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Nutritional Yeast (*Saccharomyces cerevisiae*) Spent Powder as a Clean-Label Emulsion Stabilizer: Linking pH-Dependent Interfacial Behavior, Bulk Rheology, and Emulsion Stability

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Abstract

Nutritional yeast spent powder (NYSP), a by-product of *Saccharomyces cerevisiae* processing, is rich in proteins, β -glucans, and mannoproteins, making it a promising clean-label stabilizer for food emulsions. This study evaluates NYSP as a stabilizer for oil-in-water emulsions and clarifies how pH controls interfacial and bulk stabilization. Emulsions (25% corn oil, 16.67% NYSP, and 58.33% water w/w) were prepared at pH 3.0, 5.7, 7.0, and 9.0. As pH increased, the zeta potential shifted from positive to negative values ($+6.11 \pm 0.26$ to -26.23 ± 0.53 mV), while the particle size was reduced from 881.80 ± 31.03 to 343.73 ± 23.27 nm. The surface tension peaked at pH 5.7 ($\gamma_{LV} = 38.43$ mN/m) and minimized at pH 9.0 (31.29 mN/m). Bulk rheology shifted from shear-thinning behavior: K decreased from 0.0541 to 0.0194 Pa $\cdot$ s⁻¹, and η_{50} decreased from 0.034 to 0.022 Pa $\cdot$ s (pH 3 to 9). Frequency sweeps revealed weak-gel behavior ($G'' > G'$) across all pH levels, alongside markedly reduced viscoelasticity at pH 9.0. Similarly, interfacial rheology indicated predominantly viscous interfaces ($G_i' < G_i''$) under strongly acidic and alkaline conditions, whereas elastic interfacial behavior ($G_i' > G_i''$) was observed at pH 5.7. Furthermore, maximum interface elasticity (lowest η_i^*) was achieved at pH 5.7. Long-term emulsion stability was maximal at acidic pH, where NYSP formed a weak, space-spanning gel network that increased bulk viscoelasticity and effectively suppressed coalescence, despite the higher interfacial tension. At pH 7.0–9.0, phase separation occurred due to diminished continuous-phase structuring, regardless of the higher surface charge. Overall, NYSP stands out as a promising, upcycled stabilizer that synergizes interfacial adsorption with continuous-phase structuring to yield pH-tunable (pH 3.0–6.0) emulsions.

Keywords: Nutritional yeast spent powder, clean-label emulsion stabilizer, bulk-interfacial rheology

1. Introduction

Nutritional yeast, also known as baker's or brewer's yeast, is predominantly made up of cells from carefully selected strains of *Saccharomyces cerevisiae*. This remarkable yeast is exceptionally versatile, serving as a key ingredient in the production of fuels, chemicals, pharmaceuticals, and food additives. Additionally, *S. cerevisiae* plays a crucial role in enhancing dairy productivity as a feed supplement for cattle, showcasing its invaluable contribution across multiple industries. Besides, it is undeniable that yeasts play a crucial role in the food industry. Today, the entire world, with its rapidly growing population, is facing low agricultural production, which has led to an economic focus on the production of food-grade yeasts (El-Helow et al. 2015; Sarkizi Shams Hajian et al. 2020). Yeast is currently the most widely utilized microorganism, with a global production capacity of 1.9 million tons (Kong et al. 2025). Yeast biomass powder, a by-product of yeast protein processing, is known for its low solubility, high nutritional value, and specific physiological functions (Y. Liu et al. 2021).

Additionally, due to its polysaccharide and protein content, it has the potential to serve as a natural emulsion stabilizer (Kong et al. 2025). Food-grade yeast biomass can also be produced as a by-product of industrial ethanol production on molasses (Martin and Chan 2024). Furthermore, food waste presents serious environmental and economic challenges across the globe, compelling nations to take decisive action in reducing and recycling it. Rich in nutrients, including vitamins, proteins, and carbohydrates, food waste has the potential to become a valuable resource for cultivating single-cell microorganisms like yeast, turning a problem into an opportunity (Günel-Köroğlu et al. 2025; Marson et al. 2020). Spent *Saccharomyces cerevisiae* from baker's yeast processing yields a cell wall-rich residue dominated by β -(1 $\rightarrow$ 3), (1 $\rightarrow$ 6)-glucans and mannoproteins that imparts hydrocolloid functionality in foods. β -glucan-rich fractions contribute strong water binding, viscosity build, and shear-thinning behavior, while mannoproteins supply interfacial activity that supports emulsion stabilization and improved mouthfeel; together, cell wall particles and extracts have been shown to stabilize emulsions and enhance techno-functional properties in model foods (Avramia and Amariei 2021; Baek et al. 2024). Collectively, the spent baker's yeast cell wall materials function as clean-label, multifunctional hydrocolloids that combine thickening and interfacial stabilization, making them suitable for diverse food and cosmetic matrices (Avramia and Amariei 2021; Baek et al. 2024; Günel-Köroğlu et al. 2025; Mokhtari et al. 2017; Utama et al. 2023).

The emulsions in food and cosmetic matrices are colloidal dispersions of liquid droplets in a non-miscible continuous liquid phase, exhibiting limited stability and, over time, separating

into their oil and aqueous phases. Surface-active biomolecules, low-molecular-weight emulsifiers, proteins, and specific hydrocolloids are frequently employed to formulate stable dispersed systems. These stabilizers function either by adsorbing onto interfaces to lower interfacial tension, or by thickening the continuous phase through the formation of a weak gel network. Through these distinct mechanisms, stabilizers create a protective barrier or restrict droplet mobility, thereby mitigating coalescence, flocculation, and Ostwald ripening. As a consequence, appropriate emulsion stabilizers provide robust resistance against droplet aggregation, maintaining long-term kinetic stability. Selecting the most suitable stabilizing agent is therefore a critical determinant in the effective design of stable food emulsions. Natural emulsion stabilizers, such as proteins and fibers, are increasingly preferred over synthetic additives due to their health benefits and sustainability (Pérez-Bassart et al. 2024). Despite its promising properties, research on baker's yeast spent is still in its infancy in food science, with no studies to date exploring its use as an emulsion stabilizer. Within the framework of the circular bioeconomy, the efficient recovery and reuse of such agro-industrial by-products hold significant industrial and economic value (Blasi et al. 2023; Kanai et al. 2024; Pal et al. 2024).

Given the scarcity of studies exploring the emulsifying potential of spent baker's yeast, the main objective of the present study was to hypothesize that this by-product can function as an effective natural emulsion stabilizer. Furthermore, this study investigates the feasibility of utilizing spent baker's yeast as a particulate stabilizer in oil-in-water (corn oil) emulsions. First, the spent baker's yeast powder was comprehensively characterized through ICP-MS, surface area and porosity analysis, and evaluation of its technological and physicochemical properties. Subsequently, the emulsions stabilized solely by spent baker's yeast particles were prepared and examined across a range of pH values (3, 5.7, 7, and 9) to assess their stability and performance. The stabilizing mechanism was elucidated by systematically analyzing the particle size distribution, steady-state-oscillatory (bulk rheology), and interfacial rheological behavior, as well as the microstructural features of the emulsions. Moreover, pH-dependent changes in interfacial properties of emulsions were investigated using FTIR, particle size distribution analysis, confocal laser scanning microscopy, contact angle measurements, and interfacial tension analysis. These results were complemented by an in-depth examination of particle dynamics, which clarified the mechanisms governing emulsion stability and destabilization under varying pH conditions. This work not only demonstrates the potential of spent baker's yeast as a sustainable, food-grade colloidal stabilizer but also provides a

theoretical foundation for valorizing this abundant industrial by-product, thereby enhancing its economic value within the food industry.

2. Materials and Methods

2.1. Materials

Nutritional yeast spent (*Saccharomyces cerevisiae*) sample was acquired by Özra Organik Co. from Antalya, Türkiye. Corn oil (Bizim yağ, İstanbul, Türkiye) was purchased from a local supermarket. The reagents used in this study were of analytical grade and include: Sulfuric acid (Merck, Germany), sodium carbonate (Sigma, USA), NaOH (Merck), 1-phenyl-3-methyl-5-pyrazolone (PMP, Sigma, Germany), hydrochloric acid (Merck), sodium phosphate (Sigma, Germany), acetonitrile (Merck), Nile Red (Sigma-Aldrich, Germany), Rhodamine B (Kimyalab, Türkiye), Fast Green FCF (Sigma-Aldrich, Germany), and Sodium dodecyl sulphate (SDS, Sigma-Aldrich, Germany).

2.2. Methods

2.2.1. Physicochemical properties

The color profiles of both the NYSP and the prepared emulsions were evaluated in quintuplicate using a calibrated colorimeter (CR-5, Konica Minolta, Japan). Following standard black and white calibrations, the primary color coordinates of L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were recorded. These coordinates were subsequently utilized to calculate the chroma (C^*), whiteness index (WI), browning index (BI), and total color difference (ΔE) according to the equations outlined by (Pathare et al. 2013a). Electrical conductivity of the emulsion systems was monitored directly using a portable digital dual-channel multimeter (HQ40d, Hach, Loveland, CO, USA). For the proximate composition of the raw material, the total protein content of the NYSP was quantified via the Kjeldahl method based on total nitrogen determination, while the total moisture and ash contents were assessed gravimetrically, all in accordance with the (AOAC 2000) official methods. Except for the color measurements, all analyses were performed in triplicate, and the final data were reported as mean $\pm$ standard deviation.

2.2.2. ICP-MS Analysis of NYSP

The total amount of trace elements in the spent yeast powder samples was quantified using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7900, Agilent Technologies, Tokyo, Japan), which was outfitted with an autosampler, nickel cones, Babington nebulizer, and a peristaltic sample delivery pump (Tokaloğlu et al. 2019). The powder sample was analyzed in triplicate. High-purity argon served as the plasma gas. The pulse-to-analog conversion factor was adjusted on the day of analysis, and the instrument was tuned with an Agilent ICP-MS tuning solution ($10 \mu\text{g L}^{-1}$ of Ce, Co, Li, Tl, and Y). ICP-MS operating parameters were optimized as follows: RF power at 1300 W; sample depth set to 7.5 mm; torch position adjusted to H-0.5 mm and V-0.1 mm; carrier gas flow rate at 1.11 L min^{-1} ; auxiliary gas flow rate at 0.9 L min^{-1} ; plasma gas flow rate at 15 L min^{-1} ; nebulizer pump rate of 0.12 rps; spray chamber temperature maintained at 2°C ; and nickel interface cones used. Data collection was carried out in both spectrum and full quantitative modes. The following isotopes were monitored: ^{11}B , ^{23}Na , ^{24}Mg , ^{31}P , ^{39}K , ^{43}Ca , ^{52}Cr , ^{55}Mn , ^{56}Fe , ^{63}Cu , and ^{66}Zn .

2.2.3. Surface Area and Porosity Analysis (BET Method) of NYSP

The specific surface area and porosity characteristics of the spent yeast powder samples were determined by nitrogen physisorption at 77 K using a surface area and porosimeter analyzer (Micromeritics TriStar II Plus 3.04, USA). Prior to analysis, samples were degassed under vacuum at 150°C for 360 min to remove physically adsorbed moisture and volatile components. Nitrogen (N_2) was used as the adsorptive gas. Adsorption–desorption isotherms were recorded over a wide relative pressure range at liquid nitrogen temperature (77 K). The specific surface area was calculated using the multipoint Brunauer–Emmett–Teller (BET) method in accordance with ISO 9277:2022. Pore size distribution and pore volume were evaluated from the desorption branch of the isotherm using the [BJH or t-plot] model. All calculations were performed using the instrument’s built-in analysis software.

2.2.4. Water and Oil Holding Capacities and Solubility of NYSP

The water-holding capacity (WHC) of the spent yeast powder was measured using the method described by Fu et al. (2022), with minor modifications. In this method, 100 mg of powder was mixed with 10 mL of distilled water, vortexed for 2 min, and then centrifuged (Nüve, Türkiye) at 4100 rpm for 30 min. After removing the free water, the amount of water retained by the sample was reported as grams of water absorbed per 1 g of powder. The oil-holding capacity (OHC) was measured in the same way, except that 100 mg of powder was dispersed in 10 mL of corn oil. The OHC was also expressed as grams of oil absorbed per 1 g of powder. Separately,

the water solubility index (WSI) of the NYSP was evaluated based on the method outlined by (He et al. 2019).

2.2.5. Determination of the monosaccharide composition of NYSP using HPLC

To determine the monosaccharide composition of the NYSP sample, high-performance liquid chromatography (HPLC) was employed based on the acid hydrolysis protocol outlined by Vojvodić Cebin et al. (2022), with minor adaptations. Briefly, the sample powder (approx. 25 mg) was transferred into a digestion tube and premixed intermittently with a glass rod for 30 min in 0.25 mL of 72% (v/v) sulfuric acid to achieve complete dissolution. Following the addition of 2.75 mL of distilled water, the mixture was thermally hydrolyzed in an oven at 100 °C for 2 h. Once cooled, the hydrolysate was centrifuged, and a 1 mL aliquot of the resulting supernatant was adjusted to pH 6.5–7.0 via neutralization with a molar equivalent of solid sodium carbonate. The solution was then clarified by passage through a 0.22 µm syringe filter. Derivatization was executed according to (Sun et al. 2014). For calibration, a standard reference solution containing mannose, ribose, and glucose (0.33 mg/mL each) was prepared and analyzed under identical conditions. Chromatographic quantification was performed on an Agilent 1280 series HPLC system equipped with a DAD detector and a C18 column (Agilent, USA), maintained at 25 °C, using the separation parameters described by Vojvodić Cebin et al. (2022). The mobile phase comprised 100 mM sodium phosphate buffer (pH 8.0) (A) and acetonitrile (B), running the following gradient elution: 0 min, 12% B; 35 min, 17% B; 36–45 min, 20% B; and 46–65 min, 12% B. The target monosaccharides were quantified using standard calibration curves established for mannose (1), ribose (2), and glucose (3).

$$\text{Mannose: } y = 3.332x - 38.684 \quad (R^2 = 0.998) \quad (1)$$

$$\text{Ribose: } y = 3.150x + 233.903 \quad (R^2 = 0.998) \quad (2)$$

$$\text{Glucose: } y = 2.743x + 259.134 \quad (R^2 = 0.998) \quad (3)$$

2.2.6. Preparation of Emulsions

Oil-in-water emulsions with a fixed 25% (w/w) oil content were prepared with minor modifications, following to the procedure described by Ying et al. (2021). Initially, 40 g of NYSP was dispersed into 60 g of corn oil and homogenized at 10,000 rpm for 5 minutes. Subsequently, 140 g of deionized water was introduced into the dispersion, and the mixture was

blended for an additional 10 minutes at the same speed. This composition established a precise final net NYSP concentration of 16.67% (w/w) based on the total emulsion mass. The systems were prepared under four distinct pH conditions: the natural pH of 5.7, and adjusted pH levels of 3.0, 7.0, and 9.0. The pH adjustments were performed using 3 M NaOH and HCl. All emulsions were stored at 4 °C until further analysis.

2.2.7. Characterization of NYSP-Stabilized Emulsions

2.2.7.1. Confocal laser scanning microscopy (CLSM)

The morphological analysis of emulsions stabilized with spent yeast was conducted using confocal laser scanning microscopy (LSM900 Airyscan 2, Zeiss Micro Imaging GmbH, Germany). Oil droplets in 1.0 mL of freshly prepared emulsion were stained with 25 µL of a 0.1% w/v Nile Red solution (dissolved in isopropanol). The carbohydrate and protein content in 1 mL of emulsions was stained with 20 µL of a 0.1% w/v Rhodamine B solution (dissolved in water) and 20 µL of a 0.1% w/v Fast Green FCF solution (dissolved in water), respectively. The stained emulsions were allowed to rest for 30 minutes. Emulsion preparations were made using Silane-Coated Adhesive Microscopic Slides with a positively charged surface (Objektträger, 75 x 25 mm, Isotherm, Germany). 25 µL of emulsion solution was applied to the slides, covered with a coverslip, and sealed around the edges with clear nail polish. During imaging, excitation wavelengths of 488 nm for Nile Red, 633 nm for Fast Green FCF, and 565 nm for Rhodamine B were used. Images from three different dyes were combined into a single stack to produce the final images for publication (Ako et al. 2009; Gallier et al. 2010).

2.2.7.2. Zeta potential and particle size distribution

The zeta potential, polydispersity index, and particle size distribution of the emulsions were evaluated via a dynamic light scattering (DLS) system (Zetasizer Nano ZS90, Malvern Panalytical, UK). The emulsion samples were transferred into dedicated cuvettes and analyzed at a controlled room temperature (25 °C).

2.2.7.3. Contact Angle and Surface Tension measurements

The surface tension and contact angle of the prepared emulsions were characterized at 25 °C using an Attension Theta optical goniometer/tensiometer (Biolin Scientific, Sweden) to evaluate their surface activity and wetting behavior as a function of pH. The surface tension (γ_{LV}) was determined via the pendant drop method. High-resolution profiles of the emulsion droplets suspended from a syringe needle tip were captured using an integrated camera, and γ_{LV} values were derived by fitting the experimental droplet shapes to the Young–Laplace equation (Tian et al. 2024). Separately, the contact angle (θ) was evaluated using the sessile drop method. A single droplet of the emulsion was deposited onto a solid substrate, and the resulting contact angle was precisely calculated via digital image analysis. Both measurements were performed in triplicate for each pH condition to ensure reproducibility.

2.2.7.4. Fourier-transform infrared spectroscopy (FTIR) analysis

The Attenuated Total Reflection (ATR)-FTIR spectra of both the particles and the oil were recorded using a Fourier-transform infrared spectrometer (Spectrum 400, PerkinElmer Instruments, Waltham, MA, USA) operating at a spectral resolution of 4 cm⁻¹. Each spectrum was obtained by averaging 100 scans, collected over the wavenumber range of 450–4000 cm⁻¹. This scanning protocol ensured a high signal-to-noise ratio and reliable characterization of the functional groups present in the samples. Gaussian peak deconvolution (nonlinear least-squares) was performed in the 3500–2800 cm⁻¹ and 1900–900 cm⁻¹ regions to resolve overlapping contributions; peak positions were initialized using derivative-based peak detection. Peak centers and integrated areas were extracted for comparison among pH conditions.

2.2.7.5. Emulsifying Stability Index (ESI)

The emulsifying properties of the samples were determined using the turbidity method described by Hou et al. (2022). A 100 µL sample was taken from the bottom of the centrifuge tube on days 1, 7, and 14 and diluted in 10 mL of a 0.1% sodium dodecyl sulfate (SDS) solution. The mixture's absorbance was measured promptly at 500 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan), with the 0.1% SDS solution as the blank. The emulsifying stability index (ESI) was calculated using the following equation:

$$ESI(\%) = \frac{A_{7,14}}{A_1} \times 100\% \quad (4)$$

where A_1 , A_7 , and A_{14} correspond to the absorbance values measured on days 1, 7, and 14, respectively.

270

271 **2.2.7.6. Rheological Properties**

272 **2.2.7.6.1. Steady Shear Rheology**

273 Steady shear measurements of the emulsion samples were conducted using a Peltier-controlled
274 rheometer (Thermo HAAKE Mars III, Karlsruhe, Germany). A parallel plate geometry
275 (P35TiL) with a 1 mm gap was used, and 1 mL of emulsion was loaded for each test.
276 Measurements were performed at 25°C, over a shear-rate range of 0.1–100 s⁻¹. Apparent
277 viscosity, shear stress, and shear rate data were recorded using the RheoWin Data Manager
278 software (RheoWin Pro 4.0, HAAKE, Karlsruhe, Germany). The consistency coefficient (K)
279 and flow behavior index (n) were calculated by fitting the data to the Ostwald–de Waele power-
280 law model.

281 **2.2.7.6.2. Dynamic Shear Rheology**

282 The viscoelastic properties of the emulsions were analyzed using the same rheometer (Thermo
283 HAAKE Mars III, Karlsruhe, Germany). Frequency sweep tests were performed from 0.1 to 10
284 Hz under an oscillatory stress of 0.2 Pa, previously confirmed to fall within the linear
285 viscoelastic region. Storage modulus (G') and loss modulus (G'') values were obtained using
286 RheoWin Pro v4.0 data analysis software of the instrument.

287 **2.2.7.6.3. Interfacial Rheology**

288 Interfacial rheological measurements were fulfilled using a Rheometer (Thermo HAAKE, Mars
289 III, Karlsruhe, Germany), and a bicone probe (BC 68/5Ti) was employed for interfacial
290 rheological measurements. Sample preparation for interfacial testing was performed in two
291 steps. First, the spent yeast powder intended for the emulsion system was dispersed in water
292 and mixed until a homogeneous mixture was achieved. Then, 100 mL of this aqueous phase
293 was transferred to the measurement cell, and the probe was lowered to determine the contact
294 point. The contact point was recalculated for each measurement. Afterward, corn oil was
295 carefully layered onto the aqueous phase, allowing the probe to sit directly oil–water interface.
296 Interfacial frequency sweep tests were performed within the linear range at $\gamma = 0.1\text{--}10\text{ rad}\cdot\text{s}^{-1}$
297 and $\omega = 0.1\%$.

298 **3. Results and Discussion**

299 **3.1. Physicochemical Characteristics and Porosity of NYSP**

The nutritional yeast spent powder exhibited a high protein content ($42.61 \pm 0.03\%$), low moisture ($4.75 \pm 0.09\%$), and moderate ash content ($4.72 \pm 0.11\%$). These values fall within reported ranges for dried brewer's/spent yeast biomass and are consistent with its use as a protein-rich byproduct (typically ≈ 41 – 49% protein, ash often 1.7 – 8.5%) (Delphine et al. 2023; Marson et al. 2020). The elevated protein fraction is consistent with yeast-derived biomass and is advantageous for emulsion stabilization because mannoproteins and peptide-rich fragments can contribute amphiphilicity and interfacial adsorption (Avramia and Amariei 2021; Cameron et al. 1988; Reis et al. 2023). Low residual moisture supports storage stability of the powder and limits hydrolytic degradation before use (Zeko-Pivač et al. 2023). The ash level reflects a substantive mineral load that can modulate ionic strength and, consequently, electrostatic interactions in dispersion (Adair et al., 2001; Delphine et al., 2023).

The color attributes of spent nutritional yeast powder, measured in the CIE Lab space, provide an objective description of visual appearance and a consistent basis for comparing samples and process conditions (Pathare et al. 2013b). The powder showed L^* 68.04 ± 0.08 , a^* 4.99 ± 0.04 , b^* 24.69 ± 0.26 , chroma C^* 25.19 ± 0.26 , whiteness index (WI) 59.31 ± 0.21 , total color difference (ΔE) 72.56 ± 0.05 , and browning index (BI) 49.54 ± 0.67 (Table 1). These values collectively suggest a relatively light, yet notably yellow, matching the typical yellow–brown tone of yeast-derived powders.

The material displayed a water-holding capacity (WHC) of 3.03 ± 0.08 g/g and an oil-holding capacity (OHC) of 2.21 ± 0.09 g/g. The high WHC implies substantial hydration and swelling of cell-wall polysaccharides (e.g., β -glucans and mannans) and proteinaceous components, which can increase the viscosity or weak-gel character of the aqueous phase, thereby slowing the creaming process (Avramia and Amariei 2021; Choi and McClements 2020; Kong et al. 2025; Nevara et al. 2021). The appreciable OHC indicates affinity for nonpolar phases, facilitating particle accumulation at the oil-water interface and/or partial occlusion of oil within hydrated particle networks, both of which support emulsion stabilization (Cameron et al. 1988; Qiao et al. 2022).

The Water Solubility Index (WSI) of the NYSP sample was determined to be $25.83 \pm 0.30\%$ (Table 1). This moderate solubility profile is closely related to the structural organization and solubility of its macromolecular components in their native state. Yeast proteins, which constitute $42.61 \pm 0.03\%$ of the sample, generally exhibit low water solubility under standard pH conditions (pH 2–9) due to their dense structural packaging (Amagliani et al. 2026),

although alkaline environments can enhance their dissolution (Min et al. 2024; Zidani et al. 2012). Regarding the carbohydrate fraction, the HPLC analysis following complete acid hydrolysis revealed that glucose ($65.53 \pm 0.12\%$) and mannose ($31.5 \pm 0.03\%$) are the predominant monosaccharide constituents, confirming that the polysaccharide matrix is primarily composed of glucans and mannans. In intact yeast cell walls, structural glucans (such as β -glucans) are typically insoluble in water due to their dense triple-helical conformation (Ishimoto et al. 2017; Zou et al. 2025), and the chitin-glucan complex remains highly insoluble (Araújo et al. 2020). In contrast, cell wall mannans (often associated with proteins as mannoproteins) represent the more hydrophilic and water-soluble carbohydrate fraction. Therefore, the soluble fraction contributing to the observed WSI (25.83%) is likely composed of these soluble mannoproteins/mannans and potentially minor soluble glucan fractions, while the insoluble residue is dominated by the structural insoluble β -glucans and tightly bound proteins.

Elemental analysis (ICP-MS, reported in ppm) revealed abundant monovalent cations and phosphate (Na 1,985; K 1,314; P 1,279), alongside notable levels of Mg (772.71) and Ca (317.10). Transition metals were found at lower concentrations (Zn 94.40; Fe 22.68; Cu 5.80; Mn 5.65; Cr 0.58; B 0.45). From a colloidal perspective, monovalent ions can screen electrostatic repulsion between charged particles and droplets (Adair et al. 2001; Butt et al. 2023), while divalent ions (Mg^{2+} , Ca^{2+}) may promote interparticle associations, strengthen interfacial films, or modulate interfacial rheology via salt-bridging with protein/polysaccharide functionalities (Agmo Hernández 2023; Du et al. 2021; Ye and Singh 2001; Zhou et al. 2021). These effects are expected to be pH-dependent, with stronger charge screening at higher ionic strength (Bos and van Vliet 2001). The presence of redox-active metals (e.g., Fe, Cu) suggests that antioxidant management and/or chelation strategies may be prudent for lipid-rich systems to mitigate oxidation, especially during extended storage (Keowmaneechai & McClements, 2006; McClements & Decker, 2000). This issue may affect the emulsion properties and quality.

The BET surface area of the nutritional yeast sample was determined using nitrogen physisorption at 77 K (Fig. 1), in accordance with the ISO standard (9277:2022). The analysis yielded a BET surface area of $1.20 \pm 0.13 \text{ m}^2/\text{g}$, calculated from an optimized pressure range of 0.15–0.30 P/P_0 , with an excellent correlation ($R^2 = 0.9649$). This value is characteristic of non-porous or minimally porous materials (Brunauer et al. 1938). The nitrogen adsorption-desorption isotherm exhibited Type II behavior according to IUPAC classification (Thommes et al. 2015), characteristic of non-porous solids with unrestricted monolayer-multilayer

adsorption. The minimal hysteresis (H3 type) and negligible total pore volume (0.000615 cm³/g) indicate that the yeast cell wall structure remains largely impermeable to nitrogen at 77 K. This observation is consistent with previous studies on dried microbial cell walls, which demonstrate that freeze-drying causes cell wall collapse and loss of porosity (Scherrer et al. 1977). The BJH mesopore analysis revealed only minor inter-particle voids with an average diameter of 37.7 Å, while t-plot analysis indicated negative micropore volume, confirming the absence of accessible micropores. The low surface area (1.20 m²/g) reflects only the external particle geometry and is typical for intact, dried microbial biomass with closed-cell structure (Rouquerol et al. 1994; Scherrer et al. 1977). The yeast cell wall accounts for ~15–30% of the cell's dry mass and is composed of an inner β -1,3/ β -1,6-glucan/chitin network, with an outer, densely packed mannoprotein layer that restricts permeability. This dense, rigid wall, along with the β -glucan triple-helix network, underpins the high water-binding capacity and the robustness of cell-derived particles at interfaces. Culture/processing conditions modulate wall thickness and composition (Avramia and Amariei 2021; Günal-Köroğlu et al. 2025; Lipke and Ovalle 1998).

3.2. Monosaccharide Composition of NYSP

The monosaccharide composition of nutritional yeast spent powder (NYSP) was determined by HPLC after acid hydrolysis and PMP derivatization. As shown in Table 1 and Fig. 3, glucose was the dominant monosaccharide, accounting for 65.53% of the total quantified monosaccharides, followed by mannose at 31.50% and ribose at 2.97%. This monosaccharide profile confirms that NYSP is mainly composed of glucose- and mannose-containing polysaccharide structures, which is consistent with the typical cell-wall architecture of *S. cerevisiae*.

The high glucose content is mainly associated with the β -glucan-rich fraction of the yeast cell wall. In *S. cerevisiae*, β -glucans form a major structural component of the cell wall and contribute to mechanical strength, hydration capacity, and network formation. This interpretation agrees with the high water-holding capacity of NYSP observed in the present study and with previous reports describing yeast-derived β -glucans as functional polysaccharides with strong hydration and structuring properties (Avramia and Amariei 2021; Günal-Köroğlu et al. 2025; Lipke and Ovalle 1998). Mannose was the second-most-abundant monosaccharide in NYSP. This result suggests the presence of mannans and mannoproteins, which are known constituents of the outer layer of the yeast cell wall. Mannoproteins are particularly relevant for emulsion systems because they combine hydrophilic carbohydrate

moieties with proteinaceous regions, thereby contributing to amphiphilicity, interfacial adsorption, and colloidal stabilization. Therefore, the substantial mannose content of NYSP supports its potential role as an interfacial active yeast-derived material in emulsions (Avramia and Amariei 2021; Baek et al. 2024; Kong et al. 2025).

The low ribose content (2.97%) indicates that ribose-containing components were present only in a minor fraction. Since ribose is not a principal structural monosaccharide of the yeast cell wall, its presence may be related to residual intracellular materials or nucleic-acid-derived components remaining in the spent yeast matrix after processing. Thus, the monosaccharide profile is dominated by structural cell-wall carbohydrates rather than by minor intracellular sugar residues. From a functional perspective, the glucose- and mannose-rich composition of NYSP provides chemical support for its dual role in the emulsion system. The β -glucan-rich fraction may contribute mainly to water binding, swelling, viscosity development, and weak network formation in the continuous phase, whereas mannoprotein-associated structures may enhance interfacial adsorption and support oil–water stabilization. This compositional balance is consistent with the rheological and emulsion-stability results of the present study, in which NYSP-stabilized emulsions exhibited pH-dependent differences in bulk structuring, interfacial behavior, and long-term stability. Similar functional roles of yeast-derived polysaccharides and mannoproteins have been reported for yeast-based hydrocolloids and emulsion stabilizers (Baek et al. 2024; Chraniuk and Bzducha-Wróbel 2025; Kong et al. 2025).

3.3. Colorimetric Features and Electrical Conductivity of the Emulsions

The color parameters of the oil-in-water emulsions stabilized by brewer’s spent yeast powder are summarized in Table 2. As pH rose from 3 to 9, L^* , a^* , b^* , chroma, whiteness index, and total color difference (ΔE) decreased significantly, whereas the browning index (BI) increased markedly ($p < 0.05$). This pH-dependent optical behavior is governed by two synergistic mechanisms: pH-induced microstructural evolution and alkaline-accelerated non-enzymatic browning.

At pH 3, yeast proteins and mannoproteins carry a strong net positive charge, exhibiting high solubility and surface activity. Their rapid adsorption at the oil–water interface yields small, well-dispersed droplets that maximize multiple light scattering, thereby enhancing lightness (L^*), chroma, and whiteness while minimizing BI (Chanamai and McClements 2001). Conversely, approaching the isoelectric region (evidenced by a conductivity minimum around pH 5.7) and moving into alkaline conditions, weakened electrostatic repulsion promotes droplet

flocculation or coalescence. This increases the effective optical scatterer size and reduces light scattering efficiency, lowering L^* , chroma, and whiteness index (McClements 2002). Non-adsorbing β -glucans in the yeast matrix further modulate this microstructure; while they increase continuous-phase viscosity to limit creaming, they can also induce depletion flocculation under high electrostatic screening, indirectly coarsening the emulsion (Avramia and Amariei 2021; Reis et al. 2023).

Simultaneously, alkaline conditions accelerate base-catalyzed non-enzymatic browning (Maillard and caramelization pathways) within the protein- and carbohydrate-rich yeast matrix. This generates visible-light-absorbing melanoidins, which further depress L^* , a^* , and b^* while elevating BI (Ajandouz and Puigserver 1999; P. Liu et al. 2018). These findings align with recent studies confirming that pH-dependent co-adsorption, interfacial rheology, and chemical browning collectively govern the CIELAB coordinates of yeast-stabilized systems (Kong et al. 2025; Reis et al. 2023).

The electrical conductivity of the emulsions ranged from 9.82 to 13.43 mS/cm (Table 2), confirming their oil-in-water nature with a percolating aqueous phase rich in mobile charge carriers from the spent yeast's high mineral load (Table 1) (Adair et al. 2001; Butt et al. 2023). The conductivity exhibited a non-monotonic pH dependence: it was highest at pH 3.0 (13.43 mS/cm), reached a minimum at the natural pH of the powder (pH 5.7, 9.82 mS/cm), and rose again at pH 7.0 and 9.0 (10.60 and 11.43 mS/cm, respectively).

This trend is driven by pH-induced changes in the ionization state of cell-wall polysaccharides and mannoproteins, which govern ion-binding equilibria. At pH 3.0, extensive protonation of carboxylate and phosphate groups suppresses counterion binding to the yeast matrix, keeping a larger fraction of ions free in the continuous phase to enhance conductivity. Near the natural pH (5.7), partial deprotonation coupled with strong interparticle association immobilizes a portion of these ions within a weak gel-like network, restricting their mobility and yielding the minimum conductivity (Table 5). At alkaline pH, further deprotonation increases the net negative charge density on the droplets (Table 4), thereby expanding the diffuse layer and releasing a larger population of mobile counterions into the aqueous phase, which accounts for the subsequent rise in conductivity (Agmo Hernández 2023; McClements 2002; Ravindran et al. 2018).

3.4. FTIR Characterization of the Emulsions

462 The FTIR spectra of corn oil and NYSP-stabilized emulsions (pH 3.0–9.0) recorded at
463 4000-400 cm^{-1} (Fig. 2A) confirmed the structural stability of the biopolymer-lipid systems,
464 supporting the efficacy of NYSP as an emulsion stabilizer (Bikmurzin et al. 2022; Galichet et
465 al. 2001; Herculano et al. 2021; Mahboubifar et al. 2017; Rohman et al. 2020; Rohman and Che
466 Man 2012). The high-wavenumber region (3100-2800 cm^{-1} , Fig. 2A and B) was dominated
467 by asymmetric and symmetric CH_2 stretching of fatty acyl chains at 2920 and 2850 cm^{-1} ,
468 terminal CH_3 stretching at $\sim 2953 \text{ cm}^{-1}$, and weak cis-olefinic $=\text{C}-\text{H}$ stretching shoulders at
469 3005-3010 cm^{-1} (Bunaciu et al. 2022; Rohman et al. 2020). These bands are characteristic of
470 liquid vegetable oils rich in unsaturated triacylglycerols (María D Guillén and Cabo 1997;
471 Kokalj Ladan and Kočevár Glavač 2022; Yang and Irudayaraj 2000). Notably, the sharp 2920
472 and 2850 cm^{-1} oil bands showed minor attenuation at pH 9.0 and 7.0, merged into a single
473 diffuse peak at pH 5.7, and underwent pronounced suppression at pH 3.0. This indicates that
474 acidic conditions promote a structural particulate network that entraps oil droplets, a mechanism
475 strongly supported by CLSM micrographs and stability data.

476 In the carbonyl region, the ester $\text{C}=\text{O}$ stretching band of triglycerides at 1740-1745 cm^{-1}
477 (Alshuiael and Al-Ghouti 2020; Matwijczuk et al. 2018; Zahir et al. 2017) broadened and
478 slightly decreased in the emulsions, reflecting microenvironmental heterogeneity near the
479 aqueous interface (Alshuiael and Al-Ghouti 2020; Mahboubifar et al. 2017; Matwijczuk et al.
480 2018). The absence of lower carbonyl or hydroperoxide bands ruled out significant lipid
481 hydrolysis or oxidation during processing (J. Chen et al. 2018; Embaby et al. 2022; Tsai et al.
482 2023).

483 The amide I (1650-1630 cm^{-1} , $\text{C}=\text{O}$ stretch) and amide II (1550-1530 cm^{-1} , $\text{N}-\text{H}$ bend/ $\text{C}-\text{N}$
484 stretch) regions monitored the NYSP proteinaceous fraction at the oil-water interface (Barth
485 2007; DeFlores et al. 2009; Ji et al. 2020; Schestkova et al. 2020; Tatulian 2019; Wen et al.
486 2024; Zhai et al. 2013). At pH 5.7, the amide I band broadened and shifted to lower
487 wavenumbers, indicating partial unfolding or structural reorganization under higher proton
488 concentrations (Kandasamy et al., 2025; Miller et al., 2013; Us. oltsev et al., 2019). Conversely,
489 these bands attenuated significantly at pH 7.0 and 9.0, likely due to the aggregation and
490 precipitation of water-insoluble NYSP proteins during oil phase separation (Barth 2007; Chiang
491 et al. 2023; Dai et al. 2025; DeFlores et al. 2009; Ji et al. 2020). These findings confirm that the
492 interfacial protein conformation is highly pH-modulated (De Meutter and Goormaghtigh 2021;
493 Schestkova et al. 2020; Wen et al. 2024; Zhai et al. 2013).

Below 1500 cm^{-1} , bending modes of fatty acyl chains appeared at 1465 and 1375 cm^{-1} (M. D. Guillén and Cabo 1997; María D Guillén and Cabo 1997; Mahboubifar et al. 2017). The carbohydrate "sugar region" ($1250\text{-}950\text{ cm}^{-1}$) exhibited prominent bands at $\sim 1155\text{-}1160$, $1100\text{-}1090$, $1050\text{-}1040$, and $1030\text{-}1000\text{ cm}^{-1}$ (C–O and C–O–C stretching of mannans and β -glucans), alongside anomeric features ($890\text{-}915\text{ cm}^{-1}$) confirming yeast cell wall structures at the interface (Bikmurzin et al. 2022; Bzducha Wróbel et al. 2022; Chraniuk and Bzducha-Wróbel 2025).

This macromolecular matrix exhibited pH-dependent conformational changes: lower pH enhanced protonation and hydrogen bonding, broadening the amide and carbohydrate bands (Barth 2007; De Meutter and Goormaghtigh 2021; Goormaghtigh et al. 2006). In contrast, higher pH induced electrostatic deprotonation, leading to more extended conformations and reduced band overlap (Bikmurzin et al. 2022; Galichet et al. 2001; Piotrowska and Masek 2015).

3.5. Zeta-Potential, Polydispersity Index, and Particle Size Distribution of the Emulsions

Zeta potential, Z-average hydrodynamic diameter, and polydispersity index (PDI) were used to evaluate the pH-dependent colloidal behavior of NYSP-stabilized emulsions (Table 3), with corresponding particle size distribution (PSD) profiles shown in Fig. 4. In these concentrated, particle-rich systems, the DLS signal reflects the effective hydrodynamic size of diverse NYSP-based colloidal entities (including yeast cells, fragments, aggregates, and droplet–particle complexes) rather than true oil droplet size (Choi and McClements 2020; Stetefeld et al. 2016).

The zeta potential shifted from positive to negative values as pH increased (Table 3). At pH 3.0 and 5.7, the emulsions exhibited positive values of 6.11 mV and 2.01 mV , respectively, but reached -19.60 mV at pH 7.0 and -26.23 mV at pH 9.0. This charge reversal is attributed to the gradual deprotonation of anionic functional groups (e.g., carboxylate and phosphate) on yeast cell-wall mannoproteins (Assunção Bicca et al. 2022; Lavaisse et al. 2019; Rogowska et al. 2018), which promoted net negative charges. The resulting stronger electrostatic repulsion at higher pH prevented aggregation among NYSP-coated entities, consistent with DLVO-type stabilization theories (Agmo Hernández 2023; Bos and van Vliet 2001; Butt et al. 2023; McClements 2015).

In agreement with these charge trends, the Z-average diameter decreased markedly from 881.80 nm at pH 3.0 to 343.73 nm at pH 9.0 (Table 3). The larger sizes under acidic conditions (pH 3.0 and 5.7) indicate that weaker electrostatic repulsion promoted the association of NYSP particles and complexes. Conversely, the smaller Z-averages at neutral and alkaline pH suggest improved dispersion via electrostatic stabilization (Li and Zhao 2020; Östbring et al. 2021; Ravindran et al. 2018). This pH-induced disaggregation aligns with the rheological behavior (Table 5), where the pH 9.0 emulsion exhibited the lowest viscosity, typical of weakly structured, unassociated dispersions. (Bos and van Vliet 2001; Ravindran et al. 2018).

The PDI values also decreased significantly when transitioning from acidic (0.43 at pH 3.0; 0.44 at pH 5.7) to neutral and alkaline conditions (0.29 at pH 7.0; 0.30 at pH 9.0; Tukey HSD, $p < 0.05$; Table 3). Under acidic conditions, the systems exhibited broad monomodal distributions centered at larger diameters (Fig. 4A and B), reflecting the formation of a robust particle network that successfully entrapped oil droplets, as confirmed by CLSM micrographs (Fig. 8). Conversely, neutral and alkaline environments led to narrower, sharper profiles shifted toward smaller sizes (Fig. 4C and D). However, this apparent reduction in size and polydispersity at higher pH does not indicate a fully stable, intact emulsion. Instead, due to the loss of the structural particle network (as reflected by lower viscosity), the larger oil droplets underwent rapid coalescence and upward creaming. In contrast, larger water-insoluble yeast cell-wall fragments settled out. Consequently, DLS measurements selectively captured only the remaining highly dispersed, water-soluble fractions and finely suspended particles in the middle phase. Thus, while systems at neutral and alkaline pH appeared more uniform in DLS, their overall macro-structural integrity was lost compared to the stable, oil-entrapping networks maintained under acidic conditions.

3.6. Contact Angle and Surface Tension Measurement of the Emulsions

The interfacial properties of the spent yeast-stabilized emulsions exhibited a strong dependence on pH, reflecting changes in the availability, dispersion state, and transport of yeast-derived amphiphilic components within the continuous phase. The liquid (emulsion)–air surface tension (γ_{LV}) ranged from 31 to 38 mN/m across the investigated pH range (Table 4), representing a substantial reduction compared to pure water and confirming the presence of active interfacial species under all pH conditions (visualized in Fig. 5).

As shown in Table 4, γ_{LV} reached its maximum at pH 5.7 and subsequently decreased toward alkaline pH, attaining its lowest value at pH 9.0. The decrease in γ_{LV} at higher pH suggests enhanced interfacial coverage, driven by the improved dispersion and increased aqueous solubility of proteinaceous yeast particles and soluble protein derivatives. Under these alkaline conditions, progressively more negative zeta potential values (stronger electrostatic repulsion) facilitated the diffusive transport of these solubilized amphiphilic species toward the interface, promoting adsorption and reducing interfacial tension (Moreira et al. 2016; Narsipur et al. 2025). Conversely, diminished electrostatic stabilization under other conditions can lead to particle aggregation, limiting interfacial availability and increasing surface tension (Wang et al. 2025). Importantly, however, the lower surface tension at alkaline pH did not yield more stable emulsions. Although the interfacial concentration of highly charged proteins increased, it remained insufficient on its own to establish a fully protective interfacial layer capable of stabilizing the emulsion.

The contact angle (θ) of the emulsion droplets on the solid surface followed a highly consistent non-monotonic trend with the γ_{LV} results (Table 4). The contact angle reached its peak at pH 5.7, then decreased significantly under neutral (pH 7.0) and alkaline (pH 9.0) conditions, as well as at pH 3.0. This non-monotonic response, characterized by a local maximum at pH 5.7, can be rationalized by bulk transport limitations. As shown in Fig. 6A, the emulsion viscosity reached its highest value (at shear rate $0.1-1 \text{ s}^{-1}$) at pH 5.7, which restricted the diffusive transport of both proteinaceous particles and dissolved proteins to the interfaces, thereby reducing effective surface coverage. At pH 5.7, the weaker electrostatic charges further limited the formation of a cohesive interfacial particle layer, allowing the hydrophobic character of the core oil phase to be more pronounced (higher θ , lower wettability). In contrast, at pH values away from the isoelectric point (pH 3.0, 7.0, and 9.0), the lower contact angles indicate that the oil droplets were effectively enveloped by the highly charged, surface-active NYSP components, significantly enhancing droplet wettability on hydrophilic surfaces (Meng and Nicolai 2023).

3.7. Emulsion Stability Index (ESI) of the Emulsions

The emulsion-stabilizing performance of NYSP exhibited clear pH-dependence over 14 days. Emulsions at pH 3.0 and 5.7 displayed higher ESI values (Fig. 9) and remained homogeneous, whereas pronounced phase separation occurred at pH 7.0 and 9.0 (Fig. 10). This aligns with

CLSM observations, which revealed smaller, more uniform oil droplets under acidic conditions, and larger droplets at higher pH (Fig. 8).

This behavior is directly governed by the magnitude of electrostatic interactions, as confirmed by the zeta potential values (Table 3). Under acidic conditions, the low absolute surface charges at pH 3.0 (6.11 mV) and pH 5.7 (2.01 mV) resulted in weak electrostatic repulsion. This minimized repulsion favored interparticle associations, establishing a weak, space-spanning network in the continuous phase that restricted droplet movement and suppressed coalescence and creaming (Firoozmand and Rousseau 2016).

Conversely, the higher pH levels generated stronger electrostatic repulsion, evidenced by the significantly increased absolute zeta potentials at pH 7.0 (-19.6 mV) and pH 9.0 (-26.23 mV). While these stronger repulsive forces enhanced interfacial activity, they simultaneously restricted bulk network formation and continuous-phase viscosity. Despite interfacial rheology indicating more elastic films at pH 7.0 and 9.0 (Fig. 7), this lack of bulk structuring did not prevent droplet migration. Thus, ESI results demonstrate that optimal stability required a sufficient bulk network rather than electrostatic interaction alone (Firoozmand and Rousseau 2016).

Visual variations between pH 7.0 and 9.0 highlight this balance: pH 7.0 maintained oil-rich aggregates within a heterogeneous matrix, while the stronger repulsion and lower viscosity at pH 9.0 accelerated large droplet migration, exacerbating phase separation (Fig. 10). Overall, acidic conditions optimized stability by driving continuous-phase weak-gel network formation rather than particle-mediated interfacial stabilization. Furthermore, the occurrence of phase separation in emulsions prepared with lower NYSP concentrations (data not shown) confirms that the stabilization mechanism is predominantly governed by network formation rather than interfacial activity. This finding strongly supports that NYSP-based emulsions do not conform to the conventional Pickering emulsion mechanism (Dickinson 2013).

3.8. Confocal laser scanning microscopy (CLSM) of the Emulsions

To visually elucidate the spatial organization, droplet morphology, and multi-component distribution (lipids, proteins, and polysaccharides) of NYSP-stabilized emulsions across pH levels, confocal laser scanning microscopy (CLSM) was performed using a triple-staining strategy (Fig. 8). Tri-color fluorescence imaging enabled explicit spatial differentiation among

the neutral lipid phase (Nile Red, bright yellow-gold fluorescence), proteinaceous fractions (Fast Green FCF, vibrant green fluorescence), and cell-wall polysaccharides alongside general yeast biomass (Rhodamine B, reddish-orange fluorescence). The micrographs reveal distinct structural transitions that provide direct microscopic validation for the observed colloidal, interfacial, and macro-stability trends.

Under acidic conditions (pH 3.0, Fig. 8A–C; pH 5.7, Fig. 8D–F), emulsions display polydisperse oil droplets embedded within a continuous, darker yeast-rich aqueous matrix. Rather than uniform coverage, a heterogeneous distribution is observed, where certain droplets are fully encased by NYSP particles while others exhibit partial interfacial coverage. High-resolution fluorophore mapping provides clear insight into the spatial partitioning of cellular components: the cores of the oil droplets fluoresce brightly in yellow-gold, whereas the granular yeast entities in the surrounding matrix exhibit a composite orange-green fluorescence, corresponding to their structural cell-wall polysaccharides (β -glucans and mannans) encapsulating intracellular proteins. Crucially, at pH 3.0 and 5.7, prominent bright green fluorescent rings are clearly discernable surrounding the outer perimeters of several oil droplets. This green signal directly confirms the presence of Fast Green-stained solubilized amphiphilic proteins, released from the yeast fraction, that have migrated to and adsorbed onto the oil–water interface. Consequently, interfacial stabilization under acidic regimes operates through a synergistic mechanism: a molecular film of solubilized proteins working in tandem with sterically hindering, intact particulate yeast cells.

This microstructural arrangement directly correlates with the superior macro-structural stability and high ESI values under acidic regimes (Fig. 9). Low absolute zeta potential values at pH 3.0 and pH 5.7 minimized electrostatic repulsion among NYSP cell-wall entities, promoting interparticle associations that assembled a robust, space-spanning network in the continuous phase (Y. Chen et al. 2022; Fang et al. 2025). As confirmed by CLSM, this stronger continuous gel network (greater η_{50} , G' and G'' values, Fig. 6) immobilized oil droplets and acted as a physical barrier against gravitational creaming and coalescence (Firoozmand and Rousseau 2016). Furthermore, this visualization explains the broad monomodal particle size distributions obtained via DLS; larger Z-average diameters under acidic conditions reflect these interconnected droplet–particle complexes and bulk networks rather than poorly stabilized droplets.

Conversely, transitioning to neutral (pH 7.0, Fig. 8G–I) and alkaline (pH 9.0, Fig. 8J–L) environments severely disrupted the microstructure. As pH shifted away from the point of zero charge, droplets underwent extensive coalescence, becoming larger and more irregular. This structural breakdown was most acute at pH 9.0 (Fig. 8H, I, J), where the system was dominated by massive oil domains (green arrows in Fig. 8H, I), while the granular yeast fraction inverted into discrete clusters floating within the oil phase.

These CLSM observations complement the DLS data (Table 3), which showed that neutral and alkaline conditions yielded lower Z-average sizes and reduced PDI values. Enhanced electronegativity at pH 7.0 and pH 9.0 generated strong electrostatic repulsion, which disrupted the cohesive aggregated bulk particle network. Although lower surface tension and enhanced wettability at alkaline pH (Table 4) indicate active interfacial migration of solubilized amphiphilic proteins, evidenced by the diffuse green fluorescence dispersed throughout the matrix (Fig. 9J), CLSM confirms that interfacial protein adsorption alone was insufficient. Without a structuring polysaccharide-rich bulk matrix, highly charged protein films failed to prevent coalescence. Ultimately, these microscopic insights prove that NYSP-stabilized emulsions deviate from the conventional Pickering mechanism (Dickinson 2013), as stabilization is driven primarily by an immobilizing bulk network that is favored exclusively under acidic conditions rather than by classic particulate interfacial shielding.

3.9. Rheological characteristics

3.9.1. Steady and Dynamic Behaviors

The rheological properties of emulsions stabilized with nutritional yeast spent powder (NYSP) are summarized in Table 5. An increase in pH from 3 to 9 was accompanied by a concomitant decrease in both the consistency coefficient (K) and apparent viscosity (η_{50}) values, whereas the flow behavior index (n) exhibited an upward trend. The maximum K value ($0.054 \text{ Pa} \cdot \text{s}^n$) was observed at pH 3, while the η_{50} values at pH 3 and pH 5.7 were statistically comparable ($0.034 \text{ Pa} \cdot \text{s}$; Table 5). Furthermore, the highest n value (≈ 1.05) was recorded at pH 9 (Fig. 6A). The elevated viscosity at acidic pH can be attributed to the gelling capacity of the proteinaceous material, aligning with findings by Zhang et al. (2021), who reported a similar viscosity increase in whey protein-stabilized emulsions as pH decreased.

Indeed, the NYSP utilized in this study functions as a highly versatile bio-based agent. Its substantial protein content (42.61%), notable water-holding (3.03 g/g) and oil-holding (2.21 g/g) capacities, low water solubility index (WSI: 25.83%) (Table 1), and high concentration of cell-wall polysaccharides (43.10%) corroborate established literature indicating that spent brewer's and baker's yeast powders are abundant in mannoproteins and β -glucans. These constituents act as amphiphilic, rheology-modifying biopolymers and particulate emulsion stabilizers (Jaeger et al. 2020; Vélez-Erazo et al. 2021; Worrasinchai et al. 2006).

The steady and dynamic rheological responses of the emulsions reveal a clear pH-dependent structural transition: from a densely flocculated, robust gel network (higher G') under acidic conditions to a weakly structured ($G'' > G'$), near-Newtonian fluid at pH 9. Steady-shear flow profiles (Table 5) demonstrate that at pH 3 and 5.7, the emulsions exhibit elevated consistency indices (K), comparable apparent viscosities (η_{50}), and low flow behavior indices ($n < 1$), characteristic of pronounced pseudoplastic (shear-thinning) behavior. This rheological pattern aligns closely with the colloidal measurements: at low pH, the near-zero zeta potential (+6.11 mV at pH 3 and +2.01 mV at pH 5.7; Table 3) signifies minimal electrostatic repulsion, thereby promoting droplet aggregation. This finding is consistent with the well-documented relationship between particle aggregation, weak gel network formation, and shear-thinning behavior in particle- or protein-stabilized emulsions (Boostani et al. 2020; Félix et al. 2020; H.-N. Liang and Tang 2013).

Conversely, at pH 9, enhanced electrostatic repulsion (−26.2 mV) led to reduced initial particle sizes; however, this paradoxically facilitated droplet coalescence over time, resulting in a marked reduction in both K and η_{50} , characteristic of destabilized emulsions, where bulk viscosity diminishes as a consequence of aggregate network breakdown (Aslan Türker et al. 2021; McClements 2015).

Confocal micrographs (Fig. 8) and colorimetric parameters (Table 2) further substantiate this structural interpretation. At pH 3 and 5.7, the disperse oil phase is distributed as fine droplets within a yeast-rich continuous matrix. At pH 9, however, the emergence of larger oil domains and the disruption of the interconnected aqueous network explain the macroscopic loss of viscosity. This structural coarsening is mirrored in the optical properties: as pH increases, the L^* (lightness/whiteness) parameter decreases. This trend accords with observations in other bio-

based emulsion systems, where reduced light scattering caused by droplet coarsening or phase separation lowers the whiteness index despite constant phase volumes (Chantrapornchai et al. 1999; Xiao et al. 2016).

Dynamic oscillatory shear spectra (Fig. 6B–C) complement the steady-shear results, with all systems displaying weak gel-like characteristics ($G'' > G'$). This viscoelastic behavior was particularly pronounced under acidic conditions, where higher G' and G'' values (at 1 Hz) suggest that polydisperse droplets enhance the elastic response of the matrix. This phenomenon is widely reported in yeast-derived and fiber-based Pickering emulsions, in which densely packed interfacial layers and inter-droplet bridging networks (protein-polysaccharide or polysaccharide-surfactant interactions) foster elastic network formation (Cui et al. 2021; Kong et al. 2025).

Ultimately, this rheological hierarchy directly accounts for the Emulsion Stability Index (ESI) trends (Fig. 9). Formulations characterized by higher apparent viscosities and G' values (pH 3–5.7) maintained superior ESI values. This reinforces the established paradigm that bulk viscoelasticity and physical droplet immobilization are key determinants in suppressing creaming and coalescence mechanisms in particle-stabilized food emulsions (Berton-Carabin & Schroën, 2015; Chen et al., 2020; Tadros, 2004).

3.9.2. Interfacial Behavior

Interfacial shear rheology resolves the elastic (G_i') and viscous (G_i'') components of adsorbed films at the oil–water interface, providing mechanistic insight into emulsion stability: an elastic-dominant film ($G_i' > G_i''$) resists coalescence-driving deformations, whereas a viscous-dominant film ($G_i'' > G_i'$) lacks the load-bearing capacity required for long-term stability (A. Bos and van Vliet 2001; Murray 2011). Therefore, protein-based solid particles have been demonstrated to play a pivotal role in modulating interfacial film properties, thereby conferring pH-responsive stability to emulsions (Chevalier and Bolzinger 2013). Frequency-sweep measurements (G_i' , G_i'' , η_i^*) reveal a pronounced non-monotonic pH dependence in which the NYSP interfacial film transitions from viscous to elastic character (Figure 7A-B-C).

At pH 3, the NYSP interfacial film is clearly viscous-dominant (at 1 Hz; $G_i' = 0.0013$ Pa; $G_i'' = 0.0072$ Pa), and both moduli are at their minimum values across all conditions studied,

indicating that the adsorbed layer lacks the cohesive, load-bearing character required to mechanically resist droplet coalescence (A. Bos and van Vliet 2001). The zeta potential of +6.11 mV (Table 3), while positive, falls well below the |30 mV| threshold conventionally associated with adequate colloidal repulsion (Bhattacharjee 2016); at this low charge density, the electrostatic energy barrier to interdroplet approach is insufficient to prevent coalescence, and the system cannot rely on electrostatic repulsion as a primary stabilization mode.

Despite these concurrent limitations at both the interfacial film and colloidal levels, the emulsion retains apparent macroscopic stability, an outcome that is resolved by the steady-shear rheological data rather than by any interfacial or electrostatic mechanism. At pH 3, the continuous phase exhibits the highest flow consistency index ($K = 0.0541 \text{ Pa} \cdot \text{s}^n$) and the most pronounced shear-thinning behavior ($n = 0.885$) of all pH conditions tested (Table 5), indicating the formation of a structured, viscoelastic continuous-phase network. This network constrains the translational mobility of dispersed droplets, substantially reducing collision frequency and thereby retarding coalescence and creaming in the absence of a strong interfacial or electrostatic barrier (W. Liang et al. 2024; Tadros 2004), a stabilization route best described as viscosity-mediated kinetic trapping.

While interfacial elasticity represents the primary mode of film-mediated stabilization in protein-based emulsions Tao et al. (2024), the physicochemical conditions at pH 3 preclude its operation, and the system defaults to a bulk-phase kinetic arrest regime. The comparatively large particle size ($Z\text{-avg} = 881.80 \text{ nm}$; $\text{PDI} = 0.43$; Table 3) and smaller droplets at pH 3 (Fig. 8-A) are physically consistent with this interpretation: when stability is conferred primarily by bulk-phase viscosity rather than by a cohesive interfacial film or strong electrostatic repulsion, droplets are kinetically arrested at the size generated during homogenization, and re-coalescence is retarded by viscous dissipation rather than suppressed by an energetic barrier (De Carvalho-Guimarães et al. 2022).

A qualitatively similar $G_i'' > G_i'$ pattern has been reported for modified corn fiber gum (CFG) interfacial layers, where the predominantly polysaccharidic composition of the adsorbed film yielded viscous-dominant behavior throughout the measured frequency range (Wei et al. 2021). Similarly, (Zhou et al. 2021) reported that whey protein isolate forms a viscous-dominant interface ($G_i'' > G_i'$) at pH 3, consistent with the emulsion behavior observed in the present study. The stabilization mechanism at pH 3 is therefore fundamentally bulk-phase in origin, and it is mechanistically distinct from both the interfacial-elastic regime at pH 5.7 and the electrostatic regime at alkaline pH.

A qualitatively distinct transition emerges at pH 5.7, where all three stabilization contributions converge simultaneously at their respective optima. The interfacial film transitions to elastic dominance (at 1 Hz; $G_i' = 0.0069 \text{ Pa} > G_i'' = 0.0059 \text{ Pa}$), providing a mechanically resilient barrier against coalescence. The near-zero zeta potential (+2.01 mV; Table 3), together with the charge inversion to -19.6 mV at pH 7.0, places the isoelectric point of the adsorbed protein fraction at $pI \approx 5.8$; at this condition, minimized electrostatic repulsion and enhanced hydrophobic exposure collectively facilitate the assembly of a cohesive, cross-linked interfacial network (Dickinson 2008).

The bulk consistency index remains high ($K = 0.0479 \text{ Pa} \cdot \text{s}^n$), and the apparent viscosity at 50 s^{-1} ($\eta_{50} = 0.034 \text{ Pa} \cdot \text{s}$) is comparable to that at pH 3 (Table 5), ensuring that the continuous-phase contribution to kinetic stability is preserved alongside the mechanical interfacial barrier. This conclusion is independently corroborated by the peak contact angle (θ), the corresponding detachment energy (E_d ; (Rayner et al. 2014)), and the minimum interfacial complex viscosity (η_i^*) at pH 5.7, providing a highly coherent set of findings that confirms pH 5.7 as the mechanistic optimum. Visibly, the frequency-dependent complex viscosity values increased across all pH conditions. Generally, the lowest complex viscosity value was observed at pH 5.7, followed by pH 3, 7, and 9, respectively. A lower interfacial complex viscosity confirms that the viscoelastic properties at the interface developed, rendering the emulsion more stable (Hermans and Vermant 2014; Karbaschi et al. 2014; Wei et al. 2020).

At pH 7 and pH 9, the stabilization mechanism shifts substantially toward electrostatic dominance, representing a regime mechanistically orthogonal to that at pH 3. The zeta potential of -19.6 mV at pH 7 and -26.23 mV at pH 9 (Table 3) reflects progressive deprotonation of surface-exposed carboxylate and phosphate groups, generating interdroplet electrostatic repulsion that substitutes for the mechanical and viscous contributions operative at lower pH. Concurrently, the bulk continuous-phase consistency decreases markedly with rising pH; K falls from 0.0541 (pH 3) to 0.0348 (pH 7) and $0.0194 \text{ Pa} \cdot \text{s}^n$ (pH 9), and the flow behavior index approaches or exceeds unity ($n = 0.938\text{--}1.05$), indicating a progressive transition toward near-Newtonian behavior and the dissolution of the structured continuous-phase network (Table 5). Similar trend was reported by Aslan Türker et al. (2021). The smaller particle sizes (Table 3) and larger droplets (Figure 8) at pH 7 (471.57 nm) and pH 9 (343.73 nm) further attest to the electrostatic contribution: the high negative surface charge triggers re-coalescence of oil droplets during the homogenization step, yielding coarser oil domains.

Although G_i' exhibits higher values at pH 7–9, the interfacial film remains viscous-dominant ($G_i'' > G_i'$), consistent with whey protein isolate behavior at near-neutral and alkaline pH (Zhou et al. 2021); the higher moduli reflect increased accumulation of material at the interface rather than gel-like network formation. Taken together, these observations establish that pH 3 and pH 7–9 achieve emulsion stability through mechanistically distinct routes: viscosity-mediated kinetic trapping versus electrostatic repulsion.

For pH 5.7, interfacial elasticity, the isoelectric condition, and sustained bulk viscosity collectively define the system optimum. A mechanistically analogous elasticity transition has been reported for *Saccharomyces cerevisiae*-derived dietary fiber particles (Kong et al. 2025), and the pH-mediated modulation of both interfacial and bulk rheological properties is consistent with the broader principle that charge-state transitions in food protein systems govern the balance between electrostatic, steric, and viscous stabilization routes (Lin et al. 2024; Murray 2011; Tao et al. 2024).

4. Conclusions

This study demonstrates that NYSP derived from *Saccharomyces cerevisiae* serves as an effective, clean-label, and multifunctional stabilizer for oil-in-water emulsions. Featuring a high protein content, substantial water- and oil-holding capacities, a low water solubility index, and a dense, low-porosity cell-wall architecture rich in β -glucans and mannoproteins, NYSP intrinsically combines both bulk rheological and interfacial functionalities in emulsified systems. Multiscale characterization revealed that NYSP forms pH-responsive, network-stabilized colloidal dispersions.

Despite stronger electrostatic repulsion at neutral and alkaline pH levels, long-term (14-day) emulsion stability was maximized under acidic regimes (pH 3.0 and 5.7). At these conditions, NYSP established a space-spanning aqueous network that immobilized droplets, elevated bulk viscoelasticity, and effectively suppressed coalescence, as corroborated by ESI values, macroscopic visual monitoring, and CLSM analysis. Conversely, at pH 7.0–9.0, the degradation of continuous-phase network connectivity and reduced bulk viscosity outweighed the increased interfacial charge, leading to rapid gravitational separation.

Overall, NYSP stands out as a promising, upcycled stabilizer that synergizes interfacial adsorption with continuous-phase structuring to yield pH-tunable (pH 3.0–6.0) emulsions. These findings offer crucial mechanistic insights into the valorization of yeast-derived side

streams in emulsified foods, highlighting that formulation under mildly acidic to near-native pH conditions provide an ideal balance among processability, visual appeal, and long-term storage stability. Consequently, NYSP holds strong potential as a sustainable, bio-based alternative to commercial emulsifiers and stabilizers in industrial food applications.

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Data availability

Data will be made available on request.

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.

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1331 **Table 1.** Physicochemical Characteristics of Nutritional Yeast (*Saccharomyces cerevisiae*)
1332 Spent Powder

Physicochemical parameters	Value
Protein (%)	42.61±0.03
Ash (%)	4.72±0.11
Moisture (%)	4.75±0.09
<i>L</i> *	68.04±0.08
<i>a</i> *	4.99±0.04
<i>b</i> *	24.69±0.26
<i>Chroma (C*)</i>	25.19±0.26
<i>Whiteness Index (WI)</i>	59.31±0.21
<i>Total Color (ΔE)</i>	72.56±0.05
<i>Browning Index (BI)</i>	49.54±0.67
WHC (g/g)	3.03±0.08
OHC (g/g)	2.21±0.09
Water Solubility Index (%)	25.83±0.30
Minerals and trace elements	Amount (ppm)
¹¹ B	0.45
²³ Na	1984.84
²⁴ Mg	772.71
³¹ P	1278.71
³⁹ K	1313.60
⁴³ Ca	317.10
⁵² Cr	0.58
⁵⁵ Mn	5.65
⁵⁶ Fe	22.68
⁶³ Cu	5.80
⁶⁶ Zn	94.40
Monosaccharide composition from total carbohydrates	
Mannose (%)	31.5±0.03
Ribose (%)	2.97±0.00
Glucose (%)	65.53±0.12
Total monosaccharides (%)	43.10±1.99

1334 **Table 2.** Color measurement results based on the Hunter color scale (*L*, *a*, *b*) and the electrical conductivity of emulsions.

Sample	<i>L</i> *	<i>a</i> *	<i>b</i> *	Chroma (<i>C</i> *)	Whiteness Index (<i>WI</i>)	Total Color (ΔE)	Browning Index (<i>BI</i>)	Conductivity (mS/cm)
pH 3	67.58±0.03 ^a	5.23±0.00 ^a	23.17±0.02 ^a	23.75±0.01 ^a	59.81±0.03 ^a	71.63±0.02 ^a	46.86±0.06 ^d	13.43±0.06 ^a
pH 5.7	66.64±0.05 ^b	5.09±0.01 ^b	23.05±0.02 ^b	23.61±0.02 ^b	59.13±0.05 ^b	70.70±0.04 ^b	47.24±0.09 ^c	9.82±0.07 ^d
pH 7	58.41±0.01 ^c	4.79±0.01 ^c	20.77±0.03 ^c	21.31±0.03 ^c	53.27±0.02 ^c	62.18±0.01 ^c	49.09±0.08 ^b	10.60±0.18 ^c
pH 9	56.97±0.12 ^d	4.73±0.02 ^d	20.68±0.06 ^d	21.22±0.07 ^d	52.02±0.09 ^d	60.79±0.13 ^d	50.30±0.10 ^a	11.43±0.17 ^b

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Table 3. Zeta potential (mV), Z-average hydrodynamic diameter (Z-avg, nm) for droplet size distribution, and polydispersity index (PDI) of emulsions stabilized by nutritional yeast at various pH levels (mean $\pm$ SD).

Parameters	pH 3	pH 5.7	pH 7	pH 9
Z-Average (d.nm)	881.80 $\pm$ 31.03 ^a	851.40 $\pm$ 81.90 ^a	471.57 $\pm$ 22.43 ^b	343.73 $\pm$ 23.27 ^c
PDI	0.43 $\pm$ 0.00 ^a	0.44 $\pm$ 0.01 ^a	0.29 $\pm$ 0.01 ^b	0.30 $\pm$ 0.00 ^b
Zeta Potential (mV)	6.11 $\pm$ 0.26 ^a	2.01 $\pm$ 0.95 ^b	-19.6 $\pm$ 0.49 ^c	-26.23 $\pm$ 0.53 ^d

* The uppercase letters indicate significant differences between means in the same row (Tukey HSD, $p < 0.05$).

Table 4. Surface tension (γ_{LV}) and contact angle (θ) on a hydrophilic substrate for nutritional yeast systems as a function of pH. The γ_{LV} was measured by pendant-drop tensiometry, and θ by sessile-drop goniometry.

pH	γ_{LV} (mN/m)	θ (deg)
3.0	37.81±0.28 ^{ab}	36.46±0.38 ^b
5.7	38.43±0.49 ^a	47.54±0.49 ^a
7.0	36.89±0.57 ^b	36.75±0.76 ^b
9.0	31.29±0.40 ^c	28.14±0.74 ^c

*Contact angle values reflect wetting on the test substrate and do not serve as a proxy for particle contact angle at the oil–water interface. The lowercase letters indicate significant differences between means in the same column (Tukey HSD, $p < 0.05$).

1391 **Table 5.** The parameters of the power-law model for the tested emulsions with varying pH values.

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Samples	Ostwald de Waele				1393
	K (Pa.s ⁿ)	n	η ₅₀ (Pa.s)	R ²	
pH 3	0.0541±0.001 ^a	0.885±0.006 ^a	0.034±0.0006 ^a	0.998±0.0003	1394
pH 5.7	0.0479±0.002 ^b	0.916±0.008 ^b	0.034±0.0005 ^a	0.997±0.0004	1395
pH 7	0.0348± 0.003 ^c	0.938± 0.007 ^c	0.026± 0.0005 ^b	0.999± 0.0004	
pH 9	0.0194± 0.001 ^d	1.050± 0.005 ^d	0.022± 0.0003 ^c	0.999± 0.0002	1396

1397 * Different superscript letters within the same column denote significant differences between means (Tukey HSD, p < 0.05).

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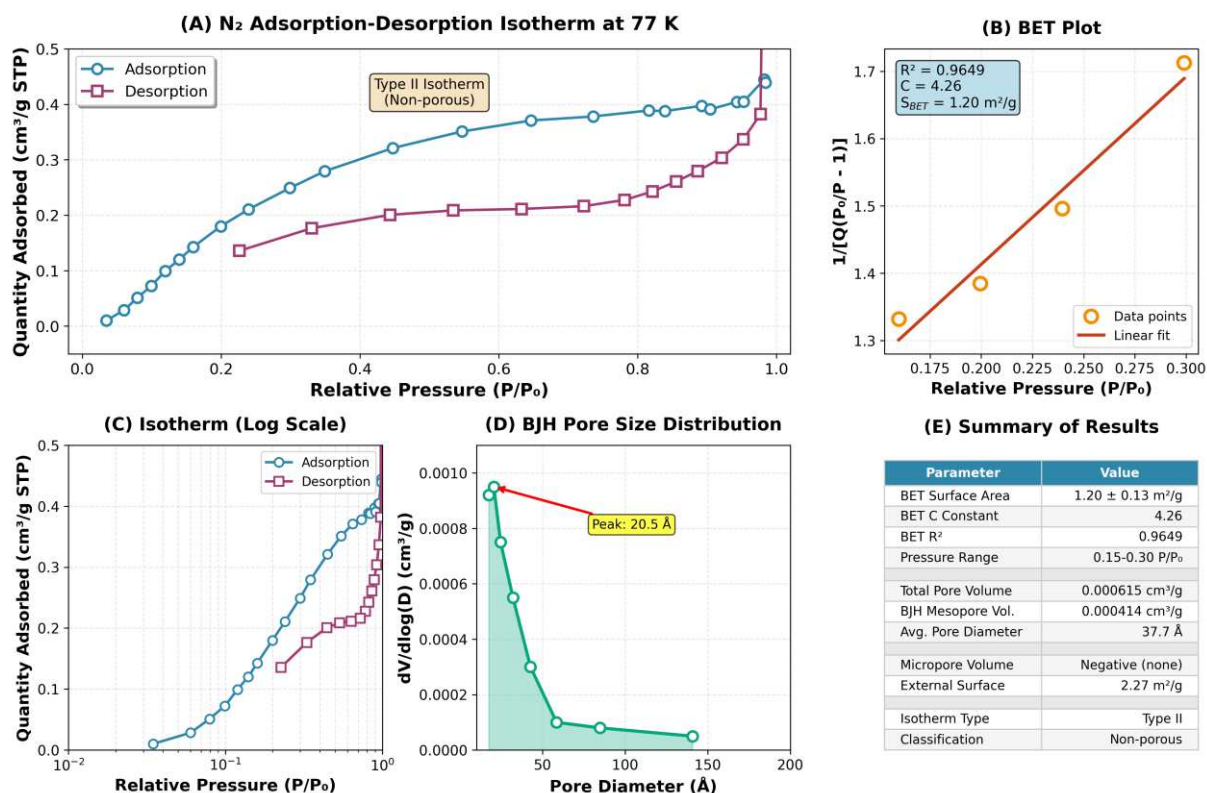


Figure 1. Nitrogen physisorption analysis of nutritional yeast spent powder at 77 K. (A) N₂ Adsorption-Desorption Isotherm. Type II isotherm with minimal hysteresis, indicating non-porous material. Low nitrogen uptake (<0.5 cm³/g STP) confirms negligible porosity. (B) BET Plot. Linear regression over the optimized pressure range (0.15–0.30 P/P₀) yields a BET surface area of 1.20 m²/g with an excellent correlation (R² = 0.9649). (C) Isotherm (Log Scale). Semi-logarithmic plot emphasizing low-pressure region, confirming absence of micropore filling and Type II classification. (D) BJH Pore Size Distribution. Minimal mesoporosity with a broad peak at ~20 Å from inter-particle voids. Total mesopore volume is negligible (0.000414 cm³/g). (E) Summary of Results. Key parameters include BET surface area (1.20 ± 0.13 m²/g), total pore volume (0.000615 cm³/g), and material classification as non-porous dried microbial biomass with intact cell walls.

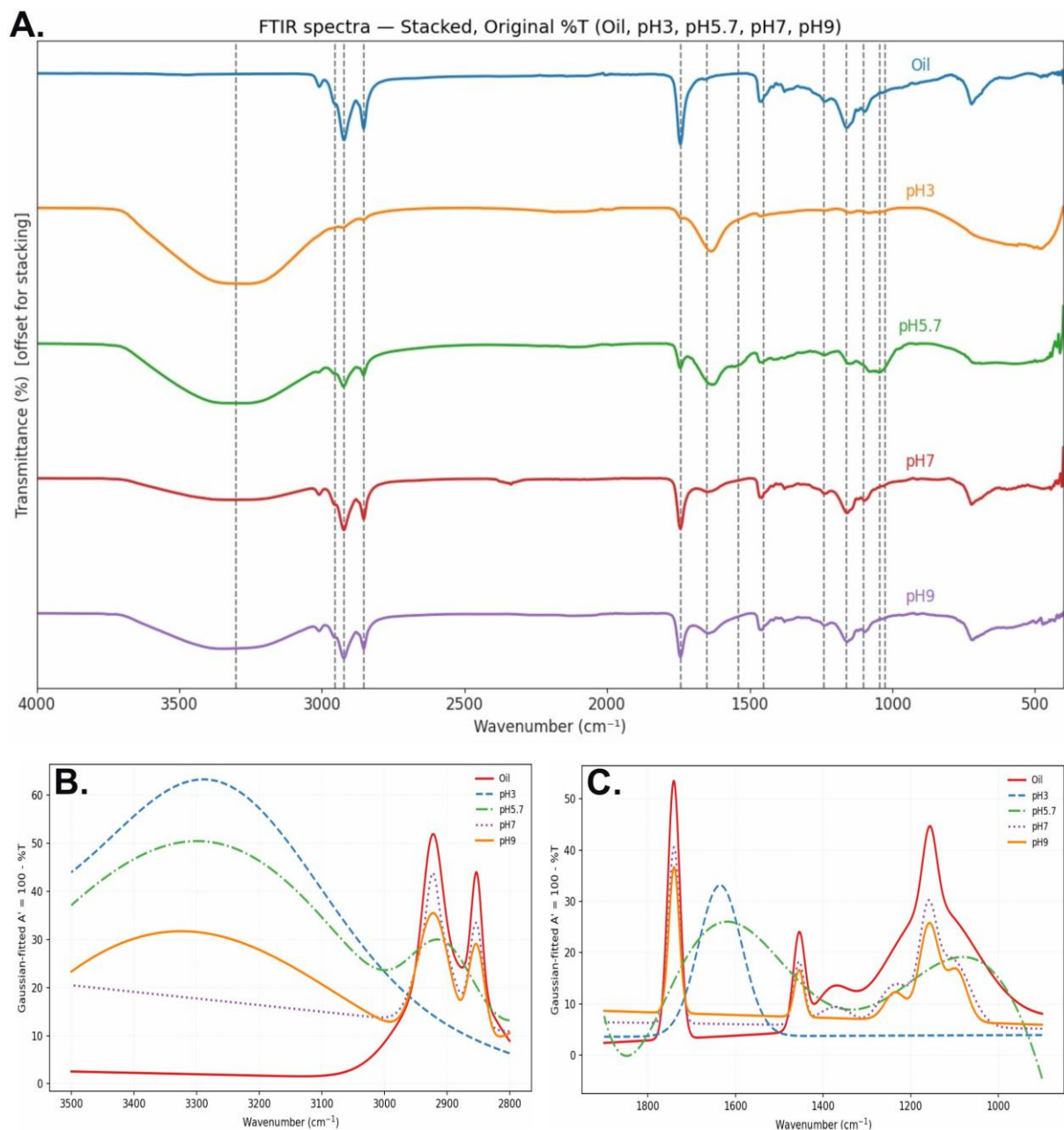


Figure 2. A: Stacked FTIR transmission spectra (raw %T) of Oil and pH-adjusted emulsion samples, arranged from top to bottom as: Oil, pH 3, pH 5.7, pH 7, and pH 9. Spectra cover the range from 4000 to 400 cm^{-1} and are vertically offset for clarity. **B and C:** Gaussian-fitted FTIR spectra ($A' = 100 - \%T$) of corn oil and nutritional yeast–oil emulsions (Oil, pH 3, 5.7, 7, 9) in the 3500–2800 cm^{-1} and in the 1900–900 cm^{-1} region, respectively.

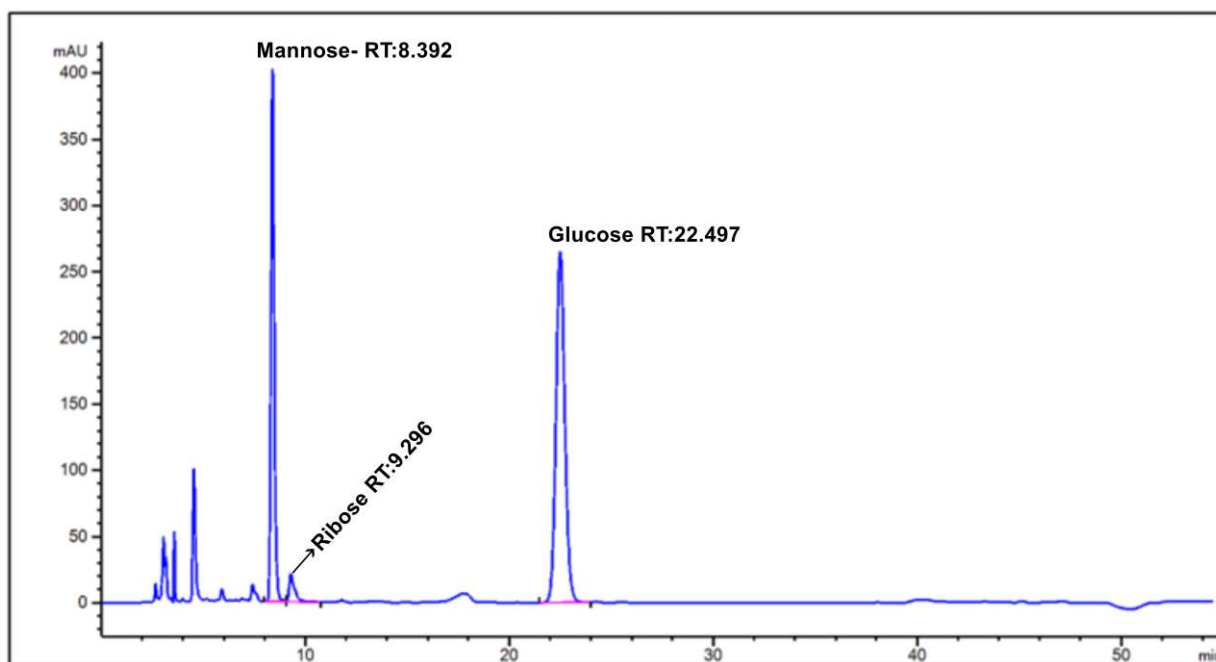


Figure 3. HPLC chromatogram showing the monosaccharide composition of Nutritional Yeast Spent Powder.

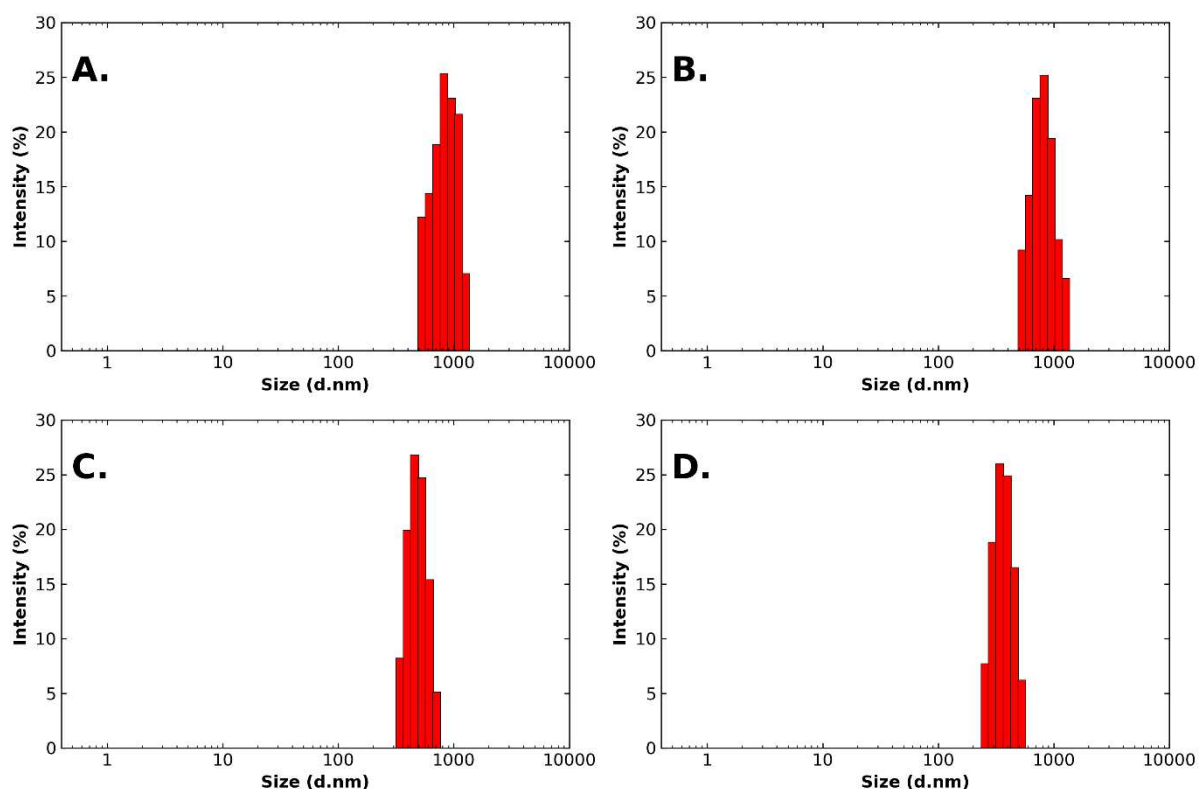


Figure 4. Particle size distribution profiles of NYSP-stabilized emulsions prepared at different pH values. The distributions show the apparent hydrodynamic size of colloidal entities detected by dynamic light scattering at pH 3.0 (A), 5.7 (B), 7.0 (C), and 9.0 (D).

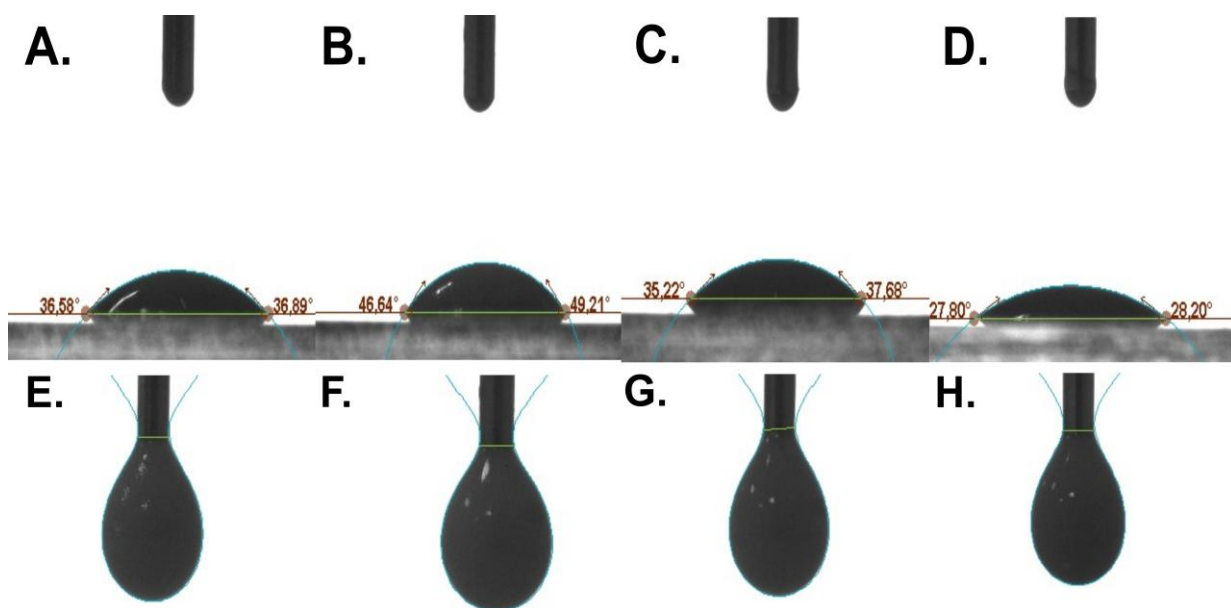
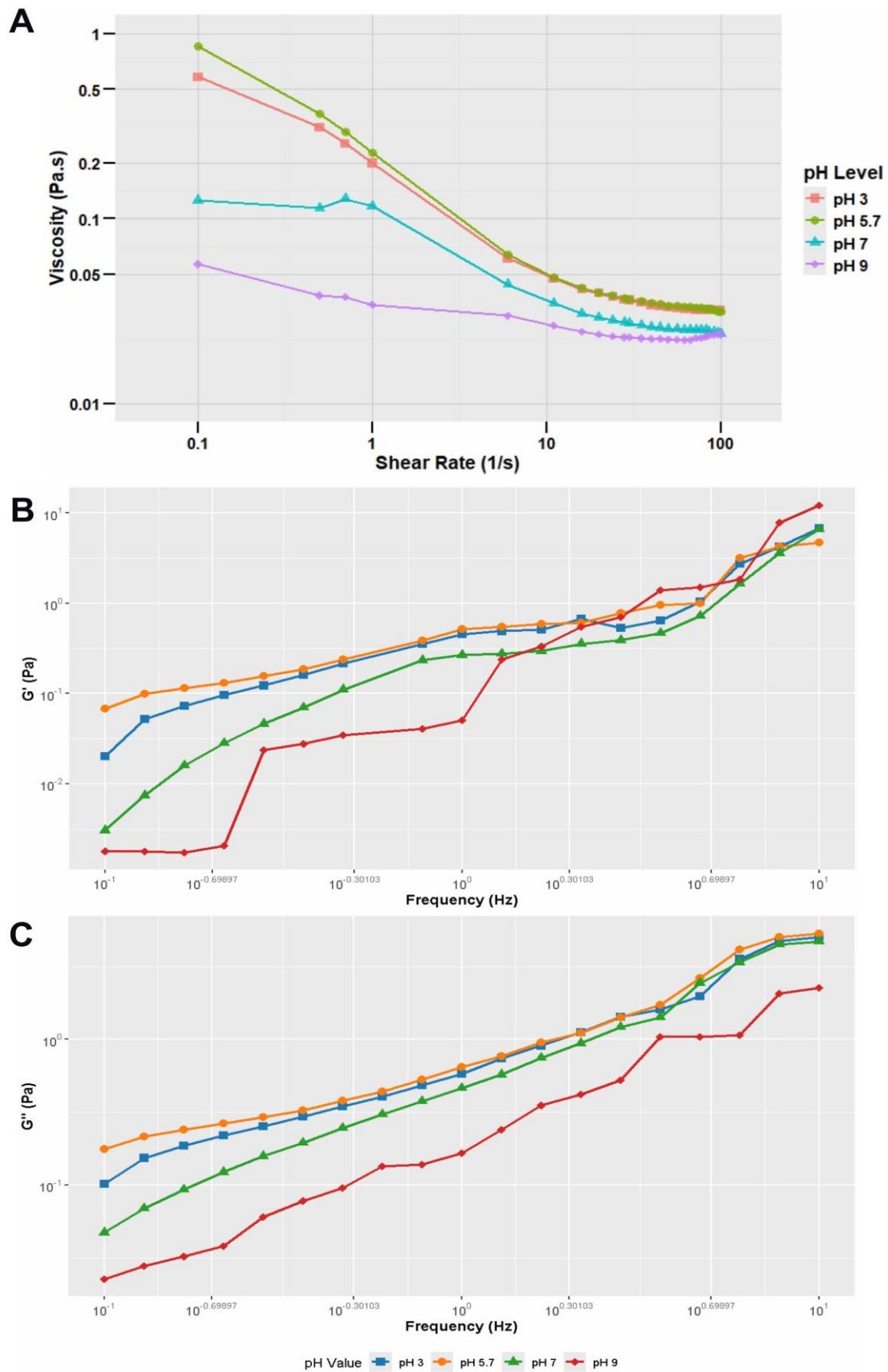
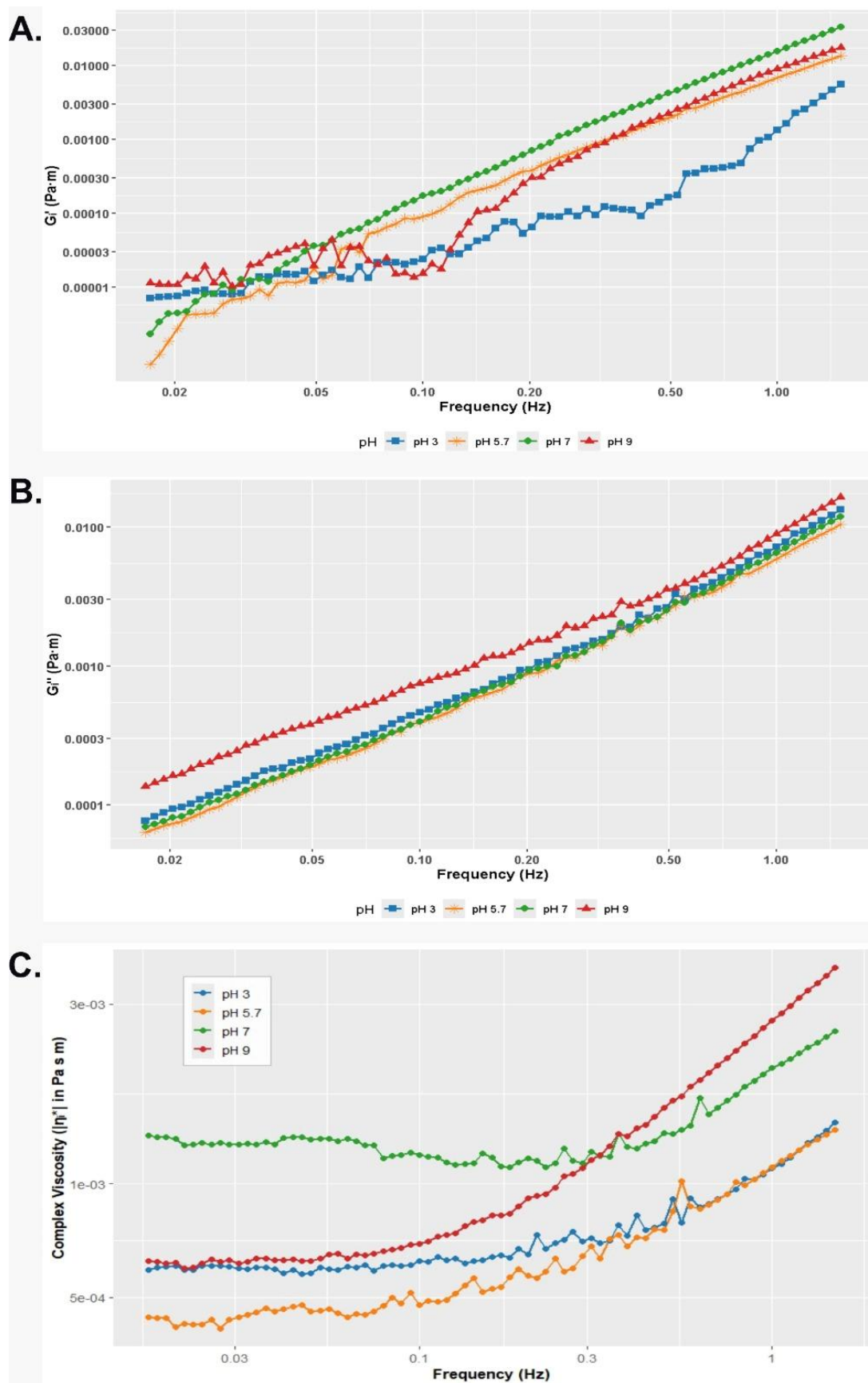


Figure 5. Images showing contact angle and surface tension measurements of emulsion droplets. A-D: Contact angle images at pH 3, 5.7, 7, and 9, respectively. E-H: Surface tension measurement images at pH 3, 5.7, 7, and 9, respectively.



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1475 **Figure 6.** Apparent viscosity of emulsions prepared with nutritional yeast spent at various pH
 1476 levels (A). Viscoelastic properties of the examined emulsions, G' (B) and G'' (C).



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1478 **Figure 7.** The interfacial dynamic frequency sweeps of the interfacial shear moduli: G'_i (A),
 1479 G''_i (B), and interfacial complex viscosity: η_i^* (C).

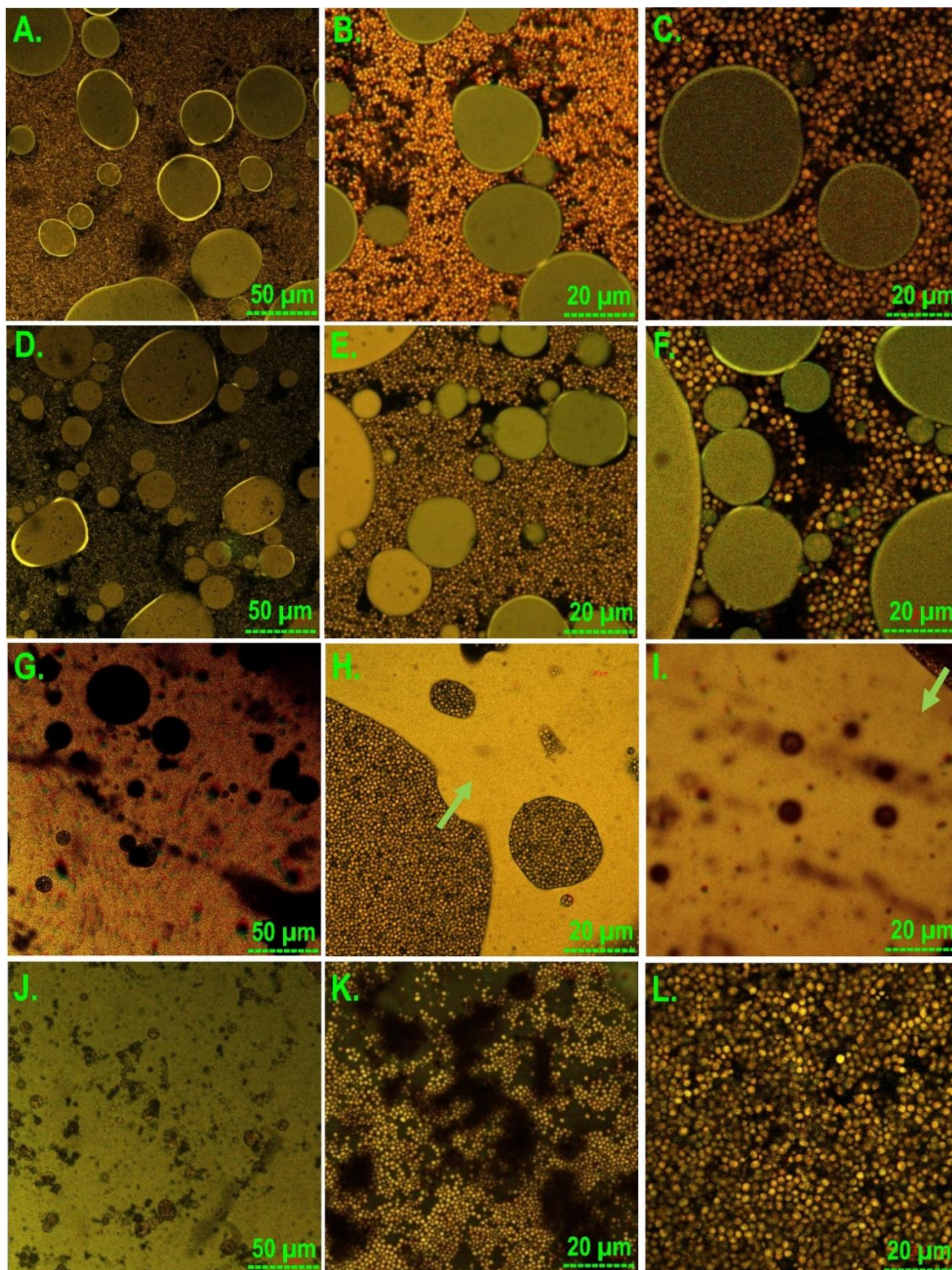


Figure 8. Confocal laser scanning micrographs (CLSM) of nutritional yeast-stabilized oil-in-water emulsions at pH 3 (A–C: 10×, 20×, 40×), pH 5.7 (D–F: 10×, 20×, 40×), pH 7 (G–I: 10×, 20×, 40×), and pH 9 (J–L: 10×, 20×, 40×). Bright round domains indicate oil droplets; darker, granular areas represent the yeast-rich aqueous phase. The green arrows in H and I indicate the location of the oil phase within the emulsion.

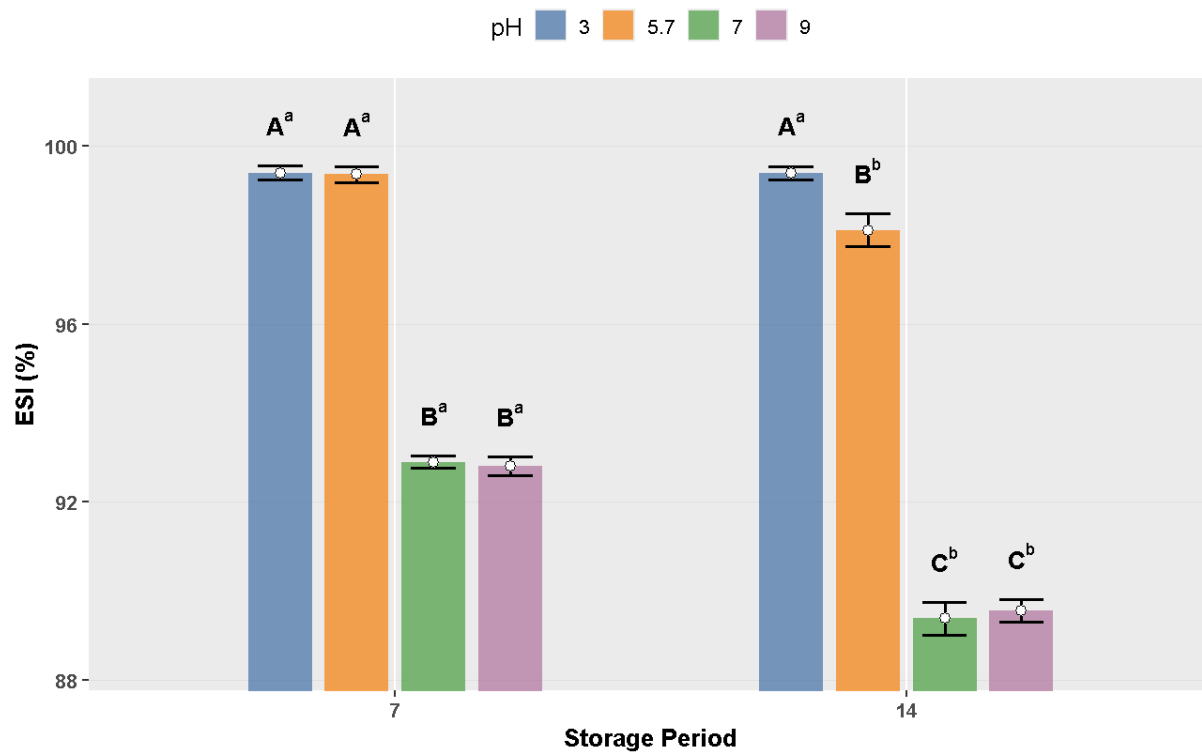


Figure 9. Emulsion stability index (ESI, %) at days 7 and 14 for pH 3.0, 5.7, 7.0, and 9.0. Bars show mean $\pm$ SD (n = 3). Uppercase letters compare pH values within the same day; lowercase letters compare days within the same pH (Tukey HSD, $p < 0.05$).

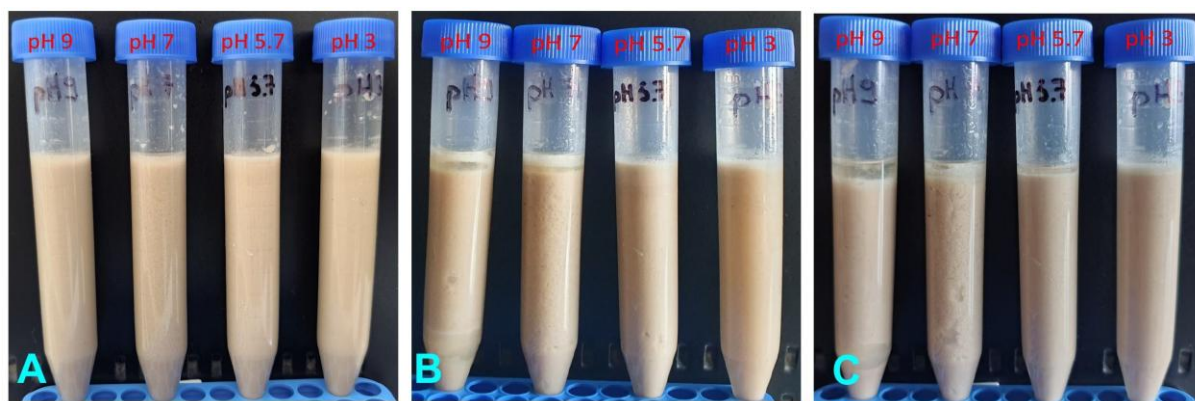


Figure 10. Visual representation of the Stability Index results during storage: A, 0th day; B, 7th day; C, 14th day.