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Development of a Novel Functional Yogurt Fortified With *Ziziphus lotus* Fruit: Enhancement of Antioxidant Capacity, Physicochemical Properties, and Sensory Quality

Chadine Hamdi[1] | Oukacha Amri[1] | Hajar Elmourabit[1] | Abdssamad Mhanni[1] | Fadma Fahmi[1] | Saida Tahrouch[1] | Khadija Elmehrach[1,2]

[1]Department of Biology, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco | [2]Department of Environment and Life Sciences, Faculty of Applied Sciences, Ibn Zohr University, Ait Melloul, Agadir, Morocco

Correspondence: Chadine Hamdi (chadine.hamdi.12@edu.uiz.ac.ma) | Khadija Elmehrach (k.elmehrach@uiz.ac.ma)

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ABSTRACT

The present paper aims to explore the potential of *Ziziphus lotus* (ZL) fruit and pulp powders as functional yogurt ingredients. Different concentrations (1%, 1.5%, and and 2%) of ZL whole fruit powder (ZE) and ZL pulp powder (ZP) were used to develop six yogurt formulations: three with 1%-2% of ZE and three with 1%-2% of ZP. Several physicochemical measurements, including pH, titratable acidity (TA), water-holding capacity (WHC), and viscosity, were conducted to assess the effects of the formulations. Yogurts fortified with ZL pulp (ZP) consistently demonstrated higher phytochemical content and antioxidant capacity, with total phenols ranging from 23.0 to 28.37 mg GAE/100 g, ABTS values between 11.6 and 27.0 mg·TE/100 g, and DPPH radical-scavenging activity of 17.99% and 25.6% over 21 days of storage, significantly exceeding those of whole fruit formulations and the control. Sensory analysis showed that yogurts fortified with 1% ZL maintained significantly higher ($p < 0.05$) scores in aroma, flavor, texture, and overall acceptability compared to higher concentrations (1.5%–2%) and remained closest to the control. These formulations were then subjected to phytochemical analysis. Results indicated that yogurts containing ZP powder had significantly higher flavonoid content and antioxidant capacity compared to those with ZE and the control. The results of this study demonstrate that ZL can be used as an additive in yogurt, boosting its phytochemical content and enhancing its health benefits, making it a more nutritionally valuable option compared to plain yogurt.

1 | Introduction

Yogurt has evolved from a simple fermented milk product into a nutrient-rich food with numerous health benefits. As one of the most important fermented dairy products, it has been part of traditional diets for centuries. It contains live microorganisms that contribute not only to its texture and flavor but also to its functional health-promoting properties [1, 2].

Today's consumers are increasingly focused on disease prevention and wellness through functional foods [3]. In response, the dairy industry has developed innovative yogurt formulations incorporating herbs, fruits, and plant-based ingredients [4]. Moreover, recent reviews highlight the increasing interest in yogurt fortification with bioactive functional foods and ingredients to enhance its nutritional and therapeutic potential [5–7]. Additionally, plant-derived bioactive compounds, such as those from cinnamon leaves or saffron, have been shown to improve yogurt's antioxidant capacity and shelf life without compromising sensory qualities [8, 9].

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Despite growing interest in plant-based enrichment, the incorporation of *Ziziphus lotus* (ZL), commonly known as jujube, into yogurt remains underexplored. ZL is a wild shrub native to arid and semiarid regions, with wide distribution across China, Iran, South Korea, parts of Africa, and southern Europe [10]. In Africa, it is particularly abundant in Mediterranean countries such as Algeria, Morocco, Tunisia, and Libya. In Morocco, ZL covers more than 110,000 ha, especially in regions like Chaouia, and is often harvested from the wild. As a medicinal and functional food resource, ZL plays a significant role in Morocco's broader wild-harvested plant economy, which was valued at approximately 300 million MAD in 2023 [11]. In response to its growing economic and therapeutic potential, agroindustrial units have increasingly engaged in the production of Ziziphus-based products including powders, extracts, jams, honey, and herbal supplements targeting both domestic consumption and export markets [11].

ZL is rich in bioactive compounds including vitamins, flavonoids, phenolics, alkaloids, carotenoids, and saponins [10, 12]. These components are unevenly distributed across plant parts: the pulp is rich in vitamins A and C and linoleic acid, seeds contain high levels of vitamin C, and the leaves are notable for vitamin E and linolenic acid [13]. ZL is associated with diverse biological effects such as antioxidant, antimicrobial, anti-inflammatory, and hypoglycemic activities. Traditionally, it has been used to treat various health conditions including fever, diarrhea, bronchitis, and insomnia and is known for its sedative properties [14–17].

Its incorporation into food products offers a natural alternative to synthetic additives while enhancing sensory qualities and shelf-life stability [18]. This study aims to develop novel yogurt formulations fortified with ZL fruit (ZE) and pulp (ZP) powders and to assess their effects on the phytochemical, physicochemical, textural, and sensory properties of yogurt during 21 days of refrigerated storage. This study will offer valuable insight into ZL's potential as a functional ingredient in the dairy sector, contributing to the growing field of dairy product fortification with plant-based bioactives to meet consumer demands for health-promoting foods.

2 | Materials and Methods

2.1 | Materials

The yogurts were prepared at SILDA dairy laboratory (Agadir, Morocco) using raw milk from Souss Massa region farms. This area is recognized for its health-promoting plants, including ZL, which was incorporated as a functional ingredient in this study. The probiotic culture (YO-MIX 883LYO 50 DCU: Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus) was sourced from DANISCO (France). Chemicals (Folin–Ciocalteu's reagent, Trolox, gallic acid, DPPH, ABTS, TPTZ) were obtained from Fluka and Merck.

2.2 | Yogurt Formulation

Six yogurt formulations were developed using ZL derived from whole fruit (ZE) and pulp (ZP) (Figure 1) at 1.0%, 1.5%, and 2.0% (w/w), calculated relative to the total weight of the fermented yogurt mixture, to evaluate their functional effects. Fresh raw milk (30 g/L fat, 15°D acidity, 1.030 density) served as the base, supplemented with sugar (10% w/v) and skim milk powder (3% w/v).

The mixture was heated (90°C, 10 min), cooled to 45°C, inoculated with starter culture (3%), and incubated (44°C ± 1°C) until pH 4.5. Postfermentation, ZL powders were incorporated postfermentation to avoid thermal degradation of bioactive compounds, preserve probiotic viability, and ensure compatibility with existing industrial yogurt production lines (Figure 2). Yogurts were stored at 4°C and analyzed on Days 7, 14, and 21, with triplicate repetitions for reliability.

2.3 | Physicochemical Properties

A range of various physicochemical attributes were determined in yogurt and plant powders. The titratable acidity (TA) of each yogurt was measured during storage time according to the guidelines described by the Association of Official Analytical Chemists [19]. The changes in pH values over the course of refrigerated storage were monitored using a pH meter (pH HI-98161, Hanna instruments, Italy). An infrared dissector (MB45, Ohaus, USA) was used to measure the humidity of the plant powders, and ash content was determined according to AOAC [20].

2.4 | Yogurt Characterization

Apparent viscosity of all formulations was recorded using a Brookfield DV-E Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) equipped with spindle C. The apparent viscosity value was recorded at a rate of 2.5 turns per minute. The viscosity of each sample was expressed as millipascal-second [mPa·s].

Measurement of WHC was determined according to Li et al. [21] by weighing the gel residue obtained after centrifugation (2000 g, 15 min) of 15 g of yogurt at room temperature (22 ± 2°C). WHC was then calculated using the following equation:

$$\mathrm{WHC}(\%) = \left(\frac{\mathrm{Weight\,of\,gel\,residue}\,(g)}{\mathrm{Weight\,of\,gel}\,(g)}\right) \times 100, \qquad (1)$$

with weight of gel residue is mass remaining after centrifugation and weight of gel is initial yogurt mass prior to centrifugation.

2.5 | Phytochemical Analysis and Extraction

2.5.1 | Extraction of Phenolic Compounds From Yogurts

Secondary metabolites present in each formulated yogurt were extracted following the guidelines described by Gurbuz and Demirci [22]. A total of 12.5 mL of methanol solution (80:20, methanol: distilled water) was added to 2.5 g of yogurt. After agitating for 3 min, the mixture was then centrifuged at 3500 g for 15 min at 4°C. The extracts were then filtered using Whatman No.1 filter paper and stored at 4°C for further analysis.

2.5.2 | Extraction of Phenolics From ZL Fruit

Fresh ZL fruits were dehydrated at 50°C for 72 h. Two preparations were made: whole fruits (including seeds and pulp) were ground, sifted, and stored in glass jars, while deseeded fruits had



FIGURE 1 | (a) *Ziziphus lotus* fruit, (b) *Z. lotus* whole fruit powder, and (c) *Z. lotus* pulp powder. (*Source*: authors own work).

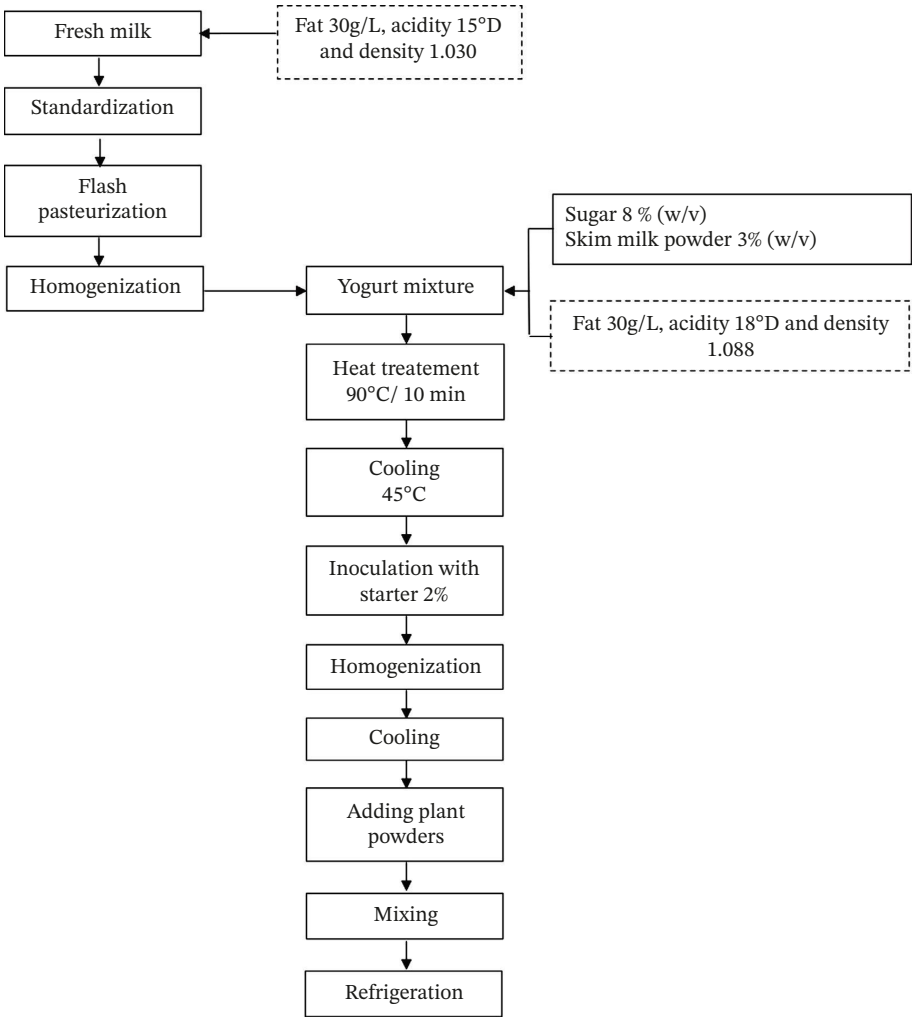


FIGURE 2 | Formulated yogurt manufacturing flowchart. (*Source*: authors own work).

their pulp coarsely ground and sifted. Methanolic extracts were prepared following Amri et al. [23]: 1 g powder was extracted with 20 mL methanol–water (80:20, v/v), ultrasonicated (20 kHz, 15 min), centrifuged (3500 g, 20 min, 4°C), filtered (Whatman No. 4), and stored at 4°C.

2.5.3 | Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC in plant extracts and the formulated yogurts were assessed by using the Folin–Ciocalteu assay according to Lim

et al. [24]. The TPC was then expressed as milligrams of gallic acid equivalent to per 100 g (mg GAE/100 g).

Total flavonoids were determined by the aluminum chloride colorimetric method according to Amri et al. [23]. The analysis was performed by taking 600 μL of the sample extract and adding 300 μL of AlCl3. The mixture was then incubated for 30 min at room temperature, and the absorbance was measured at 430 nm. The concentrations were expressed in terms of milligrams of rutin equivalents per 100 g (mg·RE/100 g).

2.5.4 | DPPH Assay

The free radical inhibition capability was measured by the decline of absorption of 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to Masuda et al. [25]. A volume of 950 μL of a methanol solution of DPPH (0.1 mM) was added to 50 μL of the sample extract. The absorbance of the mixture was measured after 30 min at 517 nm. DPPH radical scavenging potential was computed using the following formula:

$$\text{Inhibition } \% = \left(\frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}}\right) \times 100, \quad (2)$$

with A_{DPPH} is absorbance of control and A_{Sample} is absorbance of sample.

2.5.5 | ABTS Assay

The ABTS assay was carried out as described by Re et al. [26]. The ABTS radical cation ($\text{ABTS}^{\bullet+}$) was prepared by mixing a solution of 7 mM ABTS with a solution of 2.45 mM of potassium persulfate. The mixture was kept in the dark at room temperature for a period of 12–16 h and was then diluted with ethanol to reach an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of 10 μL of sample extract was mixed with 1 mL of $\text{ABTS}^{\bullet+}$ reagent, and the absorbance was measured at 734 nm after 6 min of incubation. Antioxidant activity was determined from percentage radical scavenging values and converted to Trolox equivalents using a calibration curve, with results expressed as milligrams Trolox equivalent antioxidant capacity per 100 g (mg·TEAC/100 g). The ABTS scavenging effect was calculated using the following formula:

$$\text{Inhibition } \% = \left(\frac{A_{\text{ABTS}} - A_{\text{Sample}}}{A_{\text{ABTS}}}\right) \times 100. \quad (3)$$

With:

A_{DPPH}: Absorbance of control.

A_{Sample}: Absorbance of sample.

2.5.6 | Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed according to Benzie and Strain [27]. The working FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl, and 20 mM $FeCl_3$ mixed in a 10:1:1 ratio. To 10 μL of sample was added 2 mL of FRAP solution. After an incubation of 30 min, the absorbance was measured at 593 nm. Trolox was used as the calibration standard, and results were expressed as milligrams TEAC per 100 g (mg TEAC/100 g).

2.5.7 | Phytochemical Screening

Phytochemical screening aimed to identify bioactive compounds in plant extracts. TLC was performed following Medić-Šarić et al. [28] using silica gel plates Kieselgel 60 F254-Alufolien (E. Merck, Darmstadt, Art. Nr. 5554) with fluorescent indicator. All tanks contained paper liners and equilibrated for 30 min after solvent addition. The mobile phase for flavonoid/phenolic acid separation was ethyl acetate: formic acid: water (8:1:1). Compounds were visualized by spraying with 1% methanolic diphenylboryloxyethylamine (Neu's reagent).

2.6 | Sensory Evaluation

The sensory evaluation of each formulated yogurt was conducted by 10 professionals from the dairy industry SILDA, including quality-control specialists, food technologists, and experienced sensory evaluators. According to Zedan et al. [29], each specialist evaluated the yogurts referring to the hedonic scale method. Different parameters such as color, appearance, flavor, smell, texture, and overall acceptance were taken into consideration during the analysis. Each yogurt was rated on a 1–10-point hedonic scale, where 1-2 indicated "extremely poor," 3-4 "poor," 5-6 "fair," 7-8 "good," and 9-10 "excellent." To guarantee impartiality, each sample was coded and randomly tested by the professionals. The sensory evaluation was executed on Days 7, 14, and 21.

2.7 | Statistical Analysis

All tests were subjected to analysis of variance (ANOVA) to evaluate the effect of adding plant extracts postfermentation on the yogurts during cold storage time. All analyses were conducted in triplicate ($n = 3$); however, biological replication was constrained by pilot-scale yogurt production. Future studies incorporating greater biological replication would further strengthen statistical inference. Statistical analysis was performed using IBM SPSS Statistics 26.0 (IBM SPSS, Inc.). The significance of mean differences was determined based on Duncan test at the significance level of $p < 0.05$.

3 | Results and Discussion

3.1 | pH and Acidity Characteristics

The pH variations in different yogurt formulations over a 21-day period are illustrated in Figure 3(a). By Day 7, all treatments, including the control, showed similar pH values, ranging from 4.26 to 4.33. By Day 14, the control and 1% YZP displayed identical pH values, exceeding those of 1% YZE and 1.5% YZE, which recorded the lowest values ($p < 0.05$). In contrast, the remaining formulations (2% YZE, 1.5% YZP, and 2% YZP) demonstrated higher pH levels, ranging from 4.27 to 4.30. By Day 21, all formulations showed decreased pH due to lactose fermentation. ZL powders had minimal impact on pH, which is consistent with findings from other studies where plant-based ingredients were incorporated into yogurt formulations [30, 31]. This stability may be attributed to the milk's natural buffering capacity, primarily provided by its proteins and minerals, which can help maintain a stable pH despite the mild acid contribution from the plant extracts.

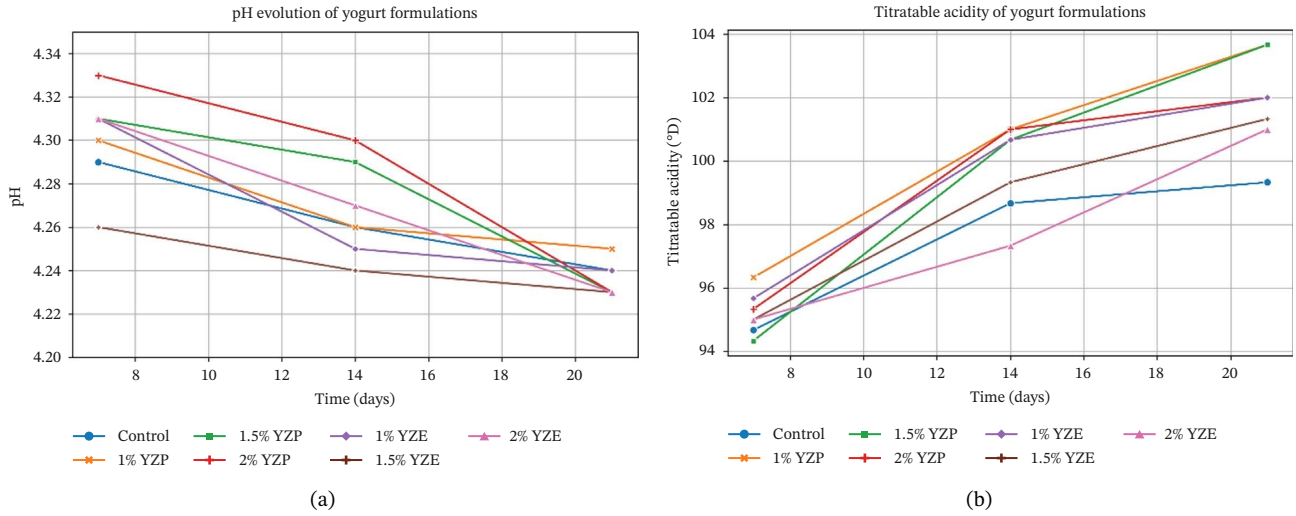


FIGURE 3 | Evolution of pH (a) and acidity (b) in different yogurt formulations during storage. (*Source*: authors own work).

In parallel, TA exhibited more significant variations (Figure 3(b)) across treatments, reflecting distinct fermentation dynamics. On Day 7, all formulations except 1.5% YZP showed significantly higher ($p < 0.05$) values compared to the control. By Day 14, 2% YZE exhibited the lowest acidity value, followed by the control and 1.5% YZE. The 1% YZE and 1.5% YZP showed identical values, while 1% YZP and 2% YZP displayed the highest value, surpassing all other formulations. By Day 21, TA increased in all treatments. ZL increased acidity, especially at higher concentrations. These findings are consistent with previous reports where fruit and herbal fortification led to enhanced TA levels compared to the control group [32]. The increase in TA may be due to enhanced LAB viability and metabolic activity, as observed by Say [33], who reported that plant-enriched yogurts exhibited higher lactic acid production compared to controls.

3.2 | Microbiological Quality Control of Yogurts

The counts of coliforms, yeast, and molds in all yogurt trails were found to be < 10 cfu/g during the 21 days of cold storage.

3.3 | Proximate Composition of ZL Powder Extracts

Table 1 presents the physicochemical and phytochemical characterization of ZL whole fruit (ZE) and pulp (ZP) powders.

According to El Maaiden et al. [34], measuring the humidity and the ash content of ZL powders (whole fruit and pulp only) ensures its shelf life and prevents microbial growth. As shown in Table 1, the higher moisture content in the ZE powder ($6.38 \pm 0.13\%$) indicates that whole fruit retains more water compared to the pulp alone ($5.57\% \pm 0.05\%$). This happens because parts like the skin and seeds usually hold onto more moisture. However, ZP powder has a higher ash content ($3.99 \pm 0.62\%$). This suggests that there are more minerals in the pulp compared to the whole fruit ($2.99\% \pm 0.35\%$). According to a detailed study by Abdoul-Azize [10], the pulp has a higher concentration of minerals, while the whole fruit includes the outer skin, which has fewer minerals.

3.4 | Phytochemical Composition of ZL Powder Extracts

Table 1 provides an overview of the phytochemistry profile of the 2 ZL powders, focusing on the TFC, TPC, and total antioxidant capacity (TAC) by DPPH and FRAP assays. The results reveal that ZP outperforms ZE in several key antioxidants and phytochemical properties. Specifically, the DPPH antioxidant activity is higher in ZP ($26.73 \pm 6.89\%$) compared to ZE ($22.34 \pm 3.76\%$), indicating that the pulp powder has a stronger antioxidant capacity. Additionally, ZP contains more flavonoids (9.68 ± 0.49 mg RE/g DM) than ZE (8.76 ± 0.70 mg RE/g DM), suggesting that the pulp powder is richer in flavonoid content. The phenolic content exhibited a similar pattern, with ZP containing 7.28 ± 0.67 mg GAE/g DM, surpassing ZE, which recorded 6.35 ± 0.31 mg GAE/g DM. Moreover, the FRAP values, are also higher for ZP (10.68 ± 0.49 mg TEAC/g DM) compared to ZE (9.13 ± 0.15 mg TEAC/g DM), reinforcing the pulp powder's superior antioxidant potential. Figure 4 highlights bioactive compounds in ZP and ZE, confirming detectable rutin and gallic acid Abcha et al. [18] identified 12 flavonoid derivatives (including rutin) in ZP, while Najjaa et al. [35] reported gallic acid as its dominant phenolic acid.

3.5 | Phytochemical Properties of ZL Yogurt Formulations

Figure 5 provides a comparative overview of the monitored characteristics in control, YZP, and YZE yogurt formulations over a 21-day storage period.

3.5.1 | TPC of the Yogurts

The TPC of the three yogurt formulations was measured. Figure 5 shows that throughout the 3 weeks of cold storage, both formulations and the control experienced a significant decrease ($p < 0.05$) in TPC. Despite this decrease, the formulations (YZP; YZE) consistently maintained higher TPC levels than the control throughout the storage period. For instance, on Day 7, YZP showed a higher TPC (28.37 ± 0.67 mg RE/100 g) followed by YZE (24.33 ± 3.21 mg RE/100 g) than the control (12.87 ± 0.55 mg RE/100 g) (Table 2). In concordance with our results, Tang et al. [36]

TABLE 1 | Physicochemical and phytochemical characterization of *Ziziphus lotus* (ZL) whole fruit (ZE) and pulp (ZP) powders.

Plant powders	Parameters						
	Moisture (%)	Ash content (%)	Flavonoids mg RE/g·DM	Phenols mg GAE/g·DM	DPPH (%)	ABTS TEAC/g·DW	FRAP mg TEAC/g·DM
ZP	$5.57 \pm 0.07^{**}$	3.99 ± 0.62	9.68 ± 0.26	7.27 ± 0.82	23.40 ± 1.64	9.5192 ± 0.3182	$10.68 \pm 0.30^{**}$
ZE	$6.38 \pm 0.27^{**}$	2.99 ± 0.35	8.76 ± 0.34	6.33 ± 0.40	22.34 ± 2.17	$10.9082 \pm 0.4166^{**}$	$9.13 \pm 0.15^{**}$

Note: (Source: authors own work). Data are expressed as mean ± standard deviation ($n = 3$). Significant significance was determined using Student's *t*-test.
**Highly significant difference at $p < 0.01$.

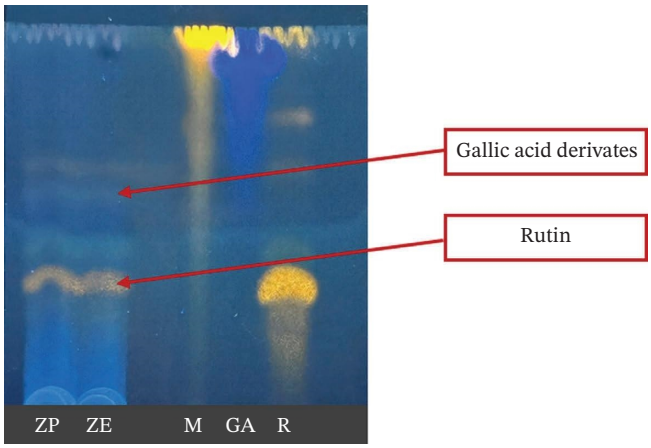


FIGURE 4 | Chromatographic profile of ZP and ZE extracts on silica gel plates (Kieselgel 60 F254, Merck 5554) using ethyl acetate: formic acid: water (8:1:1) as mobile phase. (*Source*: authors own work). Standard mixtures: M: myricetin; GA: gallic acid; R: rutin; ZE: *Ziziphus lotus* whole fruit; ZP: *Z. lotus* pulp.

found that although phenolic content decreased over time in all yogurt samples, the yogurt with mulberry leaf extract consistently maintained higher TPC levels compared to the control throughout the storage period. The phenolic content observed in the control is likely attributed to the natural presence of polyphenols in milk, most of which originate from the feed [37].

3.5.2 | TFC of the Yogurts

After 7 days of cold storage, YZE had the highest TFC (23.51 ± 1.66 mg RE/100 g), followed by YZP (21.24 ± 1.95 mg RE/100 g) and then the control (20.93 ± 1.77 mg RE/100 g). After 14 days, all three formulations demonstrated a significant increase ($p < 0.05$) in their TFC. By Day 21, the control exhibited a sudden decrease in its TFC (Figure 5), due to protein–flavonoid interactions. In contrast, YZE exhibited a significant increase in TFC compared to YZP. Likewise, Almusallam et al. [38] observed an increase in the TFC of their yogurt formulated with date palm spikelet extract. Nevertheless, the antioxidant activity decreased despite the gradual increase of flavonoids. Recent data have shown that flavonoid oxidation can affect antioxidant activity without depending on the proportional relationship between TFC. The oxidation of flavonols such as quercetin and kaempferol can alter their structure and form derivative compounds with different redox activities [39]. Therefore, the enhancement in the TFC observed during yogurt storage can be attributed to structural transformation rather than the accumulation of flavonoids, with their oxidized forms still traceable by the $AlCl_3$ assay. Since antioxidant assays involve different reaction kinetics, an increase in flavonoid content could be beyond the detection capacity of ABTS and DPPH methods. As a result, an enhancement in flavonoid content can occur despite the decrease in measured antioxidant activity.

3.6 | Antioxidant Properties of ZL Yogurt Formulations

Figure 6 provides a comparative overview of the monitored characteristics in control, YZP, and YZE yogurt formulations over a 21-day storage period.

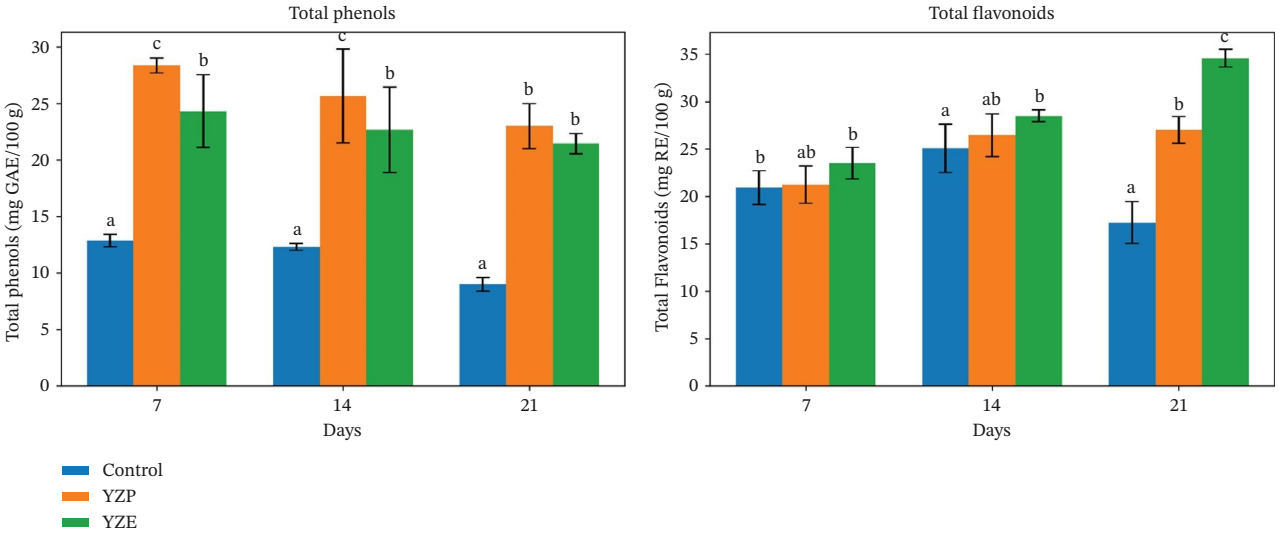


FIGURE 5 | Comparative overview of the phytochemical properties of ZL yogurt formulations. Mean values of $n = 3 \pm$ standard deviation. (a–c) Means followed by different superscript letters in each column indicate significant differences at $p < 0.05$. (*Source*: authors own work).

TABLE 2 | Characterizations of the yogurt: WHC and viscosity.

Tests	Days	Control	YZP	YZE
WHC (%)	7	78.42 ± 0.35^{a}	84.72 ± 0.26^{b}	81.09 ± 0.36^{a}
	14	76.55 ± 0.35^{a}	78.06 ± 0.83^{b}	80.09 ± 0.77^{c}
	21	75.69 ± 0.13^{a}	77.36 ± 0.43^{a}	79.78 ± 0.46^{b}
Viscosity (mPa·s)	7	20161.67 ± 23.03^{b}	20223.33 ± 10.69^{b}	19869.00 ± 89.45^{a}
	14	20578.33 ± 15.01^{c}	20474.67 ± 20.11^{b}	20134.33 ± 10.07^{a}
	21	20668.67 ± 12.66^{c}	20780.67 ± 25.54^{b}	20304.33 ± 66.46^{a}

Note: (Source: authors own work). Mean values of $n = 3 \pm$ standard deviation.
[a-c]Means followed by different superscript letters in each column indicate significant differences at $p < 0.05$.

3.6.1 | DPPH Assay

Following 7 days of cold storage, the YZP and YZE formulations showed significantly higher DPPH than the control (Figure 5), suggesting that the addition of ZL powders enhances the antioxidant activity of the yogurt. On Day 14, YZP had higher DPPH activity than both YZE and the control, indicating that adding ZP appears to be more efficient in terms of antioxidant activity than ZE. This is due to the highest antioxidant properties of ZP (Table 1). Over the 3 weeks of cold storage, the YZP preserved significantly higher DPPH activity than YZE and the control. However, throughout the storage, both YZE and the control exhibited a gradual loss in antioxidant activity (Figure 5). The decrease in antioxidant activity can be attributed to interactions between phenolic compounds with milk proteins and polysaccharides via hydrogen bonding, hydrophobic, and ionic interactions, giving rise to complexes that could reduce their accessibility and apparent in vitro antioxidant activity [40]. Besides, the phenolics and milk-derived peptides may, therefore, act as sacrificial antioxidants that decrease their availability for the DPPH or ABTS assay [41]. Microbial metabolism along with increasing acidity during storage could further stimulate oxidative or structural degradation of flavonoids [39], which contributes to the observed decline.

3.6.2 | ABTS Assay

At Week 1, both YZP and YZE yogurts showed significantly higher values for ABTS compared to the control. This suggests that the addition of ZL powders has a substantial effect on enhancing the antioxidant action in the yogurts. The highest activity was found in YZP yogurts (27.07 ± 2.03 mg TEAC/100 g) followed by YZE yogurts (20.88 ± 3.30 mg TEAC/100 g), which could be attributed to the higher presence of antioxidant compounds in the pulp powders (Table 2). After 2 weeks of cold storage, the values decreased considerably in all yogurts. However, YZP showed the highest ABTS values compared to YZE and the control. By Week 3, although the downward trend continued, YZP still retained the highest values, and the control displayed the lowest levels. The trends observed for ABTS assay were consistent with the DPPH and FRAP assays.

3.6.3 | FRAP Assay

When looking at Table 2, it is noticeable that YZP and YZE, respectively, had the highest FRAP value, while the control consistently showed the lowest values over the 3 weeks of storage. These findings are in phase with the results of the DPPH assay, showing that the addition of ZP significantly improves TAC, with YZP showing both higher initial and long-term FRAP

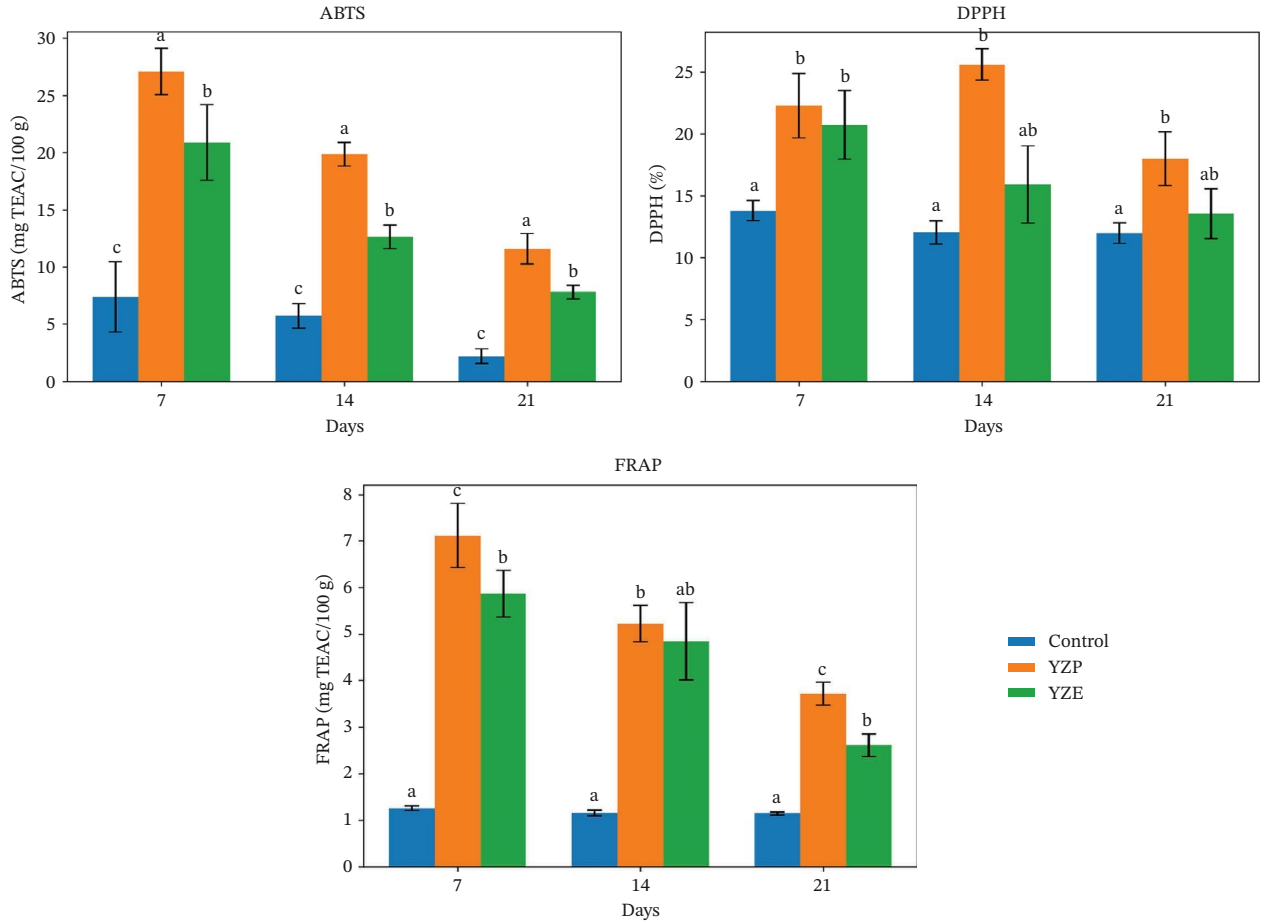


FIGURE 6 | Comparative overview of the antioxidant properties of ZL yogurt formulations. Mean values of $n = 3 \pm$ standard deviation. a–c Means followed by different superscript letters in each column indicate significant differences at $p < 0.05$. (*Source*: authors own work).

values (Figure 6). The progressive reduction in FRAP values observed across all formulations is attributed to phenolic–protein interactions that limit antioxidant availability, as well as enzymatic degradation of phenolic compounds by lactic acid bacteria during storage [42, 43].

3.7 | Textural Properties of ZL Yogurt Formulations

Table 2 presents the monitored textural characteristics of yogurt formulations, including water-holding capacity (WHC) and viscosity during storage.

3.7.1 | WHC

The ability of yogurts to retain water over time is indicated by their WHC. Higher percentages correspond to better water retention. On Day 7, YZP had the highest WHC (84.72 ± 0.26%), followed by YZE (81.09 ± 0.36%) and the control (78.42 ± 0.35%). On Day 14, both formulations, YZP and YZE, displayed better water retention than the control ($p < 0.05$). By the end of Day 21, YZE continued to exhibit the highest WHC at 79.78 ± 0.46%, followed by YZP at 77.36 ± 0.43% and the control at 75.69 ± 0.13%. Overall, the findings suggest that the YZP and YZE formulations generally exhibit higher WHCs than the control at most time points. This indicates that the addition of ZP or ZE improves yogurt's ability to retain water, which has a positive impact on texture, stability, and possibly other sensory attributes. For instance, in Table 2, YZE achieved higher results by maintaining its texture throughout the 3 weeks of storage. Similar findings have been reported in previous studies, where increased WHC was attributed to the higher fiber, protein, and total solids content [44, 45].

3.7.2 | Viscosity

By the end of the first week, YZE had a significantly reduced ($p < 0.05$) viscosity (19869.00 ± 89.45 MPa·s) compared to the control and YZP. *On* the second week, the three formulations experienced an important increase ($p < 0.05$) in their viscosity with the control exhibiting the highest value (20578.33 ± 15.01 MPa·s), followed by YZP (20474.67 ± 20.11 MPa·s) and YZE (20134.33 ± 10.07 MPa·s). After 21 days, YZP exhibited the highest viscosity (20780.67 ± 25.54 MPa·s) due to its greater sugar content, which promotes water retention and improves textural properties [34]. Similarly, previous studies, such as the one by Erkaya-Kotan [46], have shown that the addition of fiber increased yogurt viscosity for some samples.

3.8 | Correlation Analysis

The correlation heatmaps (Figure 7) reveal distinct interaction patterns between phytochemical and physicochemical parameters in yogurts fortified with ZP and ZE powders. In YZE, strong

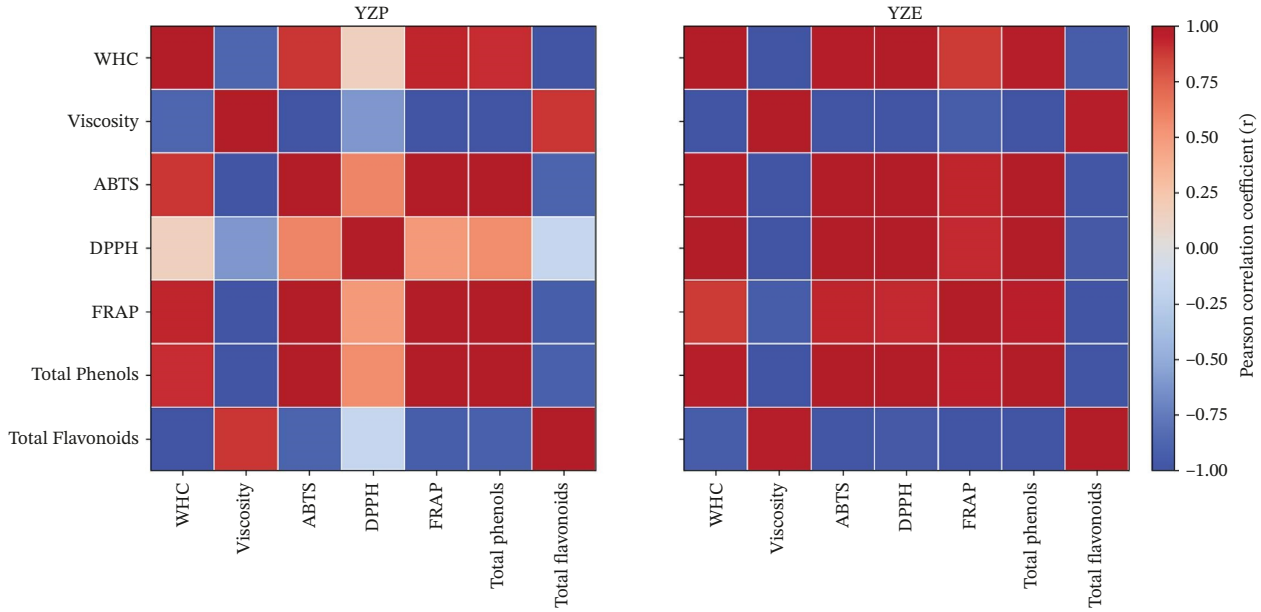


FIGURE 7 | Correlation analysis of the tested parameters of ZE and ZP fortified yogurt. (*Source*: authors own work).

positive correlations among antioxidant activities (DPPH, ABTS, and FRAP), TPC, and WHC suggest that whole fruit fortification enhances antioxidant capacity and moisture retention. On the other hand, viscosity shows a strong negative correlation with these parameters, implying that increased antioxidants and WHC properties correspond with decreased thickness. In YZP, correlations are weaker and less consistent; notably, flavonoids negatively correlate with antioxidant activity and WHC. These results suggest that YZE offers a more promising functional profile.

3.9 | Principal Component Analysis (PCA)

The 3D plot (Figure 8), where the first three components (PC1, PC2, and PC3) together explain 95.2% of the total variance in the data (65.7% for PC1, 15.7% for PC2, and 13.8% for PC3), ensures a reliable representation of the data structure. Samples are represented as colored points: blue for control samples, green for ZP samples (treatment 1), and red for ZE samples (treatment 2), each distributed according to analysis times (Day 7, Day 14, and Day 21). The vectors (arrows) represent the measured variables, including antioxidant parameters (FRAP, ABTS, DPPH, phenols, and flavonoids) and physicochemical parameters (viscosity and WHC).

The PCA highlights a clear separation between control samples and treated groups. The control samples are grouped on the negative side of the first component (PC1), indicating a low influence from the evaluated antioxidant and physicochemical variables. In contrast, ZE and ZP samples are spread over the positive components of PC1 and PC2, indicating a differentiated response based on treatment and time.

The variables FRAP, ABTS, DPPH, phenols, and flavonoids contribute strongly to PC1 and PC2, suggesting their central role in discriminating the treated samples. In particular, the ZE-21d and ZE-14d samples are strongly correlated with these variables, which may indicate a significant increase in antioxidant potential. Conversely, WHC points in the opposite direction, reflecting a negative correlation with antioxidant compounds. The ZE-7d sample, located very close to this vector, likely shows a better water retention capacity but lower antioxidant content.

The variable viscosity contributes more to the third component (PC3) and particularly distinguishes the ZP-14d and ZP-21d samples, which may be interpreted as a differential effect of the treatment on rheological properties over time. Arrows pointing in similar directions (e.g., phenols, flavonoids, DPPH, ABTS, and FRAP) indicate positive correlations between these parameters. This suggests that samples rich in phenolic compounds are also more effective in terms of antioxidant capacity.

This multivariate analysis highlights the differentiated effects of treatments on the physicochemical and antioxidant characteristics of the samples. It not only allows the grouping of samples according to their biochemical profile but also identifies the most discriminating variables, which is useful for selecting optimal formulations.

3.10 | Sensory Analysis

In the pilot study, different yogurts were prepared using ZE and ZP powders ranging from 1% to 2% (w/w). The different formulations were evaluated by 10 professionals for 21 days to choose the optimal one. The addition of ZL demonstrates significant ($p < 0.05$) differences for all parameters compared to the control as the amount added of the plant powder increased. These trends are further illustrated in Figure 9, which presents five radar charts illustrating the evolution of sensory attributes (appearance and color, texture, aroma, flavor, and overall acceptability) for the various yogurt formulations over 21 days. The charts confirm that 1% concentrations of YZP and YZE maintained sensory attributes closest to the control, particularly in terms of aroma, flavor, and overall acceptability. Higher concentrations (1.5%–2%) led to perceptible declines in these parameters, especially for YZE, while texture remained relatively stable across all samples and time points, indicating minimal impact on physical consistency. Importantly, appearance and

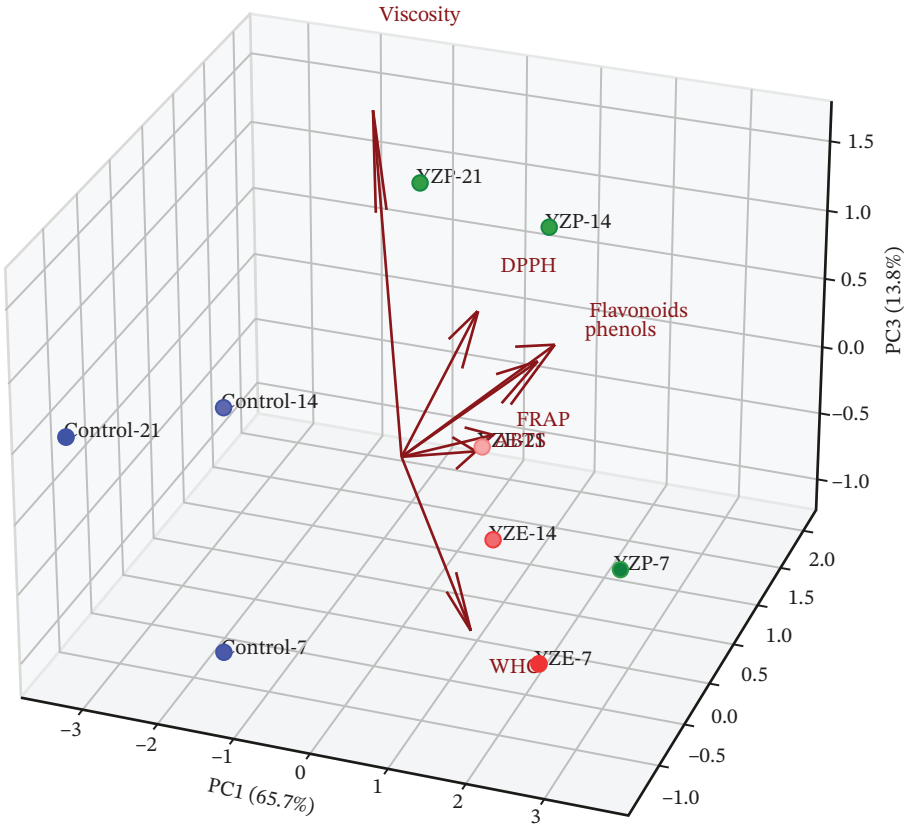


FIGURE 8 | Principal component analysis of yogurt formulations. (*Source*: authors own work).

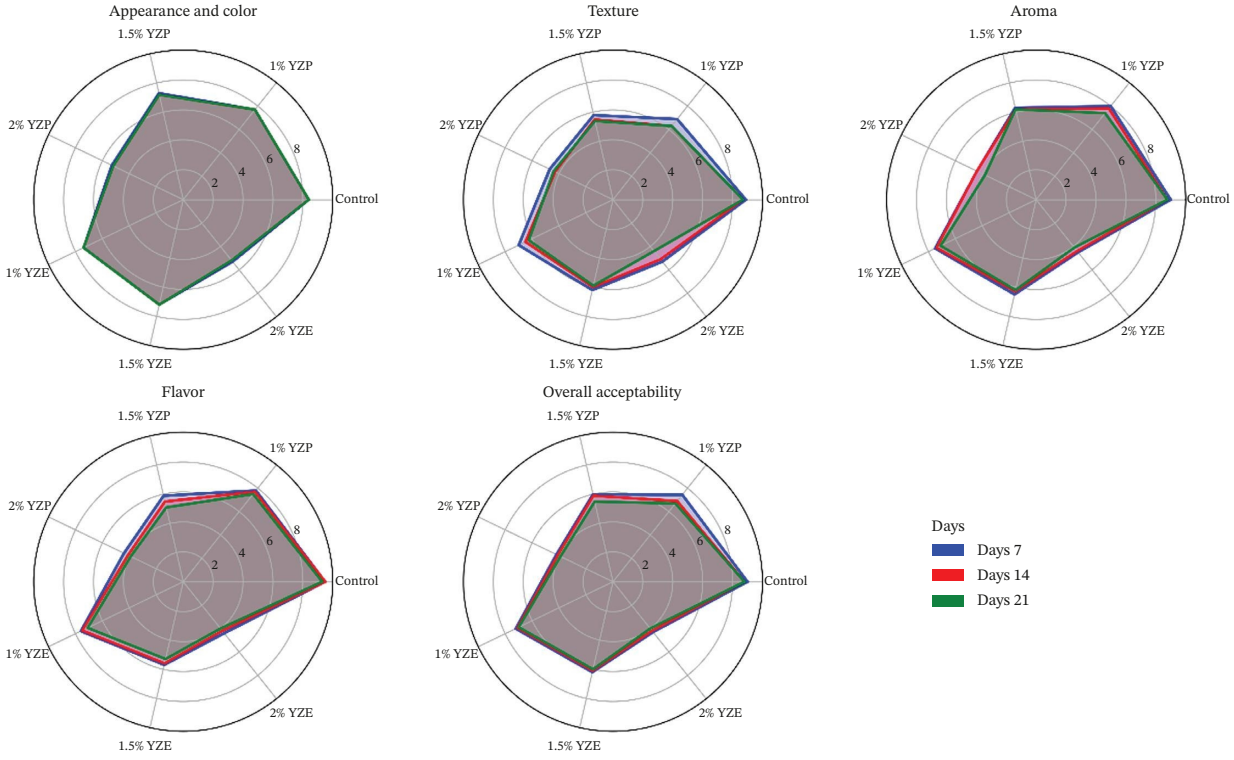


FIGURE 9 | Sensory profile radar charts of control and ZL powder–enriched yogurts. (*Source*: authors own work).

color also exhibited high stability over time, with only slight reductions in visual appeal at higher additive levels. These results align with previous findings on plant-enriched yogurts where higher additive levels were associated with reduced texture and acceptability scores, likely due to the bitter and astringent notes associated with high levels of phenolic compounds and flavonoids [47–49]. Due to the strong acceptability shown in the sensory analysis, it demonstrates that the product is well accepted and suitable for consumer use. Considering that typical daily yogurt intake of 150–180 g has been linked to improved nutrient status, better diet quality, and reduced risks of diabetes, obesity, and cardiovascular disease [50], this product demonstrates promising health benefits. The relative stability of texture over 21 days is a valuable technological outcome, indicating that ZL fortification does not compromise the consistency, which is a common barrier in plant-enriched dairy products.

4 | Conclusion

ZL fruit powders, especially ZL pulp, enhanced yogurt's functional and physicochemical properties. Despite ZL addition, fermentation followed its normal course, characterized by gradual pH decline and increasing TA during storage. Post-fermentation incorporation preserved both the yogurt's microbial integrity and the bioactive potency of ZL. Pulp-enriched yogurt displayed superior antioxidant capacity, flavonoid content, and phenolic content compared to whole fruit and control, confirming its bioactive richness. TLC analysis confirmed the presence of flavonoids, with rutin being particularly prominent. Both pulp and whole fruit improved WHC, indicating better texture. In particular, the minimal alteration of textural attributes across the storage period suggests that ZL powders can be incorporated into yogurt without negatively affecting the consistency, which represents a major advantage for industrial formulation. Sensory analysis identified 1% concentrations as optimal, balancing bioactivity and acceptability, while higher doses impaired texture and flavor. ZL emerges as a promising functional food ingredient. Future clinical trials should be conducted to validate the reported health benefits in targeted population subgroups.

Author Contributions

Chadine Hamdi: writing–original draft, writing–review and editing, methodology, investigationand formal analysis. Oukacha Amri: validation, writing–review and editing, and resources. Hajar Elmourabit and Abdssamad Mhanni: resources. Fadma Fahmi: writing–review and editing. Saida Tahrouch: validation and resources. Khadija Elmehrach: writing–review and editing, supervision, project administration, investigation, resources, methodology and conceptualization.

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Ethics Statement

This study involved a sensory evaluation conducted by 10 trained professionals from the SILDA dairy industry. According to national regulations, approval from an ethics committee was not required for this type of research. All participants were fully informed of the purpose and methodology of the study, and no personal or sensitive data were collected.

Consent

Informed consent, either written or verbal, was obtained from all participants prior to their participation. Participation was entirely voluntary, and individuals retained the right to withdraw from study at any time.

The manuscript does not contain any individual person's data in any form (including images, videos, or identifying personal information).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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