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RESEARCH

E-beam Irradiated Freeze-Dried Whey Fractions and Skimmed Camel and Cow Milk: Solubility, Water Holding Capacity, Microstructure and In Vitro α-amylase and α-glucosidase Inhibitory Activities

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Abstract

Ionizing radiation, as electron beam (E-beam), is regarded as an environmentally friendly food preserving technology with potential to enhance the bioactive content and bioactivity of food products. This study evaluated the rehydration properties, solubility, microstructure, and bioactivities (antioxidant and in vitro α-amylase and α-glucosidase inhibitory activities) of freeze-dried whey, and corresponding skimmed cow and camel milk, using or not electron beam (E-beam) irradiation at doses of 5, 10, and 20 kGy. E-beam irradiation generally caused a notable decrease in water holding capacity (from 20 to 66%) and affected solubility, with significant decline (14—77%) followed by a partial restoration at 20 kGy. Microstructure analysis suggested protein denaturation including disaggregation at 5 and 10 kGy and aggregation at 20 kGy along with a disruption of the protein network. E-beam irradiation generally increased the total phenolic content in freeze-dried cow milk but had variable effects on freeze-dried camel milk. Freeze-dried camel milk demonstrated heightened sensitivity to irradiation doses in terms of antioxidant activity, whereas cow milk exhibited resilience. Freeze-dried skimmed cow and camel milk showed dose-dependent changes in α-amylase inhibitory activity, while α-glucosidase inhibition either increased or remained stable. These findings underscore the significant effect of E-beam irradiation on the bioactivity and micro-structural properties of freeze-dried whey and milk, revealing distinct responses between cow and camel milk.

Keywords Milk · Freeze-drying · e-beam irradiation · Solubility · Microstructure · Antioxidant activity · α-amylase and α-glucosidase inhibitory activities

Introduction

Milk, with its high moisture content and nutrient-rich composition, is highly perishable and prone to microbial growth. This susceptibility has long been a concern in dairy sector, leading to the development of various methods to ensure both consumer safety and product quality. Nonthermal processing technologies, such as freeze-drying and irradiation, have emerged as promising solutions to extend shelf life and create new dairy products without compromising nutritional value or sensory quality (Neoκleous et al., 2022; Pegu & Arya, 2023). Freeze-drying, which allows sublimation under vacuum condition and low temperature, contrasts with traditional heat-based drying methods (Nowak & Jakubczyk, 2020). Irradiation, particularly electron-beam irradiation (E-beam), offers another nonthermal option, preserving or enhancing milk's bioactive content while enhancing food safety (Harizi et al., 2023a, 2023b).

Sandra Cabo Verde and Nourhène Boudhrioua contributed equally to this work.

Highlights
- Solubility of cow milk fractions is notably impacted by E-beam irradiation.
- E-beam irradiation induces a general decrease of water holding capacity.
- Lactose crystallization and protein denaturation: disaggregation at 5 and 10 kGy and aggregation at 20 kGy
- Camel milk shows sensitivity to irradiation regarding antioxidant activity
- A dose-dependent changes is observed in α-amylase inhibition and α-glucosidase inhibition.

Extended author information available on the last page of the article

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Camel and cow milk, both known for their rich nutritional profiles, differ in protein and phenolic content, resulting in distinct functional and biological properties, including α-amylase and α-glucosidase inhibitory activities and antioxidant capacity (Anwar et al., 2021; Harizi et al., 2023a, 2023b). These differences influence the techno-functional properties of milk and milk products, such as water holding capacity, and rehydration properties (solubility, wettability and dispersibility) which are critical in dairy processing (Pugliese et al., 2017). The rehydration properties, in particular, are important for producing liquid milk from powdered forms, with factors like particle size and solubility significantly affecting the ease and effectiveness of reconstitution (Crowley et al., 2016; Schuck et al., 2012).However, processing techniques such as irradiation and freeze-drying can lead to protein denaturation or aggregation, potentially impacting these properties (Pugliese et al., 2017; Thomas et al., 2004). The distinctives properties of cow and camel milk and their hydrolysates were extensively reported in literature (Bielecka et al., 2022). The effect of dehydration process on the techno-functional properties were also reported (Felfoul et al. (2020); Pugliese et al., 2017;. To the best of our knowledge this is the first work reporting the effect of E-beam on the techno-functional and antioxidant properties of freeze-dried whey and skimmed milk.

Therefore, this study aims to investigate the effect of E-beam irradiation at different doses (5, 10 and 20 KGy) on some properties of freeze-dried skimmed milk and whey, prepared from cow and camel milk, on water holding capacity, the solubility, the microstructure, as well as the in vitro antioxidant and in vitro α-amylase and α-glucosidase inhibitory activities of freeze-dried skimmed milk and whey. The outcomes of this research can contribute for milk processing technologies, ensuring quality and supporting the sustainability of the dairy industry.

Materials and Methods

Skimmed Milk and Whey Preparation and Characterization

Camel (*Camelus dromedarius*) and cow (*Bos taurus*) milk were collected aseptically from 10 to 15 milking females each, ranging from 2 to 10 months lactation stage, ensuring a representative blend of milk from multiple individuals [11]. The milk samples were immediately transported to the laboratory at 4 °C upon collection, and the pH was measured. For cow's milk, skimming was achieved through a single centrifugation step at 2000 g for 15 min at 5 °C. Acid whey was obtained using 12 N HCl following Zouari et al. (2018) and Harizi et al., (2023a, 2023b). Coagulation of milk, to obtain sweet whey, was initiated at 37 °C using rennet enzymes (Parachimic, Laboratories Arrazi, Sfax, Tunisia, strength = 1:10,000), which acted on casein proteins. After coagulation, the whey was separated from the casein fractions (Harizi et al., 2023a, 2023b). The pH and the proximate chemical composition of the liquid milk and milk were analyzed according to AFNOR methods (1993).

Freeze-drying of Whey and Skimmed Milk

Liquid whey and skimmed cow and camel milk were frozen at − 80 °C for 24 h in a sterilized glass bottle. Subsequently, the milk in the cooled bottles underwent freeze-drying using a Lab Pilot freeze dryer (USCFROID, SMH- 15, 290.94) with a 14-L capacity. The freeze-drying process occurred at − 50 °C for 48 h under a vacuum condition of 0.05 mbar (Harizi et al., 2023a, 2023b).

Irradiation Experiments

Irradiation experiments were conducted using a 10 MeV linear electron-beam accelerator (CIRCE- 3, LINAC Technology) at the Centre National des Sciences et Technologies Nucléaires in Tunisia (Barkaoui et al., 2021). Lyophilized samples were irradiated on both sides at room temperature with doses of 5, 10, and 20 kGy in 10 g graduated bottles. Conveyor speeds were adjusted to 400 cm/minute for 5 kGy, 200 cm/minute for 10 kGy, and 80 cm/minute for 20 kGy. Dosimetry was performed using a B3 DoseStix dosimeter (GEX Corporation, USA), and absorbed doses were calculated using a Genesys 20 spectrophotometer (Thermo Fisher Scientific). Unirradiated samples were used as control to elucidate the effect of e-beam on whey and skim milk properties.

Water Holding Capacity (WHC)

Water holding capacity (WHC) of whey and skimmed milk was assessed following the method by Nguyen et al. (2017), with modifications. Lyophilized samples (1 g) were hydrated in 50 mL tubes with 20 mL of distilled water for 24 h, then stirred at room temperature for 30 min. The suspension was centrifuged at 19000 × g for 10 min. After removing the supernatant, the tube weights were recorded, and WHC (grams of water per gram of powder) was calculated:

$$WHC = \frac{Pr - Pi}{Pi} \quad (1)$$

where:

- Pr = weight of sediment after decantation (g).
- Pi = weight of the powder (g).

Solubility Index

Solubility, the weight percentage of soluble matter in the powder, was assessed following Felfoul et al. (2020). Whey and skimmed milk powder (2.5 g) was mixed with 17.5 mL of distilled water at 22 °C in a Falcon tube and stirred for 30 s. The resulting suspension was centrifuged at 1800 × g at room temperature (22 °C), and the supernatant was collected. The solid residue obtained from drying the supernatant at 103 °C for 15 h was used to calculate the solubility index (SI) with the equation:

$$SI(\%) = 100 - \left(M \times \frac{100}{2.5}\right) \quad (2)$$

M represents the mass of the sediment in grams (g).

Microstructure of Freeze-dried Whey and Skimmed Camel and Cow Milk

To analyze the microstructure of the irradiated powdered samples, scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX) (SEM/EDX: Q250, Thermo Scientific™ Analytical SEM, KarlsdorfNeuthard Deutschland, Germany) was used. This equipment features a tungsten electron microscope and a robust EDS detector for detailed elemental analysis. Samples were positioned on a carbon adhesive-coated substrate and analyzed under partial vacuum conditions at 70 Pa with an acceleration voltage of 25 kV (Ben Abdallah et al., 2023).

Phenolic Content and Bioactivity Assay

To evaluate the bioactivity of irradiated whey and milk powder, the extracts were prepared using solid–liquid extraction. Sample (0.2 g) was mixed with 2 mL of solvent (deionized water/95% ethanol, 15/85 v/v) and shaken for 1 h at 30 °C. After centrifugation at 7800 × g for 15 min at 5 °C, the extracts were stored at—20 °C in the dark for further analysis.

Total Phenolic Content

Total phenolic content (TPC) was determined on the milk ethanolic extract using the Folin–Ciocalteau method as previously described (Harizi et al., 2023a, 2023b). Absorbance at 765 nm was measured, and TPC was expressed as mg of gallic acid equivalents per 100 g of whey or skimmed powder (mg GAE/100 g) using a gallic acid calibration curve (Bobo-Garcia et al., 2015).

Antioxidant Activity

DPPH-RSA and FRAP Assay DPPH radical scavenging activity (DPPH-RSA) and the ferric reducing antioxidant power (FRAP) assays were assessed in 96-well plates using an EZ Read 1200 Microplate Reader (Biochrom, Cambridge, UK) following Barkaoui et al. (2020) and Al-Duais et al. (2009), respectively. For DPPH-RSA, the absorbance was measured at 515 nm and the results were expressed as percentage of radical inhibition, whereas for FRAP assay, the absorbance was read at 593 nm and the results were presented as mmol ferric sulphate equivalents per 100 g of whey or skimmed powder (mmol FSE/100 g).

In vitro α-amylase and α-glucosidase Inhibition Assays

Samples were tested for their ability to inhibit in vitro α-amylase and α-glucosidase using methods described by Chen et al. (2020) and Silva et al. (2020), respectively. Acarbose served as positive control, and absorbances were measured in a microplate reader at 540 nm and 405 nm for α-amylase and α-glucosidase inhibitory activity, respectively.

Data Analysis

All analyses were performed in triplicate. Statistical differences were assessed using XLSTAT 19 software. A one-way analysis of variance (ANOVA) with Tukey's test was used to compare the samples, with significance determined at $p < 0.05$.

Results and Discussion

Effect of E-beam on Water Holding Capacity (WHC)

Irradiation at different doses (5, 10, and 20 kGy) demonstrated effects on the water holding capacity (WHC) of skimmed cow milk (S CoM) and skimmed camel milk (S CaM) (Fig. 1A). For S CoM, a significant decrease in WHC is observed at the highest irradiation dose. Indeed, the water holding capacity at 0 kGy is about 0.54 ± 0.02 g of water/g of powder, shows a significant decrease of 20.4% at 5 kGy (0.43 ± 0.01 g of water/g of powder). At 10 kGy, the water holding capacity reaches its maximum (0.69 ± 0.03 g of water/g of powder), then experiences a further decrease of 67.3% at 20 kGy (0.32 ± 0.01 g of water/g of powder). As for S CaM, the results also reveal a variation in WHC depending on the irradiation dose. At 0 kGy, the WHC is 0.36 ± 0.01 g of water/g of powder, then it decreases 41.7% at 5 kGy(0.21 ± 0.02 g of water/g of powder) maintaining the value at

10 kGy. After 20 kGy irradiation, the WHC reaches the highest value of 0.41 ±0.01 g of water/g of powder. These results suggest a differentiated response of skimmed cow and camel milk to irradiation treatment, thereby influencing their respective water holding capacities. The difference in WHC between skimmed cow milk (S CoM) and camel milk (S CaM) fractions under irradiation can be attributed to variations in their composition (Table S1), proteins profile (Harizi et al., 2024), structures, and interactions. Camel milk proteins seem more sensitive to irradiation, leading to greater protein network disruption compared to cow milk proteins, which indicated partial recovery at higher doses. Additionally, the higher calcium content in camel milk may promote increased protein cross-linking, resulting in reduced water retention (Arain et al., 2024).

Regarding the sweet whey (SW CoM and SW CaM) (Fig. 1B), the initial WHC of cow sweet whey, SW CoM, at 0 kGy is notably high, measured at 0.77 ±0.03 g of water/g of powder. However, following irradiation, a significant decrease of 57.1% and 86.1% in WHC is observed at 5 kGy (0.33 ±0.02 g of water/g of powder) and at 10 kGy (0.18 ±0.01 g of water/g of powder), respectively, followed by a slight increase at 20 kGy (0.23 ±0.03 g of water/g of powder). For sweet whey of camel milk (SW CaM), the initial WHC at 0 kGy is 0.48 ±0.05 g of water/g of powder. After irradiation, a slight but non-significant increase is observed at 5 kGy (0.53 ±0.03 g of water/g of powder), followed by a significant decrease at 10 kGy (0.38 ±0.03 g of water/g of powder) and at 20 kGy (0.18 ±0.01 g of water/g of powder). Irradiation affects the WHC of cow and camel sweet whey differently. SWCoM shows a tendency for decreased WHC with increasing irradiation doses (5 and 10 kGy), followed by recovery at the highest dose (20 kGy). In contrast, SWCaM exhibits a significant decrease in WHC as the irradiation dose increases (from 5 to 20 kGy). For acid whey of cow milk (AW CoM) (Fig. 1C), the WHC at 0 kGy is significantly high, reaching 0.98 ±0.2 g of water/g of powder. However, after irradiation a decreasing tendency is observed with a decrease of 56.1% in WHC at 5 kGy (0.43 ±0.1 g of water/g of powder), of 39.5% at 10 kGy (0.26 ±0.04 g of water/g of powder) and at 20 kGy (0.26 ±0.03 g of water/g of powder) compared to the control value. As for acid whey of camel milk (AW CaM), the WHC at 0 kGy is significantly lower than that of cow milk (0.32 ±0.05 g of water/g of powder). Irradiation at 5 kGy leads to an increase in WHC to 0.48 ±0.1 g of water/g of powder, remaining stable at 10 kGy (0.42 ±0.1 g of water/g of powder) and at 20 kGy (0.32 ±0.04 g of water/g of powder). The impact of irradiation at various doses is significant on the water holding capacity (WHC) of acid whey of cow milk (AW CoM) and camel milk (AW CaM). While AW CoM exhibits a substantial reduction in WHC as the dose increases, AW CaM presents an increase at 5 kGy and no variation at 10 and 20 kGy.

Water retention in milk is governed by complex interactions involving water molecules and milk proteins (Kneifel & Seiler, 1993). These interactions involve mechanisms such as protein-water affinity, hydrogen bonding, and other intermolecular forces. The amphiphilic nature of milk proteins allows them to form structures that effectively trap water, thus contributing to water retention. Camel milk proteins, which differ in amino acid profiles and molecular structure (Salmen et al., 2012; Harizi et al., 2024), may form stronger or more stable interactions with water, making them more sensitive to irradiation. Camel milk contains higher amounts of proline, glycine, and glutamine, amino acids that could affect how proteins interact with each other and with water molecules. Additionally, camel milk has a higher proportion of casein (which is more heat-stable) and different whey proteins compared to cow's milk, contributing to the increased sensitivity of its proteins to irradiation. The

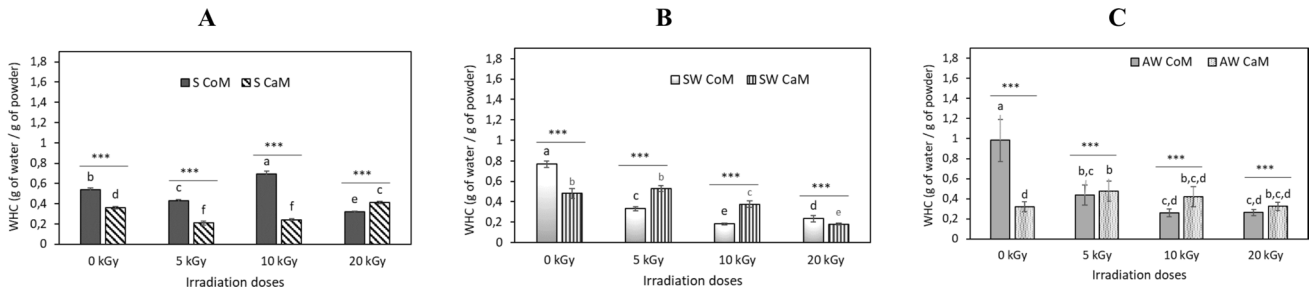

Fig. 1 Effect of e-beam irradiation (5–20 kGy) on the water holding capacity (g of water/g of powder) of lyophilized whey and skimmed cow and camel milk fractions. **A** Water retention capacity of skimmed cow milk (S CoM) and skimmed camel milk (S CaM); **B** Water retention capacity of sweet whey of cow milk (SW CoM) and sweet whey of camel milk (SW CaM); **C** Water retention capacity of acid whey of cow milk (AW CoM) and acid whey of camel milk (AW CaM). Error bars represent 95% confidence intervals around the mean values ($n = 3$). Different letters on each bar indicate significant differences between the mean values of the fractions ($p < 0.05$). The *** indicate a significant difference between the different type of milk (cow and camel milk) for each fraction ($p < 0.05$). S CoM: Skimmed cow milk, SW CoM: Sweet whey of cow milk, AW CoM: Acid whey of cow milk, S CaM: Skimmed camel milk, SW CaM: Sweet whey of camel milk, AW CaM: Acid whey of camel milk

irradiation process might cause protein denaturation (Cieśla et al., 2000), which may result in more significant changes in camel milk due to the distinct characteristics of its proteins profile. Furthermore, differences in lactose concentrations (Table S1) and lactose state could also contribute to variations in the porosity and the micro-structure of the milk powders and affect their water retention capacity.

Effect of E-beam on Solubility

The effect of e-beam irradiation (5—20 kGy) on the solubility (%) of the lyophilized whey and skimmed cow and camel milk is presented in Fig. 2.E-beam irradiation at 5, 10, and 20 kGy significantly affects the solubility (%) of skimmed cow (S CoM) and camel milk (S CaM) (Fig. 2A). For S CoM, solubility decreases with irradiation but demonstrated no dose effect (from 73.3 ± 1.2% to 22.1 ± 2.9% at 5 kGy,18.3 ± 1.4% at 10 kGy and 18.2 ± 1.1% at 20 kGy). S CaM also shows decreased solubility with irradiation, though to a lesser degree. Solubility drops from 78.66 ± 3.02% to 48.8 ± 0.5% at 5 kGy, then further decreases to 41.3 ± 0.5% at 10 kGy, before a slight rise to 54.7 ± 1.3% at 20 kGy. In summary, the impact of irradiation on solubility seems more pronounced in S CoM compared to S CaM. For sweet whey of cow milk (SW CoM) (Fig. 2B), solubility significantly decreases up to 10 kGy. This parameter shows a decrease of 45% at 5 kGy, an increase of 25.6% compared to the solubility value at 5 kGy, and after irradiation at 20 kGy a non-significant difference to non-irradiated samples. Regarding sweet whey of camel milk (SW CaM) (Fig. 2B), solubility also decreases with irradiation, although less prominently. Solubility drops from 76.1 ± 0.4% to 56.8 ± 1.8% at 5 kGy, at 10 kGy remains steady at 57.7 ± 2.3%, and after irradiation at 20 kGy decreased to 64.9 ± 1.4% at 20 kGy, comparatively to non-treated fraction. In summary, irradiation seems to trigger distinct solubility changes between the SW CoM and SW CaM fractions, with a more pronounced decline for SW CoM. Significant variations are observed for the acid whey fractions of cow (AW CoM) and camel milk (AW CaM) (Fig. 2C). For AW CoM, there is a noticeable decrease in solubility, from 58.6 ± 0.8% to 26.7 ± 0.4% at 5 kGy, to 25.9 ± 5.3% at 10 kGy, and to 38.3 ± 0.7% at 20 kGy. Regarding AW CaM, a decrease in solubility is also observed, from 68.1 ± 0.6% to 43.97 ± 1.5% at 5 kGy, to 45.5 ± 4.8% at 10 kGy, and to 64.6 ± 1.1% at 20 kGy, similar to 0 kGy. E-beam irradiation has a significant impact on the solubility of acid whey fractions of cow and camel milk, leading to a decrease in solubility at intermediate doses (5 and 10 kGy), followed by a slight increase at the highest dose (20 kGy). Both fractions react similarly to irradiation, although solubility levels differ between AW CoM and AW CaM.

Irradiation affects milk powder solubility through factors such as dose, milk type, and processing conditions. At higher doses, irradiation induces protein degradation and the formation of reactive compounds which can lead to reduced solubility due to protein agglomeration (Lee et al., 2001). These changes can break down protein molecules or promote coagulation, alter molecular interactions and functional properties (Lee et al., 2001). The extend of the impact varies depending on milk type due to the specific protein profile. Additionally, post-irradiation storage conditions, further influence solubility. Studies have shown a decrease in solubility in stored dairy products over time (Havea, 2006; Fyfe er al., 2011). For instance, milk protein concentrate (MPC85) stored for 24 months at 20 °C exhibited 32% solubility compared to 53% after 2 days at the same temperature (Havea, 2006). This reduction is attributed to the formation of particle surface films (Anema et al., 2006) or conformational changes in protein molecules (Haque et al., 2011).

Effect of E-beam Irradiation on the Microstructure of Whey and Skimmed Camel and Cow Milk Powder

The microstructure of lyophilized whey and skimmed cow and camel milk, both non-irradiated and irradiated by e-beam at three doses 5, 10, and 20 kGy, is presented in Figs. 3, 4, and 5. The e-beam irradiation effect on the microstructure of the skimmed cow milk powder (Fig. 3A-D) and skimmed camel milk powder (Fig. 3E-H), analyzed by SEM, reveals a series of dose-dependent changes. The microstructure of non-irradiated cow milk powder (0 kGy, Fig. 3A) shows a compact organization characterized by vacuole-shaped structures. In this configuration, milk particles are densely arranged, forming an overall matrix with dispersed vacuolar-shaped spaces or cavities throughout the structure.

Microscopic analysis reveals that cow milk powder consists of an amorphous matrix containing lactose and other low-molecular-weight components, including proteins (Walstra et al., 1999). In low-water environments, lactose can adopt a stable form, either as crystalline lactose monohydrate (with bound water) or anhydrous α-lactose (without bound water), depending on temperature and water activity (Roos, 2002). This variability in lactose's crystalline form highlights its sensitivity to environmental conditions such as temperature and water activity (Fox & McSweeney, 1998. At 5 kGy (Fig. 3B), irradiated cow milk powder maintains its original structure, with particles retaining integrity but showing surface modifications resembling broken glass. This suggests initial responses to irradiation while preserving overall structure resilience at this exposure level. Ebeam treatment at 10 kGy (Fig. 3C), shows a pronounced microstructural degradation of irradiated cow milk, visible ruptures in molecular structure with the formation of particle agglomerates. These changes at 10 kGy suggest substantial

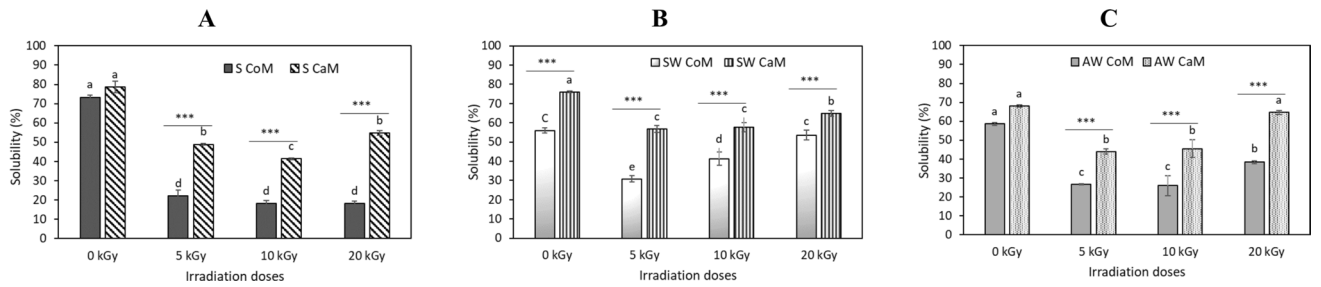

Fig. 2 Effect of e-beam irradiation (5–20 kGy) on the solubility (%) of lyophilized whey and skimmed cow and camel milk fractions. **A** Solubility of skimmed cow milk (S CoM) and skimmed camel milk (S CaM); **B** Solubility of sweet whey of cow milk (SW CoM) and sweet whey of camel milk (SW CaM); **C** Solubility of acid whey of cow milk (AW CoM) and acid whey of camel milk (AW CaM). Error bars represent 95% confidence intervals around the mean values ($n = 3$). Different letters on each bar indicate significant differences between the mean values of the fractions ($p < 0.05$). The *** indicate a significant difference between the different type of milk (cow and camel milk) for each fraction ($p < 0.05$). S CoM: Skimmed cow milk, SW CoM: Sweet whey of cow milk, AW CoM: Acid whey of cow milk, S CaM: Skimmed camel milk, SW CaM: Sweet whey of camel milk, AW CaM: Acid whey of camel milk

protein degradation reflecting, modifications in cow milk composition and molecular arrangement that can influence its properties. Higher irradiation doses significantly affect cow milk microstructure, potentially altering the final product quality and stability. Lactose is initially in a metastable glass state makes transitions towards stable crystals (Thomsen et al., 2005), influencing product characteristics and requiring careful management of irradiated dairy products. Lactose crystallization is not just a physical change; it can trigger further reactions that impact molecular structure, and water activity (Thomsen et al., 2005). At 20 kGy, significant structural disruptions occur in cow milk, including broken protein networks and may increase lactose crystallization (Fig. 3D), intensifying the effects and indicating notable microstructural degradation. Skimmed camel milk powders exhibit similar microstructural changes under e-beam irradiation doses (Fig. 3E-H). At 0 kGy, skimmed camel milk microstructure is homogeneous and leaf-like (Fig. 3E). At 5 kGy (Fig. 3F), structure is preserved with particle integrity maintained. At 10 kGy, microstructural degradation begins, evident with ruptures and assumed lactose crystallization. At 20 kGy, substantial structural degradation occurs, including intense broken protein networks and this may induce advanced lactose crystallization. These observations demonstrate dose-dependent e-beam irradiation impacts on skimmed camel milk powder microstructure, similar to those in skimmed cow milk powder, emphasizing microstructural changes induced by irradiation. Other studies reported that lactose crystallization impacts milk powder characteristics including texture, flowability, and dissolution properties (Goulart, 2021). The transition from amorphous to crystalline lactose alters its solubility, with crystalline lactose exhibiting different properties compared to the amorphous form. Indeed, the degree of crystallinity affects lactose dissolution; amorphous lactose, lacking an organized structure, is more soluble (Goulart, 2021). Crystallization dynamics are influenced by both inherent and external factors like processing conditions, including temperature and humidity controlled during milk powder production and storage, which significantly influence lactose crystallization and subsequent solubility behavior.

The microstructure of sweet whey fractions from cow (SW CoM) and camel milk (SW CaM), subjected to different irradiation doses (5—20 kGy), is presented in Fig. 4. The observation under scanning electron microscopy (SEM) of lyophilized cow sweet whey (SW CoM) before irradiation (0 kGy) reveals a configuration where whey particles are embedded in the matrix (Fig. 4A). This arrangement implies a close integration between whey particles and the overall structure of the lyophilized matrix. As the examination extends to the microstructure under various irradiation doses, it unveils two distinct phenomena. The irradiation treatment initiates a sequence of events, starting with the destabilization of proteins. This leads to noticeable structural alterations at 5 and 10 kGy (Fig. 4B and C, respectively), progressing to a significant micro-structure change at 20 kGy (Fig. 4D). Dark-shining particles visually may indicate lactose crystallization. As proteins denature, solubility decreases. Whey proteins have been associated with reduced lactose solubility (Bhargavaand Jelen, 1996). Additionally, whey proteins in the matrix promote lactose crystallization by nucleation (Huppertz & Gazi, 2015). These interactions highlight the interplay among irradiation, protein destabilization, and lactose state, shaping the microstructural characteristics of the lyophilized matrix.

Examining the sweet whey microstructure of camel milk (SW CaM) under scanning electron microscopy (Fig. 4E-H) reveals a complex architecture with a broken lamellar form. In its initial state (0 kGy, Fig. 4E), the sweet whey microstructure of camel milk, unveils a finely textured matrix in which proteins and lactose are dispersed.

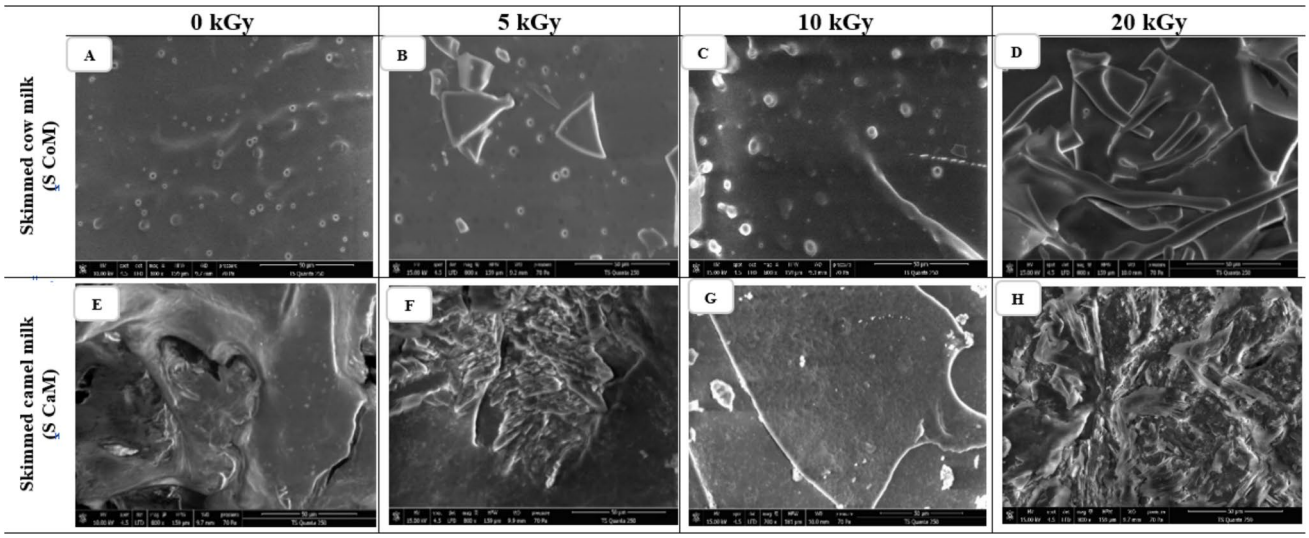

Fig. 3 Microstructure of lyophilized fractions of skimmed cow milk (S CoM) (**A**-**D**) and skimmed camel milk (S CaM) (**E**–**H**) non-irradiated, 0 kGy (**A** and **E**), and irradiated at 5 kGy (**B** and **F**), 10 kGy (**C** and **G**), and 20 kGy (**D** and **H**), at a magnification of 50 µm

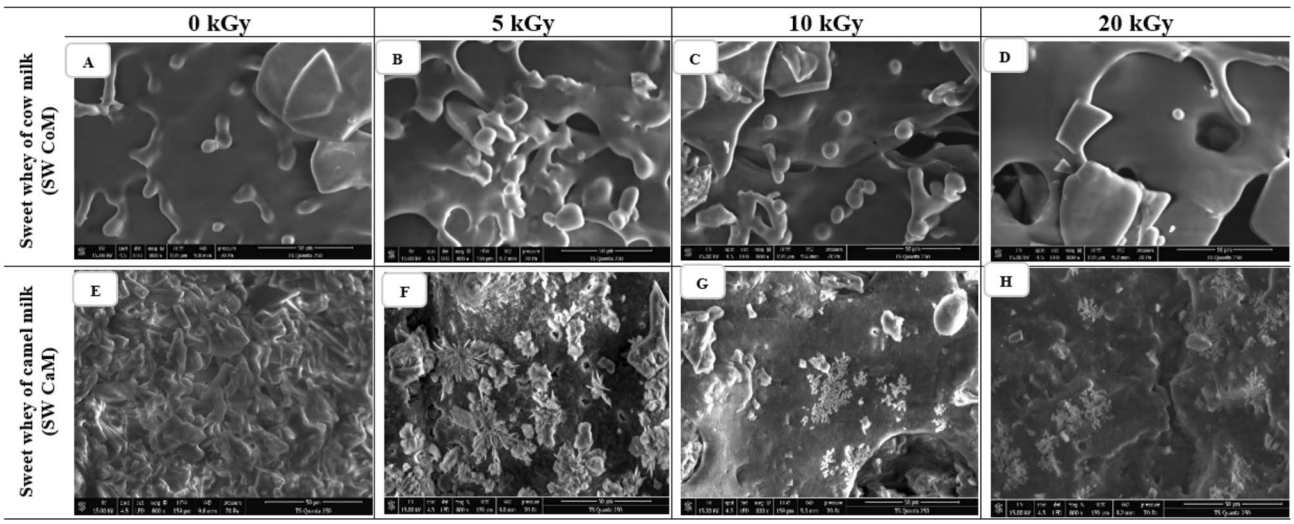

Fig. 4 Microstructure of lyophilized fractions of sweet whey of cow milk (SW CoM) (**A**-**D**) and camel milk (SW CaM) (**E**–**H**) non-irradiated, 0 kGy (**A** and **E**), and irradiated at 5 kGy (**B** and **F**), 10 kGy (**C** and **G**), and 20 kGy (**D** and **H**), at a magnification of 50 µm

Camel milk's sweet whey microstructure exhibits notable resilience, attributed to specific compositional differences compared to cow milk. The absence of beta-lactoglobulin, a major whey protein in cow milk, plays a pivotal role as it interacts with other milk components, affecting matrix organization (Seifu, 2022). Additionally, higher alpha-lactalbumin concentrations in camel milk (45.5% in acid whey and 20.1% in sweet whey) contribute to its unique microstructural characteristics (Harizi et al., 2024). As irradiation doses increase, particularly at 5 and 10 kGy (Fig. 4F-G, respectively), there is a concentration of lactose in camel milk, suggesting a distinct response to irradiation (Seifu, 2022). This observed resilience implies specific changes under irradiation, resulting in a more concentrated lactose distribution that enhances microstructural stability.

Concerning the acid whey of cow milk (AW CoM), the analysis of the microstructure reveals a complex architecture with a compact form, showing aggregates of proteins and lactose, either clustered together or dispersed across the entire surface of the micro-structure. This substantial variation in microstructure clearly suggests a direct influence of irradiation on the composition of acid whey. This

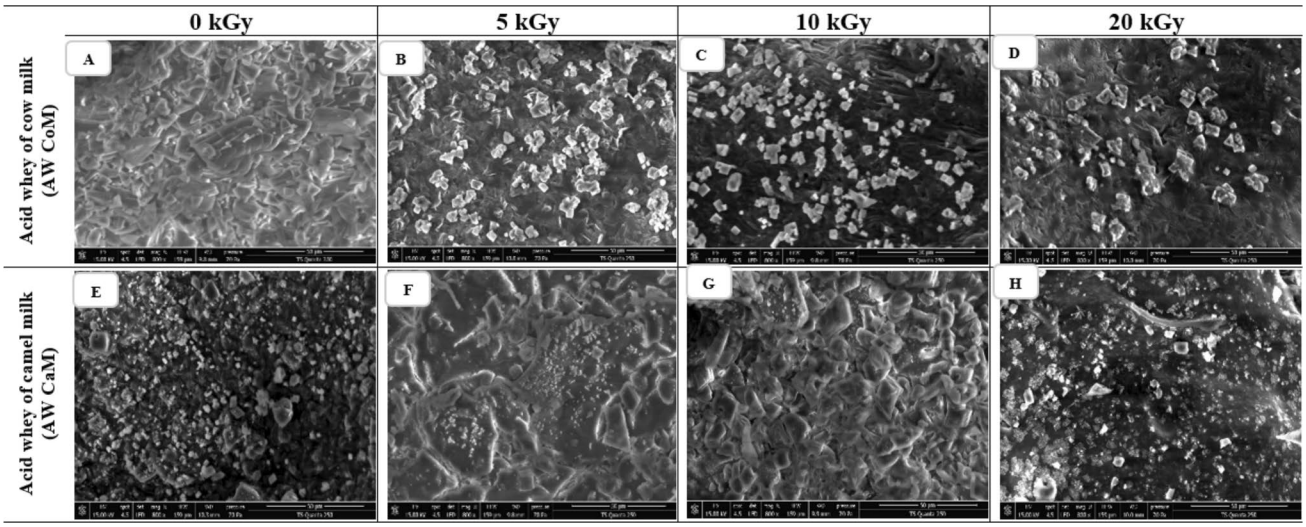

Fig. 5 Microstructure of lyophilized fractions of acid whey of cow milk (AW CoM) (A-D) and camel milk (AW CaM) (**E**–**H**) non-irradiated, 0 kGy (**A** and **E**), and irradiated at 5 kGy (**B** and **F**), 10 kGy (**C** and **G**), and 20 kGy (**D** and **H**), at a magnification of 50 µm

influence could be attributed to a reaction or chemical transformation of protein and/or lactose induced by the irradiation process. The altered structure of acid whey protein of cow milk becomes apparent from 5 kGy (Fig. 5B), resulting from the combined effect of acidification (HCl) used to produce whey and irradiation. This alteration affects the protein network, resulting in disaggregation at 5 and 10 kGy, followed by aggregation at 20 kGy. The protein structure is thus altered, with a broken protein network. As the irradiation dose increases, lactose may crystallize, leading to a decrease in solubility.

Effect of E-beam Irradiation on Total Phenolic Content and Antioxidant Activities of Freeze-dried Whey and Skimmed Milk

The total phenolic content (TPC) and antioxidant activity (DPPH and FRAP assays) of cow and camel milk, as well as the respective whey fractions, before and after irradiation are depicted in Figs. 6 and 7. Depending on the type of milk (cow or camel), TPC values in non-irradiated (0 kGy) skim milk and milk whey fractions (sweet whey and acid whey) differed significantly (Fig. 6). In fact, TPC for cow whey and skimmed milk ranges from 52.5 ± 0.9 mg GAE/100 g powder (skimmed cow milk, S CoM) to 196.5 ± 2.6 mg GAE/100 g powder (sweet whey of cow milk, SW CoM), while it ranges from 96.4 ± 4.4 mg GAE/100 g powder (skimmed camel milk, S CaM) to 195.6 ± 5.7 mg GAE/100 g powder (acid whey of camel milk fraction, AW CaM) for camel milk. The non-irradiated whey fractions exhibited higher TPC (196.5 ± 2.6 mg GAE/100 g powder and 153.8 ± 4.6 mg GAE/100 g powder for sweet whey and acid whey cow milk, respectively, and 194.2 ± 8.1 mg GAE/100 g powder and 195.6 ± 5.7 mg GAE/100 g powder for sweet whey and acid whey camel milk, respectively) than other fractions, despite the type of milk. The irradiation treatment slightly enhances the phenolic content of the majority of cow milk fractions (S CoMat 20 kGy and SW CoM at 5 kGy). The e-beam treatment of camel milk preserved (S CaM at 5 and 10 kGy and AW CaM at 20 kGy) or decreased (SW CaM at all doses and AW CaM at 5 and 10 kGy) the TPC. Irradiation at moderate doses may disrupt chemical bonds retaining phenolic compounds, releasing soluble phenolics (Alothman et al., 2009; Harizi et al., 2023a, 2023b). This release of phenolic compounds could have significant implications in food applications. Nevertheless, irradiation can lead to phenolic compound degradation, as hypothesized for the sweet whey of camel milk (SW CaM).

Regarding the DPPH radical scavenging activity, the whey fractions of cow (SW CoM: 25.6 ± 0.9% and AW CoM: 32.8 ± 0.8%), camel (SW CaM: 31.4 ± 0.5% and AW CaM: 25.9 ± 1.7%) and skimmed camel milk (20.7 ± 0.4%) showed the highest percentage of inhibition (Fig. 7a, b). Nonetheless, low DPPH RSA (percentage of inhibition < 10%) was found for the skimmed cow milk (S CoM: 8.7 ± 0.4%) (Fig. 7a). Our conclusions corroborate the findings of Harizi et al., (2023a, 2023b), who also highlighted that the whey (AW CoM and AW CaM) exhibited the highest values of antioxidant activity. The cow and camel milk fractions responded differently to e-beam irradiation doses. In fact, the percentage of inhibition at 5 kGy of almost all cow's milk fractions was maintained (S CoM and SW CoM), while for camel's milk, the majority of fractions'inhibition percentages (S CaM and SW CaM) decreased ($p < 0.05$). In contrast, significant differences ($p < 0.05$) were observed between the control and the cow

milk irradiated at 10 and 20 kGy (SW CoM: from 25.6 ± 0.9% at 0 kGy to 31.6 ± 1.1% at 10 kGy and 29.9 ± 1.6% at 20 kGy) and camel milk samples (AW CaM: from 25.9 ± 1.7% at 0 kGy to 29.9 ± 1.6% at 10 kGy and 33.7 ± 1.3% at 20 kGy), representing an improvement of antioxidant activity by DPPH by e-beam treatment. Camel milk fractions exhibit noticeable effects from e-beam treatment, while cow's milk fractions demonstrate higher resistance to this treatment. Despite ample data on e-beam irradiation's efficacy against foodborne pathogens in milk and dairy, its impact on biological activities remains underexplored. Harizi et al., (2023a, 2023b) explored gamma irradiation on antioxidant activities in lyophilized cow and camel milk fractions, aligning closely with our e-beam irradiation observations.

The analysis of the antioxidant activity by the FRAP assay revealed that the whey fractions (acid and sweet) from both cow and camel milk demonstrated the highest antioxidant activity (Fig. 7c and d). This positive trend remained consistent across all cow milk fractions following e-beam irradiation, irrespective of the applied dose (5, 10, or 20 kGy) (Fig. 7c). However, a noteworthy observation was made in the case of camel milk whey fractions (AW CaM and SW CaM) as a significant reduction ($p < 0.05$) was evident after irradiation at 5 kGy. This decrease persisted even at higher doses of 10 and 20 kGy (Fig. 7d).

Despite limited studies on irradiation applied to powdered food items (Lung et al., 2015), ionizing radiation is recognized as a non-thermal technique suitable for treating milk powder due to its ability to minimize nutrient losses compared to thermal processing (Tesfai et al., 2014). Milk's antioxidant properties, influenced by treatment methods, irradiation dose, and assessment techniques (Bielecka et al., 2022), are well-suited for electron beams, which deliver doses much faster (10^3 to -10^5 Gy/s) than gamma radiation (0.01 to 1 Gy/s), reducing processing time and food modifications [39]. Dehydrated milk powders are less prone to chemical changes induced by irradiation (Diehl, 1995), thanks to their low water activity (a_w), which limits water availability for chemical reactions and protects against amino acid degradation from water radiolysis during irradiation. While electron beam irradiation can induce water radiolysis more in foods with higher a_w, the low a_w in milk powder seem to help maintaining antioxidant activity with minimal observed variation in this study (Fig. 7a, b). Nonetheless, higher doses (> 10 kGy) may lead to distinctive changes in camel milk fractions (Fig. 7b). Irradiation-induced chemical effects involve excited molecule breakdown, cascade reactions, and the generation of reactive free radicals, particularly prolonged in dehydrated foods like milk powder due to reduced free radical mobility (Stewart, 2001).

Effect of E-beam Irradiation on the In Vitro α-amylase and α-glucosidase Inhibitory Activities of Freeze-dried Whey, Cow and Camel Milk

Figure 8 displays the in vitro antidiabetic potential (enzyme inhibition) of cow and camel milk fractions, before and after e-beam processing, utilizing α-amylase (Iα-Amyl) (Fig. 8a, b) and α-glucosidase inhibition (Iα-Gluco) (Fig. 8c, d) assays. All non-irradiated fractions of both cow and camel milk demonstrate a moderate ability to inhibit α-amylase activity. However, this capability exhibits variations among different fractions (skimmed milk or whey) and according to the type of milk (cow or camel). Among cow milk fractions, acid whey (AW CoM) displays the highest inhibition

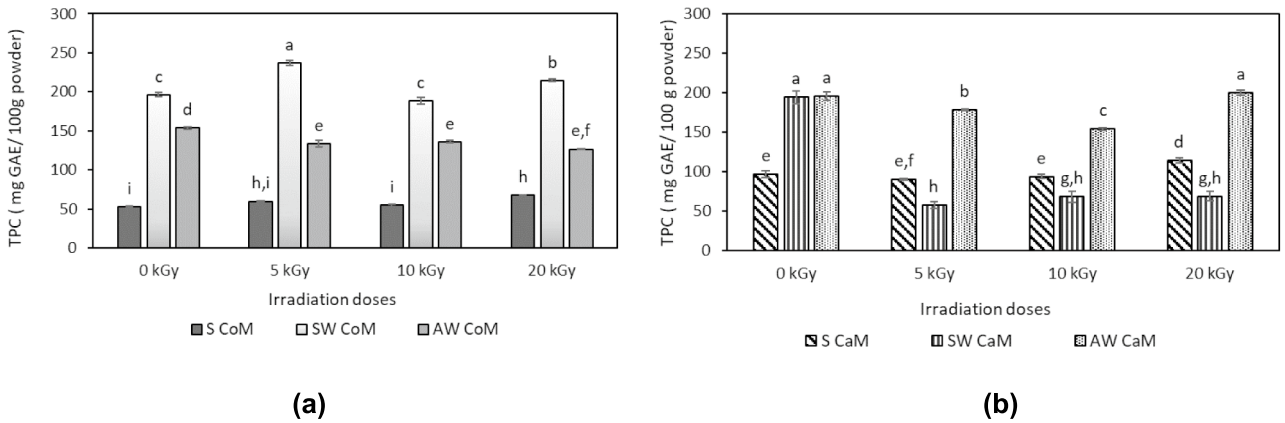

Fig. 6 Total phenolic content of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (**a**) and camel milk (**b**) fractions. S CoM: Skimmed cow milk, AW CoM:Acid whey from cow milk, SW CoM: Sweet whey from cow milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk. Error bars correspond to 95% confidence intervals about mean values ($n = 3$; $\alpha = 0.05$). In each bar, different letters mean significant differences between average values corresponding to irradiation doses: 0, 5, 10 and 20 kGy ($p < 0.05$)

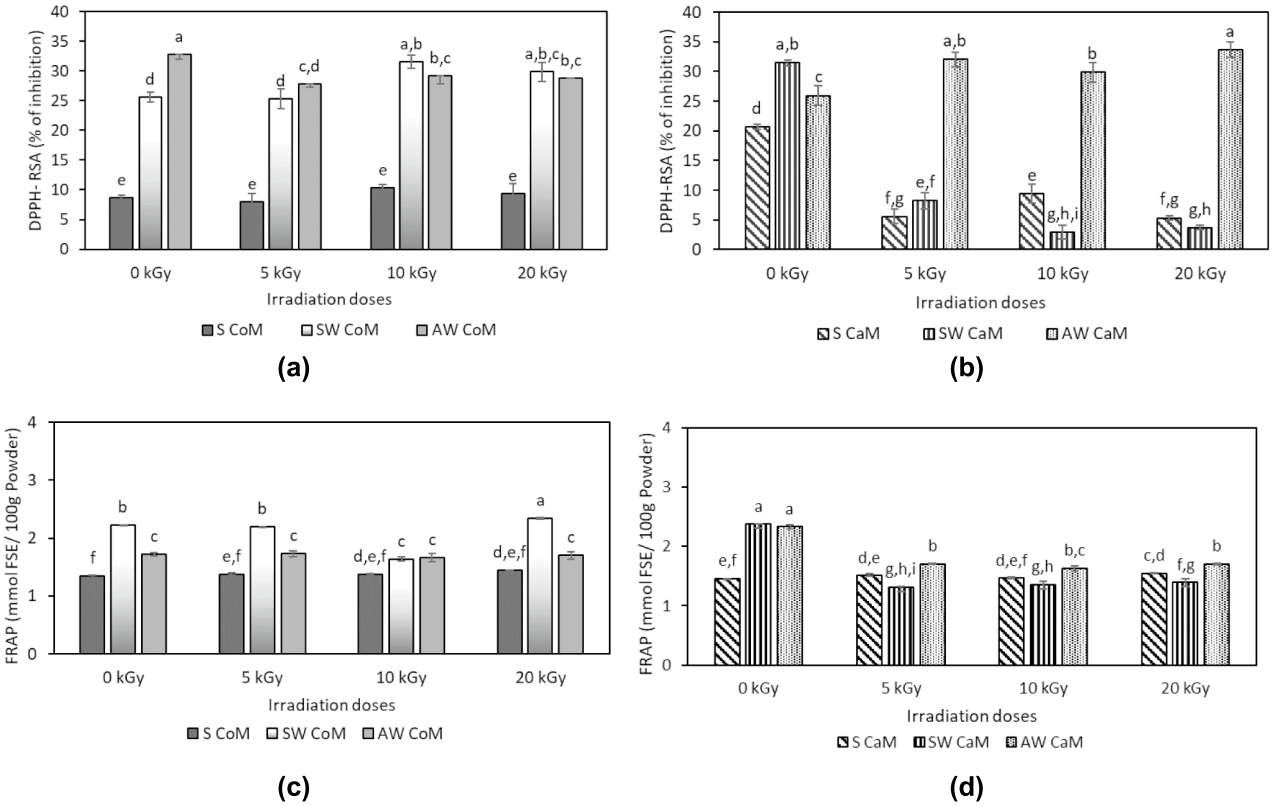

Fig. 7 DPPH radical scavenging activities (**a**, **b**), and FRAP (**c**, **d**) of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (**a**,**c**) and camel milk (**b**,**d**) fractions. S CoM: Skimmed cow milk,AW CoM: Acid whey from cow milk, SW CoM: Sweet whey from cow milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk. Error bars correspond to 95% confidence intervals about mean values ($n = 3$; $\alpha = 0.05$). In each bar, different letters mean significant differences between average values corresponding to irradiation doses: 0, 5, 10 and 20 kGy ($p < 0.05$)

percentage at 24.9 ± 3.8%, indicating a potential capacity to inhibit α-amylase activity, followed by cow milk sweet whey (SW CoM) with an inhibition percentage of 17.3 ± 4% and skimmed cow milk (S CoM) with an α-amylase inhibition of 15.2 ± 2.5% (Fig. 8a). Regarding camel milk, all fractions indicated similar α-amylase inhibition percentages ranging from 13.3 ± 3.6% to 16.3 ± 2% (Fig. 8b). Analysis of variations in the α-amylase inhibition percentage in response to different irradiation doses (5, 10, 20 kGy) across various cow and camel milk fractions reveals significant trends. Indeed, α-amylase inhibition in skimmed cow milk (S CoM) significantly increases ($p < 0.05$) at 10 kGy (25.9 ± 1.4% compared to 15.2 ± 2.5% at 0 kGy), then slightly decreases ($p < 0.05$) at 20 kGy (18.9 ± 1.2%) (Fig. 8a). The same trend was observed for sweet whey (SW CoM) after irradiation. Additionally, for the acid whey (AW CoM) the e-beam irradiation treatment indicated to decrease α-amylase inhibition potential (6.6 ± 1% at 10 kGy and 15.5 ± 2.7% at 20 kGy compared to 24.9 ± 3.8% at 0 kGy). As for camel milk fractions (Fig. 8b), skimmed camel milk (S CaM) suggested a significant increase in α-amylase inhibition after irradiation at 20 kGy. The sweet whey (SW CaM) showed a significant increase of α-amylase inhibition after 5 kGy and 10 kGy, while the acid whey (AW CaM) there is an increase in the inhibition percentage at 10 kGy (20.5 ± 2.5%) compared to 0 kGy (16.3 ± 1.2%).These complex variations suggest that the effect of irradiation on α-amylase inhibition percentage depends on the specific composition of each milk fraction, as well as the applied irradiation doses.

Comparing the inhibition rates of α-glucosidase to those of α-amylase, it becomes evident that, regardless of the type of milk studied, the specific fraction analyzed, or the applied irradiation dose, the α-glucosidase inhibition rates (Fig. 8c, d) remain significantly lower ($p < 0.05$) than those of α-amylase (Fig. 8a, b). These results highlight significant differences in the responsiveness of α-glucosidase inhibitory activity of freeze-dried whey and milk to irradiation doses, with variable responses depending on the type of milk and the fraction studied. Indeed, in the case of cow milk fractions, both skimmed cow milk (S CoM) and sweet whey (SW CoM) show an increase in α-glucosidase inhibition at 10 kGy. For example, the α-glucosidase inhibition percentage of skimmed cow milk (S CoM) gradually increases from 4.3% at 0 kGy to 12.7% at 10 kGy, and 6.7% at 20 kGy.

Similarly, sweet whey (SW CoM) and acid whey (AW CoM) reveals an increase, reaching 11.45% and 7.6% at 10 kGy, respectively (Fig. 8c). Concerning camel milk fractions, skimmed camel milk (S CaM) records a consistent increase in inhibition of α-glucosidase as irradiation doses increase, from 5.1% at 0 kGy to 15.8% at 20 kGy. In contrast, sweet whey (SW CaM) and acid whey (AW CaM) exhibit lower inhibition and minor variations, ranging from undetectable at 0 kGy to 7.3% and 9.4% inhibition for SW CaM and AW CaM, respectively, at 20 kGy.

Although limited available data on electron beam irradiation's impact on enzyme inhibitory activity in milk fractions, non-irradiated milk has shown anti-diabetic potential. The effectiveness of milk as an antidiabetic agent varies depending on test methods used for assessing inhibitory activity, targeting metabolic enzymes like α-amylase, α-glucosidase, DPP-IV, and lipase. The type and source of analyzed milk significantly affect outcomes, whether raw, fermented, or hydrolyzed from different species like cows or camels, each containing unique bioactive components and proteins that interact with metabolic enzymes to influence the antidiabetic response. Milk's bioactive compounds, including phenols, vitamins, casein, and whey proteins, contribute to various health benefits such as antioxidant, anti-diabetic, immunomodulatory, anti-inflammatory, and anticancer effects (Zhou et al., 2021). Caseins like α-casein, γ-casein, κ-casein, regulate enzymatic activity, while proteins such as β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, and lactoferrin modulate enzyme function (Chalamaiah et al., 2018). In a study by Lacroix and Li-Chan (2013), pepsin-treated bovine whey proteins, including α-lactalbumin, β-lactoglobulin, serum albumin, and lactoferrin, displayed varying inhibitory activity against DPP-IV, with α-lactalbumin hydrolysate showing the most potent activity (IC_{50} value of 0.036 mg/mL). Additionally, Shori and Baba (2013) demonstrated that fermented camel milk exhibited stronger inhibition of α-amylase and α-glucosidase compared to fermented cow milk. Harizi et al., (2023a, 2023b) showed that liquid fractions of sweet whey (SW) and acid whey (AW) from both milk types had α-amylase inhibition rates exceeding 50% and that E-beam irradiation helped maintaining α-amylase

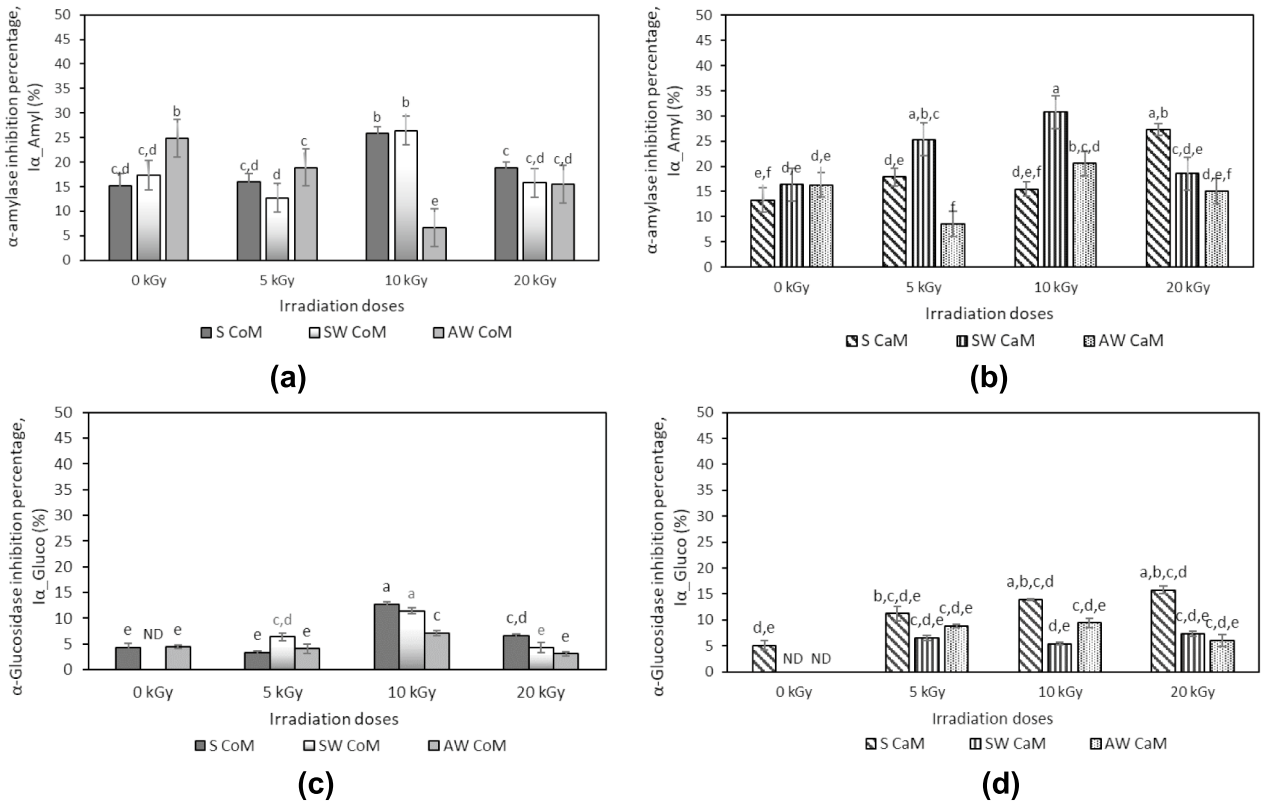

Fig. 8 Anti-diabetic potential using α-amylase inhibition (**a**, **b**) and α-glucosidase inhibition assays Percentage (**c**, **d**), Iα (%) of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (**a**, **c**) and camel milk (**b**, **d**) fractions. S CoM: Skimmed cow milk, AW CoM:Acid whey from cow milk, SW CoM: Sweet whey from cow milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk. Error bars correspond to 95% confidence intervals about mean values ($n = 3$; $\alpha = 0.05$). In each bar, different letters mean significant differences between average values corresponding to irradiation doses: 0, 5, 10 and 20 kGy ($p < 0.05$)

inhibition in the liquid fractions of sweet whey (SW) and acid whey (AW).

Conclusion

This study explored the effects of e-beam irradiation on the micro-structure, solubility, water holding capacity and in vitro bioactivity of freeze-dried whey and skimmed cow and camel milk. E-beam irradiation displayed different effects on water holding capacity and solubility index of whey and skimmed cow and camel milk with a general significant decrease at 5 and 10 kGy and a partial restoration or an increase at 20 kGy of solubility. SEM microstructure analysis showed protein microstructure alteration, disaggregation at 5 and 10 kGy followed by aggregation at 20 kGy. Further analyses (XRD or FTIR) have to be completed in order to confirm lactose crystallization at the high e-beam doses of 10 and 20 kGy. E-beam treatment generally increased phenolic content in cow milk fractions (S CoM and SW CoM) but had diverse effects on camel milk fractions, where S CaM phenolic content remained stable at 5 and 10 kGy, AWCaM phenolic content was stable at 20 kGy, and that of SW CaM decreased with irradiation with no dose effect. Antioxidant activity showed a general increase in cow milk fractions, while camel milk fractions displayed more complex responses to irradiation treatment. In particular, camel milk fractions exhibited greater sensitivity to irradiation, with some fractions maintaining stability at certain doses while others decreased the antioxidant activity. For α-amylase inhibition, cow milk fractions demonstrated an increase at 10 kGy, while camel milk fractions showed varying responses, with some maintaining or increasing antidiabetic activity at certain doses, and others a decrease. Additionally, while α-glucosidase inhibition was low across both milk types, cow milk fractions, it showed improvement at 10 kGy. Overall, e-beam irradiation was found to be a promising processing technique for preserving or enhancing bioactive content in both cow and camel milk fractions. However, a careful control of applied irradiation doses is essential to minimize undesirable changes in solubility, microstructure and bio-activity of freeze-dried whey and skimmed milk, which can help optimizing processing techniques and maintain product quality. Future research should focus on identifying optimal irradiation doses to balance these factors effectively on the basis of lactose state analysis and chromatographic analyses of protein and phenolic profiles.

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Author Contribution Authors' contributions NH: Conception, Investigation, Analysis and Review the manuscript, YZH: Data Curation and Analysis, Investigation, Write and Review the manuscript. AZ: Data Curation and Analysis, and Review the manuscript, JM: Data Curation and Analysis, Investigation, Review the manuscript, MAA: Review the manuscript, SCV Conception, Investigation, Analysis and Review the manuscript, NB: Conception, Investigation, Analysis and Review the manuscript. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interest The authors declare no competing interests.

References

AFNOR. (1993). *Contrôle de la qualité des produits alimentaires*. Lait et produits laitiers AFNOR.

Al-Duais, M., Müller, L., Böhm, V., & Jetschke, G. (2009). Antioxidant capacity and total phenolics of Cyphostemmadigitatum before and after processing: Use of different assays. *European Food Research and Technology, 228*, 813–821.

Alothman, M., Rajeev, B., & Karim, A. A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Food Science and Technology, 20*, 201–212.

Anema, S. G., Pinder, D. N., Hunter, R. J., & Hemar, Y. (2006). Effects of storage temperature on the solubility of milk protein concentrate (MPC85). *Food Hydrocolloids, 20*(2–3), 386–393.

Anwar, I., Khan, F. B., Maqsood, S., & Ayoub, M. A. (2021). Camel milk targeting insulin receptor—Toward understanding the antidiabetic effects of camel milk. *Frontiers in Nutrition Section Food Chemistry, 8*, 819278.

Arain, M. A., Salman, H. M., Mehboob, A., Khaskheli, G. B., Barham, G. S., Marghazani, I. B., & Shabbir, A. (2024). A review on camel milk composition, techno-functional properties and processing constraints. *Food Science of Animal Resources, 44*(4), 739–757.

Barkaoui, S., Madureira, J., Santos, P. M. P., Margaça, F. M. A., Miloud, N. B., Mankai, M., Boudhrioua, N. M., & Cabo Verde, S. (2020). Effect of ionizing radiation and refrigeration on the antioxidants of strawberries. *Food Bioprocess Techology, 13*(10), 1516–1527.

Barkaoui, S., Mankai, M., Miloud, N. B., Kraïem, M., Madureira, J., Cabo Verde, S., & Boudhrioua, N. (2021). E-beam irradiation of strawberries: Investigation of microbiological, physicochemical, sensory acceptance properties and bioactive content. *Innovative Food Science and Emerging Technology, 73*, 102769.

Ben Abdallah, M., Chadni, M., M'hiri, N., Brunissen, F., Rokbeni, N., Ioannou, I., Allaf, K., Besombes, C., & MihoubiBoudhrioua, N. (2023). Optimization of DIC-Tripolium ecofriendly extraction process: Recovery of Hesperidin from orange byproducts,

antioxidant and α-amylase inhibition of extracts. *Antioxidants., 12*(7), 1346.

Bhargava, A., & Jelen, P. (1996). Lactose solubility and crystal growth as affected by mineral impurities. *Journal of Food Science, 61*, 180–184.

Bielecka, M., Cichosz, G., & Czeczot, H. (2022). Antioxidant, antimicrobial and anticarcinogenic activities of bovine milk proteins and their hydrolysates—A review. *International Dairy Journal, 127*, 105208.

Bobo-García, B., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science of Food and Agriculture, 95*, 204–209.

Chalamaiah, M., Yu, W., & Wu, J. (2018). Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. *Food Chemistry, 245*, 205–222.

Chen, Z., Yin, C., Fan, X., Ma, K., Yao, F., Zho, R., Shi, U. D., Cheng, W., & Hong, G. (2020). Characterization of physicochemical and biological properties of Schizophyllum commune polysaccharide extracted with different methods. *International Journal of Biological Macromolecules, 156*, 1425–1434.

Cieśla, K., Roos, Y., & Głuszewski, W. (2000). Denaturation processes in gamma irradiated proteins studied by differential scanning calorimetry. *Radiation Physics and Chemistry, 58*(3), 233–243.

Crowley, S. V., Kelly, A. L., Schuck, P., Jeantet, R., & O'Mahony, J. A. (2016). Rehydration and solubility characteristics of high-protein dairy powders. In Advanced Dairy Chemistry: Volume 1B: Proteins: Applied Aspects, 4th ed.; McSweeney, P.L.H., O'Mahony, A.J., Eds.;Springer, 99–131.

Diehl, J. F. (1995). Safety of irradiated food 2nd Edition. https://doi.org/10.1201/9781482273168

Felfoul, I., Burgain, J., Perroud, C., Gaiani, C., Scher, J., Attia, H., & Petit, J. (2020). Impact of spray-drying conditions on physicochemical properties and rehydration ability of skim dromedary and cow's milk powders. *Drying Technology, 40*(3), 665–677.

Fox, P. F., & McSweeney, P. L. H. (1998). Lactose. In *Dairy chemistry and biochemistry*; Eds.; Blackie Academic & Professional: London, 21–66.

Fyfe, K. N., Kravchuk, O., Le, T., Deeth, H. C., Nguyen, A. V., & Bhandari, B. (2011). Storage induced changes to high protein powders: Influence on surface properties and solubility. *Journal of the Science of Food and Agriculture., 91*(14), 2566–2575.

Goulart, D. B. (2021). Principles of lactose crystallization and rheology of milk protein concentrate. *Research, Society and Development, 10*(15), e577101523028.

Haque, E., Bhandari, B. R., Gidley, M. J., Deeth, H. C., & Whittaker, A. K. (2011). Ageing-induced solubility loss in milk protein concentrate powder: Effect of protein conformational modifications and interactions with water. *Journal of the Science of Food and Agriculture, 91*(14), 2576–2581.

Harizi, N., Madureira, J., Haffani, Y., Zouari, A., Ayadi, M. A., Cabo Verde, S., & Boudhrioua, N. (2023a). E-beam irradiation of defatted liquid camel and cow milk fractions: Antiproliferative, antidiabetic and antioxidant activities. *Innovative Food Science Emerging Technology, 89*, 103457.

Harizi, N., Madureira, J., Zouari, A., Ayadi, M. A., Cabo Verde, S., & Boudhrioua, N. (2023b). Effects of spray drying, freeze drying and gamma irradiation on the antioxidant activities of camel and cow milk fractions. *Processes, 11*, 897.

Harizi, N., Zouari, A., Rokbeni, N., Ben Zid, M., M'hiri, N., Salem, A., Ayadi, M. A., & Boudhrioua, N. (2024). Amino acids and protein profiles of defatted camel and cow milk fractions: Correlation with their in vitro antioxidant and antidiabetic activities. *Frontiers in Nutrition, 10*, 1295878.

Havea, P. (2006). Protein interactions in milk protein concentrate powders. *International Dairy Journal, 16*(5), 415–422.

Huppertz, T., & Gazi, I. (2015). Lactose in dairy ingredients: Effect on processing and storage stability. *Journal of Dairy Science, 98*, 1–10.

Kneifel, W., & Seiler, A. (1993). Properties of milk protein products - water-holding properties of milk protein products-a review. *Food Structure., 12*(12), 297–308.

Lacroix, I. M. E., & Li-Chan, E. C. Y. (2013). Inhibition of dipeptidyl peptidase (DPP)-IV and α-glucosidase activities by pepsin-treated whey proteins. *Journal of Agricultural and Food Chemistry, 61*(31), 7500–7506.

Lee, J. W., Kim, J. H., Yook, H. S., Kang, K. O., Lee, S. Y., Hwang, H. J., & Byun, M. W. (2001). Effects of gamma radiation on the allergenic and antigenic properties of milk proteins. *Journal of Food Protection., 64*(2), 272–276.

Lung, M. H., Cheng, Y. C., Chang, Y. H., Huang, H. W., Yang, B. B., & Wang, C. Y. (2015). Microbial decontamination of food by electron beam irradiation. *Trends Food Science and Technology, 44*(1), 66–78.

Neoκleous, I., Tarapata, J., & Papademas, P. (2022). Non-thermal processing technologies for dairy products: Their effect on safety and quality characteristics. *Frontiers in Sustainable Food Systems., 6*, 856199.

Nguyen, D. Q., Nguyen, T. H., Mounir, S., & Allaf, K. (2017). Effect of feed concentration and inlet air temperature on the properties of soymilk powder obtained by spray drying. *Drying Technology, 36*(7), 817–829.

Nowak, D., & Jakubczyk, E. (2020). The Freeze-Drying of Foods—The characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods, 9*(10), 1488.

Pegu, K., & Arya, S. S. (2023). Non-thermal processing of milk: Principles, mechanisms, and effect on milk components. *Journal of Agriculture and Food Research, 14*, 100730.

Pugliese, A., Cabassi, G., Chiavaro, E., Paciulli, M., Carini, E., & Mucchetti, G. (2017). Physical characterization of whole and skim dried milk powders. *Journal Food Science Technology., 54*(11), 3433–3442.

Roos, Y. (2002). Importance of glass transition and water activity to spray drying and stability of dairy powders. *Le Lait, 82*, 475–484.

Salmen, S. H., Abu-Tarboush, H. M., Al-Saleh, A. A., & Metwalli, A. A. (2012). Amino acids content and electrophoretic profile of camel milk casein from different camel breeds in Saudi Arabia. *Saudi Journal of Biological Sciences, 19*(2), 177–183.

Schuck, P., Jeantet, R., & Dolivet, A. (2012). *Analytical methods for food and dairy Powders*. John Wiley & Sons.

Seifu, E. (2022). Recent advances on camel milk: Nutritional and health benefits and processing implications—A review. *AIMS Agriculture and Food., 7*(4), 777–804.

Shori, A. B., & Baba, A. S. (2013). Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes and hypertension by Azadirachta indica-yogurt. *Journal of Saudi Chemical Society, 17*(3), 295–301.

Silva, A., Pinela, J., Dias, M. I., Calhelha, R. C., Alves, M. J., Mocan, A., Garcia, P. A., Barros, L., & Ferreira, I. (2020). Exploring the phytochemical profile of Cytinushypocistis (L.) L. as a source of health-promoting biomolecules behind its in vitro bioactive and enzyme inhibitory properties. *Food and Chemical Toxicology, 136*, 111071.

Stewart, E. M. (2001). Food irradiation chemistry. In R. A. Molins (Ed.), Food irradiation: Principles and applications (pp. 37e76).

Tesfai, A., Beamer, S. K., Matak, K. E., & Jaczynski, J. (2014). Effect of electron beam on chemical changes of nutrients in infant formula. *Food Chemistry, 149*, 208–214.

Thomas, M. E. C., Scher, J., Desobry-Banon, S., & Desobry, S. (2004). Milk powders ageing: Effect on physical and functional properties. *Critical Reviews in Food Science and Nutrition., 44*, 297–322.

Thomsen, M. K., Lauridsen, L., Skibsted, L. H., & Risbo, J. (2005). Two types of radicals in whole milk powder effect of lactose crystallization, lipid oxidation, and browning reactions. *Journal of Agricultural and Food Chemistry, 53*(5), 1805–11.

Walstra, P., Geurts, T. J., Noomen, A., Jellema, M. A., & Van Boekel, J. S. (1999). Milk powder. In Dairy Technology. Principles of Milk Properties and Processes. Marcel Dekker, 445-470.

Zhou, X., Chai, L., Wu, Q., Wang, Y., Li, S., & Chen, J. (2021). Anti-diabetic properties of bioactive components from fish and milk. *Journal of FunctionalFoods, 85*, 104669.

Zouari, A., Marchesseau, S., Chevalier-Lucia, D., Raffard, G., Ayadi, M. A., & Picart-Palmade, L. (2018). Acid gelation of raw and reconstituted spray-dried dromedary milk: A dynamic approach of gel structuring. *International Dairy Journal, 81*, 95–103.

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