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Bioaccessibility and Stability Studies on Encapsulated Phenolics and Carotenoids from Olive and Tomato Pomace: Development of a Functional Fruit Beverage

Maria Katsouli, Ioanna V. Thanou, Evgenia Raftopoulou, Athina Ntzimani, Petros Taoukis and Maria C. Giannakourou *

Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, 5 Heroon Polytechniou Str., 15780 Athens, Greece; mkatsouli@chemeng.ntua.gr (M.K.); ithanou@chemeng.ntua.gr (I.V.T.); evgeniaraf8@gmail.com (E.R.); ntzimani@chemeng.ntua.gr (A.N.); taoukis@chemeng.ntua.gr (P.T.)

* Correspondence: mgian@chemeng.ntua.gr; Tel.: +30-2-107-723-166

Featured Application: This study highlights the development of a functional beverage enriched with lycopene and phenolic compounds, extracted from food industry by-products and encapsulated through nanoemulsion technology. This functional beverage can boost the bioaccessibility of bioactive compounds, contributing to health benefits and enhancing antioxidant delivery. It offers potential applications in the food industry, aligning with consumer demand for innovative, health-promoting products, providing a competitive edge in the expanding wellness sector.

Abstract: This study pertains the encapsulation of bioactive compounds, specifically phenolic compounds and lycopene, extracted from olive and tomato by-products via oil-in-water (O/W) nanoemulsions and their potential application in functional beverages. The effect of various edible oils (olive pomace oil (OPO), sunflower oil (SFO), corn oil (CO), fish oil (FO), and canola oil (CLA)) in the lipid phase and antioxidants (ascorbic acid and phenolic extracts) in the aqueous phase on the physicochemical properties of oil-in-water (O/W) nanoemulsions enriched with lycopene was evaluated, along with the bioaccessibility of the encapsulated bioactive compounds using the static INFOGEST in vitro simulation protocol for gastrointestinal food digestion. All examined edible oils led to nanoemulsions with uniform droplet sizes (droplet size < 300 nm, droplet distribution < 0.3) and high stability during storage at 4 °C, with FO being the smallest, at 259.3 ± 9.1 nm, and OPO the largest, at 286.6 ± 10.0 nm. Ascorbic acid increased the droplet size by 5–8%, improved droplet distribution, and led to a lower deterioration rate (-0.014 d^{-1}) when compared to the "control" counterparts (-0.037 d^{-1}). Lycopene bioaccessibility was significantly affected by the lipid phase, with OPO exhibiting the highest percentage (53.8 ± 2.6%) and FO the lowest (40.1 ± 2.1%). The OPO nanoemulsion was selected for the development of a functional beverage, showing excellent long-term stability. The phenolic compound concentration remained consistent during storage, and the lycopene degradation rate was minimal, at -0.0088 d^{-1}, resulting in an estimated shelf life of 165 days at 4 °C, based on a 50% reduction in lycopene content. Similarly, phenolic compounds demonstrated high bioaccessibility, without a significant dependence on the lipid phase, and stability during shelf life, enhancing the beverage's overall antioxidant profile. These results indicate that O/W nanoemulsions are effective delivery systems for functional beverages, offering improved stability and bioaccessibility of lycopene.

Keywords: o/w nanoemulsions; functional beverage; shelf-life studies; lycopene extract; phenolic compounds; bioaccessibility

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1. Introduction

The olive oil and tomato processing industries are considered the largest sectors of primary production that generate high amounts of waste (fruit peels, seeds, pomace, liquid effluents) across the entire chain; therefore, the sustainable and effective valorization of their by-products into high-value-added products is crucial from both an environmental and an economic perspective. Olive and tomato pomace, frequently discarded as animal feed or soil conditioner, are rich in polyphenols, carotenoids, fibers, and other bioactive components which possess antioxidant, anti-inflammatory, and other health benefits [1,2]. Olive and tomato pomace are important by-products of the olive oil and tomato processing industries, presenting valuable opportunities for waste valorization in the development of functional foods and beverages. The global olive oil industry generates an estimated 30 million tons of olive pomace annually, while the tomato processing industry produces around 12 million tons of tomato pomace each year. The process of olive oil extraction produces 20% olive oil as a product and both 30% of moist solid waste with 65% moisture (pomace or jift) and 50% liquid waste, called olive mill wastewater (OMW), as a by-product [3,4]. Similarly, in 2023, approximately 44.2 million tons of tomatoes were utilized in the tomato processing sector to produce different types of tomato-based products. With global tomato production exceeding 180 million tons annually, the industry produces pomace that accounts for 5%–10% of total tomato weight, contributing to extensive waste generation worldwide. Without effective management, these by-products pose significant environmental challenges. For example, the olive oil production process yields approximately 800 kg of solid pomace for each ton of olives processed, leading to the accumulation of millions of tons of waste in Mediterranean countries and other olive-growing regions. These by-products are naturally rich in bioactive compounds such as polyphenols, carotenoids, and dietary fibers. However, despite these beneficial components, much of this waste remains underutilized. Although some valorization practices are in place, such as their use as animal feed, biofuel sources, soil conditioners, and nutraceutical extracts, factors like high moisture content, perishability, and seasonal availability often limit these applications. Furthermore, these traditional uses are generally low-value and do not adequately address the large scale of waste produced. The extraction of bioactive compounds from olive and tomato pomace for direct incorporation into food systems represents a promising avenue for sustainable valorization [2,4]. These bioactive compounds can be incorporated into functional food products, increasing their nutritional value and providing additional health advantages to consumers. This strategy is consistent with the ideas of circular economy and sustainable development, encouraging resource efficiency and generating new revenue streams for the food business [5,6]. Furthermore, the incorporation of these bioactives into novel delivery systems, such as nanoemulsions, can enhance their bioaccessibility, stability and effectiveness, resulting in the development of advanced functional foods and beverages that meet the growing consumer demand for health-promoting products. Nanoencapsulation, for instance, offers a way to improve the bioavailability and stability of bioactives like lycopene and hydroxytyrosol, enhancing their functionality in beverage matrices and other food applications. By leveraging these bioactives in functional foods, companies can achieve more sustainable, high-value solutions that appeal to consumers' growing interest in eco-friendly, health-promoting products. This innovative approach aligns with circular economic principles, as it helps minimize waste while providing nutritional and economic benefits. Incorporating bioactives from olive and tomato pomace into functional beverages not only enhances waste utilization and sustainability, but also provides substantial economic returns by transforming these by-products into high-value ingredients. This valorization approach highlights the potential of olive and tomato pomace in next-generation food applications, addressing environmental concerns, supporting consumer health, and paving the way for sustainable innovation in the food industry [4,7].

Bioaccessibility refers to the amount of a compound that is released from the food matrix and becomes accessible for absorption by the human body, while bioavailability indicates the proportion of the compound that is absorbed and reaches the target tissues

to perform its bioactivity or to be stored [8]. Bioavailability is determined using in vivo methods, whereas bioaccessibility is often assessed in vitro, providing multiple benefits such as lower cost, reduced time, and the avoidance of ethical concerns associated with the use of human and/or animal subjects [9]. A widely accepted in vitro method for bioaccessibility determination is the standardized INFOGEST protocol, which simulates the oral, gastric, and small intestine phases of human food digestion [10,11].

The growing interest in functional foods and beverages arises from their potential to deliver health-enhancing bioactive compounds in innovative and appealing forms [12]. Polyphenols obtained from olive pomace and lycopene extracted from tomato pomace, among other bioactive compounds, have attracted a lot of attention due to their strong anti-inflammatory, antioxidant, and anticancer properties. However, their applicability in food and beverages is limited by bitter taste, poor water solubility, low bioaccessibility, and low oxidation stability under processing and storage conditions [13–15]. Nanoemulsions offer an effective solution to these challenges, as encapsulating bioactive compounds within nano-sized droplets enhances their solubility, stability, and bioaccessibility. This novel strategy not only increases bioactive ingredient distribution but also assures their stability during processing and storage, preserving the sensory and nutritional attributes of the final functional product [7,16–19].

In terms of nanoemulsion formulation, optimizing homogenization conditions and carefully selecting emulsion composition are critical factors in the successful production of stable nanoemulsions. Homogenization conditions (pressure, number of cycles, temperature) have a significant impact on droplet size distribution and overall stability. Effective homogenization ensures the reduction of droplet size to the nanoscale, which is critical for achieving kinetic stability and enhancing bioavailability of active compounds. Furthermore, the composition of nanoemulsions, including the type and concentration of emulsifier, co-solvents, and phase ratios, significantly impact their physical and chemical stability and bioaccessibility. The type of emulsifier affects the bioaccessibility of the encapsulated bioactive compounds, serving a dual role: providing protection during digestion and enabling controlled release of the compounds to enhance their absorption by intestinal cells. Therefore, the careful selection of these components not only determines the colloidal properties of the nanoemulsion, but also plays a pivotal role in a variety of applications, including pharmaceutical delivery systems, food, and cosmetic formulations [20–22]. Thus, a thorough understanding of the effect of both homogenization conditions and emulsion composition is required for the advancement of nanoemulsion technology and its numerous applications.

The choice of the lipid phase in nanoemulsions, typically composed of edible oils classified as GRAS (generally recognized as safe), is crucial for enhancing the solubility, stability, and bioaccessibility of hydrophobic bioactive compounds like lycopene. Different vegetable oils with varying fatty acid compositions can significantly affect the physicochemical properties, release profiles, and bioaccessibility of the encapsulated compounds. Selecting an appropriate oil is therefore key to optimizing the delivery and effectiveness of bioactives in functional beverages [23–26]. Nanoemulsions, due to their small droplet size, provide a larger surface area for digestive enzymes, which improves the solubilization and incorporation of hydrophobic compounds into mixed micelles formed in the small intestine. Mixed micelles, formed by the action of bile salts in the small intestine, are crucial for the solubilization of hydrophobic compounds, aiding their absorption in the digestive track. Nanoemulsions rapidly form mixed micelles, thus promoting the solubilization of lipophilic compounds [27]. Investigating these factors can shed light on the effectiveness of nanoemulsion-based delivery systems in overcoming bioavailability barriers, potentially leading to better nutritional results [26,28,29]. Recent studies have demonstrated the successful incorporation of polyphenols and lycopene into nanoemulsions, highlighting their potential in creating novel functional food products [7,19,25,28,30]. Recent advancements in nanoemulsion technology have demonstrated significant improvements in the bioavailability, stability, and bioaccessibility of hydrophobic bioactive compounds like

polyphenols and lycopene. Breakthroughs in the use of natural emulsifiers and innovative co-encapsulation techniques have further enhanced the release and absorption of these bioactives in the digestive system. Additionally, studies have shown that modifying the lipid phase and adjusting particle size distribution can significantly increase the bioaccessibility of these compounds, making nanoemulsion-based functional foods more effective in delivering health benefits. Looking ahead, advancements in nanoemulsion technology signify promising future trends in enhancing the bioavailability, stability, and bioaccessibility of hydrophobic and hydrophilic bioactive compounds from food by-products such as olive and tomato pomace into functional foods and beverages. Breakthroughs in the use of natural emulsifiers and innovative co-encapsulation techniques are set to improve the release and absorption of these bioactives in the digestive system. Innovations such as tailored lipid phases and precise control of particle size distribution are emerging as key strategies to maximize the release and absorption of bioactives in the digestive system. These advancements signal a transformative future for nanoemulsion-based applications, catering to the growing consumer interest in nutrient-rich, health-promoting products [27,31,32].

In this context, the present study explored the use of oil-in-water nanoemulsions enriched with polyphenols and lycopene as ingredients in a beverage model based on apple juice. The main focus was to explore the effect of lipid phase and storage conditions on the physicochemical properties and stability of the functional beverage, as well as on the bioaccessibility of encapsulated lycopene and polyphenols. The findings of this study aim at providing insights into the development of functional beverages that leverage the benefits of nanoemulsion technology to deliver health-promoting bioactive compounds effectively. A significant emphasis was placed on the stability of the o/w nanoemulsions and functional beverage at different storage conditions (4, 10, 25, and 40 °C), aiming at achieving the long-term durability required for beverage applications.

2. Materials and Methods

2.1. Materials

For the production of the nanoemulsions, olive pomace oil (OPO), sunflower oil (SFO), and corn oil (CO), produced by Minerva S.A. Greece, fish oil (FO) produced by Orkla Health AS, and canola oil (CLA) produced by Fytler Ltd. were bought from a local provider. They were selected as the lipid phase of the nanoemulsions due to their advantageous composition, cost-effectiveness, and availability. These oils, chosen for their ability to form stable nanoemulsions and their well-recognized health benefits, align with the study's focus on maximizing resource efficiency and functionality. Polyoxyethylene (20) sorbitan monopalmitate (Tween 40 (T40), PubChem: 87560417) was purchased from Acros Organics (Fair Lawn, NJ, USA). For analytical methods, Folin–Ciocalteu's reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and sodium carbonate, were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA), and petroleum ether, acetonitrile (HPLC grade), water (HPLC grade), methanol (HPLC grade), acetone, acetic acid, iso-octane, and p-anisidine were purchased by Acros Organics (Fair Lawn, NJ, USA). Enzymes and reagents used in the INFOGEST protocol were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). Lycopene extracts from tomato pomace were obtained with 100% ethyl acetate using fixed-bed semi-batch extraction, and polyphenols extracts from olive pomace were obtained using a mixture of water/methanol at a 40:60 ratio, as described by Tsevdou et al., 2024 [33], (Figure 1).

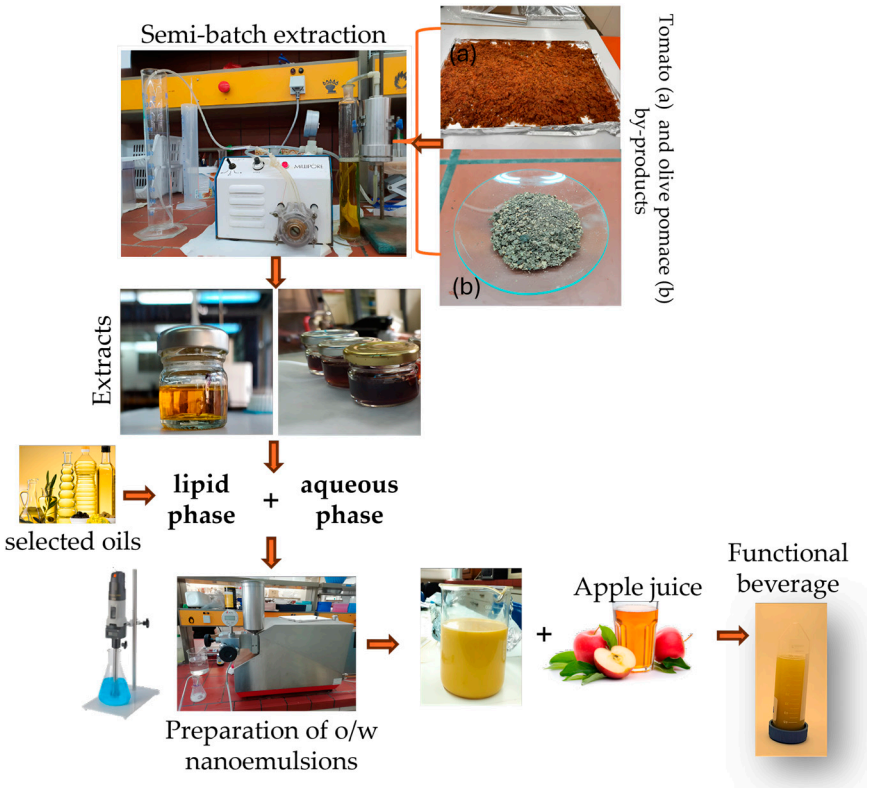


Figure 1. Flow diagram of the overall process (from semi-batch solid/liquid extraction to production of the beverage), showing also the raw materials (tomato and olive pomace by-products) and extracts used to produce o/w nanoemulsions and the final functional beverage.

2.2. Preparation of the o/w Nanoemulsions

The aqueous phase was prepared by mixing 8% wt Tween 40 and 1% wt ascorbic acid and using a magnetic stirrer. The lipid phase was prepared by dissolving 1% wt lycopene extract in the selected oils. Subsequently, the oil phase was homogenized with the aqueous phase at 9000 rpm for 10 min at 40 °C with a high-speed homogenizer (CAT Unidrive 1000, CAT Scientific, Paso Robles, CA, USA). Then, the coarse oil in water emulsion was further homogenized at 600 bar for four cycles using a high-pressure APV 1000 homogenizer (Albertslund, Denmark).

2.3. Nanoemulsion Characterization

2.3.1. DLS Measurements, Turbidity, Viscosity

A Zetasizer Nano ZS 2000 (Malvern Instruments Ltd., Malvern, UK) was used to measure the droplet size (MDD) and droplet distribution (PDI) of the nanoemulsions at 25 °C [34]. The turbidity of samples was measured using a Hitachi UV/visible spectrophotometer (U-2900 UV/Vis, 200 V) at 600 nm [34]. Water was used as the blank, and the nanoemulsions were diluted at a 1:10 ratio. Nanoemulsion viscosity was determined at 50 rpm using an S61 spindle operating at 25 °C (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) [35].

2.3.2. Color Measurements

The color of the nanoemulsions was measured using a Minolta CR-200 (Minolta Company, Chuo-Ku, Osaka, Japan) and a CIE color scale (Commission Internationale de l'Éclairage) lab system (CIE 1978). The instrumental color of the nanoemulsions was expressed as whiteness [36], calculated using Equation (1):

$$\text{Whiteness Index (WI)} = L - 3 \cdot b \quad (1)$$

where L, a, and b are the measured CIELab color parameters.

2.3.3. Determination of Lycopene Concentration

The total lycopene amount was measured according to the protocol described by Zhao et al., 2020 [25]. The absorbance of the lycopene was measured using a Hitachi spectrophotometer (Hitachi, Tokyo, Japan, U-2900 UV/Vis, 200 V) at 470 nm.

2.3.4. Total Phenolic Content (TPC) and Antioxidant Activity

The Folin–Ciocalteu method, as described by Katsouli et al. [37], was used to determine the total phenolic content, with gallic acid serving as standard, using a spectrophotometer (Hitachi, Tokyo, Japan, U-2900 UV/Vis, 200 V). The results are given as g of gallic acid equivalents (GAE)/kg of dry weight of olive pomace (dw) (g GAE/kg dw). Antioxidant activity was determined using the DPPH assay, as described by Chanioti et al. [38]. The data are expressed in terms of Trolox equivalent antioxidant capacity (g Trolox equivalents/kg dry weight of olive pomace (dw)).

2.4. Nanoemulsion Stability Study

The physical stability of nanoemulsions (droplet size and distribution monitoring) was determined by measuring changes in mean droplet diameter (MDD) and droplet distribution (PDI) during storage at 4 °C for 2 months. Regarding the chemical stability of nanoemulsions (lycopene oxidation), the samples were stored in glass containers at two different temperatures (4 °C and 25 °C) and different pH values (2, 5 and 7) for 2 months. The deterioration of the lycopene concentration was estimated at predetermined times, as described above in Section 2.3.

2.5. Bioaccessibility Determination

The standardized INFOGEST 2.0 in vitro digestion protocol [10] simulating oral, gastric, and small intestine phases was employed to assess the bioaccessibility of the bioactive compounds in nanoemulsions. Briefly, nanoemulsion samples were mixed with simulated saliva, gastric fluid, and intestinal fluid, each containing the appropriate digestive enzymes, and incubated at 37 °C for the specified times, as described by the digestion protocol. After protocol completion, samples were centrifuged at 12,000× *g* for 10 min at 4 °C to separate the micellar phase, which contained the bioaccessible compounds to be quantified. A 3:2 petroleum ether/methanol solution was used for the extraction of lycopene and phenolics from the bioaccessible fraction of the digesta. The concentration of lycopene was measured as detailed in Section 2.3. The total phenolic content (TPC) and antioxidant activity were determined using the Folin–Ciocalteu method and DPPH assay, respectively. Bioaccessibility (%) was then calculated according to Equation (2):

$$\text{Bioaccessibility}(\%) = \frac{\text{Concentration in digestible fraction}}{\text{Concentration in the undigested sample}} \times 100 \quad (2)$$

2.6. Preparation of the Functional Beverage and Shelf-Life Study

A functional beverage was prepared by mixing pasteurized apple juice and an oil-in-water (o/w) nanoemulsion at a ratio of 25:1. After good and persistent shaking and mixing, the final solutions were aseptically packaged and stored at different temperatures (4, 10, 25, and 40 °C) in dark. The o/w nanoemulsions consisted of 1% wt lycopene extracted from tomato pomace in the lipid phase and 1% wt ascorbic acid and 100 ppm polyphenols extracted from olive pomace in the aqueous phase. The composition of the lipid phase was selected based on the bioaccessibility assessment of the o/w nanoemulsions prepared with various oils enriched with lycopene extracts. Beverage controls (samples without nanoemulsions) were also stored under the same conditions. All experiments were performed in triplicate. Functional and control beverages were analyzed at predetermined

times for quality deterioration (microbiological analysis and viscosity) and lycopene and polyphenol degradation.

The temperature dependence of the deterioration rate constants and the shelf-life estimations were modelled via the Arrhenius equation, summarized as follows [39] with appropriate modifications (Equation (3)):

$$\ln k = \ln k_{ref} - \left(\frac{E_a}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) \quad (3)$$

where k_{ref} is the deterioration rate constant of lycopene concentration at a reference temperature, T_{ref} is the reference temperature in K (equal to 4 °C), T is the temperature in K, E_a is the activation energy of the studied action, which indicates the temperature dependance in kJ/mol, and R is the universal gas constant (R = 8.314 J/(mol·K)).

The shelf-life estimation was performed using the Equation (4):

$$t_{SL} = \left(\frac{C_0 - C_t}{k_{ref}\exp\left(\frac{-E_a}{R}\right)\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)}\right) \quad (4)$$

where t_{SL} is the estimated shelf-life in days, C_o is the initial concentration of lycopene in the nanoemulsions ($mg_{lycopene}/L$), and C_t represents a 50% reduction of the initial concentration of lycopene.

2.7. Microbiological Analysis

All microbiological analyses were conducted based on official methods of analysis (APHA, 1984) for total viable count (TVC), yeasts and molds, *Enterobacteriaceae* spp., *Alicyclobacillus* spp., and lactic acid bacteria [40]. Growth of all microorganisms in the beverage samples was monitored versus storage time at 4, 10, 25, and 40 °C. The microbial load of the samples was determined using the surface plating method and the successive dilution method, based on growth on agar plates. Samples (0.1 mL) of 10-fold successive dilutions of beverage were spread on the surface of the appropriate media in Petri dishes for the enumeration of different spoilage bacteria.

For the enumeration of TVC, the non-selective medium Plate Count agar (PCA, Biolife, Milan, Italy) was used, with incubation at 25 °C for 72 h, whereas the enumeration of yeasts and molds was performed using the selective medium Rose Bengal Chloramphenicol agar (RBC, Neogen, Lansing, MI, USA). The sampling procedure was identical to that of the PCA medium. The plates were incubated for 3 days at 30 °C. The growth of *Alicyclobacillus* spp. was monitored using the selective medium Potato Dextrose agar (PDA, Neogen, Lansing, MI, USA) [41]. The pH of the medium was adjusted with the addition of appropriate volumes of 0.1 N HCl and 0.1 N KOH until the pH stabilized at 3.5. The plates were incubated for 5 days at 50 °C. Finally, lactic acid bacteria were enumerated on the selective medium de Man, Rogosa, and Sharpe agar (M.R.S., Chembiotin, Greece) after incubation at 30 °C for 3–5 days. After incubation, the colonies were counted, and the microbial load was expressed as the average log CFU/mL.

2.8. Statistical Analysis

Data are described as mean value ± standard deviation (n = 3). The main effect ANOVA method was used for statistical comparisons (p-value < 0.05) (STATISTICA 7, Statsoft Inc., Tulsa, OK, USA), and Duncan's multiple range test was performed to separate the data's means when significant differences were found.

3. Results

3.1. Nanoemulsion Characterization—Particle Size (MDD), Particle Size Distribution (PDI), Viscosity, and Color (Whiteness)

Nanoemulsion technology enables the development of functional beverages by masking undesirable flavors, enhancing bioaccessibility of hydrophobic bioactives through increased solubility and absorption, and ensuring better availability of active ingredients. The small droplet size and narrow distribution improve stability, reduce oxidation, and extend shelf life, preserving ingredient efficacy while enhancing taste, texture, and consumer acceptance. This makes o/w nanoemulsions an ideal system for incorporating bioactive compounds into stable and palatable beverages.

The physicochemical properties of nanoemulsions significantly depend on the type of lipid phase, the ratio of oil/emulsifier and water in the mixture, and the addition of other soluble compounds. The non-ionic emulsifier Tween 40 (HLB value, 15.6) was selected, as it is generally recognized as safe (GRAS) and is widely used in the food industry, as well being less affected by ionic strength and pH changes than other emulsifiers. The effect of oil type and the addition of antioxidant compounds on the droplet size and distribution, viscosity, and whiteness index were examined to select the most appropriate o/w nanoemulsion as a delivery system for lycopene. Various oils were examined as the lipid phase determines the droplet size, the viscosity, the stability, and the bioaccessibility of the prepared nanoemulsions (Table 1).

Table 1. Physicochemical properties (mean droplet diameter (MDD), polydispersity index (PDI), viscosity, whiteness) of o/w nanoemulsions with or without the addition of ascorbic acid (AA) using different oils as the lipid phase; olive pomace oil (OPO), canola oil (CLA), sunflower oil (SO), corn oil (CO), and fish oil (FO).

Samples	MDD (nm)	PDI	Viscosity (cP)	Whiteness
OPO nanoemulsion	286.6 ± 10^{x}	0.276 ± 0.01^{y}	19.7 ± 1.77^{z}	16.27 ± 1.46^{a}
OPO-AA nanoemulsion	289.2 ± 10.1^{x}	0.248 ± 0.009^{y}	32.7 ± 2.62^{z}	20.91 ± 1.67^{b}
CLA nanoemulsion	275.8 ± 9.7^{x}	0.266 ± 0.009^{y}	16.1 ± 1.13^{z}	11.94 ± 0.84^{a}
CLA-AA nanoemulsion	269.8 ± 9.4^{x}	0.244 ± 0.009^{y}	22.6 ± 1.36^{z}	14.26 ± 0.86^{b}
SO nanoemulsion	258.7 ± 9.1^{x}	0.276 ± 0.009^{y}	20.4 ± 1.43^{z}	38.68 ± 2.71^{a}
SO-AA nanoemulsion	251.2 ± 8.8^{x}	0.244 ± 0.02^{y}	22.8 ± 2.28^{z}	35.42 ± 3.54^{b}
CO nanoemulsion	264.9 ± 9.3^{x}	0.263 ± 0.06^{y}	18.3 ± 1.56^{z}	47.10 ± 4.00^{a}
CO-AA nanoemulsion	274.4 ± 9.6^{x}	0.252 ± 0.07^{y}	23.9 ± 1.55^{z}	45.75 ± 2.97^{b}
FO nanoemulsion	259.3 ± 9.1^{x}	0.200 ± 0.05^{y}	18.7 ± 1.27^{z}	36.17 ± 2.46^{a}
FO-AA nanoemulsion	278.9 ± 9.8^{x}	0.190 ± 0.01^{y}	21 ± 1.58^{z}	39.2 ± 2.94^{b}

Superscript letters a and b represent significant differences ($p < 0.05$) between the addition or absence of AA in whiteness. Superscript letters x for MDD, y for PDI, and z for Viscosity represent no significant differences ($p > 0.05$) between the addition or absence of AA. Data are described as mean value ± standard deviation (n = 3).

According to size-distribution graphs based on intensity, the selected two-step homogenization condition and emulsion composition (Tween 40 and various vegetables oil) resulted in o/w nanoemulsions with uniform droplets (MDD < 300 nm, PDI < 0.3), with no significant differences among them. Previous studies used more intense conditions (higher pressure and temperature values and emulsifier concentration) to create o/w emulsions in the nano-range with narrow droplet distribution [24,42,43].

Olive pomace oil resulted in a larger droplet size, namely 286.6 ± 10.0 nm, and fish oil in the smallest droplet size, at 259.3 ± 9.1. Furthermore, the incorporation of ascorbic acid did not significantly impact the colloidal stability of the system. A minor average increase of 5% in droplet size was observed, with a more substantial increase of 8% in the case of fish oil nanoemulsions. Although ascorbic acid did not significantly affect droplet size, its presence facilitated homogenization by leading to a narrower droplet size distribution. Due to its high surface activity, ascorbic acid interacts with emulsifying agents, improving their

capacity to form a stable interfacial film around the droplets, resulting in a more uniform droplet distribution [44,45]. Figure 2 shows a representative DLS graph.

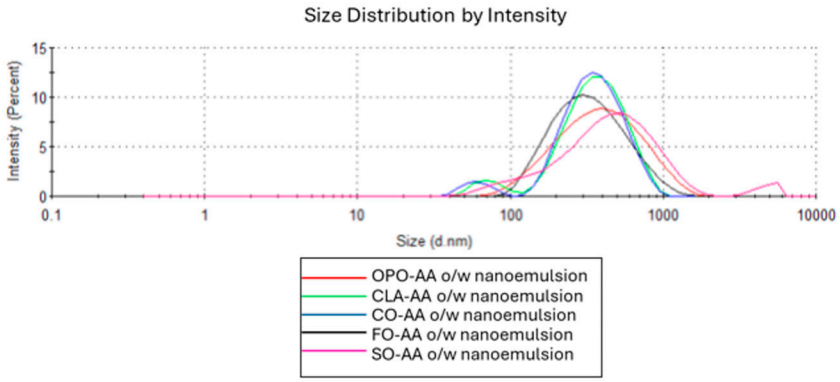


Figure 2. Typical droplet-distribution graph based on intensity for olive pomace oil (OPO), canola oil (CLA), corn oil (CO), fish oil (FO), and sunflower oil (SO) nanoemulsions enriched with lycopene and ascorbic acid (AA) after the homogenization process.

As expected, the viscosity of nanoemulsions is relatively low, ranging from 16.1 to 32.7 cP, as the viscosity of colloidal systems is strongly related to the viscosity of the continuous phase. However, it increased with increasing concentration of the dispersed phase. Moreover, viscosity values did not significantly differ (OPO 49.1 cP, CLA 48.8 cP, SO 48.8 cP, CO 31.18 and FO 69.71 cP at 25 °C), as all the examined oils have similar fatty acid composition and viscosity except fish oil [35,46,47].

The color of the nanoemulsions, expressed as whiteness, was significantly influenced by the addition of ascorbic acid ($p < 0.05$), with the blank samples having a value of approximately 20, while the addition of AA led to an increased value of 40.7. Also, the type of the lipid phase affected the color, with CO and FO nanoemulsions being whiter, which can be attributed to the lighter color of these oils as well as the different solubility of lycopene in different oils [25]. Therefore, both the lipid phase type and the addition of ascorbic acid contribute to the observed variation in color.

3.2. Nanoemulsion Stability During Storage

Nanoemulsions stabilized by non-ionic emulsifiers are characterized by good physical and chemical stability against droplet growth. However, different storage conditions and environmental factors may initiate destabilization phenomena. Therefore, the effect of different environmental factors (temperature and pH levels) on lycopene-loaded nanoemulsions are described below.

3.2.1. Physical Stability—Droplet Size During Storage

Nanoemulsions are inherently unstable colloidal systems, susceptible to various destabilization mechanisms, such as sedimentation and creaming, due to the presence of polydisperse droplet sizes and intermolecular attractive forces. Despite their enhanced stability during storage, fluctuations in temperature can significantly influence their physicochemical properties, ultimately leading to product destabilization. Therefore, the physical stability of the nanoemulsion was assessed by monitoring the mean droplet diameter (MDD) over a 2-month storage period at 4 °C.

After 2 months, all samples stored at 4 °C were found to be quite stable in the nanosized range, with a maximum MDD of 307.5 nm for the nanoemulsions with olive pomace oil and ascorbic acid (Table 2). The DLS analysis demonstrated that nanoemulsions with corn oil and ascorbic acid were subjected to significant destabilization, as the droplet size increased by 15%, compared to the other samples, which maintained their initial MDD or showed a slight increase of 5% or 6%. The satisfactory stability of the o/w nanoemulsions observed is attributed to their initial small droplet size and fairly narrow droplet distribu-

tion, which allows them to remain stable against Ostwald ripening for a period of time. Nanoemulsions are in the kinetically stable regime, where droplet growth is thermodynamically favored and the kinetic energy barrier slows this growth. However, if the particle size distribution is wide, Ostwald ripening will occur at a significantly higher rate. Other studies have also demonstrated that emulsions used in food and beverages, with around 10–20 wt.% of the oil phase, can be stabilized against Ostwald ripening by incorporating water-soluble components [45,48]. Moreover, the high stability of the nanoemulsions can be attributed to high oil concentrations and consequently to higher viscosity. When viscosity increased, the rate of Brownian motion decreased, thereby the aggregation effect caused by thermal motion was reduced [18]. This viscosity-related stability is essential for maintaining a consistent droplet size over time, as reduced movement of the droplets minimizes coalescence and phase separation, preserving the properties of the nanoemulsion.

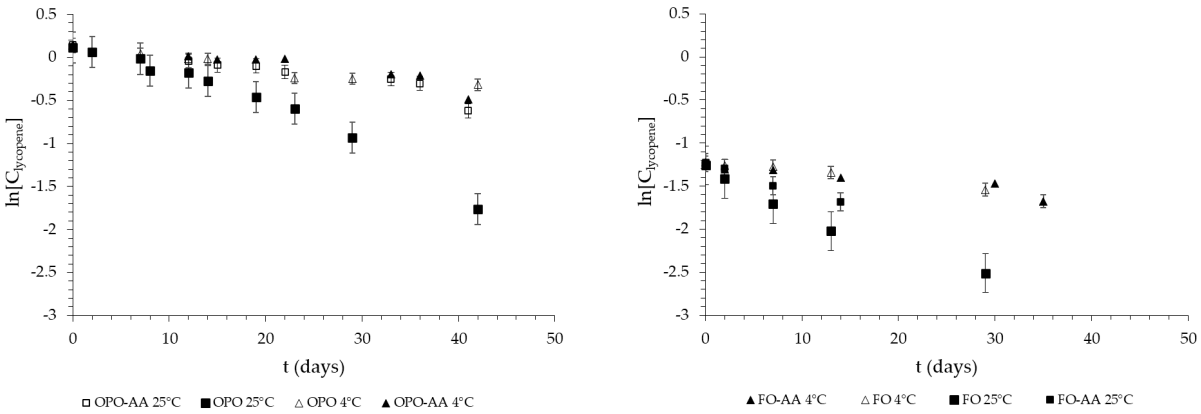
Table 2. Mean droplet diameter (MDD) and polydispersity index (PDI) of olive pomace oil (OPO), canola oil (CLA), corn oil (CO), fish oil (FO), and sunflower oil (SO) nanoemulsions enriched with lycopene and ascorbic acid (AA) after 2 months of storage at 4 °C.

Samples	MDD (nm)	PDI
OPO-AA nanoemulsion	307.5 ± 10.5 ^{x}	0.268 ± 0.006 ^{y}
CLA-AA nanoemulsion	282.2 ± 4.8	0.254 ± 0.002 ^{y}
SO-AA nanoemulsion	287.9 ± 6.3 ^{x}	0.254 ± 0.04 ^{y}
CO-AA nanoemulsion	280.4 ± 5.8 ^{x}	0.262 ± 0.08 ^{y}
FO-AA nanoemulsion	270.4 ± 10.2 ^{x}	0.200 ± 0.05 ^{y}

Superscript letters x for MDD and y for PDI represent no significant differences ($p > 0.05$) between the lipid phases. Data are described as mean value ± standard deviation (n = 3).

3.2.2. Chemical Stability—Deterioration of Lycopene Concentration and Nanoemulsion's Color During Storage

The change in lycopene concentration as a function of time in the nanoemulsions followed first-order kinetics (linear change of $\ln C_{lycopene}$ as a function of time) at all experimental conditions investigated (Figure 3), in agreement with similar studies [49,50].



(**a**) (**b**)

Figure 3. Deterioration of lycopene concentrations in (**a**) olive pomace oil (OPO) and (**b**) fish oil (FO) o/w nanoemulsions with or without the addition of ascorbic acid (AA) during storage at 25 °C and 4 °C and pH = 2.

The type of oil, the incorporation of AA, and the temperature significantly affected the deterioration rate of lycopene ($p < 0.05$), while the pH values did not show a significant effect (Table 3). Fish oil and corn oil nanoemulsions exhibited the highest average deterioration rate, namely FO -0.0249 d^{-1} and CO -0.0352 d^{-1}, while olive pomace,

canola oil, and sunflower oil led to the lowest average rates, without significant difference among them, namely OPO -0.0221 d^{-1}, CLA 0.0225 d^{-1} (Figure 3), and SO -0.0189 d^{-1}. As expected, the presence of AA reduced the deterioration rate of lycopene by 57% due to its strong antioxidant activity. Moreover, nanoemulsions exhibited higher chemical stability at refrigerated storage, with an average deterioration rate of -0.0185 d^{-1} compared to -0.0334 d^{-1} at 25 °C. Although pH did not significantly affect the deterioration rate of lycopene, at pH = 7 slightly lower rates (-0.0231 d^{-1}) were found compared to at pH = 2 and pH = 5; -0.0298 d^{-1} and -0.0247 d^{-1}, respectively (Table 2). Others studies also reported an increased stability of encapsulated lycopene in the nanoemulsions at 4 °C compared to ambient temperature or abusive conditions of accelerated testing [51,52].

Table 3. Deterioration rate of lycopene concentrations (k, in d^{-1}) in o/w nanoemulsions with or without ascorbic acid (AA) during storage at 4 °C and 25 °C and different pH values (2, 5, and 7).

Samples	Deterioration Rate, k (d^{-1})					
	pH = 2		pH = 5		pH = 7	
	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C
OPO nanoemulsion	$-4.30 \times 10^{-2\,axi}$	$-9.94 \times 10^{-3\,axii}$	$-6.93 \times 10^{-2\,axi}$	$-1.64 \times 10^{-2\,axii}$	$-2.44 \times 10^{-2\,axi}$	$-6.14 \times 10^{-3\,axii}$
OPO-AA nanoemulsion	$-1.51 \times 10^{-2\,ayi}$	$-1.29 \times 10^{-2\,ayii}$	$-1.87 \times 10^{-2\,ayi}$	$-1.71 \times 10^{-2\,ayii}$	$-1.56 \times 10^{-2\,ayi}$	$-1.39 \times 10^{-2\,ayii}$
CLA nanoemulsion	$-5.47 \times 10^{-2\,a\,xi}$	$-3.72 \times 10^{-2\,axii}$	$-6.26 \times 10^{-2\,axi}$	$-1.34 \times 10^{-2\,axii}$	$-4.79 \times 10^{-2\,ax\,i}$	$-1.68 \times 10^{-2\,axii}$
CLA-AA nanoemulsion	$-1.84 \times 10^{-2\,ayi}$	$-8.22 \times 10^{-3\,ayii}$	$-8.59 \times 10^{-3\,ayi}$	$-9.24 \times 10^{-3\,ayii}$	$-8.22 \times 10^{-3\,ayi}$	$-5.67 \times 10^{-3\,ayii}$
SO nanoemulsion	$-5.63 \times 10^{-2\,axi}$	$-1.64 \times 10^{-2\,axii}$	$-3.46 \times 10^{-2\,axi}$	$-1.80 \times 10^{-2\,axii}$	$-2.93 \times 10^{-2\,axi}$	$-1.41 \times 10^{-2\,axii}$
SO-AA nanoemulsion	$-1.25 \times 10^{-2\,ayi}$	$-1.16 \times 10^{-2\,ayii}$	$-1.78 \times 10^{-2\,ayi}$	$-1.24 \times 10^{-2\,ayii}$	$-1.65 \times 10^{-2\,ayi}$	$-9.61 \times 10^{-3\,ayi}$
CO nanoemulsion	$-7.42 \times 10^{-2\,bxi}$	$-6.54 \times 10^{-2\,bxii}$	$-1.63 \times 10^{-2\,bxi}$	$-6.96 \times 10^{-2\,bxii}$	$-8.57 \times 10^{-2\,bxi}$	$-3.17 \times 10^{-2\,bxii}$
CO-AA nanoemulsion	$-1.66 \times 10^{-2\,byi}$	$-1.12 \times 10^{-2\,byii}$	$-1.06 \times 10^{-2\,byi}$	$-2.01 \times 10^{-2\,byii}$	$-3.17 \times 10^{-2\,byi}$	$-1.36 \times 10^{-2\,byii}$
FO nanoemulsion	$-5.78 \times 10^{-2\,bxi}$	$-1.24 \times 10^{-2\,bxii}$	$-4.29 \times 10^{-2\,bxi}$	$-1.33 \times 10^{-2\,bxii}$	$-5.79 \times 10^{-2\,bxi}$	$-1.16 \times 10^{-2\,bxii}$
FO-AA nanoemulsion	$-3.32 \times 10^{-2\,byi}$	$-9.85 \times 10^{-3\,byii}$	$-1.52 \times 10^{-2\,byi}$	$-1.08 \times 10^{-2\,byii}$	$-1.17 \times 10^{-2\,byi}$	$-9.14 \times 10^{-3\,byii}$

Superscript letters a and b represent significant differences ($p < 0.05$) between lipid phases; superscript letters x and y present significant differences ($p < 0.05$) between the addition or absence of AA; superscript letters i and ii represent significant differences ($p < 0.05$) between storage temperatures.

Lycopene degradation can also be visually monitored by the change in color of lycopene nanoemulsions. The increase in whiteness can be correlated with the deterioration of lycopene concentrations in nanoemulsions, as samples underwent oxidative degradation, which disrupts the conjugated double bonds of lycopene. This breakdown causes a shift in the absorption maxima, leading to a fading of its characteristic red/orange hue [25,51,52]. The whiteness index was significantly affected by lipid phase, storage parameters (temperature, storage time), and the presence of ascorbic acid in the nanoemulsions ($p < 0.05$), while pH value did not significantly affect the color of the nanoemulsions. Ascorbic acid protected the color, as a milder increase in the whiteness index was observed during storage, with the greatest protection being observed under refrigerated storage conditions. Other studies also demonstrated that lycopene degradation degree, based on visual color observation, was more prominent at higher storage temperatures, due the production of the free radicals following oil oxidation, which accelerates the degradation of lycopene at higher temperatures [45]. An increase in the whiteness index of lycopene nanoemulsions was observed at all experimental conditions investigated, with the increase being more intense (reaching a two-fold increase) in the nanoemulsion with fish oil and corn oil (Table 4). As the degree of fading of the characteristic lycopene color can be correlated to the different lipid phase under the same storage conditions, this indicates that the choice of oil phase played an important role in maintaining lycopene stability. This may be due to the fact that fish oil, corn oil, and sunflower oil are more susceptible to oxidation compared to monounsaturated lipids such as olive pomace oil and canola oil [35,53]. The change in color can reflect directly the degradation of lycopene, which was closely related to the encapsulation efficiency of lycopene in the nanoemulsions, as well as to the oxidation stability of the oil phase; the free radicals produced by lipid oxidation during storage can cause degradation of lycopene, and thus fading [25]. Consequently, the choice of lipid phase is critical not only for the physical stability of the nanoemulsions but also for preserving lycopene's visual and

functional integrity, as oils with higher oxidative stability, such as olive pomace and canola oils, which are rich in monounsaturated fatty acids, can mitigate color loss and maintain the red/orange hue of lycopene more effectively over time.

Table 4. Whiteness index of o/w nanoemulsions enriched with lycopene, with or without ascorbic acid (AA), after 2 months of storage at 4 °C and 25 °C and different pH values (2, 5, and 7).

Whiteness Index						
Samples	**pH = 2**		**pH = 5**		**pH = 7**	
	25 °C	**4 °C**	**25 °C**	**4 °C**	**25 °C**	**4 °C**
OPO nanoemulsion	49.30 ± 2.7^{axi}	23.92 ± 1.3^{axii}	58.21 ± 3.2^{axi}	28.46 ± 1.6^{axii}	28.40 ± 1.6^{axi}	39.79 ± 2.2^{axii}
OPO-AA nanoemulsion	23.69 ± 1.3^{ayi}	26.79 ± 3.2^{ayi}	24.16 ± 1.3^{ayi}	27.52 ± 1.5^{ayii}	27.06 ± 1.5^{ayi}	22.75 ± 1.3^{ayii}
CLA nanoemulsion	54.65 ± 3.0^{axi}	20.67 ± 2.4^{axii}	43.35 ± 2.4^{axi}	23.25 ± 1.3^{axii}	47.30 ± 2.6^{axi}	21.38 ± 1.2^{axii}
CLA-AA nanoemulsion	32.31 ± 1.8^{ayi}	25.91 ± 1.1^{ayii}	20.45 ± 1.1^{ayi}	24.18 ± 1.3^{ayii}	23.96 ± 1.3^{ayi}	26.10 ± 1.4^{ayii}
SO nanoemulsion	58.72 ± 3.2^{bxi}	36.59 ± 1.4^{bxii}	45.37 ± 2.5^{bxi}	41.29 ± 2.3^{bxii}	43.85 ± 2.4^{bxi}	42.21 ± 2.3^{bxii}
SO-AA nanoemulsion	37.44 ± 2.1^{byi}	21.18 ± 2.0^{byii}	31.9 ± 1.8^{byi}	38.43 ± 2.1^{byii}	27.59 ± 1.5^{byi}	43.35 ± 2.4^{byii}
CO nanoemulsion	60.10 ± 3.3^{cxi}	44.66 ± 1.2^{cxii}	53.39 ± 2.9^{cxi}	43.31 ± 2.4^{cxii}	62.90 ± 3.5^{cxi}	45.58 ± 2.5^{cxii}
CO-AA nanoemulsion	50.06 ± 2.8^{cyi}	48.40 ± 2.5^{cyii}	48.14 ± 2.6^{cyi}	50.52 ± 2.8^{cyii}	49.29 ± 2.7^{cyi}	54.15 ± 3.0^{cyii}
FO nanoemulsion	44.28 ± 2.4^{cxi}	37.36 ± 2.7^{cxii}	59.16 ± 3.3^{cxi}	36.55 ± 2.0^{cxii}	53.68 ± 3.0^{cxi}	50.30 ± 2.8^{cxii}
FO-AA nanoemulsion	40.37 ± 2.2^{cyi}	42.30 ± 2.1^{cyii}	39.31 ± 2.2^{cyi}	44.70 ± 2.5^{cyii}	44.39 ± 2.4^{cyi}	41.25 ± 2.3^{cyii}

Superscript letters a, b, and c represent significant differences ($p < 0.05$) between lipid phases; superscript letters x and y represent significant differences ($p < 0.05$) between the addition or absence of AA; superscript letters i and ii represent significant differences ($p < 0.05$) between storage temperatures.

3.3. Characterization and Bioaccessibility Study of o/w Nanoemulsions Enriched with Lycopene and Phenolic Extracts

Phenolic compounds extracted from olive pomace were added to evaluate their effect on nanoemulsion characteristics and to enhance the functional beverage's value by introducing additional bioactive compounds. These phenolics could potentially provide synergistic benefits when combined with lycopene.

3.3.1. Characterization of Nanoemulsions Enriched with Lycopene and Phenolic Extracts

Oil-in-water nanoemulsions were prepared using the previously studied edible oils (OPO, CLA, SFO, CO, and FO) enriched with lycopene, ascorbic acid, and phenolic extracts. These emulsions were subsequently characterized in terms of droplet size and distribution, viscosity, turbidity, and whiteness, in an attempt to correlate the bioaccessibility of lycopene and phenolic compounds with the physicochemical properties of nanoemulsions, as well as to select the adequate lipid phase for the preparation of the nanoemulsion–apple juice functional beverage. The initial concentration of lycopene was calculated as 10.65 ± 0.2 mg lycopene/mL for OPO, 10.70 ± 0.30 mg lycopene/mL for CLA, 13.4 ± 0.8 mg lycopene/mL for CO, 12.0 ± 0.5 mg lycopene/mL for SO, and 11.1 ± 0.3 mg lycopene/mL for FO, and the initial TPC was 7.85 ± 0.85 mg GAE/mL for OPO, 7.31 ± 0.15 mg GAE/mL for CLA, 7.58 ± 0.24 mg GAE/mL for CO, 7.77 ± 0.35 mg GAE/mL for SO, and 7.79 ± 0.33 mg GAE/mL for FO.

In contrast to the previously examined nanoemulsions, the droplet size of those enriched with lycopene and phenolic extracts was significantly influenced by the type of oil used ($p < 0.05$), with corn oil and fish oil resulting in the largest droplet size, as can be seen in Table 5. Also, the incorporation of phenolics in the nanoemulsion significantly increased the droplet diameter, to ca. 297.02 nm compared to 269.56 nm for the control and 279.1 nm for the nanoemulsion with ascorbic acid. Furthermore, the presence of ascorbic acid and phenolic compounds broadened the droplet size distribution, with droplets smaller than 100 nm and larger than 1000 nm appearing in the DLS graph (Figure 4). The addition of ascorbic acid and phenolics significantly influenced the whiteness of the samples ($p < 0.05$): the whiteness of blank samples was approximately 20, while with the addition of AA whiteness increased to 40.7 and further increased to 41.8 with the addition of phenolics. However, no significant differences were observed among the samples regarding the viscosity and turbidity values (Table 5). Turbidity values were relatively low, in agreement with

the low droplet size diameter and narrow distribution, which likely will not significantly alter the appearance of apple juice. Also, the viscosity of the nanoemulsions remained low, ensuring ease of flow and stability, which is favorable for maintaining the desired texture and mouthfeel of the apple juice.

Table 5. Mean droplet diameter (MDD), polydispersity index (PDI), turbidity, viscosity, and whiteness of lycopene o/w nanoemulsions enriched with ascorbic acid (AA) and phenolic compounds using different lipid phases; olive pomace oil (OPO), canola oil (CLA), sunflower oil (SO), corn oil (CO), and fish oil (FO).

Samples	MDD (nm)	PDI	Turbidity	Viscosity (cP)	Whiteness
OPO-AA_ph	307.9 ± 15.0^{a}	0.276 ± 0.01^{x}	0.506 ± 0.046^{y}	19.7 ± 1.77^{z}	16.27 ± 1.46^{w}
CLA-AA_ph	331.8 ± 11.4^{a}	0.266 ± 0.009^{x}	0.535 ± 0.037^{y}	16.1 ± 1.13^{z}	11.94 ± 0.84^{w}
SO-AA_ph	317.7 ± 7.7^{a}	0.276 ± 0.009^{x}	0.404 ± 0.028^{y}	20.4 ± 1.43^{z}	38.68 ± 2.71^{w}
CO-AA_ph	259.5 ± 5.5^{b}	0.263 ± 0.06^{x}	0.401 ± 0.034^{y}	18.3 ± 1.56^{z}	47.10 ± 4.00^{w}
FO-AA_ph	246.4 ± 8.4^{b}	0.200 ± 0.05^{x}	0.387 ± 0.026^{y}	18.7 ± 1.27^{z}	36.17 ± 2.46^{w}

Superscript letters a and b represent significant differences in MDD ($p < 0.05$) between lipid phases. Superscript letters x for PDI, y for Turbidity, z for Viscosity, and w for whiteness represent no significant differences ($p > 0.05$) between lipid phases.

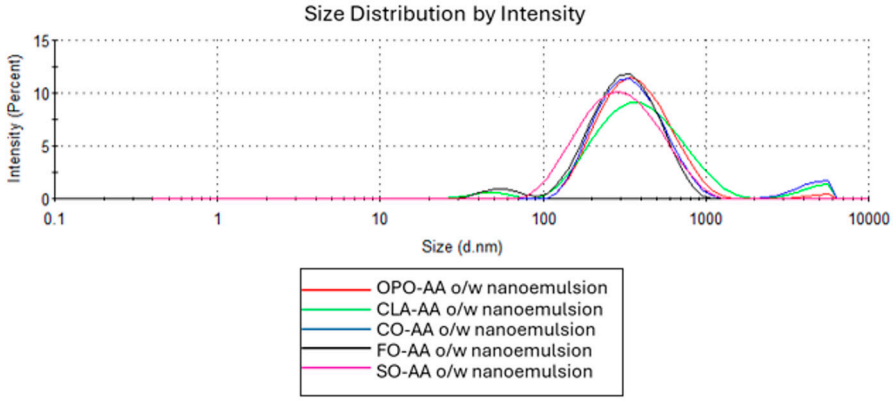


Figure 4. Typical droplet-distribution graph based on intensity for olive pomace oil (OPO), canola oil (CLA), corn oil (CO), fish oil (FO), and sunflower oil (SO) nanoemulsions enriched with lycopene, ascorbic acid (AA), and phenolic compounds (ph).

3.3.2. Bioaccessibility Study of Nanoemulsions

The standardized static INFOGEST 2.0 in vitro protocol [10] was applied to study the effect of each lipid phase on the bioaccessibility of the bioactive compounds. Lycopene and phenolic compound (TPC) bioaccessibility (%) values for each nanoemulsion lipid phase are shown in Figure 5.

The bioaccessibility percentages of lycopene in raw tomato pulp reported in literature range from undetected to as low as 0.1%, with some studies reporting values between 1.5% and 3% [54–56]. Therefore, it is evident that the nanoemulsions tested in this study exhibit significantly higher lycopene bioaccessibility values. Numerous studies have demonstrated that nanoemulsions enhance the bioaccessibility of lipophilic compounds [57–61]. The small droplets in nanoemulsions provide a larger surface area for digestive enzymes to act upon, and penetrate and remain longer in the mucus layer lining the epithelial cells—thus increasing the chances of absorption—while they can also enhance the absorption of lipophilic components due to their high water solubility [62]. Moreover, the formulation of these nanoemulsions allows for improved stability and protection of lycopene from degradation during gastrointestinal transit, which further contributes to its enhanced bioaccessibility. This characteristic is particularly important given the susceptibility of lycopene to oxidation and degradation in harsh digestive environments.

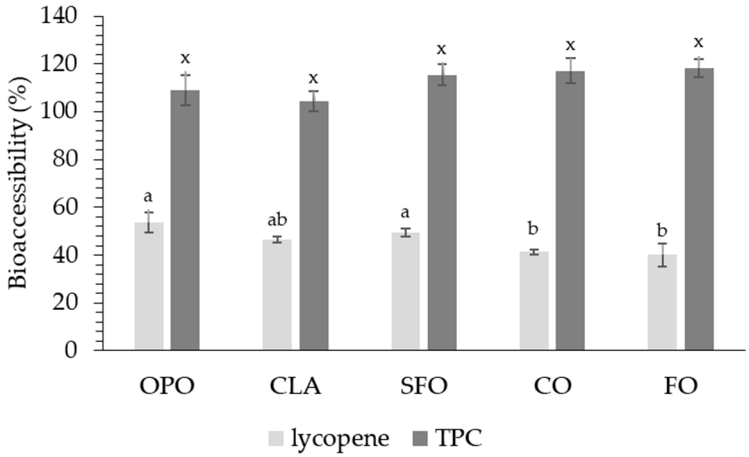


Figure 5. Lycopene and total phenolic compound (TPC) bioaccessibility (%) in different nanoemulsions containing olive pomace oil (OPO), canola oil (CLA), sunflower oil (SFO), corn oil (CO), and fish oil (FO). Superscript letters a and b represent significant differences ($p < 0.05$) in lycopene between lipid phases. Superscript letters x for TPC represent no significant differences ($p > 0.05$) between lipid phases. Data are described as mean value ± standard deviation (n = 3).

Our results indicate that the nanoemulsion containing olive pomace oil achieved the highest bioaccessibility percentage (53.8 ± 2.6%), while the nanoemulsion with fish oil had the lowest (40.1 ± 2.1%) (Figure 5). The lipid phase of the nanoemulsion significantly affected bioaccessibility percentages ($p < 0.05$). The values reported are in agreement with the literature, though differences may be attributed to variations in emulsifiers, homogenization conditions, or the amount of oil used in the lipid phase of the emulsions. Particularly, Liang et al. [56] supported that using olive oil in emulsions with sodium alginate improved lycopene bioaccessibility (61.5%) compared to corn oil (52.2%). Nemli et al. [31] observed up to 85% lycopene bioaccessibility in olive oil emulsions, highlighting the role of emulsifier type and homogenization conditions in carotenoid release. Moreover, the composition of the fatty acids of the oils used in the lipid phase of nanoemulsions is reported to have a significant impact on carotenoid bioaccessibility [60]. Oils with long-chain fatty acids can improve bioaccessibility by forming mixed micelles with high solubility for lipophilic compounds, while also stimulating the formation of chylomicrons in the epithelial cells, facilitating absorption and reducing first-pass metabolism [61]. In vivo studies with animal subjects have shown that monounsaturated fatty acids (MUFAs) and n-6 polyunsaturated fatty acids (PUFAs) enhance carotenoid absorption through the postprandial lipemic response, whereas long-chain n-3 PUFAs show a lower increase in blood fat levels [63,64]. This indicates that oils rich in MUFAs, such as olive pomace and canola oil, can improve carotenoid bioavailability, while PUFAs in fish oil are expected to show lower values. Furthermore, oils with a high concentration of PUFAs, such as fish and corn oil, can form larger mixed micelles, which may reduce their diffusion through the unstirred water layer, thereby decreasing their uptake in the intestine. PUFAs are also more prone to oxidation, which can lead to lycopene oxidation in the intestinal chyme, resulting in lower bioaccessibility [54].

The bioaccessibility of phenolic compounds was found to be high, with values exceeding 100%, indicating effective release and solubilization during digestion. These results are in agreement with the existing literature, as many researchers have reported similar results where TPC was higher after simulated digestion [31,32,65,66]. This phenomenon may be attributed to the hydrolysis of polymerized polyphenols into monomers and aglycones as they pass through the gastrointestinal tract, leading to an increase in free phenolic components [67]. The lipid phase did not have a significant effect on TPC bioaccessibility ($p > 0.05$), indicating that the intestinal absorption of olive pomace polyphenols is independent of the oil composition. This contrasts with the results observed for lycopene

bioaccessibility. Consequently, the selection of the appropriate lipid phase was primarily focused on enhancing lycopene bioaccessibility rather than TPC.

The nanoemulsion containing olive pomace oil was chosen for incorporation into the apple juice beverage due to its higher lycopene bioaccessibility and other favorable characteristics, such as stability during storage. In this sample, the DPPH assay was used to evaluate antioxidant activity, which was reduced to 63.6 ± 2.3% after digestion. This result contrasts with the TPC of the same sample, which increased to 109.0 ± 2.5%, indicating that the observed antioxidant activity was primarily attributed to the added ascorbic acid rather than the olive pomace polyphenols. It has been reported that ascorbic acid can degrade during digestion to dehydroascorbic acid, which retains some antioxidant properties, or even further to diketogulonic acid, which possesses no antioxidant activity, leading to the observed decrease in antioxidant capacity post-digestion [67].

3.4. Bioaccessibility and Shelf-Life Study of the Functional Beverage

The beverage containing apple juice (as the basis) and olive pomace oil nanoemulsion underwent in vitro digestion using the INFOGEST 2.0 protocol [10]. The results for lycopene bioaccessibility were markedly different compared to the nanoemulsion alone, with values of 6.2 ± 1.8% for the beverage in contrast to 53.8 ± 4.2% for the pure nanoemulsion. The reduction in lycopene bioaccessibility could be attributed to the dilution of the nanoemulsion with apple juice, which may have slowed down the solubilization of lycopene into mixed micelles due to its lower concentration in the overall sample. Additionally, apple juice is rich in fibers such as pectin, which can hinder lycopene incorporation into mixed micelles or restrict the activity of pancreatic enzymes, resulting in lower extraction of lycopene from lipid droplets [68]. In contrast, the TPC bioaccessibility and antioxidant activity in the beverage showed no statistically significant difference ($p > 0.05$) compared to the nanoemulsion values, with TPC bioaccessibility at 119.1 ± 5.7% for the beverage and 109.0 ± 2.5% for the nanoemulsion, and antioxidant capacity at 62.0 ± 3.5% compared to 63.6 ± 2.3%, respectively.

For an aseptically packaged lycopene-fortified apple juice beverage the considerations for shelf-life stability extend beyond microbiological safety to include the stability of the emulsion and the preservation of bioactive compounds. The overall shelf life is also influenced by factors such as the stability of the emulsion, lycopene degradation, and changes in sensory attributes.

Similarly to the nanoemulsion lycopene stability, the degradation of lycopene in the functional beverage followed first-order kinetics (Figure 6) as a function of time and temperature, as observed in similar studies [49,50].

The functional beverage enriched with lycopene and phenolic compounds was proven to be a stable system. Specifically, the phenolic compound concentration remained stable during storage, with no significant differences among storage temperature, as the TPC at the end of storage was 0.30 mg GAE/mL beverage and 0.33 mg GAE/mL beverage at 4 °C and 40 °C, respectively. However, lycopene appeared to be more prone to deterioration, as increased storage temperature led to higher degradation rates: -0.0088 d^{-1} at 4 °C and -0.105 d^{-1} at 40 °C (Figure 6). The initial concentration of lycopene was reduced by 32% after 47 days at 4 °C and by 53% after 19 days at 25 °C. Taking into consideration the degradation rates, lycopene in the functional beverage can be protected for longer storage periods at 4 and 10 °C. The functional properties of the proposed beverage are depended on lycopene content; thus, the shelf life of the product is determined by the point at which a 50% reduction in lycopene was observed. The estimated activation energy (Ea) was calculated to be 50.68 KJ/mol, which is a relatively moderate value. This implies that lycopene degradation is temperature-sensitive, meaning that small increases in temperature can lead to significantly faster degradation, yet it does not require high energy levels to occur. This observation aligns with the estimated shelf life (t_{50}), which is 165 days at 4 °C, 103 days at 10 °C, 35 days at 25 °C, and 13 days at 40 °C, highlighting the im-

portance of low-temperature storage to preserve the beverage's lycopene content and functional properties.

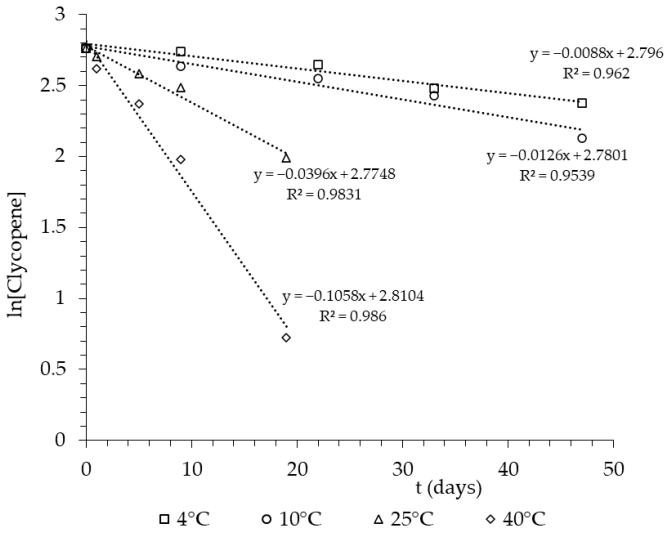


Figure 6. Deterioration of lycopene concentrations in the functional beverage during storage as a function of temperature (4, 10, 25, and 40 °C).

Emulsions are inherently sensitive to destabilization mechanisms like creaming, flocculation, and coalescence, which can be accelerated by variations in temperature or pH. Therefore, maintaining the physicochemical stability of the lycopene emulsion is critical to ensuring the product's consistency, appearance, and nutritional quality.

The functional beverage, during storage at all temperatures, exhibited similar rheological behavior to control apple juice, which remained almost stable throughout storage. The initial viscosity of functional beverage was slightly increased, at 4.88 cP, compared to the control, at 2.72 cP (due to the presence of the o/w nanoemulsion with MDD 307 ± 15 nm and 19.7 ± 1.77 cP).

The initial viscosity of the functional beverage was higher (4.88 cP) than the control (2.72 cP) due to the addition of the o/w nanoemulsion characterized by a MDD of 307 ± 15 nm and a viscosity of 19.7 ± 1.77 cP at 25 °C. The slight increase in the functional beverage's viscosity is attributed to the presence of nanoemulsion, which alters the structural density and increases resistance to flow. The nanoemulsion droplets interact with the beverage matrix, creating a more cohesive structure that slows down molecular movement, thereby increasing the overall viscosity. During storage at 4, 10, 25, and 40 °C, the functional beverage displayed similar rheological behavior to the control apple juice, remaining mostly stable throughout shelf-life.

Microbiological analysis of the beverage samples stored at 4, 10, 25, and 40 °C was also performed. The results of the microbiological analysis showed that, at time 0 of storage, the Total Viable Count (TVC) was below the detection limit (<1 log CFU/mL) in all beverage samples studied and remained below the detection limit in all samples stored at 4 and 10 °C at the end of the beverage shelf life (50% lycopene reduction). Acceptable Total Viable Count (TVC) levels in fruit juices can vary depending on the regulatory standards of different countries. Generally, TVC levels are used to assess the microbiological quality and safety of the juice. The European Commission (EC) Regulation No 2073/2005 on microbiological criteria for foodstuffs suggests that fruit juices should have a TVC of less than 10^4 CFU/mL (colony-forming units per milliliter) at the end of their shelf life [69].

Similarly to the TVC, the growth of yeasts/molds and of *Enterobacteriaceae* spp. was below the detection limit in all samples at the end of beverage shelf life for all examined temperatures. *Enterobacteria* are considered hygiene indicators, and their absence in all beverage samples suggests proper handling and production processes, during which hygiene conditions were adequately maintained, leading to inhibition of the growth of

the studied microorganisms. The results of the microbiological analysis showed that all microorganisms studied remained below the detection limit in all samples stored at 4, 10, 25, and 40 °C throughout the respective storage period. It is evident that the packaging of the final product was performed under aseptic conditions, indicating that, in all stages of the preparation of the final product, the hygiene conditions were adequate.

The results of the current study indicate that refrigeration is recommended for long-term storage of this product, as it can help slow down lycopene degradation. Implementing refrigeration, therefore, not only extends the microbiological shelf life but also ensures that the product retains its nutritional value over an extended period, making it more appealing and beneficial for consumers. This is particularly important for products rich in antioxidants like lycopene, as their effectiveness can diminish rapidly at higher temperatures. However, the sensory attributes of the beverage (flavor, mouthfeel, visual appeal) may be altered if the emulsion destabilizes or if chemical reactions occur. As a result, even in the case where microbiological quality is preserved, the sensory characteristics should be monitored, as they impact consumer acceptance.

4. Conclusions

This study demonstrates the potential of oil-in-water (O/W) nanoemulsions as effective delivery systems for bioactive compounds, particularly lycopene and phenolic compounds, for functional beverage development. By employing various oils and incorporating ascorbic acid, nanoemulsions were shown to present improved stability and bioaccessibility of the bioactive compounds. The addition of ascorbic acid enhanced droplet distribution and contributed to better colloidal stability, while olive pomace and fish oil produced stable nanoemulsions with favorable droplet sizes (259.3 to 286.6 nm). The stability of lycopene in the nanoemulsions was influenced by storage temperature, with refrigerated samples (4 °C) maintaining better stability than those stored at higher temperatures, as demonstrated by a lower degradation rate (-0.0185 d^{-1} at 4 °C vs. -0.0334 d^{-1} at 25 °C). Although pH had a minimal effect on lycopene degradation, a slightly lower deterioration rate was observed at pH 7. Bioaccessibility determination showed that the olive pomace oil nanoemulsion had the highest lycopene bioaccessibility (53.8 ± 2.6%), while the nanoemulsion with fish oil had the lowest (40.1 ± 2.1%). Lipid phase did not affect the bioaccessibility of phenolic compounds, with all tested oils achieving bioaccessibility values over 100%. The developed functional beverage, containing the olive pomace oil nanoemulsion, showed decreased lycopene bioaccessibility (6.2 ± 1.8%) compared to the pure nanoemulsion (53.8 ± 2.6%), possibly due to the presence of fibers in apple juice which may have a hindering effect. Moreover, the study confirmed that beverage storage at 4 °C preserved lycopene stability and led to a shelf life of approximately 165 days, based on a 50% reduction in lycopene concentration, while no growth of microorganisms responsible for spoilage of the beverage was noted. The findings indicate that nanoemulsion technology is an effective strategy to enhance the shelf-life, physical stability, and bioaccessibility of bioactives in functional beverages. Additionally, the study supports circular economy practices by valorizing food industry by-products, such as olive and tomato pomace, transforming waste into valuable nutritional resources and promoting more sustainable product development. This approach not only adds nutritional and functional value but also aligns with industry goals for sustainability and innovation, making it a practical and environmentally beneficial solution. These results should be further validated through sensory evaluations to ensure commercial appeal and consumer acceptance.

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