α-tocopherol and γ-tocopherol) contained in the non-stripped rapeseed oil used in this study. Kiralan et al. (2014) observe that tocopherols naturally occurring in refined oils have their antioxidant efficiency enhanced by forming co-micelles with Tween 20. The authors suggest that surfactant micelles would solubilize tocopherols out of droplet core, but also that surfactant-tocopherol co-micelles could act as both vehicles and reservoir to replace very quickly oxidized tocopherol at lipid interface. Therefore, it is possible that a similar behavior could have occurred between tocopherols and surfactant micelles in the present emulsion system, promoting an increase in antioxidant efficiency of endogenous tocopherols in the rapeseed oil droplets.

4. Conclusions

In this study, medium to long chain length gallate alkyl esters were more efficient to prevent lipid oxidation than the parent unesterified molecule. Overall, our results highlighted the importance of interactions occurring between antioxidants and surfactant micelles, demonstrating that they might modify antioxidants behavior in the emulsion system affecting their solubility and partition. The effect of the mode of incorporation was less important and modulated by the presence of surfactant micelles in the aqueous phase. In the absence of micelles, only G8 and G16 were affected by the initial distribution (mode of incorporation), and they were more effective antioxidants when added pre-homogenization. Upon the presence of micelles, this effect vanishes and the antioxidants could be incorporated in any phases without efficiency loss. This suggests that using surfactant concentrations above its CMC can be an interesting strategy towards the optimization of emulsion formulations and processing. This result can be used by the food industry, where food emulsions and nanoemulsions are usually prepared with large excess of surfactant and with addition of antioxidants with different polarities.

CRediT authorship contribution statement

Tayse Ferreira Ferreira da Silveira: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Mickaël Laguerre:** Conceptualization, Methodology, Investigation, Writing and Editing draft. **Claire Bourlieu-Lacanal:** Conceptualization, Methodology, Investigation, Writing and Editing draft, Supervision. **Jérôme Lecomte:** Conceptualization, Methodology, draft review & editing. **Erwann Durand:** Conceptualization, Methodology, Investigation, draft review & editing. **Maria Cruz Figueroa-Espinoza:** Methodology, draft review & editing. **Bruno Baréa:** Investigation, Formal analysis. **Nathalie Barouh:** Investigation, Formal analysis. **Inar Alves Castro:** Methodology, draft review & editing. **Pierre Villeneuve:** Conceptualization, Methodology, Investigation, Writing and Editing draft, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.110892.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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