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Incorporation of curcumin-loaded solid lipid nanoparticles into yogurt: Tribo-rheological properties and dynamic *in vitro* digestion

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ABSTRACT

The incorporation of nanostructures loaded with bioactive compounds into food matrices is a promising approach to develop new functional foods with improved nutritional, health profiles and good sensorial properties. The rheological and tribological properties of yogurt enriched with curcumin-loaded solid lipid nanoparticles (SLN) were evaluated. Also, the TCA solubility index, the bioaccessibility of curcumin and cell viability were assessed after dynamic *in vitro* digestion. The presence of SLN in yogurt did not affect its rheological properties; however, SLN addition increased the lubrication capability of yogurt. After *in vitro* digestion, yogurt with added SLN (yogurt_SLN) presented a lower TCA solubility index (22 %) than the plain yogurt (39 %). The bioaccessibility and stability of curcumin were statistically similar for yogurt_SLN (30 % and 42 %, respectively) and SLN alone (20 % and 39 %, respectively). Regarding cell viability results, the intestinal digesta filtrates of both controls (i.e., SLN alone and plain yogurt) did not affect significantly the cell viability, while the yogurt_SLN presented a possible cytotoxic effect at the concentrations tested. In general, the incorporation of SLN into yogurt seemed to promote the mouthfeel of the yogurt and did not adversely affect the bioaccessibility of curcumin. However, the interaction of SLN and yogurt matrix seemed to have a cytotoxic effect after *in vitro* digestion, which should be further investigated. Despite that, SLN has a high potential to be used as nanostructure in a functional food as a strategy to increase the bioactive compounds' bioaccessibility.

1. Introduction

The development of new functional foods with improved nutritional value, health properties and good sensorial properties is the current goal of food industry. Yogurt is a semi-solid food produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* through milk fermentation (Helal et al., 2022). This product is a very relevant source of protein, calcium, magnesium, phosphorus, riboflavin, zinc, among others. Yogurt is highly consumed around the world by people of all ages once its consumption is linked to healthy habits (Das et al., 2019; Helal et al., 2022). Therefore, the fortification of yogurt with bioactive compounds is a great approach for enhancing nutrient intake (Gahruie et al., 2015). Curcumin is a lipophilic compound extracted from the rhizome of *Curcuma longa*, which is already used as a spice and colorant in the food industry. Curcumin presents several health-promoting benefits, such as antioxidant, anti-inflammatory, anticarcinogenic and antimicrobial activities. However, the main drawbacks of curcumin's application in the food industry are its poor solubility in aqueous solutions and sensitivity to light, oxidation, heat and alkaline solutions (Munekata et al., 2021). One of the strategies to overcome these drawbacks is its encapsulation.

Lipid-based nanostructures are a class of nanostructures where curcumin can be easily encapsulated due to their lipophilic character. Solid lipid nanoparticles (SLN) are a category of lipid-based nanostructures, which can be formed using one or more solid lipids, such as fatty acids, waxes, glycerides and triacylglycerol. These nanostructures are fully crystallized at room temperature, granting protection against pH, ionic strength and high temperature conditions during the processing, storage and intake of foods (Ban et al., 2020). Furthermore, the application of lipid-based nanostructures in food products, namely dairy products, can prevent the chemical degradation of bioactive compounds by the

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exposure of the food to several operational and environmental conditions, the impact on the organoleptic properties of the food product, and the possible molecular interactions between the bioactive compound and food ingredients changing the bioactivity of the bioactive compounds (Nejatian et al., 2022).

The incorporation of nanostructures into the food matrices can affect foods' and nanostructures' properties, presenting some possible challenges on functional food development (Dima et al., 2020). Furthermore, incorporation of nanostructures can alter the texture and mouthfeel of the product, being perceived during the complex oral processing, which are two of the most important factors in food acceptability by the consumer.

Oral processing is a complex process which consists in the transport, manipulation and breaking down the food in the mouth before being swallowed. Depending on the type of food product, oral processing styles and the emotions of consumers, this process can change (Sethupathy et al., 2021). While the oral process of solid food consists in biting, chewing, mixing with saliva, lubrication, bolus formation, swallowing and clearing, the oral process of liquid and semi-solid foods only includes the oral manipulation, tongue rolling and swallowing with little or no chewing requirement (Nguyen et al., 2016; Sethupathy et al., 2021). Therefore, the oral processing for solid foods has a longer residence time than that of liquid or semi-solid foods, allowing the food to interact with more oral receptors thus improving the mouthfeel. Furthermore, the oral contact of solid food can occur through the lips, tongue, teeth, cheeks and palate while the food is broken down, allowing to a greater extent the processing of textural information. On the other hand, due to shorter retention time of liquid and semi-solid foods in the mouth, the mouthfeel is mainly attributed to the tongue movements and characterized by the flow behavior of the food and the after-feeling sensation when the food is swallowed (Nguyen et al., 2016; Sethupathy et al., 2021). During oral processing, different textural properties can be perceived such as softness, hardness, adhesiveness, thickness, brittleness, springiness, sponginess, crispiness, creaminess, slipperiness, smoothness, among others (Prakash et al., 2013; Sethupathy et al., 2021). Many of these attributes, such as hardness, springiness and thickness, can already be analyzed using rheology and texture analysis, since those attributes are linked directly to the bulk phase deformation. However, the mouthfeel and after-feeling sensations (e.g., creaminess, slipperiness and smoothness) cannot be assessed by rheology, because these attributes are not described by bulk deformation but by lubrication behavior (Nguyen et al., 2016; Prakash et al., 2013). Therefore, tribology has gained increasing attention by food researchers as it can be used to analyze the sensory attributes associated to the lubrication properties of a food product, such as creaminess, slipperiness and smoothness, by simulating in-mouth lubrication and squeeze actions between the tongue and oral surfaces such as palate (Laiho et al., 2017; Nguyen et al., 2016). In this way, the combination of rheology and tribology has shown as a very promising approach to assess the oral perception of food (Huang et al., 2021). Nonetheless, such studies applied to food products fortified with bio-based nanoparticles have not been performed before.

Furthermore, food matrices are complex systems containing protein, fats, carbohydrates, minerals among others, with different interactions between them which originate various types of structures at the nano-, micro- and macro-scale, conferring specific nutritional and sensorial properties (Dima et al., 2020). These interactions may affect the gastrointestinal (GI) fate of the bioactive compound-loaded nanostructures and, therefore, the bioactive compound bioaccessibility, stability and safety.

In our previous work, curcumin-loaded SLN were incorporated into stirred yogurt and the stability of yogurt during its shelf-life was evaluated. In general, the presence of SLN in yogurt did not affect the stability of the yogurt during its shelf-life, regarding physicochemical and rheological properties at 4 °C (Gonçalves et al., 2022). However, the effect of SLN in yogurt in terms of the oral processing, namely their rheological and tribological properties, *in vitro* digestion and cytotoxicity were not assessed.

The main goal of this work is to understand the effects of curcumin-loaded SLN incorporated in yogurt on oral processing and sensory perception, through the assessment of rheological and tribological properties of the yogurt. The bioaccessibility of curcumin after *in vitro* digestion as well as the yogurt digestibility through TCA solubility index of yogurt were equally evaluated. Furthermore, potential cytotoxicity of SLN incorporated into yogurt was also assessed after *in vitro* digestion.

2. Materials and methods

2.1. Materials

PHOSPHOLIPON® 90G, composed by 90 % of phosphatidylcholine from soybean, was kindly provided by Lipoid (Switzerland). Beeswax produced by *Apis mellifera* was purchased from QUIMIND (Portugal) and Tween® 80 was obtained from Panreac (Spain). Plain yogurts were purchased at a local supermarket (Braga, Portugal). Polydimethylsiloxane (PDMS) was produced from a Sylgard 184 silicone elastomer kit (Dow Corning Corporation, U.S.A.). Curcumin from *Curcuma longa* (Turmeric, purity of ≥ 65 %), pepsin (≥2 500 U/mg, P7012), lipase (30–90 U/mg, L3126), pancreatin (8x USP, P7545), bile salts (B8631), bovine serum albumin and Folin reagent were obtained from Merck (Portugal). Chloroform (≥99.5 %) and dimethyl sulfoxide (≥99.0 %) (DMSO) were purchased from Fisher Scientific (NJ, USA). Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids and phosphate-buffered saline (PBS) were obtained from Lonza (Basel, Switzerland). Penicillin/streptomycin (PS), trypsin-EDTA, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trichloroacetic acid (≥99.0 %) (TCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA); fetal bovine serum (FBS) was obtained from Merck (Darmstadt, Germany). Human colon Caco-2 cell line (American Type Culture Collection, ATCC) was kindly provided by the Department of Biology of the University of Minho (Braga, Portugal).

2.2. SLN production

SLN were produced as described by (Gonçalves et al., 2021a). The lipid phase was composed by curcumin (0.1 %), beeswax (3 %) and lecithin (1.5 %), and was heated at 80 ℃ in a water bath under magnetic stirring. The aqueous phase composed by Tween® 80 (1.5 %) and distilled water was also heated at 80 ℃ in a water bath and mixed for 2 min at 3 400 rpm using an Ultra-Turrax homogenizer (T18, Ika-Werke, Germany). The aqueous phase was quickly added to the lipid phase and mixed in an Ultra-Turrax homogenizer for 8 min at 18 000 rpm. Then, the mixture was slowly dispersed in cold water at 2 ℃, to a volume ratio of 1:10, under agitation at 2 000 rpm. To ensure crystallization and stability, the SLN were stirred for another 35 min at 800 rpm. Finally, the SLN were freeze-dried using 2 % of sucrose as a cryoprotectant.

2.3. Yogurt preparation

The yogurt was prepared according to Gonçalves et al., (2022). Curcumin-loaded SLN (6.67 g) were added into 100 g of plain yogurt and mixed manually until a homogeneous mixture was obtained. The curcumin amount incorporated into the yogurt corresponds to 20 mg of curcumin per 100 g of food product, respecting the acceptable daily intake value (i.e., 3 mg/kg body weight/day) (EFSA, 2014). A plain yogurt mixed at the same time as the fortified yogurt was used as a control. Both sample and control were stored in glass containers protected from light at 4 ℃. The samples were prepared in three different batches using the same pack. The nutritional composition of yogurt provided by the manufacturer and of yogurt with curcumin-loaded SLN is presented in Table 1.

Table 1
Nutritional composition of plain yogurt and yogurt_SLN.

Nutritional composition in 100 g	Plain Yogurt	Yogurt_SLN
Energy (kcal)	45	48
Lipids (g)	1.5	1.8
Carbohydrates (g)	4.4	4.5
Sugars (g)	2.9	3.0
Proteins (g)	3.5	3.5
Salt (g)	0.09	0.09

2.4. Rheological characterization

Rheological measurements were performed in triplicate at 25 ℃ in a TA Instruments HR-1 rheometer equipped with a Peltier plate (TA Instruments, New Castle, DE USA), which was configured with a parallel plate geometry (Ø 40 mm) and gap of 1 000 μm. The viscoelastic properties of the yogurts were assessed by the frequency sweep determination in the linear viscoelastic region, between 0.1 and 10 Hz at 0.5 % strain. The storage modulus (G') and loss modulus (G'') were recorded as a function of frequency. Additionally, the flow curves were obtained through a 3 steps program sequence (up-down-up) using a continuous ramp and shear rate range from 0.1 to 300 s^{-1}. The three steps program was performed in order to eliminate time-dependence. The flow behavior was characterized by the Herschel-Bulkley model (Equation 1) that was fitted to the upward flow curve:

$\sigma = \sigma_0 + k \times \dot{\gamma}^n$(Eq. 1).

where σ represents the shear stress (Pa), σ_0 is the yield stress (Pa), k is the consistency index (Pa.s^n), $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the flow index (i.e., $n = 1$ for Newtonian, $n < 1$ for pseudoplastic, and $n > 1$ for dilatant fluids).

2.5. Tribological characterization

Tribological measurements were carried out in triplicate at 25 ℃ using a UMT-2 (CETR, Bruker, UK) tribometer equipped with a reciprocating ball-on-plate contact geometry. A stainless-steel ball (Ø 10 mm) was used to represent the palate and a polydimethylsiloxane (PDMS) disc to mimic the human tongue surface. Comparable Stribeck curves were obtained through a similar ball-on-plate setup, however imposing a rotational motion of the plate to simulate an equivalent ball-on-disc sliding geometry. The triboexperiments were performed with constant applied force of 1 N and linear increasing sliding speeds, up to 105 mm/s. The coefficient of friction (*CoF*) was plotted as a function of the sliding speed to obtain look-alike Stribeck curves.

2.6. Dynamic in vitro digestion

The yogurt_SLN, control (i.e., plain yogurt) and SLN alone (i.e., produced SLN) samples were subjected to a dynamic *in vitro* digestion using the dynamic *in vitro* gastrointestinal system (DIVGIS) simulating the gastric, duodenal, jejunal and ileal phases of the GI tract, as described by Pinheiro et al. (2016). The digestion parameters were determined through the procedure described by Fernandes et al. (2021). Simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) compositions are described in Table 2. Once DIVGIS does not include the oral phase, it was performed separately. Briefly, the samples were mixed with SSF in a final ratio of 1:1 (w/v) of dry weight of the food and incubated for 2 min at 37 ℃ under magnetic stirring. The obtained bolus was added to the stomach reactor of DIVGIS, and the following procedure was conducted under dynamic conditions where the simulated fluids and enzymes were added continuously using syringe infusion pumps (KDS-100-CE, KD Scientific, Holliston, MA). The simulated fluids and enzyme solutions for each phase were added in a volume that assures a final ratio of 1:1 (v/v) of oral and gastric phases' contents and SGF and SIF mixtures, respectively. In order to simulate basal level of secretion that the stomach retains in the fasted state, 10 % of the amount of SGF without enzymes was placed in the stomach reactor. SGF and simulated intestinal fluids (SIF) delivering rates were calculated considering the caloric content of the sample and the gastric emptying time as described in Fernandes et al. (2021). The volume of HCl (1 mol/L) and NaOH (2 mol/L) needed to reach pH 2 at gastric phase and pH 7 at intestinal phase, respectively, were added to SGF and SIF solutions to be infused, respectively, in order to adjust the pH continuously during the digestion procedure as found *in vivo*. The jejunal and ileal phases were simulated in reactors controlled by peristaltic pumps (120S, Watson-Marlow, Concord, Canada). The reactors were coupled to hollow fiber filters MiniKros Sampler (S02-E001-05-N, Repligen, Amsterdam, Netherlands). At the end of each phase, endpoint samples were collected, as well as the filtrated portions from the membranes (i.e. jejunum and ileum filtrates) and non-filtrated portion (i.e., undigested sample). For each sample, the experiments were carried out in triplicate.

Table 2
Composition of simulated digestion fluids.

Salt Solution	SSF	SGF	SIF
KCl (mmol/L)	15.1	6.9	6.8
KH_2PO_4 (mmol/L)	3.7	0.9	0.8
$NaHCO_3$ (mmol/L)	13.6	25	85
NaCl (mmol/L)	–	47.2	38.4
$MgCl_2(H_2O)_6$ (mmol/L)	0.15	0.12	0.33
$(NH_4)_2CO_3$ (mmol/L)	0.06	0.5	–
$CaCl_2(H_2O)_2$ (mmol/L)	1.5	0.15	0.6
Pepsin (U/mL)	–	4000	–
Lipase (U/mL)	–	120	–
Pancreatin (U/mL)	–	–	100
Bile salts (mmol/mL)	–	–	10

SSF – Salivary simulated fluid; SGF – Simulated gastric fluid; SIF – Simulated intestinal fluid.

2.7. Bioaccessibility and stability of curcumin

The bioaccessibility and stability of curcumin were determined at the end of the digestion, through the methodology described by (Gonçalves et al., 2021a) with some modifications. The jejunum and ileum filtrates were considered as the micellar phase. Briefly, jejunum and ileum filtrates and non-filtrated portions (5 mL) were mixed with 5 mL of chloroform using a vortex and centrifuged at 700 *g* for 10 min at room temperature. The bottom layer was collected, and the top layer was subjected to a second extraction procedure. The second bottom layer was combined with the first one and analyzed in an UV–VIS spectrophotometer (V-560, Jasco, USA) at 420 nm. Curcumin concentration was determined through a calibration curve (absorbance *versus* curcumin concentration) in chloroform.

Curcumin bioaccessibility (B) was defined as the curcumin fraction present inside the micellar phase, while stability (S) was defined as the curcumin present in the whole digesta at the end of digestion. These parameters were calculated using equations (2) and (3), respectively.

$B(\%) = C_{Filtrates}/C_{Digesta} \times 100$(Eq. 2).

$S(\%) = C_{Digesta}/C_{Initial} \times 100$(Eq. 3).

where $C_{Filtrates}$ is the sum of curcumin concentrations determined at the end of digestion in the jejunum and ileum filtrates and $C_{Digesta}$ is the sum of curcumin concentrations determined at the end of digestion in the jejunum and ileum filtrates and non-filtrated portions. $C_{Initial}$ is the nominal concentration of curcumin present in the sample at the beginning of digestion process.

2.8. TCA solubility index

The TCA solubility index was determined from samples collected in the DIVGIS at different timepoints based on Silvestre et al. (2013)

procedure, with some modifications. In order to remove the insoluble protein, the samples were vortexed with TCA 20 % in a ratio of 1:1 (v/v) and incubated for 30 min at room temperature. After that, the mixture was centrifuged at 3 000 *g* for 15 min at room temperature. The supernatant was collected and the soluble protein was determined based on Sousa et al. (2022) procedure, with some modifications. The samples (500 μL) were mixed with Lowry reagent (1.25 mL) and incubated at room temperature for 10 min in the dark. After that, 250 μL of Folin reagent was added to the mixture and incubated for 30 min at room temperature in the dark. The samples were transferred to a 96-well plate and the absorbance was measured at a 750 nm in a Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments, Inc., USA). The soluble protein content was determined using bovine serum albumin as the standard. TCA solubility index was calculated using equation (4):

$$TCA solubility index(\%) = m_{solubleprotein}/m_{totalprotein} \times 100 \text{(Eq. 4)}.$$

where $m_{soluble\ protein}$ is the soluble protein content in TCA (mg) and $m_{total\ protein}$ is the total protein content (mg).

2.9. Cell viability assay

Caco-2 cells were grown in DMEM supplemented with 10 % (v/v) FBS, 1 % (v/v) PS and 1 % (v/v) of non-essential amino acid solution, in a humidified 5 % CO_2 incubator at 37 ℃. Cell viability assay was carried out using the metabolic activity MTT assay based on (Gonçalves et al., 2021b) and DeLoid et al. (2017). Cells were cultured in a 96-well culture plate at 2×10^5 cells/well for 24 h at 37 ℃ in 5 % CO_2. After that, the culture medium was replaced by 200 μL of fresh culture medium containing digested samples (i.e., filtrated jejunum and filtrated ileum samples) of yogurt, SLN alone and yogurt_SLN at different dilution factors (i.e., 1:10, 1:15 and 1:20). After 4 h of incubation, the medium was removed and washed with 200 μL of PBS. Then, 100 μL of MTT (0.5 mg/mL in PBS) was added to each well and incubated for 3 h at 37 ℃ in 5 % CO_2. After incubation, 200 μL DMSO was added to solubilize the formazan crystals, which is formed in the presence of viable cells with active metabolism. Cell viability was determined by spectrophotometry at 570 nm (reference wavelength 630 nm) and calculated using equation (5):

$$Cell viability(\%) = Abs_{sample}/Abs_{control} \times 100 \text{(Eq. 5)}.$$

where Abs_{sample} and $Abs_{control}$ are the absorbances obtained from the wells containing treated cells and the absorbance of untreated cells (i.e., cells in DMEM medium), respectively. Four replicates of each sample were analyzed.

2.10. Statistical analyses

All assays were performed at least in triplicate and results were presented as mean ± standard deviation (SD). Statistical analyses of the experimental data were carried out using OriginPro 2018 Statistic software (version b9.5.1.195; OriginLab Corporation, Northampton, MA, USA). The acquired data was processed using One-Way analysis of variance (ANOVA), and Tukey's test was used to evaluate statistically significant differences between the mean values ($p < 0.05$).

3. Results and discussion

3.1. Tribo-rheometric characterization

Rheological and tribological properties of food products can be studied to predict the mouthfeel attributes. Furthermore, the flow behavior and viscoelastic profile constitute major parameters in order to understand the rheological properties of food under oral processing (Zheng, 2019).

3.1.1. Rheological properties

Curcumin-loaded SLN were developed in our previous work presenting a particle size of 172.4 ± 6.0 nm, a polydispersity index (PDI) value of 0.282 ± 0.006 and a ζ-potential value of −13.5 ± 0.8 mV, after resuspension. Furthermore, curcumin-loaded SLN maintained its stability over 1 month (Gonçalves et al., 2022; Gonçalves et al., 2021a).

Representative flow curves (Fig. 1a) of both yogurt_SLN and control samples presented a very similar behavior, where the shear stress increases with growing shear rate and it is possible to observe the existence of yield stress and shear-thinning behavior. This result shows that the biopolymeric molecules are quickly aligned in the direction of the shear force, thus reducing the interactions between biopolymeric molecules chains, demonstrating that the viscosity is dependent on the shear rate (Tan, 2019; Yu et al., 2016). Other authors also reported a shear-thinning behavior for the yogurts with nanostructures and plain yogurts (Molina et al., 2019; Osorio-Arias et al., 2020). The rheological behavior of both samples was characterized using the Herschel-Bulkley model, fitting parameters associated with that model and respective yield stress values are presented in Table 3. Yield stress represents the force/stress that needs to be applied to the material to make it start flowing; its presence signals the existence of a structural network (Tan, 2019). The Herschel-Bulkley model showed a very strong fitting to the experimental data, with $R^2 > 0.99$. The yogurt_SLN showed a yield stress slightly lower than the control yogurt, but not statistically different ($p > 0.05$). Regarding the consistency index (k), yogurt_SLN samples showed a slightly increased consistency compared to the control yogurt, but once again not statistically different ($p > 0.05$). Both samples exhibited similar flow index (n) values ($p > 0.05$) and $n < 1$, thus confirming their shear-thinning behavior. Complex viscosity at a shear rate of 50 rad/s has been widely proposed as a good correlation to some attributes of the sensory mouthfeel (such as thickness, sliminess and stickiness) for a wide range of food products between Newtonian fluids and thick emulsions (Huang et al., 2021; Shama & Sherman, 1973). The presence of curcumin-loaded SLN did not affect the complex viscosity (Table 3, $p > 0.05$) and therefore, the yogurt_SLN are expected to show similar thickness, sliminess and stickiness properties as control yogurt. Regarding the viscoelastic profile, both samples presented a similar behavior, where G' was higher than G'' over the frequency sweep, showing a typical weak viscoelastic gel with an elastic structure (Fig. 1b). Moreover, both samples also showed an increase of G' and G'' moduli with increasing frequency, indicating a typical physical gel behavior. Other authors also reported a similar behaviors for fortified and plain yogurts (Bakry et al., 2019; Osorio-Arias et al., 2020).

In conclusion, the incorporation of SLN into yogurt did not affect the flow behavior and the viscoelastic profile of the product.

3.1.2. Tribological properties

Recently, tribology has gained scientific attention in food science, once some relevant mouthfeel attributes (such as oiliness, fatty feel, creaminess and smoothness) are not correlated with viscosity but are correlated to lubrication properties, instead (Nguyen et al., 2017). Furthermore, these mouthfeel attributes are the most commonly perceived in dairy products, such as yogurt (Corvera-Paredes et al., 2022). The applied normal force used in our study was 1 N, in order to simulate a moderate normal force present during oral processing, once it has been reported that the in-mouth force varies between 0.01 and 10 N (Nguyen et al., 2017).

Representative Stribeck analogue curves of both samples analyzed are presented in Fig. 2. At the boundary lubrication regime, both samples showed a rapid increase of the *CoF* with increasing sliding speed, which might be associated to the movement of the yogurt gel into the contact zone to form a lubricating film. In this region, the *CoF* of yogurt_SLN was higher than the *CoF* of control yogurt, which might be attributed to the presence of SLN. However, in mixed lubrication regime, the *CoF* of yogurt_SLN decrease to values lower than those obtained for control yogurt with the increase of sliding speed. This could be caused by the presence of lipid-based nanoparticles which could slightly increase the lipid and surfactant content, facilitating the formation of a

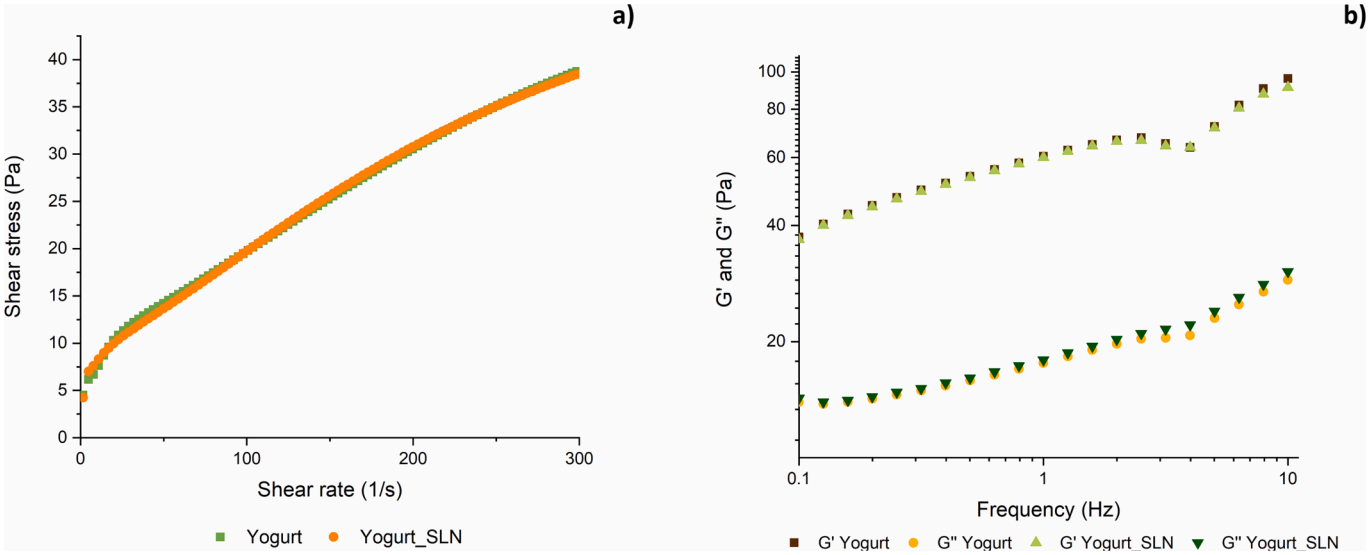

Fig. 1. Rheological properties of control yogurt and yogurt containing SLN (Yogurt_SLN): a) Flow curves and b) Frequency sweeps.

Table 3
Rheological properties (*k*, *n* and $\eta^*_{50\ rad/s}$) of control yogurt and yogurt added with SLN (Yogurt_SLN).

Sample	σ_0 (Pa)	k (Pa.s^n)	n	R^2	$\eta^*_{50\ rad/s}$ (Pa.s)
Yogurt	5.35 ± 0.46^a	0.428 ± 0.071^a	0.767 ± 0.024^a	0.998	1.89 ± 0.26^a
Yogurt_SLN	4.73 ± 0.15^a	0.514 ± 0.053^a	0.736 ± 0.011^a	0.998	1.85 ± 0.30^a

σ_0 = yield stress; *k* = consistency index; *n* = flow index; $\eta^*_{50\ rad/s}$ = complex viscosity at shear rate of 50 rad/s. Mean values with different superscript letters within the same column are significantly different from each other ($p < 0.05$).

continuous stable and thin film in the contact zone. Other authors reported similar findings for yogurts with different fat levels (Laguna et al., 2017; Nguyen et al., 2017). Therefore, the yogurt_SLN showed higher lubrication properties, allowing to conclude that SLN have positive effects on the yogurt mouthfeel properties.

In conclusion, despite the rheological properties were not affected by SLN addition, the yogurt creaminess, fatty feel and smoothness potentially increased by SNLs incorporation, improving the mouthfeel of the yogurt.

3.2. *In vitro digestion*

3.2.1. *TCA solubility index*

Protein digestion mainly occurs in the stomach due to the presence of proteases, namely pepsin, and continues in the small intestine as a result of α-chymotrypsin and trypsin action (Cao et al., 2019). The digestibility of yogurt_SLN was analyzed over the *in vitro* digestion, through the quantification of soluble protein. Fig. 3 shows TCA solubility index of control yogurt and yogurt_SLN at the end of gastric phase, during the duodenal phase and at the end of the digestion. In the gastric phase, the yogurt_SLN presented a higher TCA solubility index (26.1 ± 2.1 %) than the control yogurt (0.17.6 ± 2.8 %) at the end of the gastric phase ($p < 0.05$). On the other hand, control yogurt showed a significantly higher TCA solubility index (38.2—56.6 %) than the yogurt_SLN (21.8—31.4 %) during the duodenal phase and at the end of the digestion. The reduction of the proteins' digestibility of yogurt_SLN can be related with the presence of curcumin released from SLN during the

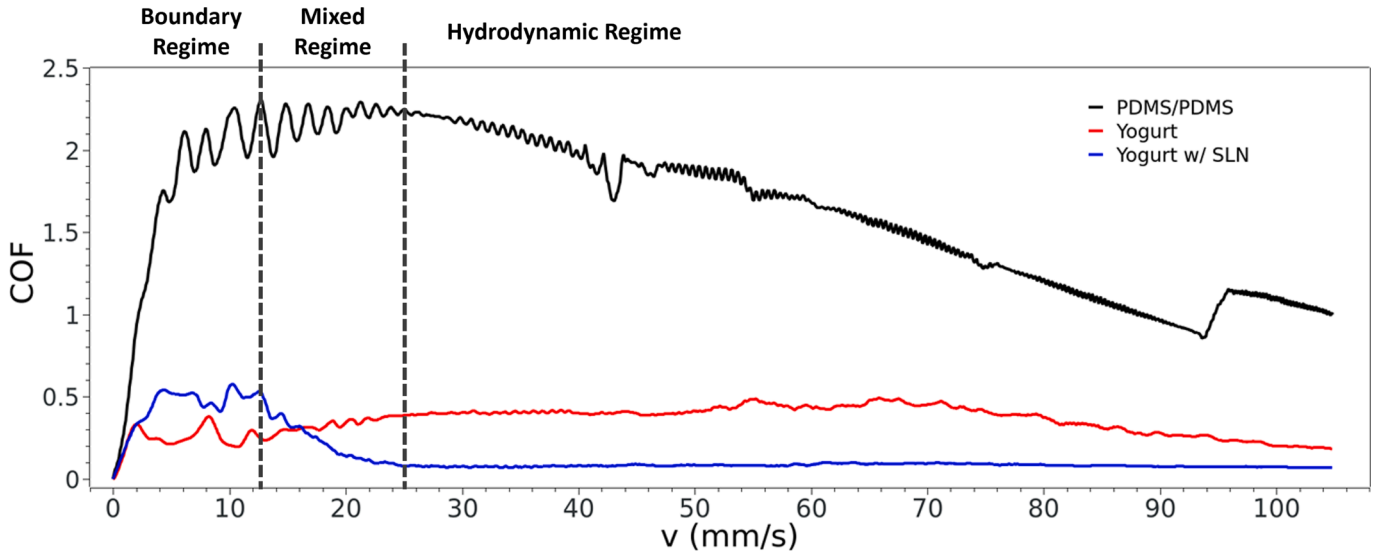

Fig. 2. Coefficient of friction (*COF*) *versus* sliding speed curves of yogurt containing SLN, control yogurt and polydimethylsiloxane (PDMS) systems.

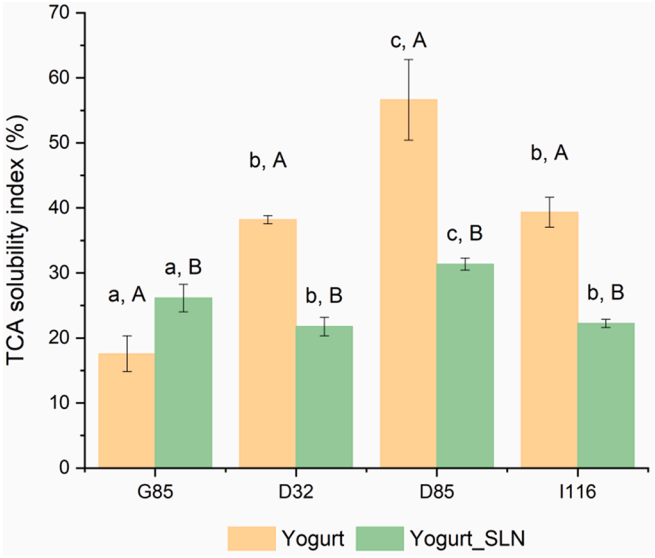

Fig. 3. TCA solubility index of digested samples at gastric (i.e., 85 min (G85)), duodenal (i.e., 32 min (D32) and 85 min (D85)) and final (i.e., 116 min (I116)) phases for the yogurt containing SLN (yogurt_SLN) and control yogurt. Error bars represent the standard deviation of $n = 3$ replicates. [a-c] Different lower-case letters indicate significant differences between endpoints for the same sample ($p < 0.05$). [A-B] Different upper-case letters indicate significant differences between samples for each endpoint ($p < 0.05$).

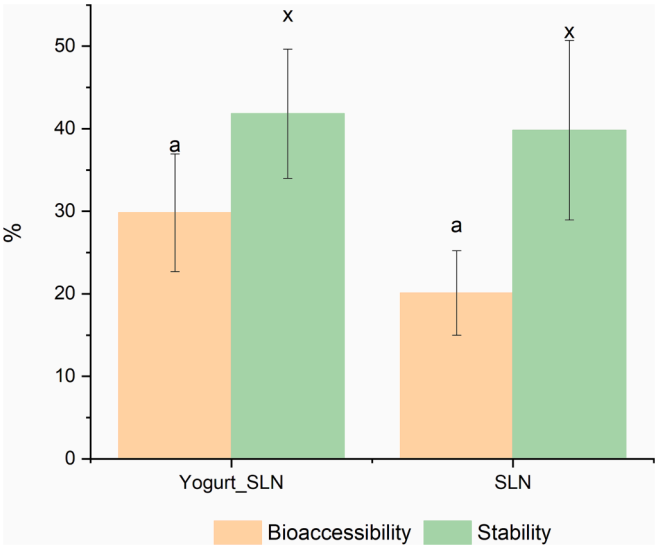

Fig. 4. Bioaccessibility (%) and stability (%) of curcumin in the yogurt_SLN and SLN alone after *in vitro* digestion process. Error bars represent the standard deviation of $n = 6$ replicates. Different letters indicate significant differences between samples ($p < 0.05$).

intestinal phase. A higher amount of curcumin could be released during the intestinal phase, probably due to SLN digestion, which could interact with the proteins forming complexes, reducing or inhibiting enzymatic action (Stojadinovic et al., 2013). Zygmantaitė et al. (2021) studied the digestibility of yogurt fortified with an extract from cranberry pomace as a functional ingredient, and they also observed a reduction of the hydrolysis degree on this product due to the presence of polyphenols on the extract. Furthermore, the SLN present in the yogurt could also have contributed to slow down the hydrolysis of protein, due to electrostatic interactions between SLN and proteins. Other authors have reported similar results (Cao et al., 2019; Hashemi et al., 2017).

Therefore, both curcumin and SLN may interfere with proteases' adsorption on proteins, decreasing the rate and extent of protein hydrolysis.

3.2.2. *Bioaccessibility and stability of curcumin*

Bioaccessibility and stability of curcumin were determined at the end of *in vitro* digestion for yogurt_SLN and SLN alone (Fig. 4). Yogurt_SLN showed values of bioaccessibility (29.8 ± 7.1 %) and stability (41.8 ± 7.8 %) not statistically different ($p > 0.05$) from those values of SLN alone (i.e., 20.1 ± 5.1 % and 39.8 ± 10.9 %, respectively). As previously discussed, part of the curcumin released from SLN probably interacted with the proteins present in the yogurt matrix through electrostatic interactions, hydrogen bonding, hydrophobic interactions, and ionic bonds, forming polyphenol-protein complexes (Yilmaz et al., 2022). These polyphenol-protein interactions may have some effect on the bioaccessibility and stability of curcumin. Other authors reported some improvement of bioaccessibility and stability of free polyphenols when incorporated into yogurt (Helal et al., 2022; Ye et al., 2021; Zygmantaitė et al., 2021). On the other hand, these curcumin-protein interactions could also prevent the curcumin to be bioaccessible. For example, Donhowe et al., (2014) encapsulated β-carotene through different microencapsulation methods the using maltodextrin, alginate and chitosan and evaluated the bioaccessibility of encapsulated β-carotene in yogurt and pudding. The authors observed a high impact of food matrix on the release and bioaccessibility of β-carotene, where the yogurt showed a lower bioaccessibility than pudding and both matrices presented lower bioaccessiblity when compared to the digestion without food matrix. The authors concluded that the presence of a food matrix could delay the contact of the enzymes with the structures or inhibit the transfer of β-carotene into the micelles. In other work, de Campo et al., (2019) evaluated the bioaccessiblity of zeaxanthin-loaded nanoemulsions and nanoparticles in yogurt, where also observed a low bioaccessibility of zeaxanthin for nanoemulsions (4.46 ± 0.25 %) and nanoparticles (3.66 ± 0.07 %). Furthermore, the authors concluded that the relatively low stability of curcumin could be caused by the expulsion of curcumin from the fat crystals, leading to a high concentration on the surface of the SLN. Consequently, the curcumin becomes more prone to degradation (Qian et al., 2013).

Therefore, these results indicate that the yogurt matrix did not affect the bioaccessiblity and stability of curcumin.

3.2.3. *Cell viability*

In order to eliminate the possible cytotoxic effect caused by GI fluids, different dilutions (i.e., 1:3, 1:5 and 1:10) of GI fluids were tested (data not shown). Only the lowest dilution factor (1:10) had shown a reduced cytotoxic effect when compared to the other dilutions. This result can be due the fact that the enzymes, bile salts and other digestion products are present at sufficiently high concentrations to cause the observed cytotoxic effects. Therefore, the effect of the filtrates, at different dilutions (i.e., 1:10, 1:15 and 1:20) obtained from *in vitro* digestion of SLN alone, control yogurt and yogurt_SLN on cell viability was determined (Fig. 5).

Regarding SLN alone, both jejunum and ileum filtrates did not produce a negative effect on cell viability (i.e., ≥ 80 %) for the range of concentrations tested, and no statistical differences were observed ($p > 0.05$) between the two samples. On the other hand, as expected, high cell viability (i.e., ≥ 80 %) was observed after cells incubation with yogurt filtrates at 1:15 and 1:20 dilution factors. However, yogurt_SLN jejunum filtrate decreased cell viability (i.e., < 20 %) at both dilution factors ($p < 0.05$). Also, low cell viability (10.3 ± 4.6 %) at the dilution factor of 1:15 ($p < 0.05$) was observed for ileum filtrate of yogurt_SLN while for the dilution factor of 1:20, an increase of cell viability to 79.2 ± 13.7 % ($p < 0.05$) was observed. This result could be due to the removal of possible toxic digestion products and aggregates during the second filtration (ileum filtrate). These unexpected results could be linked to several hypotheses related to the synergetic cytotoxic effect between yogurt matrix and SLN. The first hypothesis is the formation of curcumin-

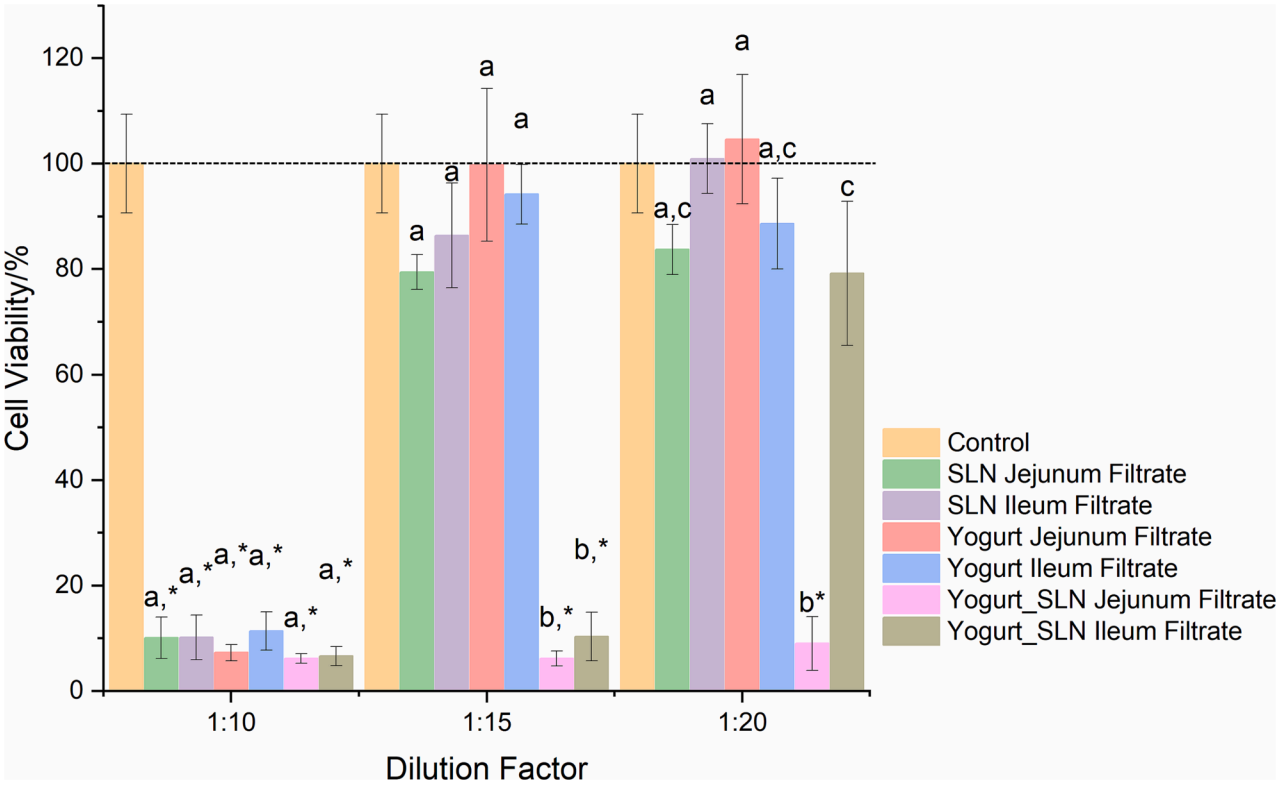

Fig. 5. Cell viability of digested samples from jejunum and ileum filtrates for yogurt_SLN, control yogurt, and SLN alone on Caco-2 cells measured by MTT assay. Error bars represent the standard deviation of $n = 4$ replicates. Different letters indicate significant differences between samples for each concentration ($p < 0.05$). *Asterisks indicate significant differences relative to the control group ($p < 0.05$).

protein systems or aggregates, which could potentiate the curcumin cytotoxicity against Caco-2 cells. Mirzaee et al. (2019) reported that curcumin-casein and curcumin-albumin systems increased the cytotoxicity against MCF7 cells, probably due to a correlation between bonding affinity and biological activity of curcumin. Another hypothesis is the possible formation of a protein-corona around the SLN due to the yogurt proteins which could change SLN physicochemical properties and therefore, their biological effects (Nishihira et al., 2019; Wang et al., 2021). Finally, the interactions between the lipid digestion products and the yogurt digestion products could form aggregates that promote the cytotoxic effects. However, more studies need to be done in order to understand which mechanisms are responsible for the cytotoxic effect observed here.

4. Conclusions

This work evaluated the influence of the incorporation of SLN into yogurts in terms of their rheological and tribological properties as well as its correlation with mouthfeel attributes. Moreover, *in vitro* digestion was performed to assess bioaccessibility and stability of curcumin, the TCA solubility index and the *in vitro* cytotoxicity of the digestion products obtained.

The incorporation of SLN into the yogurt did not affect the rheological properties of the yogurt. However, the presence of SLN led to an increase of the lubrication capacity of yogurt. Therefore, a potential increase of its creaminess, fatty feel and smoothness is expected, improving the mouthfeel of the yogurts. During *in vitro* digestion, the TCA solubility index was lower for the yogurt_SLN, which may be attributed to curcumin's bonding to the yogurt proteins. Apparently, these possible curcumin-protein interactions did not negatively affect bioaccessibility and stability of curcumin. After *in vitro* digestion, the jejunum and ileum filtrates of yogurt_SLN reduced cell viability, whereas same filtrates for the control yogurt and SLN alone presented no cytotoxic effect.

Overall, the results obtained suggest that SLN has a high potential to be used as delivery system in functional foods, as a strategy to increase the bioaccessibility of bioactive compounds. Further studies should be conducted to evaluate potential mechanisms responsible for SLN cytotoxicity, as well as the application of SLN in other type of food products should be explored, in order to increase the state-of-art on the development of functional foods using SLN as carrier systems for bioactive compounds.

CRediT authorship contribution statement

Raquel F.S. Gonçalves: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jean-Michel Fernandes:** Writing – review & editing, Methodology. **Joana T. Martins:** Writing – review & editing, Methodology. **Jorge M. Vieira:** Writing – review & editing, Methodology. **Cristiano S. Abreu:** Writing – review & editing, Methodology. **José R. Gomes:** Writing – review & editing, Methodology. **António A. Vicente:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Ana C. Pinheiro:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Project administration, Validation.

Declaration of competing 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.

Data availability

Data will be made available on request.

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