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Olive Oil-Enhanced Yoghurt: An Innovative Method for Developing Functional Foods Using Sodium Caseinate and Emulsification Technique

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Abstract

This study investigates the formulation and functional properties of yoghurt in which milk fat is completely replaced with extra-virgin olive oil (EVOO), excluding the control sample, which contained 4% milk fat, with the aim of improving its nutritional profile and textural quality. Emulsions were prepared using a high-shear mixer to homogenise skim milk as the dispersion medium, with different concentrations of EVOO (2.5% w/w, 5% w/w, 7.5% w/w, and 10% w/w) as the dispersed phase, stabilised with 1% w/w sodium caseinate used in yoghurt preparations. Emulsions containing lower concentrations (2.5% w/w and 5% w/w) remained stable during 21 days of storage without creaming; in contrast, higher concentrations (7.5% w/w and 10% w/w) exhibited instability due to creaming and were excluded from further analysis. The resulting functional yoghurts were assessed over 21 days for their physicochemical properties, including pH, texture profile, and water-holding capacity (WHC). Results demonstrated that yoghurts containing EVOO exhibited improved pH, hardness and WHC compared to the control, with 5% EVOO yoghurt showing the highest textural integrity.

Keyword: Olive oil, Emulsion, Milk, Functional Yoghurt, Fat substitution

I. Introduction

Public health issues are increasingly focused on chronic, non-communicable diseases like cardiovascular disease (CVD), obesity, diabetes, and certain cancers. Nutrition science now emphasises not only basic nourishment but also disease prevention and health promotion. In this context, functional foods- foods that offer health benefits beyond basic nutrition have become central to modern dietary strategies (Das et al., 2020). Dairy products are particularly promising carriers for functional ingredients due to their high consumption rates, rich nutrient profiles, and versatility in formulation (Playne et al., 2003). One notable functional ingredient with well- documented health advantages is olive oil, especially extra virgin olive oil (EVOO) (Yubero-Serrano et al., 2019). The replacement of milk and yoghurt fat with extra-virgin olive oil (EVOO) represents a promising strategy for improving the lipid profile of dairy products. EVOO is rich in monounsaturated fatty acids and bioactive compounds, which may contribute to enhanced nutritional quality. However, substituting milk fat with plant-based oils can influence milk stability, fermentation behavior, and the physicochemical and textural properties of yoghurt. Therefore, evaluating the technological and quality-related effects of EVOO incorporation is essential to determine its suitability as a milk fat replacer in yoghurt production (Schwingshackl et al., 2014). Numerous studies show that regular olive oil intake can lower oxidative stress, influence inflammatory pathways, and reduce blood pressure, all contributing to better cardiovascular health (Estruch et al., 2018, Schwingshackl et al., 2019). In contrast, traditional dairy products made from whole milk are high in saturated fatty acids (SFAs) and cholesterol, which are linked to increased serum LDL-cholesterol, a known risk factor for atherosclerosis and cardiovascular disease (Astrup et al.,

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2020). Although recent research has nuanced the understanding of dairy fats' health effects, excessive saturated fat intake remains a concern, especially for individuals with preexisting metabolic conditions (Timon et al., 2020). Therefore, reducing saturated fats and cholesterol in dairy products without compromising taste and quality has become a key goal in research and product development. This has sparked growing interest in reformulating dairy products, particularly yoghurt, a popular fermented milk product with healthier fat alternatives (Abbas et al., 2024). Replacing animal fat with olive oil in yoghurt provides several benefits, including improved lipid profiles, increased antioxidant capacity, and enhanced functional properties (Frankel and Chemistry, 2011).

II. Material and Methods

This study was conducted in the Food Science field, the Food Science and Quality Control Department, College of Agricultural Engineering Sciences, University of Sulaimani. Homogeniser: ULTRA-TURRAX T25 (IKA, Germany), Mastersizer: 3000(Malvern, UK). Milk: Commercially available milk (Almarai) was obtained from the local market. Olive Oil: Extra virgin olive oil was sourced from Rasan (Halabja, Kurdistan Region, Iraq). Sodium Caseinate: used as a stabilising agent in the yoghurt formulation.

Emulsion Preparation:

Skimmed milk served as the base for emulsion preparation. First, 1% w/w sodium caseinate was added to the milk, excluding the control sample, to improve stability, then mixed thoroughly with a high-shear homogeniser equipped with an S25N-18G probe at 24,000 rpm for 3 minutes for even dispersion. Olive oil was gradually added at four different concentrations—2.5% w/w, 5% w/w, 7.5% w/w, and 10% w/w—while continuous homogenisation at 24,000 rpm was maintained for 15 minutes to create stable emulsions. This process produced four distinct emulsions, each with a different olive oil concentration (McClements, 2004).

Emulsion Storage and Stability Analysis:

After preparation, all emulsions were stored at 5°C for 21 days to evaluate their stability over time. Visual inspections were performed to check for phase separation or creaming. Photos of the emulsions were taken on the first day,then every 7 days, until day 21, to document physical changes during storage. To further assess stability, droplet size distribution was measured at the same intervals using a Mastersizer 3000.

Yoghurt Preparation:

The yoghurt preparation process involved heating the emulsions to 90°C ± 1°C for 10 minutes to eliminate microbes. Following heating, the mixtures were cooled to 43°C and then inoculated with a starter culture (3% w/w). Fermentation took place at 43°C for 3 to 4 hours (Ibrahim and Al Saaid, 2018). After fermentation, the yoghurts were stored at 4 to 5°C for further testing. Physical and textural properties such as pH, hardness, and water-holding capacity were assessed on days 1, 7, 14, and 21 across all formulations (Tamime and Robinson, 2007).

Texture determination:

Texture Profile Analysis (TPA) was employed to assess the textural properties of the yoghurt samples. The measurements were carried out using a texture analyser (CT3-1000, Ametek Brookfield, USA) fitted with a 20mm diameter cylindrical probe. Before testing, the device was calibrated in accordance with the manufacturer’s instructions for force and distance. The procedure involved a test speed of 1.0 mm/s, a trigger force of 5.0 g, and a deformation depth of 20 mm (Bonczar et al., 2002).

PH determination:

The pH of the yoghurt samples was determined using a digital pH metre (Inolab pH/Ion/Cond 750, WTW, Germany) with a glass electrode. The device was calibrated beforehand with standard buffer solutions at pH 4.0 and pH 7.0, according to the manufacturer’s guidelines. For each reading, the electrode was immersed

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directly into the yoghurt, and the pH was recorded once the value stabilised. All measurements were conducted in triplicate, and the mean pH for each sample was calculated and documented.

Water-holding capacity determination:

The water-holding capacity (WHC) of yoghurt samples was determined using the centrifugation method described by (Harte et al., 2003). Approximately 10g of yoghurt was placed in a pre- weighted centrifuge tube and centrifuged at 5000 rpm for 10 minutes at 5 °C using a refrigerated centrifuge (Z 36 HK, Hermle, Germany). After centrifugation, the separated whey was gently decanted to avoid disturbing the pellet, and the tube was weighed again.

WHC was calculated using the following formula:

$$\mathrm{WHC}(\%) = \left(1 - \frac{\text{Weigt of separaed whey}}{\text{Initial weight of yogurt}}\right) \times 100$$

Statistical Analysis:

The statistical analysis was performed using SPSS software (version 25). Statistical differences were identified using Duncan's test after analysis of variance (ANOVA). $P \leq 0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

Visual observation:

All emulsions were stored at 5°C to evaluate stability over time. Visual inspections for phase separation or creaming were conducted, with photographs taken on Day 1 and every 7 days (Days 7, 14, and 21) to record physical changes, as shown in Figures 1 through 4. Variations in creaming behaviour with olive oil concentrations from 2.5% w/w to 10% w/w highlight the significance of the oil-to-emulsifier ratio in emulsion stability. Sodium caseinate at 1% w/w, combined with the original skimmed milk protein (including whey and casein), adsorbed at the oil-water interface, forming a protective layer. This layer effectively stabilised low to moderate oil contents (up to 5% w/w) over 21 days, aligning with its known interfacial properties (Dickinson, 2003, McClements, 2004). The absence of creaming after 21 days indicates that sodium caseinate can develop viscoelastic interfacial layers that prevent droplet coalescence and aggregation (Dickinson, 2003). When oil content ranges from 2.5% to 5% w/w, and the emulsifier-to-oil ratio varies from 0.4 to 0.2 w/w, sufficient caseinate coats the oil droplets fully, creating electrostatic and steric barriers (EUSTON et al., 1995). Conversely, the delayed creaming observed in the emulsion with 7.5% w/w olive oil and 1% w/w SC is related to the lower emulsifier-to-oil ratio of 0.13, compared to lower oil concentration ratios. A critical amount of SC is needed to fully coat droplets during homogenization. If this threshold isn't reached, the interfacial film becomes thinner or incomplete, weakening repulsive forces and increasing van der Waals attractions between droplets, which promotes aggregation (Tcholakova et al., 2004). (Dickinson and Golding, 1997) report that the surface load of sodium caseinate is about 2–3 mg/m². In this study, increasing the oil concentration to 7.5-10% w/w seems to diminish the emulsifier's ability to fully cover the surface, potentially creating bare spots that facilitate droplet coalescence.

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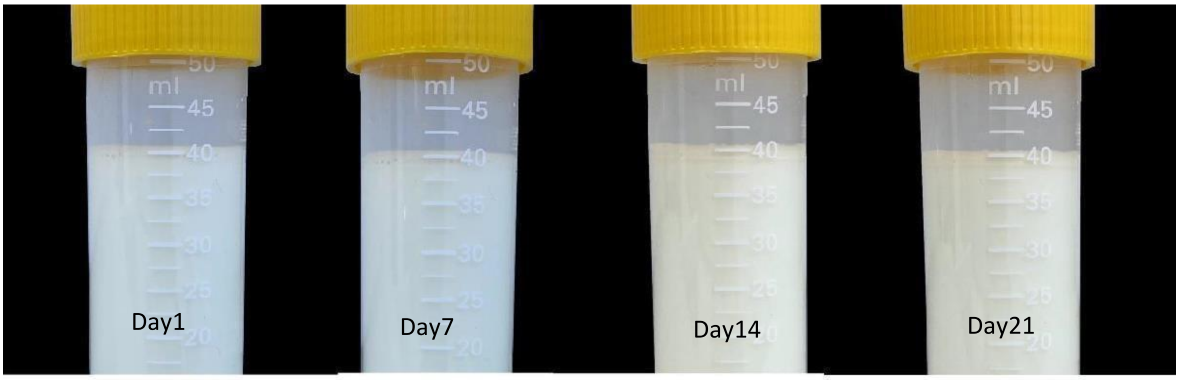

Fig. 1: Skimmed milk emulsion with 2.5% w/w EVOO over 21days of storage.

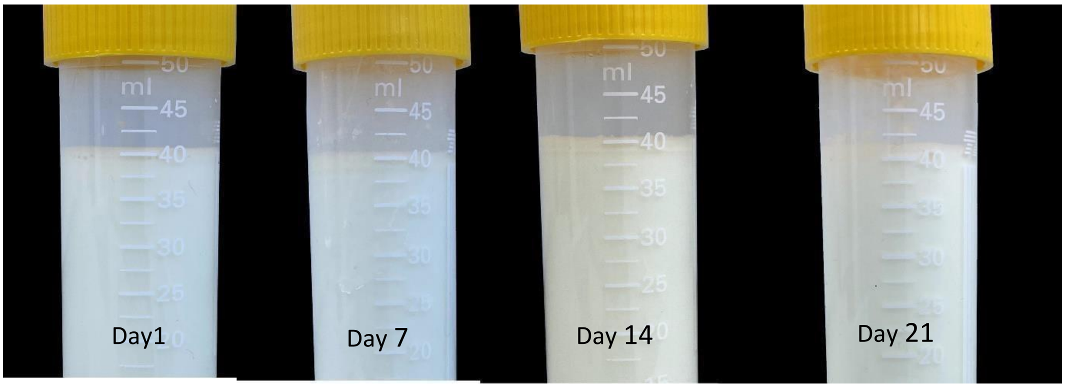

Fig. 2: Skimmed milk emulsion with 5% w/w EVOO over 21days of storage.

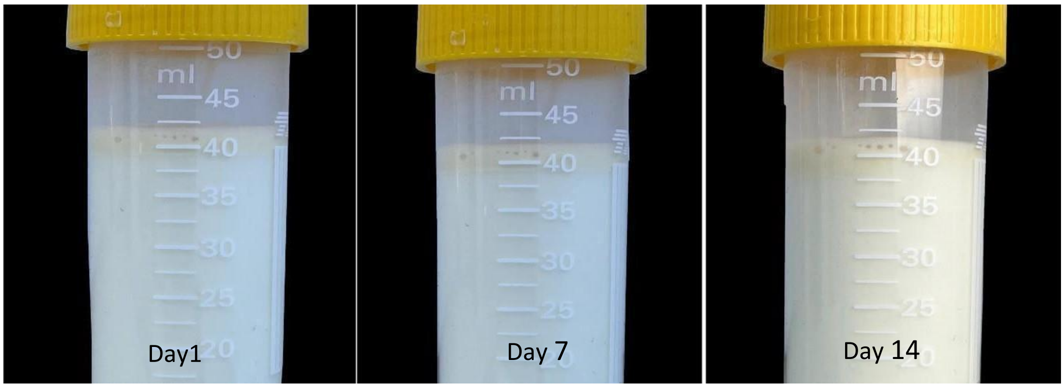

Fig. 3: Skimmed milk emulsion with 7.5% w/w EVOO over 14 days of storage.

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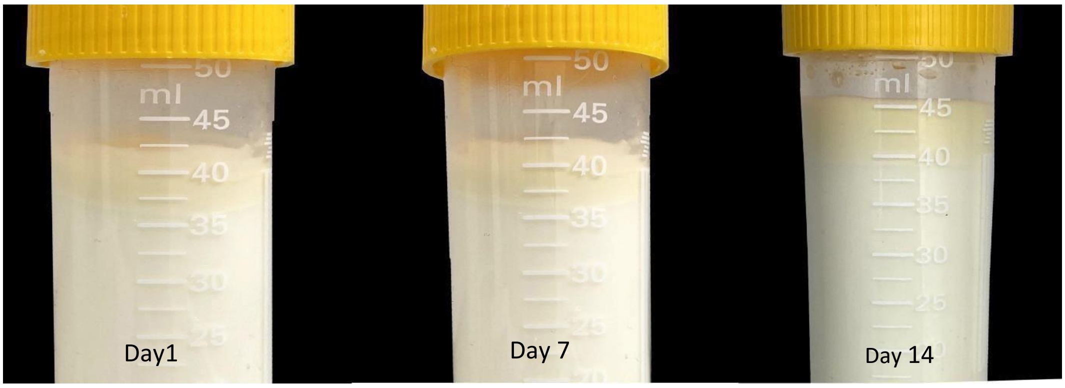

Fig. 4: Skimmed milk emulsion with 10% w/w EVOO over 14 days of storage.

Droplet size determination

This overview describes the anticipated changes in droplet size for olive oil-in-skimmed milk emulsions stabilised by 1% w/w sodium caseinate across different oil concentrations during storage, based on the experimental setup and supporting literature. Droplet size analysis was focused on lower oil concentrations (2.5% w/w and 5% w/w), which remained stable, as confirmed visually. It was also confirmed that the droplet sizes of these lower-concentration emulsions remained within an acceptable range after 21 days, as measured by the Mastersizer 3000, as shown in Figure 5. The diameter was 0.43µm initially and slightly grew to 1.06µm by day 21 in emulsions with 2.5% w/w EVOO. When the EVOO content increased to 5% w/w, a minor size increase was observed, likely due to the decreased sodium caseinate ratio, which was 0.66µm initially and increased to 1.24µm by day 21, as shown in Figure 6. Instability factors, such as flocculation, played a significant role in increasing the droplet size of these droplets. These droplet sizes, typically 0.1 to 5 µm, are common in food emulsions, as they contribute to stability and an appealing mouthfeel. (Tadros and stability, 2013). The oil content significantly influences droplet size; higher oil levels cause more collisions during homogenization, resulting in larger droplets (Walstra, 2002).

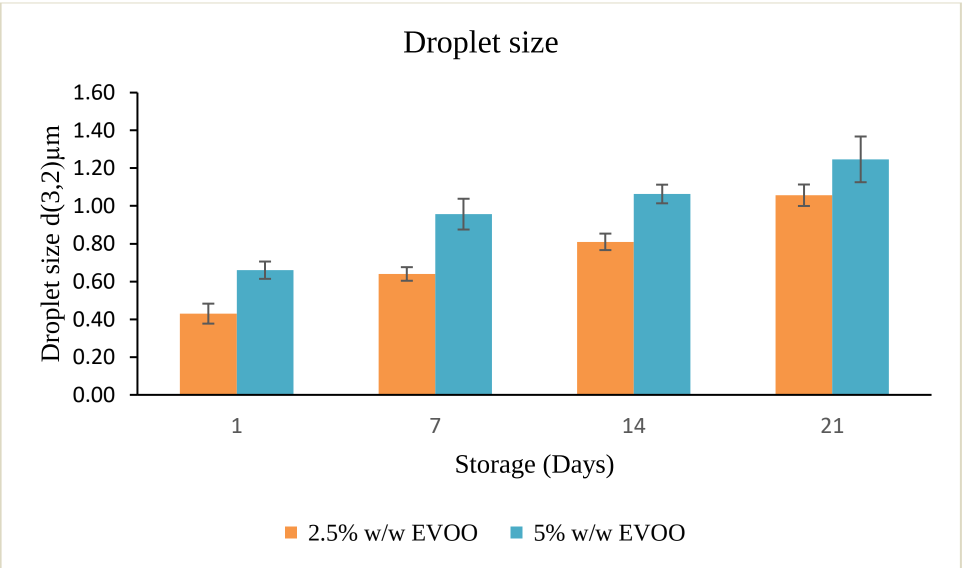

Fig. 5: Droplet size distribution of 2.5 and 5% w/w olive oil emulsion in skimmed milk during 21 days of storage.

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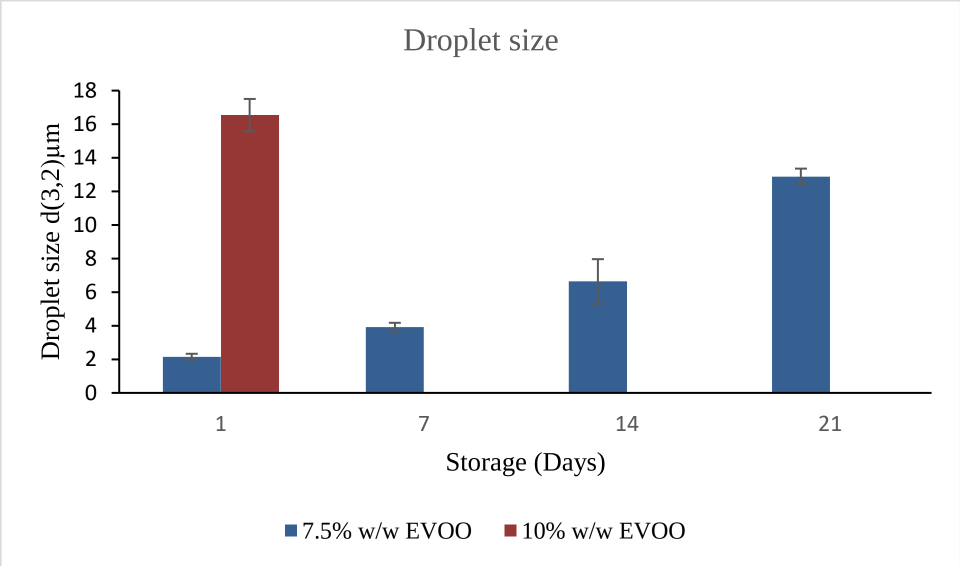

Fig. 6: Droplet size distribution of 7.5% and 10%w/w olive oil and emulsion in skimmed milk during 21 days of storage.

Visual observation showed that emulsions containing 7.5% w/w and 10% w/w olive oil became unstable within 10 hours, with creaming at the surface. In addition to visual observation, droplet size also confirmed this trend; an increase in droplet diameter was observed as the oil concentration increased to 7.5% w/w, resulting in droplets of 2.15µm on day 1 and 12.87µm on day 21 as showing in figure 6. Moreover, a 10% w/w EVOO concentration resulted in larger droplets reaching 16.54 µm and forming a separate oil layer at the top of the emulsion, providing clear evidence of Ostwald ripening. This phase separation indicated poor stability for these formulations. Conversely, emulsions with 2.5% w/w and 5% w/w olive oil remained stable over the 21-day storage period, with no visible separation. Based on these results, emulsions containing 2.5% and 5% w/w olive oil were selected for further testing in yoghurt formulation.

The pH of the produced yoghurts:

Adding olive oil at 2.5% w/w and 5% w/w influenced the pH values compared to the control. On day 1, the control yoghurt had the lowest pH (4.42), while those with 2.5% and 5% olive oil showed higher pH levels of 4.52 and 4.43, respectively. This pattern continued during storage. By day 21, pH decreased slightly across all samples, with the 2.5% and 5% olive oil yoghurts reaching 4.09 and 4.04, whereas the control fell to 3.95. These findings suggest that replacing milk fat with olive oil at these levels results in yoghurts with higher initial pH, as shown in Figure 7. Similar results were reported by (Dou et al., 2022). These results are consistent with those from (St, 2015), who studied cheese yoghurt with 1-2% EVOO and observed higher initial pH (+0.3 to 0.4 units), likely due to lipid buffering. They also reported less post-acidification during 21 days of storage, helping maintain pH in EVOO-treated samples. Likewise, (Barukčić et al., 2022) found that yoghurt with 1.5–5% olive leaf extract (OLE) started at a lower fermentation pH of 4.35 because of organic acids, which contributed to greater antioxidant stability throughout storage.

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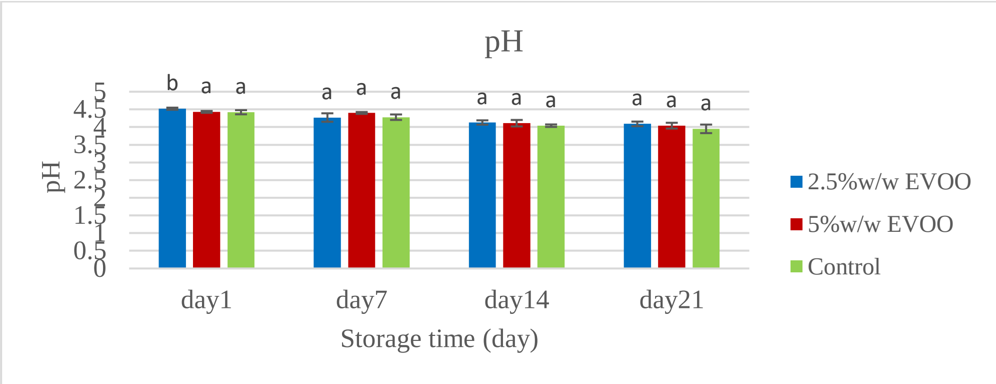
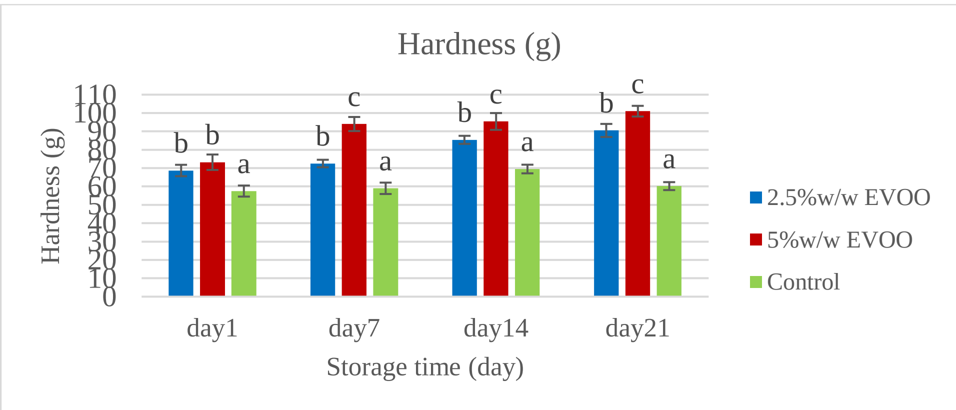


Values are means ± SD (n = 3). Different letters indicate significant differences (p < 0.05) per Duncan's test.

Fig. 7: The pH of various yoghurt types containing 2.5% w/w and 5% w/w olive oil, compared to the control, over 21 days of storage time.

Hardness determination of the produced yoghurts:

Incorporating 2.5–5% w/w olive oil and 1% w/w sodium caseinate significantly enhances yoghurt hardness, with effects depending on concentration. On day 1, control yoghurt had a hardness of 57.5 g, while samples with 2.5% and 5% olive oil reached 68.7 g and 73.2 g. Over 21 days of refrigerated storage, hardness increased in all samples, reaching 60.2 g (control), 90.5 g (2.5% oil), and 101.0 g (5% oil). The combination of olive oil and sodium caseinate improved the gel matrix, with the highest hardness at 5% oil, as shown in Figure 8. These results agree with those reported by (Bonczar et al., 2002). The enhancement is more pronounced during storage and peaks at the 5% level. Olive oil serves as a filler and interacts with proteins, strengthening the gel (Farjami et al., 2019). Researchers like (Dickinson, 2012) explain that increased hardness is due to olive oil droplets acting as active fillers, embedding within the casein network and serving as cross-linking points that resist deformation. (Grasberger et al., 2024) observed that interactions between oil droplets and proteins (whey, casein, sodium caseinate), via covalent and non-covalent bonds, lead to a denser gel formation. (Li et al., 2021) added that reduced water mobility decreases whey separation and syneresis, paradoxically increasing hardness.



Values are means ± SD (n = 3). Different letters indicate significant differences (p < 0.05) per Duncan's test.

Fig. 8: The hardness of various yoghurt types containing 2.5% w/w and 5% w/w olive oil, compared to the control over 21 days of storage time.

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Water holding capacity of the formed yoghurts:

The water holding capacity of the yoghurt samples varied significantly depending on the olive oil concentration and storage time. The yoghurt with 5% w/w olive oil consistently demonstrated the highest WHC, starting at 73.99 on day 1 and maintaining above 72.5 throughout the storage period. The 2.5% w/w olive oil yoghurt showed moderate WHC values, ranging from 61 to 61.7. In contrast, the control sample had the lowest WHC, dropping from 57.4 on day 1 to 51 by day21, as show in Figure 9. These results are similar to the result founded by (Dou et al., 2022). This substantial improvement in WHC with olive oil addition can be explained by the emulsion stabilising role of sodium caseinate and the effect of olive oil droplets in reinforcing the gel matrix (Ma and Chatterton, 2021). (Huck-Iriart et al., 2011) emphasised the synergistic role of sodium caseinate (1%) on stabilising olive oil in the emulsions, firming up the gel matrix. This is due to the ability of SC to form a membrane around oil droplets, interacting with casein networks, reducing the mobility of water and retaining the moisture. These findings align with (Li et al., 2022) who confirmed improved WHC via yoghurt fortification with oil.

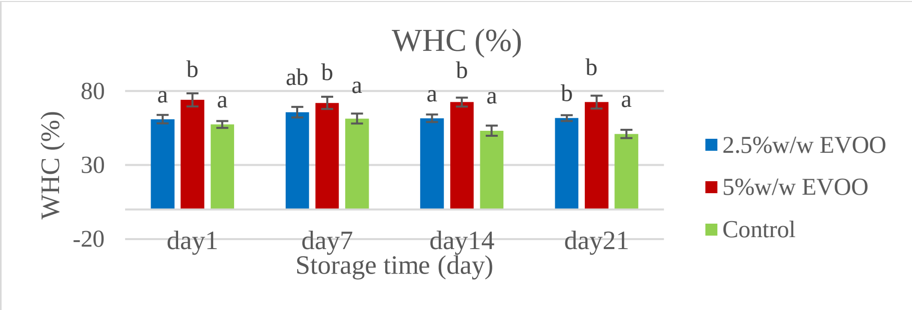

Fig. 9: The WHC of various yoghurt types containing 2.5% w/w and 5% w/w olive oil, compared to the control, over 21 days of storage time.

Values are means ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$) per Duncan's test.

IV. STATISTICAL ANALYSIS

Table 1: Physicochemical and Textural Properties of Yoghurt Formulated with Different Levels of Extra Virgin Olive Oil (Mean ± SE)

Treatment	pH*	Hardness*	WHC*
Control	4.17±0.1079^{a}	61.55±2.7069^{a}	55.75±2.304^{a}
2.5%	4.25±0.0971^{a}	79.27±5.1740^{ab}	62.50 ±1.077^{b}
5%	4.24±0.0993^{a}	90.9±6.0907^{b}	72.74 ±0.430^{c}

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- Data presented as mean ± S.E.
- The same letters mean no statistical differences
- The different letters mean statistical differences
- * =P<0.05 n=12 for each group

IV. CONCLUSIONS

The incorporation of extra virgin olive oil (EVOO) at concentrations of 2.5% w/w and 5% w/w in yoghurt formulations, stabilised with sodium caseinate, significantly enhanced the physicochemical and functional properties of the final product. Emulsions with higher olive oil levels (7.5% w/w and 10% w/w) were unstable, whereas those with 2.5% w/w and 5% w/w remained physically stable during 21 days of storage. Yoghurts enriched with EVOO demonstrated improved hardness, water-holding capacity and pH compared to the control.

V. RECOMMENDATION

1. investigate the substitution of dairy fat with omega-3 fatty acids and fat-soluble vitamins to improve yoghurt quality and nutritional value.

2. Apply alternative homogenisation techniques to improve emulsion stability and texture.

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