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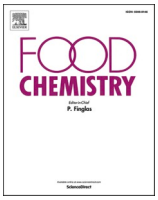

Non-emulsion-based encapsulation of fish oil by coaxial electrospraying assisted by pressurized gas enhances the oxidative stability of a capsule-fortified salad dressing

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ABSTRACT

The influence of the encapsulation technology (spray-drying, mono- or coaxial electrospraying assisted by pressurized gas, EAPG) and the oil load (13, 26 or 39 wt%) on the oxidative stability of: i) fish oil-loaded capsules, and ii) capsule-fortified salad dressings were investigated. The highest encapsulation efficiency (EE > 83%) was achieved by the emulsion-based encapsulation methods (e.g., spray-drying and monoaxial EAPG), irrespective of the oil load. Nonetheless, monoaxially EAPG capsules were the most oxidized during storage due to their increased surface-to-volume ratio. On the contrary, non-emulsion-based coaxial EAPG resulted in low lipid oxidation after processing and subsequent storage. The oxidative stability of the capsule-fortified salad dressings correlated well with that of the encapsulates, with the dressing fortified with the coaxially EAPG capsules showing significantly lower levels of oxidation. Our results show that the fortification approach (e.g., emulsion or non-emulsion-based delivery systems) significantly influenced the oxidative stability of the enriched food matrix.

1. Introduction

The proven health benefits related to the intake of omega-3 polyunsaturated fatty acids (PUFAs), particularly EPA (C20:5n-3) and DHA (C22:6n-3), have driven the development of fortified food products rich in these bioactive compounds (Calder, 2021; Ghelichi et al., 2021). However, due to their polyunsaturated nature, they are very prone to lipid oxidation. Thus, they are generally introduced into the food matrix in the form of a delivery system (Ghelichi et al., 2021; Sørensen et al., 2021). Therefore, in the last decades, the production of stable micro/nanocapsules loaded with oils rich in omega-3 PUFAs (e.g., fish oil) to be used as delivery systems has become a focus of scientific research.

Traditionally, spray-drying has been the technique of choice of the food industry to produce oil-loaded encapsulates due to its multiple advantages (e.g., high throughput) (Rahmani-Manglano, García-Moreno, et al., 2020). Nonetheless, it also presents some drawbacks that need to be considered when it comes to food fortification. Particularly, it has been demonstrated that emulsification of omega-3 rich oils followed by drying at high temperatures (e.g., 160–210 °C) results in an initial lipid oxidation if the oil is not properly stabilized and/or the process is not well designed (Serfert et al., 2009). Besides, the use of spray-dried capsules might be restrained for certain commercial food applications. By spray-drying, relatively large particles (~5–100 μm) with a polydisperse particle size distribution are produced, which may affect the reproducible performance of the encapsulates (Rahmani-Manglano et al., 2023). In the literature, fish oil-loaded capsules produced by spray-drying have been used as omega-3 delivery systems in a wide range of food products to investigate their influence on the oxidative, physical and/or sensory stability of the fortified matrix (e.g., dressings, baked, spread or meat products) (Aquilani et al., 2018; Davidov-Pardo et al., 2008; Jeyakumari et al., 2016; Nielsen & Jacobsen, 2013; Rahmani-Manglano, González-Sánchez, et al., 2020; Solomando, Antequera, & Perez-Palacios, 2020a, 2020b; Solomando, Antequera, González-Mohíno, et al., 2020). However, in the aforementioned studies, the influence of the oil load of such delivery systems on the physicochemical properties (e.g., physical and oxidative stability) of the

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enriched foodstuff was not investigated.

Recently, electrospraying technology has emerged as a promising alternative to spray-drying for the production of omega-3-loaded capsules aimed as delivery systems (García-Moreno et al., 2021). In conventional electrospraying, the oil-loaded solution/emulsion is subjected to a high electric field which forms a spray of ultrathin droplets allowing solvent evaporation at room temperature (García-Moreno et al., 2021). Therefore, contrary to spray-drying, heat is not required at any point of the process, which prevents from thermal degradation and enables the use of a wider variety of (bio)polymeric wall materials (Prieto & Lagaron, 2020). In addition, by electrospraying in the coaxial configuration, two physically separated liquids can be infused simultaneously allowing to produce oil-loaded capsules without emulsifying or dispersing the oil first within the (bio)polymer-based encapsulating solution (Rahmani-Manglano et al., 2023). Thus, by electrospraying technology, initial lipid oxidation due to processing could be reduced in the monoaxial configuration, which does not use hot air to dry but requires a homogenization step, or even theoretically be avoided in the coaxial configuration, which might not require previous homogenization. Moreover, as a result of electrohydrodynamic atomization, smaller and monodispersed encapsulates are produced resulting in a more reproducible performance of the capsules, enhanced bioaccessibility and fewer modifications of the original food structure when added as delivery systems when compared to those produced by spray-drying (García-Moreno et al., 2021). Nonetheless, it should be borne in mind that the higher surface-to-volume ratio of these systems may result in lower oxidative stability of the encapsulated oil, due to the higher contact area with prooxidants species such as oxygen (Boerekamp et al., 2019), which will further affect the quality of the enriched food product.

Despite the multiple advantages of electrospraying over spray-drying, its industrial use is restrained due to the low productivity of the process (Busolo et al., 2019). In this regard, a novel high-throughput encapsulation technology based on electrospraying, and referred to as electrospraying assisted by pressurized gas (EAPG), has been recently developed (Busolo et al., 2019). This novel technology is already available at an industrial scale (Lagaron et al., 2017). The EAPG technology increases the throughput of conventional electrospraying by introducing a pneumatic injector that atomizes the oil-loaded solution/emulsion within a high electric field. Again, due to the high voltage applied, the atomized droplets are further disrupted allowing that the solvent evaporates at room temperature. The dry material is then collected as a free-flowing powder in the collection unit (Prieto et al., 2021). This technology has been previously used to produce omega-3 delivery systems (Busolo et al., 2019; García-Moreno et al., 2021; Miguel et al., 2019; Prieto & Lagaron, 2020) and their impact on the properties of selected enriched foodstuffs has been also investigated (e.g., powdered milk or low-fat mayonnaise) (Busolo et al., 2019; Hermund et al., 2019; Miguel et al., 2019). However, the influence of the capsules' oil-load on the physicochemical properties of the enriched matrix was not studied. Moreover, to our knowledge, the potential of EAPG technology in the coaxial configuration to produce neat fish oil-loaded capsules aimed for food fortification purposes has not been reported to date.

In light of the above, the aim of the study was to investigate the influence of the encapsulation technology and the fish oil load on: i) the physicochemical properties of the dried omega-3 delivery systems, and ii) the physical and oxidative stabilities of a capsule-fortified food matrix. Salad dressing was selected as the food model system due to its high fat content, with potential for replacement with fish oil (Let et al., 2007a). First, the fish oil-loaded capsules were produced by spray-drying and EAPG technology in the monoaxial and coaxial configuration at three different fixed fish oil loads (e.g., 13, 26 or 39 wt%). The capsules' morphology and encapsulation efficiency were subsequently characterized and their oxidative stability was monitored during 6 weeks of storage at 25 °C. Selected fish oil-loaded capsules with varying oil loads and produced by different encapsulation techniques were further used for omega-3 enrichment of a salad dressing. Finally, the physical (e.g., oil droplet size, viscosity) and the oxidative (e.g., peroxide value and formation of volatile compounds) stabilities of the fortified salad dressings were studied during 28 days at ambient temperature.

2. Materials and methods

2.1. Materials

The fish oil (Omega Oil 1812 TG Gold) was acquired from BASF Personal Care and Nutrition GmbH (Illertissen, Germany). Cargill Germany GmbH (Krefeld, Germany) provided the glucose syrup (GS; DE38, C*Dry 1934) and the whey protein concentrate (~35 wt% protein content) was supplied by Abbott Laboratories S.A. (Granada, Spain). The whey protein concentrate hydrolysate (WPCH), used as an emulsifier, was produced by enzymatic hydrolysis as described elsewhere (Rahmani-Manglano, González-Sánchez, et al., 2020). For the production of the salad dressings, refined rapeseed oil (RSO) was provided by AAK Sweden AB (Malmö, Sweeden). The stabilizer Grinsted FF5128 was donated by DuPont (DuPont Nutrition Biosciences Aps, Haderslev, Denmark). The rest of the ingredients used were purchased in the local market. The reagents used for the analysis were of analytical grade.

The peroxide value (PV) and the tocopherol content of the fresh oils (fish oil and rapeseed oil) were measured as described in Section 2.4.3.1. The fish oil had a PV of 0.33 ± 0.06 meq O_2/kg oil and alpha-, gamma- and delta-tocopherol content of 500.8 ± 1.3, 2108.8 ± 5.3 and 677.4 ± 2.0 μg/g oil, respectively. The rapeseed oil had a PV of 0.17 ± 0.07 meq O_2/kg oil and alpha- and gamma-tocopherol content of 260.5 ± 6.0 and 378.9 ± 1.1 μg/g oil, respectively.

2.2. Production of fish oil-loaded capsules

2.2.1. Emulsions preparation

The fish oil (5, 10 or 15 wt%) was dispersed in the aqueous phase containing the encapsulating agent (GS; 28, 17 or 7 wt%) and the emulsifier (WPCH; 6, 12 or 17 wt%). The protein/oil ratio (P/O ratio) was fixed to 0.4 and the solids content of the emulsions was kept constant at 39 wt%, irrespective of the oil load. First, an Ultraturrax T-25 homogenizer (IKA, Staufen, Germany) was used to produce the coarse emulsions. The fish oil was added during the first minute of mixing, and the total mixing time was 2 min. The coarse emulsions were then homogenized by applying three passes at 450/75 bar in a high-pressure homogenizer (PandaPLUS 2000; GEA Niro Soavi, Lübeck, Germany) (Rahmani-Manglano, González-Sánchez, et al., 2020).

2.2.2. Production by spray-drying

The fish oil-in-water emulsions (Section 2.2.1) were processed by spray-drying in a pilot plant scale spray-drier (Mobile Minor; Niro A/S, Copenhagen, Denmark) (Rahmani-Manglano, González-Sánchez, et al., 2020). The inlet and outlet air temperature were set to 190 °C and 80 °C, respectively. The rotary atomizer's activation pressure was fixed to 4 bar, which corresponds to a 22,000 rpm rotational speed. After drying, the capsules were stored in airtight flasks at − 80 °C in the dark until analysis.

2.2.3. Production by electrospraying assisted by pressurized gas (EAPG) in monoaxial configuration

The fish oil-in-water emulsions (Section 2.2.1) were processed by EAPG using the pilot plant equipment Capsultek™ from Bioinicia S.L. (Valencia, Spain) consisting of a nebulizer, a drying chamber and a cyclonic collector (Prieto & Lagaron, 2020). During processing, the emulsions were kept under constant nitrogen bubbling to minimize lipid oxidation and the ambient conditions were monitored (30 °C and 25% relative humidity; RH). The emulsions flow rate was fixed to 1 mL/min and the injector worked with an assisted air pressure of 10 L/min. The

electric voltage was set to 10 kV. Every 20 min, the capsules were collected from the cyclone and stored in airtight flasks at –20 °C in the dark until analysis.

2.2.4. Production by electrospraying assisted by pressurized gas (EAPG) in coaxial configuration

For coaxial EAPG, neat fish oil (NFO) was infused as the core solution whilst through the annular gap, the water-based encapsulating agent solution consisting of a mixture GS:WPCH (~80:20, w/w) was infused. The ratio GS:WPCH was fixed in a way that the capsules with a 13 wt% of fish oil load had the same composition irrespective of the technology used for their production (e.g., spray-drying, monoaxial or coaxial EAPG). The EAPG process was carried out in the equipment described in Section 2.2.3, but this time a coaxial nebulizer was used. The core flow rate was fixed to 1 mL/min and the shell flow rate varied between 1 mL/min and 10 mL/min to achieve the desired load capacity (~13 wt%). The injector worked with an assisted air pressure of 16 L/min and the electric voltage was set to 10 kV. Every 20 min, the capsules were collected from the cyclone and stored in airtight flasks at –20 °C in the dark until analysis.

2.3. Physicochemical characterization of the capsules

2.3.1. Oil droplet size of the parent and the reconstituted emulsions

The oil droplet size of the parent and reconstituted emulsions was measured using a Mastersizer 3000 (Malvern Instruments, Ltd., Worcestershire, UK). For the reconstituted emulsions, the capsules were dissolved in distilled water in order to achieve the same solids content as the original emulsions. For the measurements, the samples were diluted in circulating water (3000 rpm) until an obscuration level between 12 and 15% was reached. For particle and dispersant, respectively, the refractive indexes of fish oil (1.481) and water (1.330) were used. The measurements were made in triplicate.

2.3.2. Morphology and size

A Field Emission Scanning Electron Microscope (FESEM, LEO 1500 GEMINI, Zeiss, Germany) was used to investigate the morphology and size of the capsules (Rahmani-Manglano et al., 2023). For this purpose, a thin layer of powder was placed on a carbon tape and subsequently carbon-coated using an EMITECHK975X Turbo-Pumped Thermal Evaporator (Quorum Technologies, UK). The Scanning Electron Microscopy (SEM) images were captured at magnifications ranging from 500X to 15 KX, with a 5 kV accelerating voltage. The mean particle size and the particle size distribution were determined by measuring 160 randomly-selected capsules using the ImageJ software (National Institute of Health, USA).

2.3.3. Load capacity (LC) and encapsulation efficiency (EE)

The load capacity (LC) and the encapsulation efficiency (EE) of the capsules were determined as described in our previous work (Rahmani-Manglano et al., 2023), with some modifications. For determining the LC, 150 mg of powder was dissolved by adding 1 mL of distilled water. Then, the fish oil was extracted using a hexane/2-propanol (1:1, v/v) solvent and the total oil load was determined by measuring the absorbance of the lipid extract at 250 nm in a UV4000 spectrophotometer (Dinko Instruments, Barcelona, Spain). To measure the EE, 25 mg of powder was immersed in 10 mL of hexane and gently shaken for 30 s. Then, the mixture was filtered into a pyrex tube and the absorbance of the filtrate was measured at 250 nm. The EE was calculated as described elsewhere (Rahmani-Manglano et al., 2023). The measurements were carried out in triplicate.

2.3.4. Oxidative stability of the capsules

To investigate the oxidative stability of the capsules, these were stored for 6 weeks at ambient temperature (25 °C) in the dark to quantify the content of selected secondary volatile oxidation products (SVOPs). For the study, 4 g of capsules were stored in closed brown bottles (30 mL). Samples were taken every week (one bottle per sampling time) and overlaid with nitrogen before storage at –40 °C until analysis.

2.3.4.1. Secondary volatile oxidation products (SVOPs) – Dynamic headspace GC–MS.

The content of selected secondary volatile oxidation products (SVOPs) was determined as described in our previous work (Rahmani-Manglano, González-Sánchez, et al., 2020). In brief, approximately 1 g of powder and 30 mg of internal standard (4-methyl-1-pentanol, 30 μg/g water) were mixed with 10 mL of distilled water in a pear-shaped flask. Then, the volatile compounds were released by heating the flask content while purging with nitrogen (45 °C; flow rate 150 mL/min) for 30 min to a Tenax GR tube. The released volatile compounds were identified by MS-library searches (Wiley 138 K, John Wiley and Sons, Hoboken, New Jersey, USA and Hewlett-Packard, San Jose, California, USA) and quantified through calibration curves using external standards dissolved in 96% ethanol. The external standards employed were: 3-methylbutanal, pentanal, 1-penten-3-ol, hexanal, (*E*)-2-hexenal, heptanal, (*Z*)-4-heptenal, 2-pentyl-furan, octanal, (*E,E*)-2,4-heptadienal, 1-Octanol, 2-nonanone and decanal (Sigma-Aldrich, Brøndby, Denmark). The standards solution was diluted to concentrations of approximately 25, 50, 100, 200, 500, 1000 and 2000 μg/mL, and 1 μL of each was directly injected into the Tenax tubes. Measurements were made in triplicate. The content of the SVOPs identified in the fish oil-loaded capsules are shown in Table S1 of the Supplementary Material.

2.4. Production and characterization of fortified salad dressing

2.4.1. Production of fortified salad dressing

The fortified salad dressings (2.5 wt% of fish oil) were produced following two different approaches: i) adding the neat fish oil (SD-NFO) or ii) adding the fish oil-loaded capsules produced either by spray-drying (SD-spd-13 or SD-spd-39), monoaxial EAPG (SD-mo-13 or SD-mo-39) or coaxial EAPG (SD-co-13). In all cases, 300 g of salad dressing was produced containing: 2.5 wt% of fish oil, 22.5 wt% of RSO, 6 wt% of vinegar, 1.2 wt% stabilizer (Grindsted FF5128, consisting of a mixture of xanthan and guar gums) and 0.08 wt% of whey protein concentrate, following the procedure described by Let et al. (2007a) with some modifications. First, the stabilizer was dispersed in the RSO (1/3 of the total RSO) and mixed into the water phase in a Stephan Universal Mixer (Stephan, Hameln, Germany) for 2 min under vacuum. Then, the whey protein concentrate was dissolved in deionized water and added to the mixture. Fish oil (in case of SD-NFO sample), the remaining rapeseed oil, and vinegar were added slowly during mixing for 3 min and the dressing was mixed for an additional 2 min under vacuum. In case of the salad dressings fortified with the encapsulated fish oil, the capsules were dispersed into the matrix using a 4-bladed propeller stirrer for 45 s. As the last step, sodium azide solution (10 wt%) was added to all the samples to have a final concentration in the fortified salad dressings of 0.05 wt%. The solution was dispersed manually. Sodium azide was added to prevent microbial growth during storage.

2.4.2. Physical stability: Oil droplet size, viscosity and color

The oil droplet size of the salad dressing samples was measured by laser diffraction as described in Section 2.3.1 at days 0 and 28. The samples were pretreated by dissolving 1 g of salad dressing in SDS buffer (10 mM NaH_2PO_4, 5 mM SDS) to a ratio 1:9 (w/w) and then sonicated for 15 min in a water bath at ambient temperature. The refractive index of sunflower oil (1.4694) was used for the dispersed phase. The measurements were made in triplicate.

The viscosity of the salad dressing samples was measured at 25 °C using a Discovery Hybrid Rheometer HR-2 (Waters TA Instruments, New Castle, USA) at days 0 and 28. Plain bottom base and upper cone plate (Peltier plate Steel – 113935) were used, with a gap of 1.5 mm. An

increasing gradient of stress was applied up to 200 Pa. Measurements were carried out in duplicate.

The color of the salad dressing samples was measured at days 0 and 28 of storage using a Konica Minolta CR-300 Chroma colorimeter (Minolta, Tokyo, Japan). The data were recorded by the instrument using the CIEL*a*b* color system space. Yellowness index (YI) was calculated as described by Miguel et al. (2019). All measurements were carried out in duplicate.

2.4.3. Oxidative stability

2.4.3.1. Peroxide value (PV) and tocopherol content.

The peroxide value

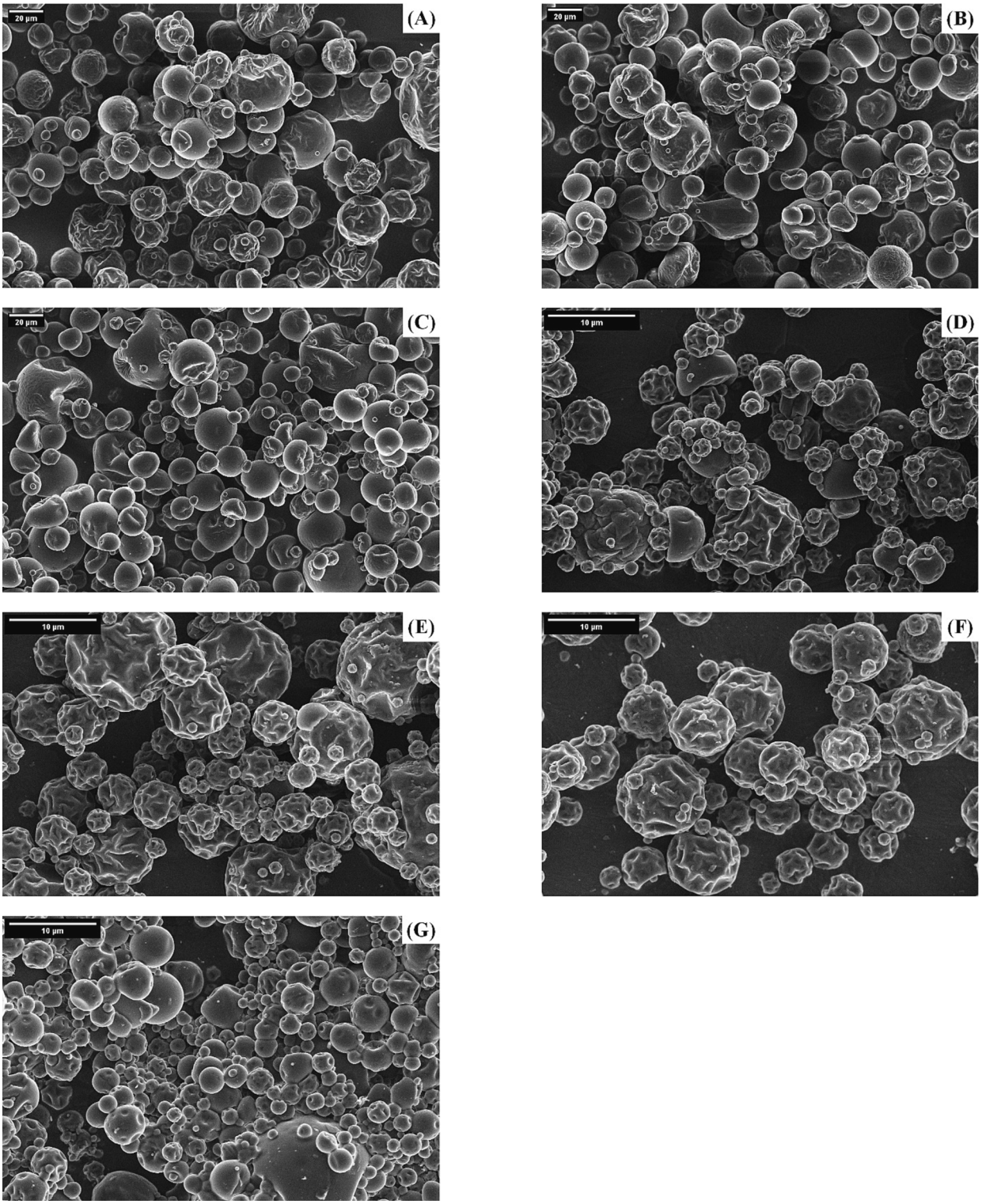

Fig. 1. SEM images of the fish oil-loaded capsules (13, 26 and 39 wt% fish oil) produced by spray-drying (spd-), EAPG monoaxial (EAPG-mo-) and EAPG coaxial (EAPG-co): spd-13 **(A)**, spd-26 **(B)**, spd-39 **(C)**, EAPG-mo-13 **(D)**, EAPG-mo-26 **(E)**, EAPG-mo-39 **(F)**, EAPG-co-13 **(G)**. The scale bar of the spray-dried capsules (A, B, C) corresponds to 20 μm. The scale bar of the EAPG capsules (monoaxial; D, E, F or coaxial; G) corresponds to 10 μm.

(PV) and the tocopherol content of the fortified salad dressing samples were measured as previously described elsewhere (Rahmani-Manglano, González-Sánchez, et al., 2020). Briefly, the lipids were extracted from the salad dressing samples (~9g) using a reduced amount of chloroform/methanol (1:1, w/w) solvent. Then, the peroxide value (PV) was quantified on the lipid extracts using the colorimetric ferric-thiocyanate method at 500 nm. The tocopherol content was also quantified on the lipid extract by HPLC (Agilent 1100 Series) according to the American Oil Chemists' Society (AOCS) official method (1998).

2.4.3.2. Secondary volatile oxidation products (SVOPs) – Dynamic headspace GC–MS. The content of secondary volatile oxidation products (SVOPs) of the salad dressing samples was measured as described in Section 2.3.4.1. Approximately 5 g of sample and 30 mg of internal standard (4-methyl-1-pentanol, 30 μg/g water) were mixed with 15 mL of distilled water and 2 mL of antifoam Synperonic PE/L 61 (Croda Chocques, Chocques, France; 3.2 mL/L water). The volatile compounds were released by heating the samples in a water bath at 45 °C for 30 min while purging with nitrogen (flow rate 150 mL/min) through a S-tube filled with powdered KOH (200 mg) to a Tenax GR tube. The volatiles were desorbed in the gas chromatograph as described above. The temperature program can be found elsewhere (Let et al., 2007a). The individual volatiles were analyzed by MS, identified by both library and external standards and quantified through calibration curves. The external standards employed were: 2-ethyl-furan, pentanal, 1-penten-3-ol, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, heptanal, 2-pentyl-furan, octanal, (*E,E*)-2,4-heptadienal and nonanal (Sigma-Aldrich, Brøndby, Denmark). The standards solution was diluted to concentrations of approximately 0.25, 0.50, 1, 5, 10, 25, 50 and 100 μg/g, and 30 mg of each were added to a fresh salad dressing prepared with RSO. Measurements were made in triplicate. The content of the SVOPs identified in the fortified salad dressing samples are shown in Table S2 of the Supplementary Material.

2.5. Statistical analysis

For the statistical analysis, one-way analysis of variance (ANOVA) and Tukeýs HSD multiple range test were carried out to determine significant differences between means at 95% confidence level ($p < 0.05$). Statgraphics software (version 5.1; Statistical Graphics Corp., Rockville, MD, USA) was used.

3. Results and discussion

3.1. Physicochemical characterization of the capsules

3.1.1. Morphology and size

The morphology and the particle size distribution of the fish oil-loaded capsules are shown in Fig. 1 and Fig. 2, respectively. Regardless of the encapsulation technology (spray-drying or monoaxial/coaxial EAPG) and the oil load of the capsules (13, 26 or 39 wt%), a discrete distribution of spherical particles was observed for all the samples (Fig. 1). However, significant differences were observed on the capsules' morphology and size, depending on the encapsulation technique used to produce the encapsulated systems. In case of the spray-dried capsules (spd- samples), the typical morphology of such powders was noted with particles showing both smooth and wrinkled surfaces (Fig. 1A-C). Interestingly, less wrinkled particles were observed as the oil load of the spray-dried capsules increased, which could be related to the higher whey protein content (WPCH) of the infeed emulsions (the ratio P/O was fixed to 0.4) at the same drying conditions (Both et al., 2018). Nonetheless, the latter was not observed in case of the capsules produced by EAPG technology in the monoaxial configuration although the composition of the infeed emulsions was the same (Fig. 1D-F). For these systems (EAPG-mo samples), wrinkled capsules of rather similar morphology were observed irrespective of the oil load (Fig. 1D-F). The morphology development of particles upon drying depends on several factors such as the composition of the dispersion/emulsion to be dried, the droplet size or the drying temperature (Both et al., 2018). At low temperature, the water evaporation rate is slower, and the structures formed during drying have more time to deform, shrink and collapse (Alamilla-Beltrán et al., 2005). Therefore, the difference between the morphology of the emulsion-based capsules produced by spray-drying or EAPG technology might be a consequence of the different solvent evaporation rate influenced by the temperature of the drying air for each process (e.g., 190 °C and ambient temperature, respectively). On the other hand, wrinkles were not observed on the surface of the coaxially electrosprayed capsules (Fig. 1G) although drying also occurred at ambient temperature. This time, a water-based solution consisting of a GS:WPCH mixture was infused as the outer fluid, while NFO was infused as the core solution. Thus, during drying in the coaxial configuration, water did not have to evaporate from the core of the droplets (which consisted of neat oil) allowing a faster crust formation and, therefore, leading to more spherical particles after drying compared to those produced by monoaxial EAPG (Maria Leena et al., 2020).

As expected, the particle size and the particle size distribution of the encapsulated systems (Fig. 2) were significantly influenced by the encapsulation technology. A polydisperse particle size distribution of large capsules ($17.0 \pm 8.1 – 17.3 \pm 7.6$ μm; $p > 0.05$) was obtained for the spray-dried samples (Fig. 2A-C), whilst the capsules produced by EAPG technology (monoaxial or coaxial) showed a narrow particle size distribution of significantly smaller capsules ($4.2 \pm 2.6 – 4.9 \pm 2.4$ μm; $p \leq 0.05$) (Fig. 2D-G). Mechanical atomization followed by electrohydrodynamic atomization, contrary to mechanical atomization alone (as is the case of EAPG technology contrary to spray-drying) leads to the formation of smaller droplets and, therefore, smaller capsules are produced after drying. Nonetheless, it was also noted that, contrary to the spray-dried capsules, a slight but significant ($p \leq 0.05$) increase of the size of the capsules occurred for the EAPG-mo systems as the oil load of the samples increased (e.g., 29% of the capsules > 10 μm for EAPG-mo-13 sample over 40% of the capsules > 10 μm for EAPG-mo-39 sample). This phenomenon was already observed by Prieto and Lagaron (2020) when increasing the algae oil load of whey protein- or maltodextrin-based capsules produced by monoaxial EAPG.

3.1.2. Load capacity (LC) and encapsulation efficiency (EE)

Encapsulation of fish oil was successfully achieved at the assayed conditions as confirmed by the load capacity (LC) values of the different systems produced (Fig. 3A). Slight deviations from the theoretical oil load (13, 26 and 39 wt%) and differences in the oil load between capsules produced by the technologies studied (e.g., spd-39 vs EAPG-mo-39) might be related to differences in the moisture content of the dry components (emulsifier and encapsulating agent) at the time of the samples' preparation.

Our results show that emulsion-based encapsulation methods (e.g., spray-drying and EAPG monoaxial) resulted in significantly higher EE values (EE > 83%), compared to coaxial EAPG (EE ~50%) (Fig. 3B, $p \leq 0.05$). Drying physically stable emulsions results in high EE values (Ramakrishnan et al., 2014), which correlates with our results. As can be seen in Fig. S1A of the Supplementary Material, all the emulsions fed to the dryers showed a monomodal droplet size distribution of small oil droplets ($D[4,3] = 0.4–0.5$ μm) regardless of the oil load. Moreover, the reconstituted emulsions showed a droplet size distribution similar to that of the parent emulsions and these were also fairly similar among the samples ($D[4,3] = 0.5–0.7$ μm), except for the sample EAPG-mo-39 ($D[4,3] = 1.9$ μm) (Fig. S1B of the Supplementary Material). The latter is an indication that the emulsions behaved similarly upon drying irrespective of the technology used and that the integrity of the oil–water interface was retained irrespective of the atomization method (e.g., mechanical atomization or mechanical atomization followed by electrohydrodynamic atomization) (Taboada et al., 2021). Hence, the larger

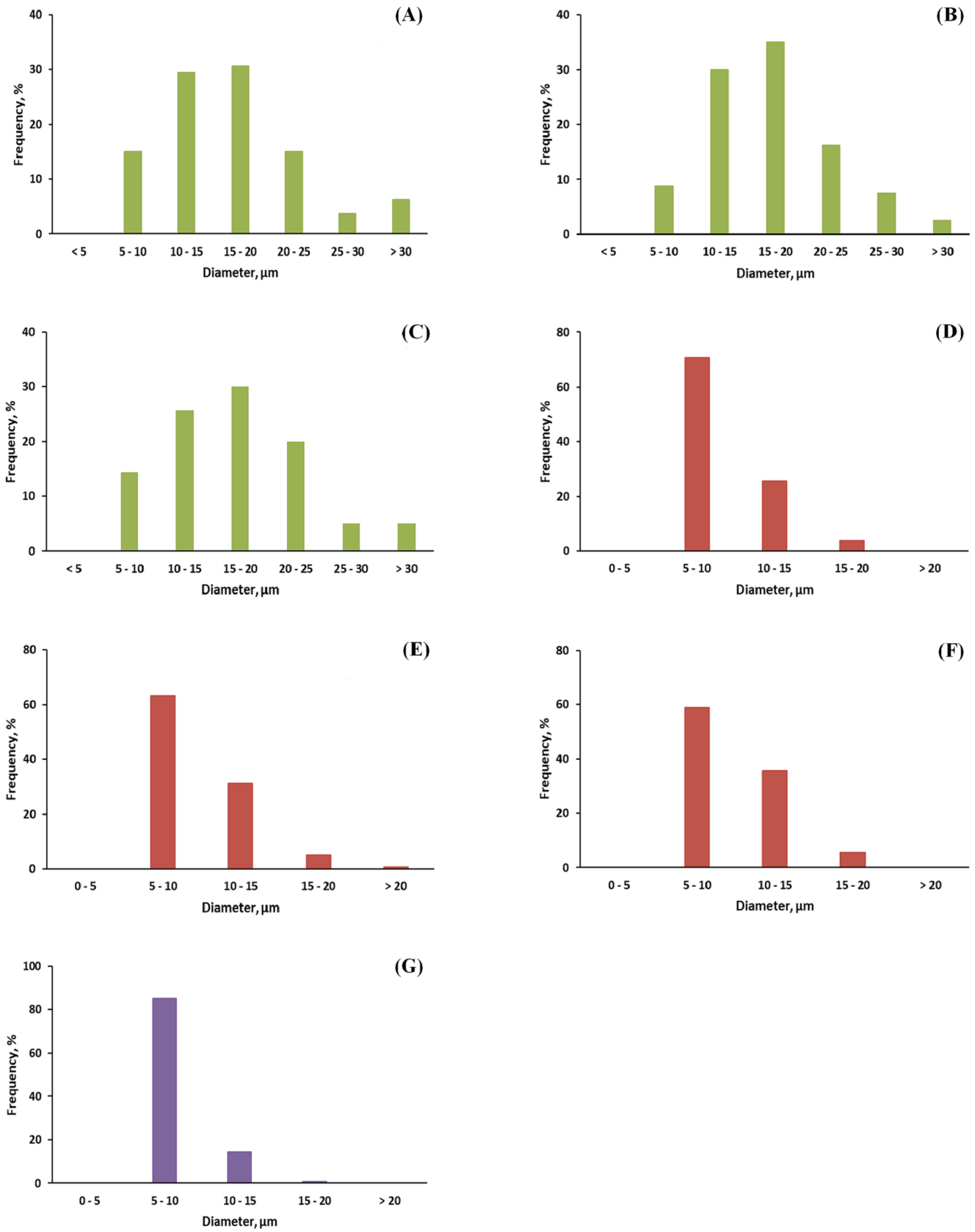

Fig. 2. Particle size distribution of the fish oil-loaded capsules (13, 26 and 39 wt% fish oil) produced by spray-drying (spd-), EAPG monoaxial (EAPG-mo-) and EAPG coaxial (EAPG-co): spd-13 **(A)**, spd-26 **(B)**, spd-39 **(C)**, EAPG-mo-13 **(D)**, EAPG-mo-26 **(E)**, EAPG-mo-39 **(F)**, EAPG-co-13 **(G)**.

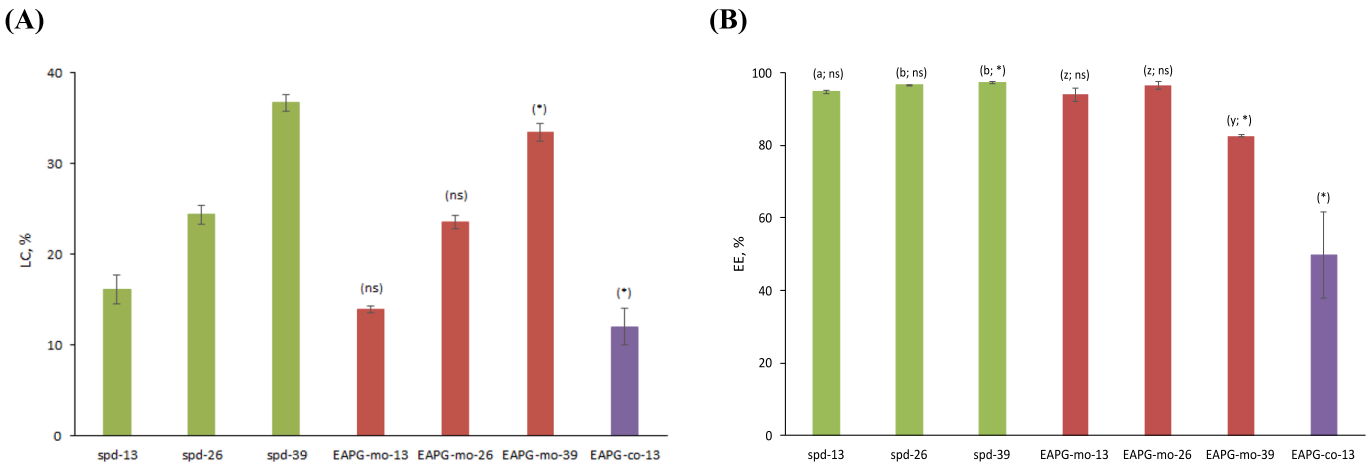

Fig. 3. Load capacity (LC) **(A)** and encapsulation efficiency (EE) **(B)** of the fish oil-loaded capsules (13, 26 and 39 wt% fish oil) produced by spray-drying (spd-), EAPG monoaxial (EAPG-mo-) and EAPG coaxial (EAPG-co-). Letters (a–b) or (y-z) indicate statistical differences ($p \leq 0.05$) between capsules produced by spray-drying or monoaxial electrospraying, respectively. For capsules with the same oil content, an asterisk (*) indicate statistical differences ($p \leq 0.05$), whereas "ns" indicate no statistical differences ($p > 0.05$) between capsules.

oil droplet size of the reconstituted emulsion for sample EAPG-mo-39 might be related to its lower EE (EE = 83%, Fig. 3B), meaning that more non-encapsulated oil was available to coalesce after the redispersion of the capsules. Indeed, at a fixed particle size, increasing the oil load might result in a reduced entrapment of the oil within the encapsulating matrix (Drosou et al., 2017), which explains our observations for small capsules as those obtained in monoaxial EAPG (e.g., thinner encapsulating wall). Nonetheless, the EE of the spray-dried capsules was not affected by their oil load ($p > 0.05$, Fig. 3B) due to their large particle size (Fig. 2A-C). Overall, our results are in line with other studies reported in the literature on the encapsulation of omega-3 PUFAs rich oils by EAPG in the monoaxial configuration within carbohydrates or proteins-based matrices (e.g., maltodextrin or whey protein concentrate; EE = 65–85%) (Prieto & Lagaron, 2020) and mixtures carbohydrate: protein-based matrices (e.g., glucose syrup:whey protein concentrate; EE = 78–86%) (García-Moreno et al., 2018). This could be related to the highly-physically stable emulsions produced in the current study with considerably low droplet size (Fig. S1A of the Supplementary Material).

It should be noted that the lowest EE value was observed for the capsules produced by coaxial EAPG (EE ~50%) (Fig. 3B). As reported in the literature, extractable oil fractions in encapsulated systems comprises both non-encapsulated surface oil and encapsulated oil that can be reached by the extracting solvent trough capillary or cracks present on the surface of the capsules and, depending on the extraction method, different fractions of the encapsulated oil can be reached (Drusch & Berg, 2008). Theoretically, optimal coaxial electrospraying leads to capsules consisting of an oil droplet located at the core of the structure surrounded by the encapsulating wall when neat fish oil is infused. Therefore, the extraction of a portion of the encapsulated oily core might be likely to occur if the dried encapsulating shell presents some capillary for solvent diffusion. In the current study, the capsules were immersed in the extracting solvent and thoroughly washed by shaking the mixture for a certain time, thus extraction of the encapsulated oil through capillary or cracks cannot be ruled out. This might explain the low EE values reported for this system (EAPG-co-13 sample), which do not correlate with its high oxidative stability, as will be further discussed below.

3.1.3. Oxidative stability

To investigate the oxidative stability of the capsules obtained, the development of secondary volatile oxidation products (SVOPs) derived from the oxidation of omega-3 PUFAs (e.g., 1-penten-3-ol) was monitored throughout 6 weeks of storage at 25 °C (Fig. 4). Interestingly, our results showed that the encapsulation technology used to produce the capsules, but not the oil load, significantly influenced ($p \leq 0.05$) their oxidative stability. The most oxidized capsules during storage were those produced by monoaxial EAPG (EAPG-mo systems), whereas the spray-dried (spd-) and the coaxially EAPG capsules (EAPG-co-13 sample) were significantly less oxidized. Furthermore, for the different oil loads evaluated, the development and the final content of the selected SVOPs found in the encapsulated systems produced either by spray-drying or monoaxial EAPG were not statistically different among the samples ($p > 0.05$; Fig. 4). These results are in agreement with those previously reported by Linke et al. (2021), who found that the oil load of the capsules obtained by spray-drying (e.g., 10, 15 or 20 wt% fish oil load) did not influence the lipid oxidation rate and extent, as indicated by the hydroperoxides content and anisidine value of the samples (expressed per kg of total oil). These authors concluded that the oxygen availability and supply played a major role on the lipid oxidation of the fish oil-loaded capsules rather than the oil load (Linke et al., 2021). In fact, oxygen solubility and diffusivity through the dried encapsulating matrix has been extensively reported to be a key factor promoting lipid oxidation of encapsulated systems (Boerekamp et al., 2019; Linke et al., 2020, 2021; Rahmani-Manglano et al., 2023) and its supply will vary depending on the physicochemical properties of the capsules including EE, permeability/thickness/porosity of the encapsulating wall or the particle size. Therefore, taking into account that the formulation of the emulsion-based capsules was the same for a fixed oil load and that the EE values were not significantly different among the samples ($p > 0.05$; Fig. 3B), the lower oxidative stability reported for the EAPG-mo systems compared to the spray-dried capsules could be discussed on the basis of a higher oxygen uptake during storage influenced by: i) their smaller particle size, which implies a high contact area with environmental oxygen, and ii) their thinner encapsulating walls, derived from the same oil load but lower particle size, which implies a favored contact between encapsulated oil and oxygen diffusing through the dried matrix. Based on the latter, low oxidative stability could be also expected for the capsules produced by coaxial EAPG (EAPG-co-13 sample). As previously mentioned, by coaxial electrospraying the oil is theoretically located at the core of the dried matrix, contrarily to emulsion-based encapsulation methods (e.g., EAPG monoaxial) where a random distribution of oil droplets within the encapsulating wall is achieved. Our results suggest that the coaxially-encapsulated neat fish oil was more efficiently protected from environmental oxygen (i.e., the oxygen needs to penetrate throughout a thicker encapsulating wall before reaching the oily core) compared to monoaxially-encapsulated fish oil where lipid oxidation could be more easily initiated by diffusing oxygen encountering oil

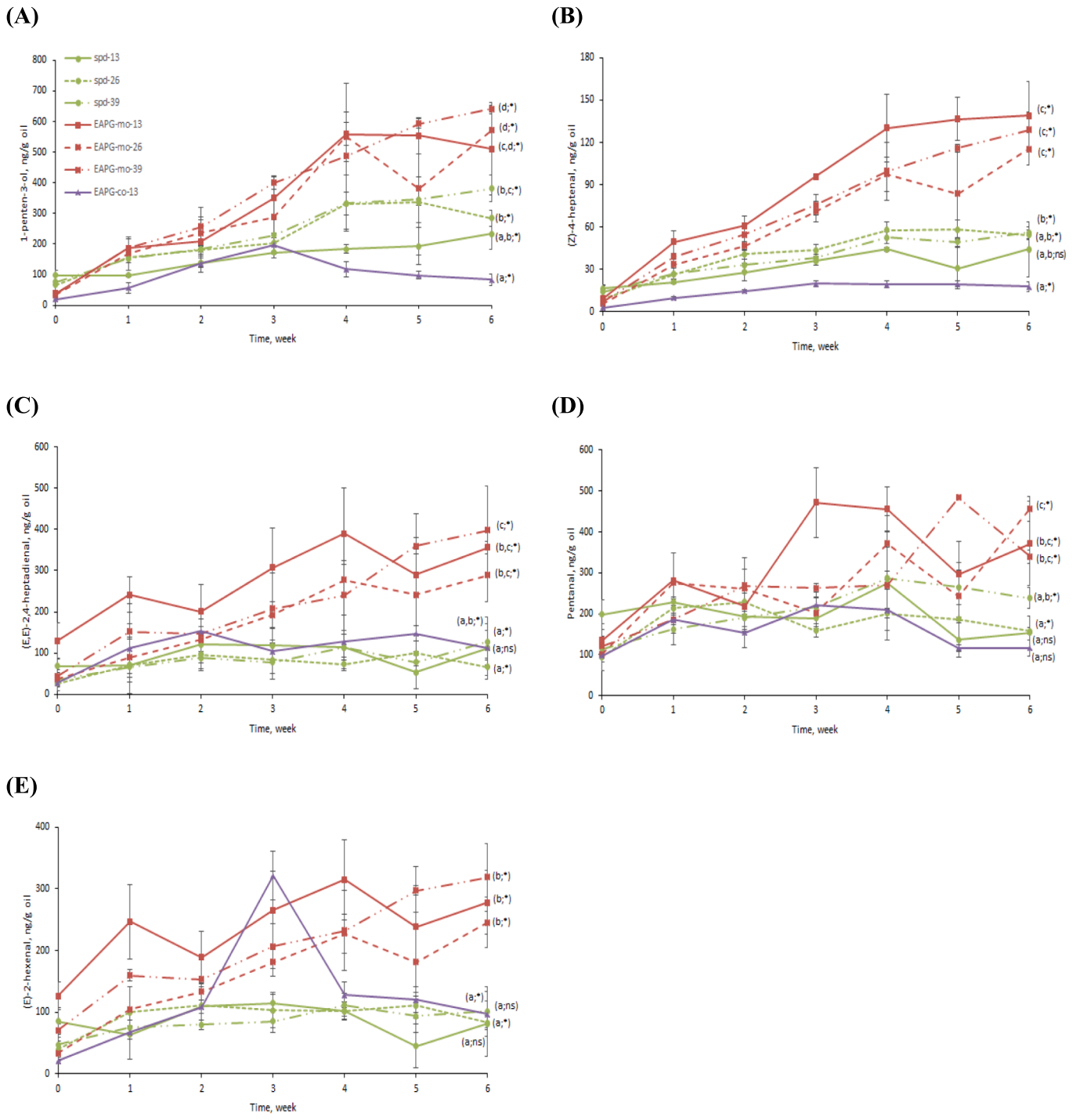

Fig. 4. Secondary volatile oxidation products (SVOPs) of the fish oil-loaded capsules (13, 26 and 39 wt% fish oil) produced by spray-drying (spd-), EAPG monoaxial (EAPG-mo-) and EAPG coaxial (EAPG-co) during storage: 1-penten-3-ol **(A)**; (*Z*)-4-heptenal **(B)**; (*E,E*)-2,4-heptadienal **(C)**; Pentanal **(D)**; (*E*)-2-hexenal **(E)**. Samples followed by a letter (a–e) indicate statistical differences ($p \leq 0.05$) between capsules. Means within the same sample followed by an asterisk (*) indicate statistical differences ($p \leq 0.05$) between week 0 and week 6. Means within the same sample followed by "ns" indicate no statistical differences ($p > 0.05$) between week 0 and week 6.

droplets dispersed in the encapsulating matrix closely to the capsule surface. It is also worth noting that the oxidation rate and extent of the coaxially EAPG capsules (EAPG-co-13 sample) was similar to or better than those of the spray-dried capsules (spd- systems) for all the selected SVOPs studied (Fig. 4) despite their significantly different EE values ($p \leq 0.05$; Fig. 3). A low oxidative stability of encapsulated systems is often related to low EE values due to the direct contact of the unprotected non-encapsulated surface oil with environmental prooxidants (e.g., oxygen). However, it has been reported that the contribution of this non-encapsulated oil fraction to the overall lipid oxidation is minor when the fraction of the non-encapsulated surface oil is rather low compared to the fraction of the encapsulated oil (Linke et al., 2020). This is also an indication that, for the coaxially EAPG systems, a fraction of the encapsulated oil was extracted during the EE measurements, thus

overestimating the non-encapsulated oil fraction (EE ~50%, Fig. 3), since a high oxidative stability was found for this system. In addition, it is particularly interesting to note that the lowest content of the selected SVOPs immediately after production was found for the coaxially EAPG capsules (EAPG-co-13 sample; Fig. 4A-E). This finding further confirms that initial lipid oxidation was significantly reduced by producing the capsules in the coaxial configuration since: i) the fish oil was not emulsified (it was infused through the core as NFO), which avoids an increase in temperature and the inclusion of oxygen during homogenization, and ii) the drying process occurred at ambient temperature.

3.2. Physicochemical characterization of the fortified salad dressing

Based on the results presented, the salad dressing samples (SD-) were fortified with the selected capsules produced by the three technologies studied: spray-drying (spd-) and monoaxial EAPG (EAPG-mo) with 13 or 39 wt% fish oil loads, and coaxial EAPG (EAPG-co) with 13 wt% fish oil load. A salad dressing sample fortified with NFO (SD-NFO) was also produced as a control.

3.2.1. Physical stability: Oil droplet size, viscosity and color

The physical stability of the fortified salad dressing samples was investigated by monitoring the changes in the oil droplet size, the viscosity and the color at the beginning (day 0) and at the end (day 28) of the storage time.

After production, the salad dressing samples showed different oil droplet size distributions depending on the fortification approach (e.g., emulsion or non-emulsion-based delivery systems; Fig. S2A of the Supplementary Material). The samples enriched with the capsules produced by the emulsion-based encapsulation methods (e.g., SD-spd or SD-mo samples) showed the highest proportion of small oil droplets (first peak centered at ~0.3 μm), irrespective of the oil load of the capsules (Fig. S2A of the Supplementary Material). Indeed, when comparing these curves to that of the reconstituted emulsions of the emulsion-based capsules after drying (peak centered at ~0.3–0.4 μm; Fig. S1B of the Supplementary Material), it can be concluded that the proportion of small oil droplets of such fortified dressings represents the droplet size of the encapsulated fish oil. Hence, the other peaks observed at larger diameter values (from 1 up to 200 μm) are a consequence of the RSO droplets dispersed in the food matrix. Overall, these results are indicating that the integrity of the water-soluble encapsulating wall was retained after fortification of a food matrix with a relatively high water content (i.e., 25 wt% fat), thus the production process was efficiently optimized. Conversely, the proportion of small oil droplets was rather low (<0.4% volume) for the samples enriched with the coaxially EAPG capsules (SD-co-13) or the NFO (SD-NFO) since in both cases the fish oil was not emulsified prior to food fortification (Fig. S2A of the Supplementary Material). In Table 1, the D[3,2] and the D[4,3] values of the enriched salad dressing samples are shown. In the fortified salad dressings, the D[3,2] value is more influenced by the size of small droplets present in the matrix whilst the D[4,3] value is more affected by the size of the bulk oil droplets (Miguel et al., 2019). As expected, higher D[3,2] values were found in the samples fortified with the non-emulsified delivery systems (e.g., SD-co-13 and SD-NFO samples) after production compared to those enriched with the emulsion-based capsules (Table 1). Furthermore, significant differences were observed in the D[4,3] values among the samples at day 0 (Table 1). High D[4,3] values of emulsion-like food products fortified with dried delivery systems (e.g., mayonnaise) have been related to a limited reduction of the oil droplet size during sample preparation in presence of the encapsulates (Hermund et al., 2019; Miguel et al., 2019). However, the opposite was found in the current study. This is explained on the basis that the dried delivery systems were added to a previously produced salad dressing and mixed for a certain time until complete dispersion, which allowed a better reduction of the RSO oil droplets size during preparation of the food matrix. During storage, no creaming or phase separation was observed. However, changes in the oil droplet size of the samples occurred (Table 1 and Fig. S2B of the Supplementary Material). After storage, a small but significant increase in the D[3,2] and D[4,3] values was observed for the dressing samples fortified with the emulsion-based capsules, contrary to SD-co-13 and SD-NFO samples ($p \leq 0.05$, Table 1). This suggests that, for SD-co-13 and SD-NFO samples, potential oil floccules present after production disintegrated during storage (Miguel et al., 2019). Nonetheless, for the salad dressings fortified with the emulsion-based delivery systems, flocculation and/or coalescence of the oil droplets occurred, which can be in part attributed to a partial, although limited, disintegration of the encapsulating shell throughout storage (Miguel et al., 2019; Rahmani-Manglano, González-Sánchez, et al., 2020).

It should be noted that all the fortified dressings presented a pseudoplastic behavior (Fig. S3 of the Supplementary Material) and, depending on the delivery system used, different apparent viscosities were observed (Table 1). After production, the lower apparent viscosity corresponded to the sample fortified with NFO (SD-NFO sample) and it could be observed that the viscosity of the samples significantly increased as the oil load of the dried delivery systems decreased ($p \leq 0.05$; Table 1). The higher viscosity of the dressing samples fortified with the dried delivery systems is attributed to the thickening effect of the intact capsules dispersed in the food matrix (Miguel et al., 2019; Rahmani-Manglano, González-Sánchez, et al., 2020). Therefore, since more capsules with a lower oil load are required to achieve the fixed fish oil load of the food matrix, the viscosity of such samples was expected to be higher. During storage, slight, and in most cases, non-significant ($p > 0.05$) changes in the apparent viscosity values were observed (Table 1). In the literature, changes in the apparent viscosity of emulsion-like food products during storage have been related to changes in the oil droplet size of the matrices (Miguel et al., 2019; Rahmani-Manglano, González-Sánchez, et al., 2020). Overall, a reduced specific surface area of the

Table 1
Oil droplet size, apparent viscosity ($\gamma = 10\ s^{-1}$) and yellowness index (YI) of the fortified salad dressing with encapsulated fish oil (SD-spd-, spray-drying; SD-mo-, EAPG monoxial; SD-co-, EAPG coaxial) at different oil loads (13 or 39 wt%) or neat fish oil (SD-NFO).

	Oil droplet size				Apparent Viscosity		Yellowness Index	
	Day 0		Day 28		($\gamma = 10\ s^{-1}$), Pa·s		(YI)	
Sample	D[3,2], μm	D[4,3], μm	D[3,2], μm	D[4,3], μm	Day 0	Day 28	Day 0	Day 28
SD-spd-13	0.85 ± 0.02^{a}	16.31 ± 0.55^{a}	$0.94 \pm 0.02^{a;*}$	$19.45 \pm 0.90^{b;ns}$	9.80 ± 0.34^{c}	$10.01 \pm 0.36^{d;ns}$	16.00 ± 0.23^{b}	$20.14 \pm 1.44^{b,ns}$
SD-spd-39	0.86 ± 0.03^{a}	20.85 ± 0.41^{b}	$0.97 \pm 0.03^{a;*}$	$24.57 \pm 0.30^{c;*}$	6.45 ± 0.13^{b}	$6.11 \pm 0.10^{b,ns}$	$14.83 \pm 0.25^{a,b}$	$17.87 \pm 0.50^{a,b;ns}$
SD-mo-13	0.89 ± 0.04^{a}	20.35 ± 0.29^{b}	$0.99 \pm 0.02^{a;*}$	$14.69 \pm 0.04^{a;*}$	9.94 ± 0.07^{c}	$8.67 \pm 0.09^{c,*}$	16.15 ± 0.46^{b}	$24.23 \pm 0.88^{c,*}$
SD-mo-39	0.86 ± 0.01^{a}	22.04 ± 0.26^{c}	$1.10 \pm 0.03^{b;*}$	$24.85 \pm 1.09^{c;ns}$	$6.34 \pm 0.07^{a,b}$	$5.83 \pm 0.01^{b,*}$	$14.81 \pm 0.12^{a,b}$	$20.08 \pm 1.08^{b,*}$
SD-co-13	2.07 ± 0.03^{c}	24.47 ± 0.25^{d}	$1.59 \pm 0.06^{c;*}$	$23.88 \pm 0.49^{c;ns}$	8.79 ± 0.65^{c}	$8.73 \pm 0.28^{c,ns}$	19.16 ± 0.61^{c}	$20.20 \pm 0.92^{b,c;ns}$
SD-NFO	1.81 ± 0.10^{b}	25.62 ± 0.40^{e}	$1.66 \pm 0.02^{c;ns}$	$22.93 \pm 0.41^{c;*}$	5.21 ± 0.02^{a}	$4.14 \pm 0.04^{a,*}$	14.24 ± 0.03^{a}	$15.00 \pm 1.04^{a,ns}$

Means within the same column followed by a letter, a-e, indicates statistical differences ($p \leq 0.05$) between samples. Means within the same sample followed by an asterisk, *, indicates statistical differences ($p \leq 0.05$) between day 0 and day 28. Means within the same sample followed by "ns" indicates no statistical differences ($p > 0.05$) between day 0 and day 28.

dispersed phase leads to less friction between the droplets, hence the larger the oil droplet size, the lower the apparent viscosity, which correlates well with our findings (Table 1).

Color measurement is a common analysis carried out in the food industry to address the quality changes of a food product as a result of processing and/or storage (León et al., 2006). As shown in Table 1, the yellowness index (YI) of the fortified dressings after production were in the same magnitude (YI = 14–19), although the value reported for the sample enriched with the coaxially EAPG capsules (SD-co-13 sample) was significantly higher ($p \leq 0.05$). After 28 days of storage, the YI of all the samples only increased slightly, which implies a high chemical stability of the food matrices (e.g., non-enzymatic browning reactions did

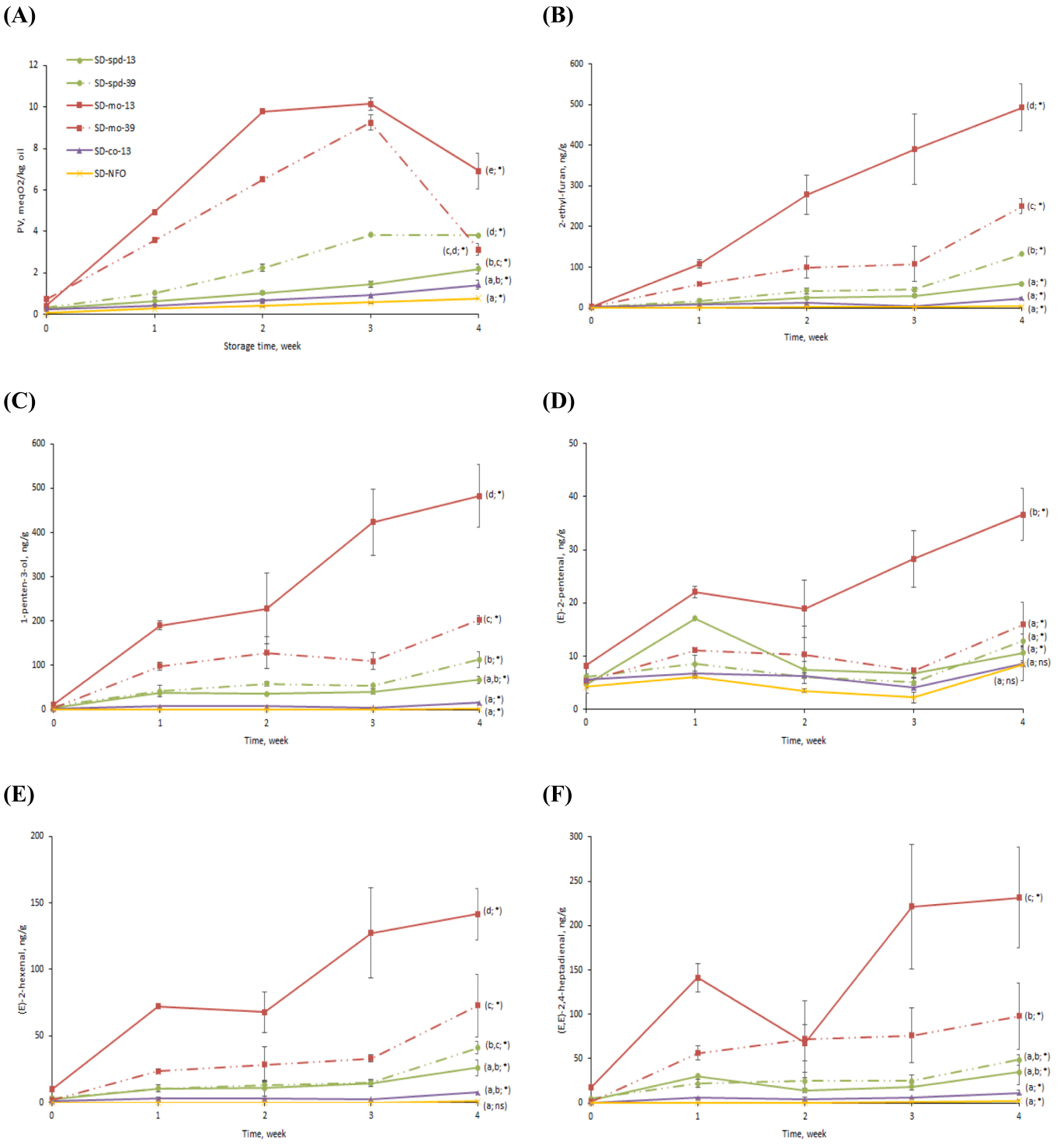

Fig. 5. Peroxide value (PV) **(A)** and secondary volatile oxidation products (SVOPs) **(B-F)** of fortified salad dressing enriched with encapsulated (SD-spd-, spray-drying; SD-mo-, EAPG monoaxial; SD-co-, EAPG coaxial) at different oil loads (13 or 39 wt%) or neat fish oil (SD-NFO) during storage. Means within the same sampling point followed by a letter, a-e, indicates statistical differences ($p \leq 0.05$) between samples. Means within the same sample followed by an asterisk (*) indicates statistical differences ($p \leq 0.05$) between week 0 and week 4. Means within the same sample followed by "ns" indicate no statistical differences ($p > 0.05$) between week 0 and week 4.

not occur during the storage time). This was further confirmed by the pH value of the dressing samples, which was constant (pH = 4.7) during the storage time.

3.2.2. Oxidative stability

3.2.2.1. Peroxide value (PV) and tocopherol content. Fig. 5A shows the evolution of the peroxide value (PV) of the fortified dressing samples during storage. After production, a low PV was found in all the samples (PV = 0.1–0.7 meq O_2/kg oil) irrespective of the fortification strategy used. However, significantly different oxidation rates could be observed during storage depending on the processing of the fish oil prior to food fortification (e.g., emulsion or non-emulsion-based delivery systems). Moreover, among the dressing samples enriched with the emulsion-based capsules, also different trends could be distinguished depending on the technology used to produce such capsules (e.g., spray-drying or EAPG monoaxial). For instance, a sharp increase in the PV occurred for the samples fortified with the capsules produced by EAPG technology in the monoaxial configuration (SD-mo-13 and SD-mo-39 samples) up to the third week of storage, followed by a sharp decrease. Conversely, a sustained increase in the PV was observed for the samples fortified with the spray-dried capsules (SD-spd-13 and SD-spd-39 samples) until the end of the storage time (Fig. 5A). These results are in line with those reported for the oxidative stability of the emulsion-based capsules, being those produced by monoaxial EAPG the less oxidatively stable throughout storage (Fig. 4). The latter is also an indication that the emulsion-based capsules were not disrupted after food fortification since different oxidation trends were observed depending on the technology used to produce such encapsulates despite the formulation was the same for a fixed oil load and the EE and the droplet size of the encapsulated fish oil was not significantly different ($p > 0.05$) among the samples (e.g., spd-13 and EAPG-mo-13 or spd-39 and EAPG-mo-39; Fig. 3B and Fig. S1 of the Supplementary Material).

In case of the salad dressing samples fortified with the non-emulsified delivery systems (e.g., coaxially EAPG capsules, SD-co-13 or NFO, SD-NFO) a slight increase in the PV was also noted during storage. However, although the final values were significantly higher compared to that of the beginning of the storage experiment ($p \leq 0.05$, Fig. 5A), they were still very low (PV < 1.5 meq O_2/kg oil). Surprisingly, the lowest PV was found for the dressing sample fortified with the "non-protected" NFO. In a previous study, Let at al. (2007a) produced a fish oil-enriched salad dressing (10 wt% fish oil) and also found that the highest oxidative stability corresponded to the sample fortified with NFO compared to the sample fortified with a fish oil-in-water emulsion. The authors argued that this finding could be attributed to the protective effect that the tocopherols present in the RSO exerted when it was mixed with the NFO before processing. Nonetheless, it seems that this is not the case in the current study. Firstly, the final PV of the salad dressing samples fortified with the non-emulsified delivery systems were comparable ($p > 0.05$) despite their different fortification approach (e.g., encapsulated fish oil by coaxial EAPG or NFO) and preparation procedures (e.g., mixing of the NFO with the RSO before processing or dispersion of the dried fish oil-loaded capsules to an already produced salad dressing). Secondly, and considering the tocopherol content of the fortified dressing samples, it can be observed that their concentration was rather constant during storage and similar among the samples, irrespective of the delivery system used (Fig. S3 of the Supplementary Material). Therefore, this led us to conclude that the oxidative stability of the dressing samples was not influenced by their tocopherol content, but it was most likely to be influenced by the fish oil oxidative status prior to food fortification (e.g., emulsified or non-emulsified), as will be further discussed below.

3.2.2.2. Secondary volatile oxidation products (SVOPs) – Dynamic headspace GC–MS. The content of selected SVOPs of the fortified dressing samples (e.g., 2-ethyl-furan) during storage is shown in Fig. 5B-F. In line with the PV of the salad dressings, the highest content of the selected SVOPs was found in the samples fortified with the emulsion-based capsules (e.g., SD-spd and SD-mo samples), being those enriched with the capsules produced by monoaxial EAPG the most oxidized at the end of the storage time (SD-mo-13 and SD-mo-39 samples; Fig. 5B-F). Therefore, taking into account that the integrity of the capsules was retained after processing, the different oxidation trends of the salad dressing samples fortified with the emulsion-based delivery systems could also be attributed to the different physicochemical properties of the capsules influencing the diffusivity of prooxidant species present in the food matrix through the encapsulating wall. Nonetheless, contrary to what we observed during storage of the dried delivery systems (Fig. 4), the oxidation rate and extent of the dressing samples enriched with the emulsion-based capsules were influenced by their oil load (13 or 39 wt %). Moreover, this influence was different depending on the technology used for their production (spray-drying or monoaxial EAPG) (Fig. 5B-F). For instance, whilst a sharp increase in the content of all the selected SVOPs was observed for the sample SD-mo-13 from the beginning of the storage time, the oxidation rate and extent were significantly lower for the sample SD-mo-39 (Fig. 5B-F). Conversely, the dressing samples fortified with the spray-dried capsules oxidized similarly throughout storage irrespective of their oil load (SD-spd-13 and SD-spd-39 samples), leading to final concentrations of the SVOPs not significantly different between the samples ($p > 0.05$; Fig. 5B-F). Increasing the oil load of EAPG-mo capsules resulted in a lower proportion of small particles, approximately ~10% fewer small particles between 5 and 10 μm of sample EAPG-mo-39 compared to EAPG-mo-13 (Fig. 2D,F), whereas the particle size distribution of the spray-dried capsules was the same irrespective of their oil load (13 or 39 wt%, Fig. 2A,C). Thus, our results suggest that although the size of the capsules had a low impact on the oxidative stability of the EAPG-mo-based delivery systems (Fig. 4), it more notably influenced the differences observed on the oxidative stability of the fortified dressing samples (Fig. 5B-F). In any case, it should be borne in mind that the capsules are found in different environments (e.g., stored in a bottle or dispersed in a food matrix) and therefore exposed to different prooxidant species (e.g., oxygen and/or metal ions). Prooxidants present in food promote lipid oxidation, with transition metals being one of the most important prooxidants of food systems (Ghelichi et al., 2021). Metal ions are excellent prooxidants since they can trigger the initiation phase of lipid autoxidation and also promote the decomposition of lipid hydroperoxides giving rise to SVOPs (Jacobsen et al., 2013). Although the content of metal ions in salad dressings is suggested to be low (Jacobsen et al., 2008), it has been demonstrated that lipid oxidation mediated by transition metals is a very important factor affecting their oxidative stability (Jacobsen et al., 2008; Let et al., 2007b). In the literature, the diffusion of molecules in carbohydrate-based systems has been investigated, highlighting the importance of the molecular diameter of the diffusing molecule travelling through the cavities of the glassy matrix (Orlien et al., 2000). Furthermore, it has been reported that transition metal ions are capable to diffuse through the peptide-based interfacial layer of oil-in-water emulsions due to their small molecular size (Berton-Carabin et al., 2014). Thus, our results suggest that the higher surface-to-volume ratio of the EAPG-mo-13 capsules, compared to EAPG-mo-39 capsules, might have favored the contact and diffusion of the small-sized metal ions present in the food matrix through the encapsulating wall.

On the other hand, and also in line with the PV of the dressing samples (Fig. 4), the low content of selected SVOPs observed for the samples fortified with the non-emulsified delivery systems (e.g., SD-co-13 and SD-NFO samples) was rather constant throughout storage (Fig. 5B-F). These results further confirm that the oxidative stability of the fortified dressing samples was strongly influenced by the fish oil oxidative status prior to food fortification, as well as by the specific surface area of the fish oil droplets dispersed within the food matrix. Firstly, for the non-emulsion-based delivery systems, the inclusion of

oxygen and the temperature increase that occurs as a result of the emulsification step, both promoting lipid oxidation during processing, was avoided (Serfert et al., 2009). Furthermore, as a result of the emulsification step, the fish oil droplet size was significantly reduced (Fig. S2A of the Supplementary Material), implying an increased contact area with prooxidants species. In addition, in case of EAPG-co-13 capsules, drying was carried out at ambient temperature. In this line, it is worth noting that the emulsion-based delivery systems (e.g., spd- and EAPG-mo capsules) presented a poorer initial oxidative status than that of the coaxially EAPG capsules (EAPG-co capsules; Fig. 4A-E) or the NFO ($PV = 0.33 \pm 0.06$ meq O_2/kg oil), which in turn confirms that the onset of lipid oxidation of these systems occurred during the production of the encapsulated systems (e.g., air inclusion and high temperature). This further affects the oxidative stability of the food system since, once the lipid radicals are present in the oil, lipid oxidation occurs as a chain reaction (Rahmani-Manglano, García-Moreno, et al., 2020).

4. Conclusions

Our results show that the physicochemical properties of the capsules (e.g., particle size or EE) and their oxidative stability were significantly influenced by the encapsulation technique, rather than by the oil load. Despite their high EE (EE = 83–97%), the most oxidized capsules were those produced by monoaxial EAPG, which may be attributed to their small particle size influencing the oxygen diffusivity through the encapsulating wall together with the initial degree of lipid oxidation of the encapsulated fish oil after processing due to emulsification. On the other hand, the non-emulsion-based encapsulation approach by coaxial EAPG resulted in an enhanced oxidative stability of the encapsulated fish oil during and after processing. The oxidative stability of the fortified salad dressings was in line with the oxidative stability of the dried delivery systems. The salad dressing samples fortified with the non-emulsified delivery systems (e.g., coaxially EAPG capsules or neat fish oil) showed the highest oxidative stability through storage, as confirmed by their low PV and the low content of the selected volatiles (e.g., 2-ethyl-furan). This finding can be attributed to the better oxidative status of the fish oil since it was not emulsified prior to food fortification. Taken altogether, our results showed that coaxial EAPG is a promising technique to produce neat fish oil-loaded capsules aimed as omega-3 delivery systems to produce fortified food matrices with omega-3 PUFAs.

CRediT authorship contribution statement

Nor E. Rahmani-Manglano: Methodology, Formal analysis, Writing – original draft. **Emilia M. Guadix:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition. **Betül Yesiltas:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Cristina Prieto:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Jose M. Lagaron:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Charlotte Jacobsen:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Pedro J. García-Moreno:** Conceptualization, Methodology, Supervision, Writing – review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.137157.

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