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Nanoemulsions and acidified milk gels as a strategy for improving stability and antioxidant activity of yarrow phenolic compounds after gastrointestinal digestion

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Key words: encapsulation efficiency, gastrointestinal digestion, milk gelation, phenolic compounds, nanoemulsions, sodium caseinate, yarrow extract.

Abstract

The aim of this study was to improve the stability and antioxidant activity of yarrow phenolic compounds upon an *in vitro* simulated gastrointestinal digestion. Therefore, two types of caseins-based delivery systems, sodium caseinate stabilized nanoemulsions (NEs) and glucono delta-lactone acidified milk gels (MGs), were formulated containing an ultrasound-assisted yarrow extract (YE) at two concentrations (1 and 2.5 mg/mL). Formulations with 1 mg/mL of YE were chosen based on their higher encapsulation efficiency to perform the *in vitro* digestion experiments. After digestion, YE-loaded NEs only partially protected phenolic compounds from degradation; meanwhile the phenolic composition of YE including in MGs after digestion was quite similar to undigested YE. Moreover, the antioxidant activity of MGs after digestion was higher than NEs digested samples, which confirms the higher protection of YE phenolic compound by the milk gels systems.

This research demonstrated the potential use of acidified MGs as carriers to improve the stability and antioxidant activity of yarrow phenolic compounds. Therefore, these matrices could be employed to develop new dairy products enriched with phenolic compounds.

Abbreviations

NEs, nanoemulsions, MGs, milk gels; YE, yarrow extract; EtOH; ethanol; UAE, Ultrasound-Assisted Extraction; HPH, high pressure homogenization; NaCas, sodium caseinate; GDL; glucono delta-lactone; G′, storage modulus; G″; loss modulus; tan δ, loss tangent value; EE, encapsulation efficiency; PVDF, polyvinylidene fluoride; TEAC, Trolox equivalent antioxidant capacity; LSD, least significant difference; EGCG, epigallocatechin-gallate; DCQA, dicaffeoylquinic acid.

1. Introduction

Achillea millefolium L., commonly known as yarrow, has been traditionally used to treat gastrointestinal and hepatobiliary disorders, inflammation or diabetes (Akram, 2013). The bioactivity of aqueous and ethanolic yarrow extracts has been associated with the presence of phenolic compounds, mainly phenolic acids and flavones (Villalva et al., 2019). Nevertheless, after oral consumption, the potential health benefits of phenolics compounds could be limited due to instability under certain pH conditions, poor water solubility, gastrointestinal enzyme degradation, low intestinal permeability or metabolism during digestion (Karakaya, 2004). Thus, phenolic stability during gastrointestinal digestion has been related to their chemical structure; for instance, the presence or absence of glycosylation, the glycosylation patterns, etc. (Czubinski et al., 2019). Besides, alkaline medium of small intestine has been also described as an important parameter in phenolic compounds stability reduction (Chen et al., 2014). Moreover, digestive enzymes were reported to accelerate the hydroxylation of phenolics after gastric digestion phase (Barak, Celep, Inan & Yesilada, 2019). Therefore, oral administration of these compounds requires strategies to preserve their chemical integrity and successful delivery in physiological targets.

In recent years, the encapsulation of phenolics compounds as a strategy to reduce the undesirable changes in these compounds during gastrointestinal transit has been extensively studied (Ghayour et al., 2019; Velderrain-Rodríguez, Acevedo-Fani, González-Aguilar & Martín-Belloso, 2019). Thus, several encapsulation techniques (e.g., spray drying, emulsion, inclusion complexation), as well as carrier materials (e.g., chitosan, soy proteins, glucan, etc.), have already been proposed for phenolic compounds (Chen, Gnanaraj, Aurulselvan, El-Seedi & Teng, 2019; Esfanjani, Assadpour & Jarafi, 2018; Ozkan, Franco, De Marco, Xiao & Capanoglu, 2019). The encapsulation of phenolic compounds like quercetin, oleuropein, resveratrol and tea polyphenols by different techniques and using several carriers, provided an

increase in their stability and bioaccessibility after gastrointestinal process (González et al., 2019; Jayan, Leena, Sundari & Moses, 2019; Liang et al., 2017; Yan et al., 2018).

Ideally, food-grade encapsulation materials, as proteins, are selected to formulate food products. Proteins have multiple applications in encapsulation matrices of phenolic compounds, as emulsifiers, gelling agents and also as entities to formulate micro and nano-particles (Jia, Dumont & Orsat, 2016; Foegeding, Plundrich, Schneider, Campell & Lila, 2017). The ability of phenolic compounds to associate with milk proteins is well documented, particularly to proline-rich proteins such as α- and β-caseins through hydrophobic interactions and hydrogen bonds (Chanphai, Bourassa, Kanakis, Tarantilis & Polissiou, 2018). Thus, this ability has been used to develop delivery carriers. Ghayour at al. (2019) proposed the nanoencapsulation of quercetin and curcumin by using caseins in order to design protein-based delivery systems to incorporate these compounds to beverages. Arranz et al. (2019) also employed casein matrices as carriers for rosmarinic acid delivery in food product development..

Nanoemulsion-based encapsulation systems for phenolic compounds have been well established, due to its high efficacy encapsulation, maintenance of chemical stability and controlled release (Artiga-Artigas, Lanjari-Pérez & Martín-Belloso, 2018; Lu, Kelly & Miao, 2016). In order to develop these systems, sodium caseinate has been described as a food grade emulsifier that improves delivery of phenolic compounds. Sabouri, Arranz, Guri & Corredig (2018) indicated that the incorporation of epigallocatechin-gallate (EGCG) in sodium caseinate emulsions improved its bioaccessibility and preserved its functional properties during gastrointestinal digestion. Besides, Casanova et al. (2018) also reported a complexation of cyaniding-3-O-glucoside with sodium caseinate, suggesting that sodium caseinate is a potential carrier for anthocyanins.

The gelation of protein matrices has also been indicated as an alternative method to encapsulate phenolic compounds (Jia et al., 2016). Caseins have excellent gelation properties and have been proposed as carriers for phenolic compounds since they are able to interact with phenolics, form complexes and entrap them through their gelation process (Ozdal, Capanoglu & Alta, 2013). Thus, Bayraktar, Harbourne & Fagan (2019) showed that polyphenols extracts (green tea and white grape) and simple phenolic compounds (tannic and gallic acids) could be successfully used to develop acidified dairy products with antioxidant properties. Moreover, Trigueros, Wojdylo & Sendra (2014) also reported that in yogurts containing pomegranate juice, most of anthocyanins were bound to casein fraction.

The aim of this study was first to evaluate the stability of yarrow phenolic compounds after *in vitro* simulated gastrointestinal digestion. Then, two different casein-based delivery systems were developed and characterized; as a strategy to improve yarrow phenolic compounds stability and antioxidant activity after simulated digestion.

2. Materials and methods

2.1 Reagents and chemicals

Ethanol (EtOH) (99.5% purity) was obtained from Panreac (Barcelona, Spain). Formic acid (99%) was obtained from Acros Organics (Madrid, Spain) and acetonitrile HPLC grade from Macron Fine Chemicals (Madrid, Spain). (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%) and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) were purchased from Sigma-Aldrich (Madrid, Spain). Reference standards (HPLC purity ≥95%) for phenolic compounds identification such as chlorogenic acid, diosmetin and vitexin were purchased from Sigma-Aldrich. 1,5-Diccafeoylquinic acid (DCQA), 3,4-DCQA, 3,5-DCQA, 4,5-DCQA, apigenin, luteolin, orientin, schaftoside and vicenin 2 were obtained from Phytolab (Madrid, Spain). Apigenin-7-*O*-glucoside, caffeic acid, homoorientin, luteolin-7-*O*-*β*-glucoside

and rutin were from Extrasynthese S.A. (Genay, France). The water used in this study was ultrapure type 1 (Millipore, Madrid, Spain).

2.2. Yarrow samples and Ultrasound-Assisted Extraction (UAE)

Achillea millefollium L. (yarrow) was obtained from an herbalist´s local supplier (León, Spain). The sample included inflorescences and upper dried leaves of the plant (water content less than 5%). The plant was ground using a Premill 250 hammer mill (Leal S.A., Granollers, Spain) and sieved to the appropriate size (<500 μm). The extraction process was performed by UAE in an ultrasonic device (Branson digital sonifier 450, Danbury, USA) at 400 W and 60 kHz. Ethanol was used as extraction solvent (1:10 plant/solvent ratio) during 30 min at 40 ºC and an output of 50% nominal amplitude. Ethanol was evaporated in a rotary evaporator (IKA®, Werke GmbH and Co., KG, Germany) and samples were stored at −20ºC until use.

2.3. Chemical characterization of phenolic compounds

Phenolic chromatographic analyses were conducted in an Agilent 1260 Infinity series HPLC-PAD (Santa Clara, CA, USA). A reverse phase ACE Excel 3 Super C18 column (150 mm x 4.6 mm, 3 μm particle size) from ATC (Aberdeen, Scotland, UK) equipped with a pre-column from the same material was employed according to the chromatographic method reported by Villalva et al. (2018). Besides, accurate mass from HPLC-PAD-QTOF-MS/MS in a negative mode analysis was used for compounds identification (Villalva et al., 2018). Quantification of identified compounds was performed using calibration curves of pure analytical standards. In addition, luteolin-di-hexoside and 6-hydroxyluteolin-7-*O*-glucoside were quantified by the calibration curves of orientin and luteolin-7-*O*-glucoside respectively. Correspondingly, apigenin glycosylated derivative were quantified using apigenin-7-*O*-glucoside calibration

curve and schaftoside isomer and apigenin-hexoside-pentoside respect to schaftoside calibration curve.

2.4. Preparation and characterization of yarrow extract (YE)-loaded nanoemulsions (NEs).

NEs were prepared using high pressure homogenization (HPH). Oil in water emulsions, containing 7% of soybean oil (w/w) (Sigma Aldrich, Oakville, ON, Canada) and a YE stock solution (dissolved in less than 1% of EtOH) to achieve 0, 1 or 2.5 mg/mL in final emulsion, were formulated with 2% sodium caseinate (NaCas) (New Zealand Milk Proteins, Mississauga, ON, Canada) as emulsifier. Initially, NaCas was dispersed in ultrapure water with magnetic stirrer and stored overnight at 4ºC to allow complete hydration. Emulsions were prepared by addition of YE to soybean oil and subsequent incorporation of NaCas solution. Both phases were pre-homogenized using a Polytron mixer (Brinkmann Inst. Corp., Mississauga, ON, Canada) at 30,000 RPM for 2 min, and then HPH was performed at 450 kPa for five passes using a microfluidizer (model M-110Y, Microfluidics Corporation, Newton, MA, USA). Then NEs were characterized (particle size and ζ-potential) and stored under refrigeration at 4ºC for two weeks in order to evaluate its stability.

The particle size distribution was measured using static light scattering (Mastersizer 2000, Malvern Instruments, Worcestershire, UK), with water as dispersant. The refractive indices were 1.33 and 1.47, for water and soybean oil, respectively. Mean particle diameter was reported as the volume-weighted mean diameter ($d_{4,3}$) calculated as the average of triplicate measurements. Zeta (ζ)-potential was determined by dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments, Malvern, UK) in samples diluted in ultrapure grade water at 1:500 ratio. All measures were done by triplicate.

2.5. Preparation and characterization of milk gels (MGs) of yarrow extract

Pasteurised skimmed milk, acidified with 2% (w/w) of glucono delta-lactone (GDL) (Sigma Aldrich) and containing a YE stock solution to obtain 0, 1 or 2.5 mg/mL in final gels, was used to prepare MGs. Initially, a suspension of heated (85ºC for 30 min) or non-heated milk with YE was stirred for 1 hour at room temperature and protected from direct light. Then, this suspension was warmed up to 30ºC, the GDL was added, and the mixture was kept at 30ºC for 180 min. After milk gelation, the samples were stored at 4ºC until analyses.

The dynamic rheological measurements were performed by applying a constant strain of 0.05% at a frequency of 1 Hz by means of a Physica MCR301 stress-controlled rheometer (Anton-Paar, Graz, Austria) using a concentric cylinder with solvent trap cover, at 30°C. The gelation point was taken at the time of cross-over between G′ (storage modulus) and G″ (loss modulus) (Haratifar & Corredig, 2014). In parallel, the pH was monitored during milk gel formation using an Accumet pH meter (Fisher Scientific, Edmonton, Canada). All experiments were done by triplicate.

2.6. Encapsulation efficiency of phenolic compounds

The encapsulation efficiency (EE) of yarrow phenolic compounds in NEs and MGs was measured after centrifugation of fresh samples for 1 h (60.000 g (NEs) and 20.500 g (MGs), respectively). Aqueous phase were recovered and filtered using 0.22 μm polyvinylidene fluoride (PVDF) filters prior injection in a HPLC-PAD. Analysis was performed as previously described in section 2.3.

Encapsulation efficiency of individual phenolic compounds (individual EE%) was determined by Eq. 1 and the total encapsulation efficiency (total EE%) of the formulation was calculated by Eq. 2, as followed:

Eq. (1)
$$\text{Individual } EE(\%) = \left[1 - \frac{Individual\ \ phenolic\ compound\ in\ aqueus\ phase}{Individual\ \ phenolic\ compound\ quantified\ in\ extract}\right] x\ 100$$

Eq. (2)

$$\text{Total } EE(\%) = \left[\frac{\sum Encapsulated\ phenolic\ compounds}{Total\ phenolic\ compounds\ in\ extract}\right] x\ 100$$

2.7. *In vitro* simulated gastrointestinal digestion

A two steps *in vitro* simulated digestion, gastric and intestinal, was performed according to a standardised protocol (Minekus et al., 2014), with slight modifications, suited to the food matrix being analysed (Arranz et al., 2017). Briefly, 10 mL of each sample were mixed with 10 mL of simulated gastric fluids containing 25,000 U/mL of pepsin, 0.2 mL of HCL (1 M) and 5 µL of $CaCl_2$ (0.3 M) at a final pH 3 in amber jars. Samples were incubated in a shaking water bath (220 strokes/min) (New Brunswick Scientific Co., Inc., NJ, USA) for 30 min at 37°C. Then, 20 mL of simulated intestinal fluids were incorporated containing 5.0 mg/mL of pancreatin (800 U/mL), 160 mM bile salts, 40 µL of $CaCl_2$, (0.3 M), 0.15 ml of NaOH (1 M), 1 mM phospholipids and 5 µL phospholipase A2 (stock solution 6.7 mg/mL), and the mixture was incubated for 2 h at 37 °C and pH 7. At the end of the digestion, the enzyme reaction was immediately stopped by keeping the samples on ice and stored at −20 °C. Samples (4 mL) were then freeze-dried for further experiments. Digestion process was performed by triplicate for every formulation (free YE, YE-NEs and YE-MGs). In addition, control digestions without YE (water, NEs and MGs) were performed.

For quantification of phenolic compounds after digestion, digested freeze-dried samples were re-suspended in 1 mL of EtOH and acidified water with 0.1% formic acid (60:40, v/v). Later, the mixture was placed into an ultrasound water-bath (5 min) and centrifuged at room temperature (4,500 RPM, 5 min). The clear supernatant was filtered using 0.45 µm PVDF filters prior analysis by HPLC-PAD as described in 2.3 section.

2.8. Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) method was applied according to Brand-Williams, Cuvelier & Berset (1995), with some modifications. Briefly, a volume of 975 µL of 6.1 x 10^{-5} M DPPH$^{\bullet}$ methanol solution was used. The reaction was started by adding 25 µL of sample or blank. The bleaching of DPPH$^{\bullet}$ was followed at 517 nm at room temperature until the reaction was completed (3 h). All the samples were prepared at 4 different concentrations, by triplicate, to obtain the inhibition curve of DPPH$^{\bullet}$. The results were calculated as mmol Trolox equivalent/g extract (TEAC value). Analysis was done by triplicate.

2.9. Statistical analysis

For each sample, experiments were performed at least by triplicate and expressed as mean values and standard deviation. A one-way analysis of variance (ANOVA) followed by LSD test (Fisher's least significant difference) post-test were used to discriminated significant differences between means at $p < 0.05$. Analysis was performed using Statgraphic v. Centurion XVI package for Windows (Statpoint Inc., Warrenton, VA, USA).

3. Results and discussion

3.1. Effect of *in vitro* simulated gastrointestinal digestion on YE phenolic compounds

Phenolic compounds characterization of YE, before and after *in vitro* simulated gastrointestinal digestion, was performed by HPLC-PAD analysis (Table 1). Therefore, twenty-one phenolic compounds were identified in YE, mainly glycosylated flavonoids and phenolic acids. The main compounds were 3,5-dicaffeoylquinic acid (DCQA) and luteolin-7-*O*-glucoside, followed by vicenin 2 and an apigenin glycosylated derivate. These phenols represented 49% of the total phenolic compounds quantified in YE. Other caffeoylquinic acid derivatives like chlorogenic acid and 4,5-DCQA were found in representative amounts within the phenolic acids in YE.

Glycosylated flavones like 6-hydroxyluteolin-*7*-*O*-glucoside and schaftoside, besides its isomers, were also identified. These results were consistent with previously reported studies of yarrow extracts (Benedek, Gjoncaj, Saukei & Kopp, 2007; Pereira et al., 2018).

The quantitative analysis of phenolic fraction of YE after digestion showed that the concentration of almost all detected compounds was modified. Several authors indicated that the stability of phenolic compounds during the gastrointestinal digestion process is strongly influenced by their chemical structure, since phenolics have a different sensitivity to pH variations and digestive enzymes activity (Goulas & Hadjisolomou, 2019; Lima et al., 2019). Regarding the main components of the extract, 3,5-DCQA concentration was highly reduced after digestion (from 17.0 ± 0.47 to 5.84 ± 0.28 mg/g of extract), while 3,4-DCQA and 4,5-DCQA were increased. In fact, 4,5-DCQA was the main compound in the digested extract. These results suggest an isomerization of 3,5-DCQA towards 3,4-DCQA and 4,5-DCQA in pH conditions occurring during digestion. Similarly, D'Antuono, Garbetta, Linsalata, Minervini & Cardinali (2015) also detected an isomerization effect after gastrointestinal digestion of 3,5-DCQA (pure individual compound) with the presence of 4,5- and 3,4-DCQAs after intestinal step. Luteolin-7-*O*-glucoside, the second most abundant compound in YE, was also reduced in approx. 25%, after digestion. This reduction has been generally attributed to the instability of flavonoid glycosides under pH values of small intestine, where several modifications such as oxidation and polymerization might occur (Celep, Akyüz, Inan & Yesilada, 2018). Therefore, the encapsulation of YE phenolic compounds in delivery systems could be an useful strategy to improve their stability during gastrointestinal digestion.

3.2. Formulation and characterization of YE-loaded nanoemulsions (NEs)

YE-loaded NEs with two different concentrations of YE, 1mg/mL (NE-1.0) and 2.5 mg/mL (NE-2.5), were formulated in soy oil in NaCas water emulsions. NEs control, without YE, was also formulated.

The particle size distribution (Fig. 1) indicated that the control emulsion had a monomodal distribution. This distribution did not change after YE incorporation, although NE-2.5 showed a wider size distribution than control and NE-1.0. Freshly prepared control emulsion (day 0) had a mean particle diameter ($d_{4,3}$) of 248 ± 1.0 nm (Table 2). The addition of YE did not modify the emulsions particle diameter. All NEs had a negative net surface charge (ζ-potential), with no statistical differences between the control and YE-loaded NEs.

The physical stability of YE-loaded NEs was monitored during two weeks. The values of mean particle diameter and ζ-potential after 7 and 14 days of storage at 4ºC are shown in Table 2. After 14 days, no statistical differences were observed neither in the mean particle diameter nor in the ζ-potential of any formulation. These data denote the stability of YE-loaded NEs. Thus, caseinate stabilized nanoemulsions containing tea polyphenols (epigallocatechin-gallate) showed a mean particle diameter and ζ-potential values quite similar to those found in this study (Sabouri et al., 2015). Besides, Sharma et al. (2017) also reported that a clove nanoemulsion stabilized with sodium caseinate presented a similar mean particle diameter and ζ-potential. This emulsion was found to be stable for 20 days.

3.3. Formulation and characterization of acidified milk gels (MGs) including YE

Milk gels including two different concentrations of YE, 1mg/mL (MG-1.0) and 2.5 mg/mL (MG-2.5), were prepared using heated or unheated milk, acidified with GDL. Control MGs (without YE) were also formulated.

MGs formulated with heated milk showed a shorter gelation time and higher pH values at gelation time than those obtained with unheated milk (Table 3). Heated MGs also showed higher final storage modulus (G′) values than those formulated with unheated milk (Table 3), indicating that heated gels presented a higher stiffness at the end of the acidification process. Besides, the final loss tangent values (tan δ) were significantly lower for heated gels. At moderate heating temperatures (70-90ºC), whey proteins unfold and associate with casein micelles. During acidification, these unfolded-associated proteins act as a cross-link between protein particles and increase the number (and strength) of bonds in the gel network, consequently higher G′ values are obtained, pH increases at gelation and shorter gelation times are required (Donato, Alexander & Dalgleish, 2007).

Despite the differences found in the rheological properties between heated and unheated formulations, the addition of different concentration of YE did not significantly modify the gelation time, the pH at gelation time, the final G´ value or the final tan δ value (Table 3). Similarly, Vega & Grover (2011) formulated acidified MGs including a commercial cocoa extract, rich in flavanols, showing that final G′ values and pH values at gelation time were not modified with extract addition, compared to the control gel. However, the addition of tannic and gallic acids to milk have resulted in faster gelation times and gels with G′ values higher than control samples (Harbourne, Jacquier & O'Riordan, 2011). These different results could be explained by interactions between milk proteins and phenolic compounds and it was described that these interactions are highly affected by the concentration, type and structure of phenolic compounds employed (Ozdal et al., 2013).

3.4. Encapsulation efficiency (EE) of YE phenolic compounds in NEs and MGs.

The encapsulation efficiency of YE individual phenolic compounds in NEs and MGs is shown in Table 4. Regarding NEs, the encapsulation efficiency was related to the YE concentration, NE-1.0 showed higher EE for all the compounds than NE-2.5. Encapsulation efficiency of all phenolic compounds in NE-1.0 ranged from 88.1 to 100%. However, in NE-2.5 the values were under 55%, except for luteolin, apigenin and diosmetin. Hence, the total encapsulation efficiency calculated in NE-1.0 was significantly higher than in NE-2.5 (92.3 ± 4.6% vs. 33.7 ± 0.9% respectively).These results could be explained since at a concentration of 1 mg/mL, most YE phenolic compounds were binding sodium caseinate at the oil-water interface. However, the concentration of 2.5 mg/mL could saturated interaction sites in protein chain, due to the polyphenol/protein ratio was too high. Consequently, a high quantity of phenolic compounds was non-encapsulated and presented in the aqueous phase.

Regarding MGs, the encapsulation efficiency was also affected by the concentration of YE, but in a lesser extent than in NEs. Thus, MG-1.0 had a total encapsulation efficiency of 72.0 ± 2.7%, meanwhile MG-2.5 showed a 63.1 ± 2.6%. In MGs formulations, apigenin and luteolin glycosylated forms presented a lower EE than their aglycone forms. Thus, Xiao et al. (2011) also indicated that the glycosylation of flavonoids lowered their affinity for milk proteins. These authors endorsed the decrease of affinity after glycosylation to the non-planar structure, since molecules with near-planar structure can easily enter the hydrophobic pockets in proteins. However, this decrease in EE of glycosylated forms could be also related to their lower hydrophobicity, with respect to non-glycosylated forms. Among phenolic acids, the DCQAs showed higher encapsulation efficiency than chlorogenic and caffeic acids. DCQAs higher encapsulation could be related to the higher hydrophobicity of DCQAs (with respect to chlorogenic and caffeic acids), that induces a stronger association between proteins and polyphenols (Adrar, Madani & Adrar, 2019). Nevertheless, their different structures (size, length and flexibility) could also affect protein-phenolic compounds interactions.

3.5 Effect of *in vitro* digestion on YE phenolic compounds included NEs and MGs.

Formulations with 1 mg/mL of YE (NE-1.0 and MG-1.0) were chosen based on their higher EE to perform *in vitro* digestion experiments. The quantitative analysis of individual phenolic compounds was performed after gastrointestinal digestion (Table 1). The quantification of phenolic compounds after NE-1.0 digestion showed that the digestion process modifies their concentration, but in a lower extent than when free yarrow extract was digested. Moreover, 10 of the 21 phenolic compounds detected in digested NE-1.0 decreased their concentration (compared to undigested YE), meanwhile after free YE digestion the quantity of 16 compounds were reduced. Regarding 3,5-DCQA, the most abundant compound in YE, a 3.4 times decreased was observed when free YE was digested *versus* 1.6 times reduction with NE-1.0. Luteolin-7-*O*-glucoside's concentration was only reduced from 7.57 ± 0.17 to 6.51 ± 0.10 mg/g of sample when NE-1.0 was employed. These results suggested that some encapsulated compounds in NEs were partially released and exposed to digestion conditions, indicating that the formulation of YE-loaded NEs only partially protected the YE phenolic compounds during gastrointestinal transit. The effect of gastric digestion on NaCas stabilized emulsions was previously reported. Li, Ye, Lee, & Singh (2012) described that caseinate-stabilized emulsions flocculate after digestion at acidic conditions (pH 1-3) due to pepsin hydrolysis over interfacial proteins in the droplets surface. Nevertheless, Sabouri et al. (2018) reported a higher recovery of EGCG (but not 100%) after digestion, when it was formulated with NaCas stabilized emulsions compared to water solutions. They concluded that the complexation of EGCG and NaCas at the interface of emulsions protected EGCG from digestive degradation.

After MG-1.0 digestion process, most of the phenolic compounds remained stable, comparing to undigested YE (Table 1), except chlorogenic acid, 3,4-DCQA and 3,5-DCQA. Regarding 3,5-DCQA, the concentration detected after MG-1.0 digestion was 15.9 ± 0.23 versus 17.0 ± 0.26 mg/g of sample from the undigested extract. Moreover, 3,4-DCQA concentration

increased from 1.79 ± 0.26 to 2.62 ± 0.25 mg/g of sample after digestion. These data indicated a possible isomerization of 3,5-DCQA to 3,4-DCQA during digestion. This isomerization was also found in the digestion of the free YE (non-encapsulated), but in a greater extent.

These findings are in line with the protection effect of YE phenolic compounds against degradation during digestion by MGs. Indeed, Lamothe, Azimy, Bazinet, Couillard & Britten (2014) described that the addition of a green tea extract to a yogurt matrix significantly decreased the rate and extent of proteolysis in the gastric phase. They suggested that the formation of complexes between milk proteins and green tea polyphenols increase protein stability during pepsin digestion. However, they also reported that the presence of tea extract did not influence protein hydrolysis in the intestinal phase, with the release of 100% of the polyphenols initially added. They concluded that interactions between green tea polyphenols and milk proteins help to maintain the integrity of polyphenols during digestion. Recently, Adrar et al. (2019) reported that polyphenols-proteins interactions would play a certain role in the preservation of polyphenols structure and function through the gastrointestinal tract transit. Thus, MGs offered an almost complete protection of phenolic compounds during gastrointestinal digestion; meanwhile this protection is only partial when employing NEs.

3.6 Effect of *in vitro* digestion on free and encapsulated YE antioxidant activity

The antioxidant activity of undigested YE and digested samples: free YE, NE-1.0 and MG-1.0 was evaluated (Table 5). Empty (without YE) NEs and MGs were also digested and its antioxidant activity was determined (0.050±0.002 and 0.078±0.003 mmol Trolox/g sample respectively). The values obtained with these empty formulations were subtracted from the formulations with the extract. The digestion process reduced almost 50% of the antioxidant activity of free YE samples, which could be related with the reduction in phenolic compounds found (Table 1). The gastrointestinal digestion of NE-1.0 also decreased the antioxidant activity

of the samples, however, in a lesser extent than free YE solutions. These results agree with the partial protection provided by the NE to YE phenolic compounds. Moreover, the antioxidant activity of MG-1.0 after digestion was the highest compared to free YE and NE-1.0, which confirms the higher protection of YE phenolic compounds by the milk gels systems. Thus, several authors previously reported that protein-polyphenol complexes formation preserved polyphenols bioactivities, including antioxidant activity (Foegeding, et al, 2017).

4. Conclusions

The quantitative analysis of YE phenolic fraction after *in vitro* gastrointestinal digestion showed that the concentration of all phenolic compounds was modified. Two types of casein-based delivery systems, sodium caseinate stabilized NEs and GDL-acidified MGs, were proposed to improve stability of YE phenolic compounds during digestion process. Recovery of YE phenolic compounds after digestion was significantly higher in MGs than NEs. Similarly, the antioxidant activity of MGs after digestion was higher compared to NEs. Thus, the encapsulation of yarrow phenolic compounds using acidified MGs can be proposed as a strategy to improve their stability during gastrointestinal digestion and consequently, enhanced their bioaccessibility. In addition, such matrices could be employed to develop new dairy products enriched with phenolic compounds.

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References

Adrar, N.S., Madani, K., & Adrar, S. (2019). Impact of the inhibition of protein activities and the chemical aspect of polyphenols-proteins interactions. *PharmaNutrition, 7*, 100142.

Akram, M. (2013). Minireview on *Achillea millefolium* Linn. *The Journal of membrane biology, 246*(9), 661-663.

Arranz, E., Guri, A., Fornari, T., Mendiola, J.A., Reglero, G., & Correding, M. (2017). *In vitro* uptake and immune functionality of digested Rosemary extract delivered through food grade vehicles. *Food Research International, 97*, 71-77.

Arranz, E., Villalva, M., Guri, A., Ortego-Hernández, E., Jaime, L., Reglero, G., Santoyo, S., & Corredig, M. (2019). Protein matrices ensure safe and functional delivery of rosmarinic acid from marjoram (*Origanum majorana*) extracts. *Journal of the Science of Food and Agriculture, 99*(5), 2629-2635.

Artiga-Artigas, M., Lanjari-Pérez, Y., & Martín-Belloso, O. (2018). Curcumin-loaded nanoemulsions stability as affected by the nature and concentration of surfactant. *Food Chemistry, 266*, 466-474.

Barak, T.H., Celep, E., Inan, Y., & Yersilada, E. (2019). Influence of in vitro human digestion on the bioavailability of phenolic content and antioxidant activity of Viburnun opulus L. (European cranberry) fruit extracts. *Industrial corps and Products 131*, 62-65.

Bayraktar, M.K., Harbourne N.H., & Fagan, C.C. (2019). Impact of heat treatment and acid gelation on polyphenol enriched milk samples. *LWT-Food Science and Technology 113*, 108282. Doi.org/10.1016.j.lwt.2019.108282.

Benedek, B., Gjoncaj, N., Saukei, J., & Kopp, B. (2007). Distribution of phenolic compounds in Middleeuropean taxa of the *Achillea millefollium* L., aggregate. *Chemistry & Biodiversity, 4*(5), 849-857.

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28(1)*, 25-30.

Casanova, F., Chapeau, A.L., Hamon, P., F. de Carvalho, A., Croguennec, T., & Bouhallab, S. (2018). pH and ionic strength-dependent interaction between cyanidin-3-O-glucoside and sodium caseinate. *Food Chemistry, 267*, 52-59.

Celep, E., Akyüz, S., Inan, Y., & Yesilada, E. (2018). Assessment of potential bioavailability of major phenolic compounds in *Lavandula stoechas* L. ssp. *Stoechas. Industrial Crops & Products, 118*, 111-117.

Chanphai, P., Bourassa P., Kanakis, C.D., Tarantilis, P.A., & Polissiou, M.G. (2018). Review on the loading efficacy of dietary polyphenols with milk proteins. *Food hydrocolloids, 77*, 322-328.

Chen, G., Chen, S., Zhao, Y., Luo, C., Li, J., & Gao, Y. (2014). Total phenolic content of 33 fruits and their antioxidant capacities before and after digestion. *Industrial corps and Products 57*, 150-157.

Chen, L., Gnanaraj, C., Arulselvan, P., El-Seedi, H., & Teng, H. (2019). A review on advances microencapsulation technology to enhance bioavailability of phenolic compounds: Based on its activity in the treatment of Type 2 Diabetes. *Trends in Food Science & Technology 85*, 149-162.

Czubinski, J., Wrobleswska, K., Czyzniejewski, M., Górnas, P. Kachlicki, P., & Siger, A. (2019). Bioaccesibility of defatted lupin seed phenolic compounds in a standardized static in vitro digestion. *Food Research International, 116*, 1126-1134.

D'Antuono, I., Garbetta, A., Linsalata, V., Minervini, F., & Cardinali, A. (2015). Polyphenols from artichoke heads (*Cynara cardunculus* (L.) subsp. *scolymus* Hayek): *in vitro* bio-accessibility, intestinal uptake and bioavailability. *Food & Function, 6* (4), 1268-1277.

Donato, L., Alexander, M., & Dalgleish, D.G. (2007). Acid gelation in heated and unheated milks: Interactions between serum proteins complexes and the surfaces of casein micelles. *Journal of Agricultural and Food Chemistry, 55*(10), 4160-4168.

Esfanjani, A.F., Assadpour, E., & Jafari, S.M. (2018). Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers. *Trends in Food Science & Technology 76*, 56-66.

Foegeding, E. A., Plundrich, N., Schneider, M., Campbell, C., & Lila, M. A. (2017). Protein-polyphenol particles for delivering structural and health functionality. *Food Hydrocolloids, 72*, 163-173.

Ghayour, N., Hosseini, S.M.H., Eskandari, M.H., Esteghlal, S., Nekoei, A.R., Gahruie, H.H., Tartar, M., & Naghibalhossaini, F. (2019). Nanoencapsulation of quercetin and curcumin in casin-based delivery systems. *Food Hydrocolloids, 87*, 394-403.

González, E., Gómez-Caravaca, A.M., Gimenez, B., Cebrián, R., Maqueda, M., Martinez-Férez, A., Segura-Carretero, A., & Robert, P. (2019). Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during in vitro gastrointestinal digestion. *Food Chemistry, 279*, 40-48.

Goulas, V., & Hadjisolomou, A. (2019). Dynamic changes in targeted phenolic compounds and antioxidant potency of carob fruit (*Ceratonia siliqua* L.) products during *in vitro* digestion. *LWT-Food Science and Technology, 101*, 269-275.

Harbourne, N., Jacquier, J.C., & O'Riordan, D. (2011). Effects of addition of phenolic compounds on the acid gelation of milk. *International Dairy Journal, 21*(3), 185-191.

Haratifar, S., & Corredig, M. (2014). Interactions between tea catechins and casein micelles and their impact on renneting functionality. *Food Chemistry, 143*, 27-32

Jayan, H., Leena, M.M., Sundari, S.K.S., Moses, J.A., & Anandharamajrishnan, C. (2019). Improvement of bioavailability for resveratrol through encapsulation in zein using electrospraying technique. *Journal of Functional Foods, 57*, 417-424.

Jia, Z., Dumont, M.J., & Orsat, V. (2016). Encapsulation of phenolic compounds present in plats using protein matrices. *Food Bioscience, 15*, 87-104.

Karakaya, S. (2004). Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition, 44*, 453-464.

Lamothe, S., Azimy, N., Bazinet, L., Couillard, C., & Britten, M. (2014). Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. *Food & Function, 5*(10), 2621-6231.

Li, J., Ye, A., Lee, S.J., & Singh, H. (2012). Influence of gastric digestive reaction on subsequent *in vitro* intestinal digestion of sodium caseinate-stabilized emulsions. *Food & Function, 3*(3), 320-326.

Liang, J., Yan, H., Puligundla, P., Gao, X., Zhou, Y., & Wan., X. (2017). Applications of chitosan nanoparticles to enhance absorption and bioavailability of tea polyphenols: A review. *Food hydrocolloids, 69,* 286-292.

Lima, K., Silva, O., Figueira, M. E., Pires, C., Cruz, D., Gomes, S., Mauricio, E. M., & Duarte, M. P. (2019). Influence of the *in vitro* gastrointestinal digestion on the antioxidant activity of *Artemisia gorgorum* Webb and *Hyptis pectinata* (L.) Poit. Infusions from Cape Verde. *Food Research International, 115*, 150-159.

Lu, W., Kelly, A.L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science and Technology, 47*, 1-9.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014). A standardized static in vitro digestion method suitable for food- An International consensus. *Food and Function, 5*, 1113-1124.

Ozkan, G., Franco, P., De Marco, I., Xiao, J., & Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drwbacks and applications. *Food Chemistry 272,* 494-506.

Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein-phenolic interactions and associated changes. *Food Research International, 51*(2), 954-970.

Pereira, J.M., Peixoto, V., Teixeira, A., Sousa, D., Barros, L., Ferreira, I.C.F.R., & Vasconcelos, M.H. (2018). *Achillea millefolium* L. hydroethanolic extract inhibits growth of human tumor cells lines by interfering with cell cycle and inducing apoptosis. *Food and Chemical Toxicology, 118*, 635-644.

Sabouri, S, Geng, J., & Correding, M. (2015). Tea polyphenols association to caseinate-stabilized oil-water interfaces. *Food Hydrocolloids, 51*, 95-100.

Sabouri, S., Arranz, E., Guri, A., & Correding, M. (2018). Sodium caseinate stabilized emulsions as a delivery system for epigallocatechin-gallate: Bioaccessibility, anti-proliferative activity and intestinal absorption. *Journal of Functional Foods, 44*, 166-172.

Sharma, M., Mann, B., Sharma, R., Bajaj, R., Athira S., Sarkar, P., & Pothuraju, R. (2017). Sodium caseinate stabilized clove oil nanoemulsion: Physicochemical properties. *Journal of Food Engineering, 212*, 38-46.

Trigueros, L., Wojdylo, A., & Sendra, E. (2014). Antioxidant activity and protein-polyphenol interactions in a pomegranate (Punica granatum L.) yogurt. *Journal of Agricultural and Food Chemistry, 62*, 6417-6425.

Vega, C., & Grover, M.K. (2011). Physicochemical properties of acidified milk gels containing cocoa flavanols. *Journal of Agricultural and Food Chemistry, 59*(12), 6740-6747.

Velderrain-Rodríguez, G.R., Acevedo-Fani, A., González-Aguilar, G.A., & Martín-Belloso, O. (2019). Encapsulation and stability of a phenolic-rich extract from mango peel within water-in-oil-in water emulsions. *Journal of Functional Foods, 56*, 65-73.

Villalva, M., Jaime, L., Aguado, E., Nieto, J. A., Reglero, G., & Santoyo, S. (2018). Anti-Inflammatory and antioxidant activities from the basolateral fraction of Caco-2 cells exposed to a rosmarinic acid enriched extract. *Journal of Agricultural and Food Chemistry, 66*(5), 1167-1174.

Villalva, M., Jaime, L., Villanueva-Bermejo, D., Lara, B., Fornari, T., Reglero, G., & Santoyo, S. (2019). Supercritical anti-solvent fractionation for improving antioxidant and anti-inflammatory activities of an *Achillea millefolium* L. extract. *Food Research International, 115*, 128-134.

Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C., & Yamamoto, K. (2011). Interaction of dietary polyphenols with bovine milk proteins: molecular structure-affinity relationship and influencing bioactivity aspects. *Molecular Nutrition and Food Research, 55*(11), 1673-1645.

Yan, L., Wang, R., Wang, H., Sheng, K., Liu, C., Qu, H., Ma, A., & Zheng, L. (2018). Formulation and characterization of chitosan hydrochloride and carboxymethyl chitosan encapsulated quercetin nanoparticles for controlled applications in food system and simulated gastrointestinal condition. *Food hydrocolloids, 84*, 450-457

Table 1. Phenolic composition of yarrow extract (YE) and digested samples: YE, NE-1.0 and MG-1.0.

Compound	Concentration (mg /g sample)			
	YE undigested	**YE digested**	**NE-1.0 digested**	**MG-1.0 digested**
Chlorogenic acid [S]	4.49 ± 0.20	1.24 ± 0.31*	3.03 ± 0.19*	3.28 ± 0.40*
Vicenin 2 [S]	5.33 ± 0.25	4.93 ± 0.24	5.03 ± 0.32	5.09 ± 0.60
Caffeic acid [S]	0.68 ± 0.22	1.09 ± 0.13*	0.63 ± 0.11	0.69 ± 0.14
Schaftoside isomer	3.50 ± 0.07	3.27 ± 0.17	3.38 ± 0.46	3.36 ± 0.45
Schaftoside [S]	3.49 ± 0.22	3.25 ± 0.30	3.43 ± 0.56	3.52 ± 0.38
Homoorientin [S]	0.46 ± 0.07	0.25 ± 0.01*	0.28 ± 0.03*	0.35 ± 0.04
Apigenin-hexoside-pentoside	2.63 ± 0.16	2.28 ± 0.19	2.54 ± 0.28	2.68 ± 0.21
Luteolin-dihexoside	2.72 ± 0.21	2.08 ± 0.10*	2.29 ± 0.14*	2.60 ± 0.24
6-Hydroxyluteolin-7-*O*-glucoside	3.67 ± 0.31	1.08 ± 0.13*	1.14 ± 0.25*	3.51 ± 0.29
Rutin [S]	1.37 ± 0.28	0.86 ± 0.14*	0.98 ± 0.31	1.20 ± 0.15
Vitexin [S]	0.71 ± 0.03	0.41 ± 0.05*	0.57 ± 0.02*	0.70 ± 0.07
Apigenin glycosylated derivative	4.79 ± 0.16	4.09 ± 0.19*	4.78 ± 0.23	4.88 ± 0.20
Luteolin-7-*O*-*β*-glucoside [S]	7.57 ± 0.17	5.71 ± 0.14*	6.51 ± 0.10*	7.36 ± 0.30
3,4-diccafeoylquinic acid [S]	1.79 ± 0.26	4.59 ± 0.34*	3.54 ± 0.19*	2.62 ± 0.25*
1,5-diccafeoylquinic acid [S]	1.91 ± 0.11	1.43 ± 0.18	1.73 ± 0.19	1.88 ± 0.25
3,5-diccafeoylquinic acid [S]	17.0 ± 0.47	5.84 ± 0.28*	10.8 ± 0.11*	15.9 ± 0.23*
Apigenin-7-*O*-glucoside [S]	2.20 ± 0.15	1.41 ± 0.08*	1.40 ± 0.25*	2.16 ± 0.26
4,5-diccafeoylquinic acid [S]	3.36 ± 0.19	7.48 ± 0.13*	3.96 ± 0.14*	3.23 ± 0.28
Luteolin [S]	2.15 ± 0.27	1.22 ± 0.13*	2.10 ± 0.13	2.13 ± 0.10
Apigenin [S]	0.61 ± 0.13	0.24 ± 0.20*	0.50 ± 0.26	0.58 ± 0.04
Diosmetin [S]	0.49 ± 0.07	<LOQ	0.35 ± 0.05*	0.44 ± 0.06
Total phenolics	70.52 ± 4.34	52.75 ± 2.21*	57.99 ± 3.28*	68.97 ± 3.31

YE: yarrow extract. NE-1.0: YE-loaded nanoemulsions at 1 mg/mL. MG-1.0: milk gels including 1 mg/ mL of YE. <LOQ: below limit of quantification. [S] Phenolic compound identified and quantified *via* comparison with its authentic standard. * An asterisk indicates statistical differences of digested samples with respect to the YE undigested extract (first column). Significance level at $p<0.05$ with Fisher's Least Significant Difference (LSD) test.

Table 2. Characterization of fresh nanoemulsions (0 day) and throughout storage (7 and 14 days).

	Mean particle diameter ($d_{4,3}$) (nm)			**ζ– potential (mV)**		
Sample	*0 d*	*7 d*	*14 d*	*0 d*	*7 d*	*14 d*
Control	248 ± 1.0	247 ± 1.0	247 ± 1.2	-39.1 ± 0.9	-39.2 ± 1.1	-38.7 ± 0.2
NE-1.0	250 ± 0.8	249 ± 1.2	249 ± 1.6	-38.6 ± 0.5	-38.2 ± 0.6	-37.9 ± 0.7
NE-2.5	251 ± 2.0	251 ± 1.0	250 ± 1.0	-38.2 ± 0.7	-38.0 ± 0.9	-37.8 ± 0.7

NE-1.0: YE-loaded NEs at 1 mg/mL.
NE-2.5: YE-loaded NEs at 2.5 mg/mL.
ζ–potential: zeta potential.

Table 3. Properties of acidified skim milk gels formulated with heated and unheated milk.

	Gelation time (min)		pH at gelation time		Final G′ value (Pa)		Final tan δ	
Sample	**Heated**	**Unheated**	**Heated**	**Unheated**	**Heated**	**Unheated**	**Heated**	**Unheated**
Control	27 ± 2.1[a]	74 ± 1.6[a]	5.51 ± 0.11[a]	5.11 ± 0.09[a]	424 ± 23[a]	15.6 ± 0.0[b]	0.28 ± 0.01[a]	0.31 ± 0.01[a]
MG-1.0	27 ± 0.6[a]	74 ± 1.1[a]	5.37 ± 0.12[a]	5.08 ± 0.16[a]	397 ± 18[a]	15.5 ± 0.1[b]	0.28 ± 0.01[a]	0.31 ± 0.01[a]
MG-2.5	26 ± 2.7[a]	75 ± 0.6[a]	5.33 ± 0.09[a]	5.03 ± 0.23[a]	389 ± 13[b]	14.1 ± 1.0[a]	0.29 ± 0.01[a]	0.32 ± 0.01[a]

MG-1.0: milk gels with 1.0 mg/mL of YE. MG-2.5: milk gels with 2.5 mg/mL of YE. G′: storage modulus values. tan δ loss tangent values. [a,b] Different superscript letters mean statistical difference among different formulations for each evaluated property. Significance level at $p<0.05$ with Fisher's Least Significant Difference (LSD) test.

Table 4. Encapsulation efficiency (EE) of individual yarrow phenolic compounds presented in yarrow extract (YE).

Compound	Encapsulation Efficiency (%)			
	NE-1.0	**NE-2.5**	**MG-1.0**	**MG-2.5**
Chlorogenic acid [S]	88.5 ± 6.8	6.6 ± 1.2	47.1 ± 7.0	28.3 ± 6.7
Vicenin 2 [S]	88.3 ± 6.7	17.5 ± 1.1	34.3 ± 8.0	8.2 ± 2.3
Caffeic acid [S]	89.7 ± 6.1	20.5 ± 6.9	55.0 ± 7.5	11.0 ± 5.0
Schaftoside isomer	89.4 ± 5.9	18.9 ± 5.0	56.8 ± 2.9	25.7 ± 7.3
Schaftoside [S]	88.1 ± 7.9	17.0 ± 4.7	40.9 ± 3.6	19.8 ± 2.1
Homoorientin [S]	94.6 ± 4.1	34.5 ± 7.7	65.3 ± 7.0	29.5 ± 6.5
Apigenin-hexoside-pentoside	89.9 ± 6.6	13.4 ± 2.5	33.8 ± 9.8	12.5 ± 4.8
Luteolin-dihexoside	92.3 ± 4.8	23.5 ± 7.0	56.6 ± 2.6	25.1 ± 6.0
6-Hydroxyluteolin-7-*O*-glucoside	97.0 ± 1.4	42.0 ± 5.9	74.0 ± 0.8	79.6 ± 4.5
Rutin [S]	93.1 ± 3.4	20.0 ± 6.6	47.4 ± 5.4	34.0 ± 7.8
Vitexin [S]	93.2 ± 3.6	36.6 ± 7.8	51.3 ± 0.5	31.8 ± 7.0
Apigenin glycosylated derivative	91.1 ± 5.2	34.7 ± 7.9	55.0 ± 7.4	48.4 ± 4.6
Luteolin-7-*O*-*β*-glucoside [S]	95.4 ± 2.8	44.5 ± 5.7	80.0 ± 3.3	73.5 ± 3.3
3,4-diccafeoylquinic acid [S]	91.0 ± 7.0	22.2 ± 7.6	81.4 ± 6.1	61.9 ± 6.7
1,5-diccafeoylquinic acid [S]	92.3 ± 4.9	43.0 ± 7.1	91.6 ± 1.6	85.0 ± 5.9
3,5-diccafeoylquinic acid [S]	93.2 ± 4.4	36.4 ± 6.0	98.0 ± 0.2	97.0 ± 1.0
Apigenin-7-*O*-glucoside [S]	94.4 ± 1.9	54.4 ± 7.8	89.4 ± 2.3	85.0 ± 2.9
4,5-diccafeoylquinic acid [S]	93.9 ± 3.7	43.8 ± 5.0	91.6 ± 1.6	87.5 ± 1.1
Luteolin [S]	97.6 ± 2.9	92.6 ± 1.1	100 ± 0.0	100 ± 0.0
Apigenin [S]	99.3 ± 0.3	93.4 ± 2.8	100 ± 0.0	100 ± 0.0
Diosmetin [S]	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Total %EE	92.3 ± 4.6 [a]	33.7 ± 0.9 [b]	72.0 ± 2.7 [A]	63.1 ± 2.6 [B]

NE-1.0: YE-loaded nanoemulsion at 1.0 mg/mL. NE-2.5: YE-loaded nanoemulsion at 2.5 mg/mL. MG-1.0.: milk gels with 1.0 mg/mL of YE. MG-2.5: milk gels with 2.5 mg/mL of YE. [S] Phenolic compound identified and quantified *via* comparison with its authentic standard. [a,b] Different lower case letters mean statistical difference within nanoemulsions. [A,B] Different super case letters mean statistical difference within milk gels. Significance level at $p<0.05$ with Fisher's Least Significant Difference (LSD) test.

Table 5. Antioxidant activity of yarrow extract (YE) and digested samples: free YE, NE-1.0 and MG-1.0.

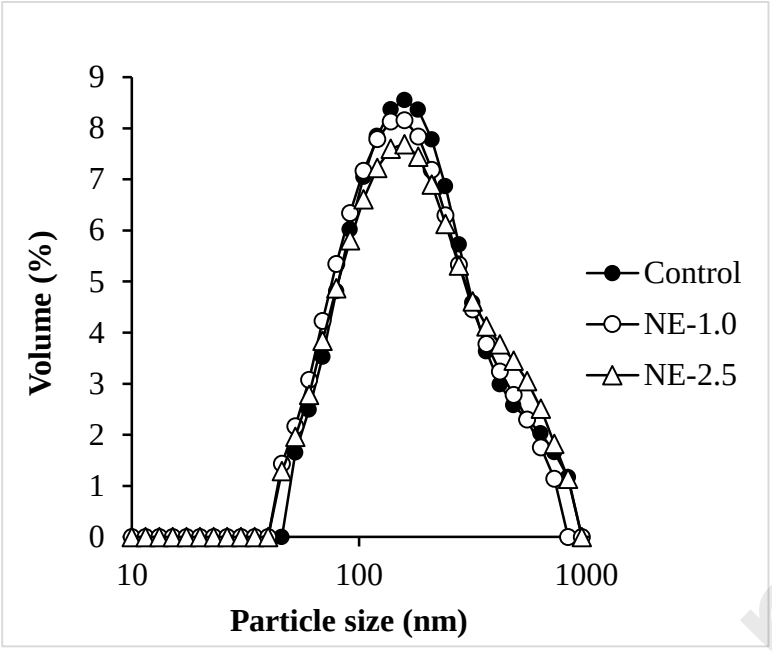
TEAC Value (mmol Trolox/g sample)	
YE undigested	0.349 ± 0.007^{a}
Free YE digested	0.186 ± 0.002^{d}
NE-1.0 digested	0.202 ± 0.003^{c}
MG-1.0 digested	0.270 ± 0.005^{b}

NE-1.0: YE-loaded nanoemulsions at 1 mg/mL. MG-1.0: milk gels including 1 mg/ mL of YE. $^{a-d}$ Different letters mean statistical difference between samples with Fisher's LSD test ($p<0.05$).

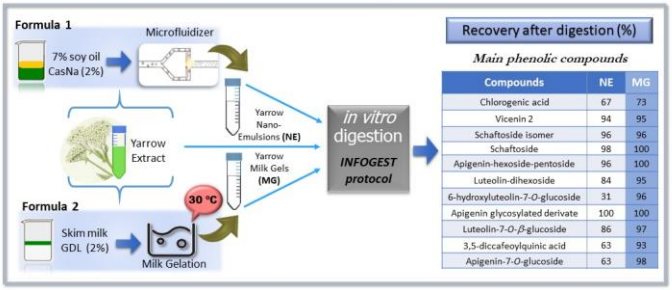
Figure captions

Figure 1. Particle size distribution of freshly (day 0) nanoemulsions in the presence or not of yarrow extract (YE). Control: without YE. NE-1.0: YE-loaded nanoemulsions at 1 mg/mL. NE-2.5: YE-loaded nanoemulsions at 2.5 mg/mL.

Figure 1.



Graphical abstract



Compounds	NE	MG
Chlorogenic acid	67	73
Vicenin 2	94	95
Schaftoside isomer	96	96
Schaftoside	98	100
Apigenin-hexoside-pentoside	96	100
Luteolin-dihexoside	84	95
6-hydroxyluteolin-7-*O*-glucoside	31	96
Apigenin glycosylated derivate	100	100
Luteolin-7-*O*-*β*-glucoside	86	97
3,5-diccafeoylquinic acid	63	93
Apigenin-7-*O*-glucoside	63	98

Highlights

- Casein-based delivery systems improved yarrow phenolic compounds stability
- Nanoemulsions partially protected yarrow phenolic compounds during digestion
- Yarrow phenolics included in acidified milk gels were protected during digestion
- After digestion, phenolics in milk gels showed the highest antioxidant activity
- Milk gels could be used to develop dairy products enriched with phenolic compounds.

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-M. Villalva**:** Investigation, Formal Analysis and Writing-Original Draft

-L. Jaime: Conceptualization, Methodology and Writing-Original Draft

-E. Arranz: Conceptualization, Methodology

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-G. Reglero: Resources and Funding acquisition

-S. Santoyo: Conceptualization, Supervision, Writing-Original Draft, Writing-Review & Editing