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E-beam irradiation of defatted liquid camel and cow milk fractions: Antiproliferative, antidiabetic and antioxidant activities

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ABSTRACT

The aim of this work was to assess the suitability of electron beam (e-beam) irradiation (at 5, 10 and 20 kGy) to treat defatted liquid cow and camel milk and their whey and casein fractions. The different milk fractions were investigated for their antiproliferative, antidiabetic and antioxidant activities. Irradiated milk fractions did not indicate any cytotoxic effect on HEK293T cell line, except for sweet whey of cow milk treated at 20 kGy, the acid whey of camel milk treated at 5, 10 and 20 kGy and β casein of cow and camel milk treated at 10 and 20 kGy. The non-irradiated and irradiated whey fractions of camel milk and the irradiated β-casein of cow milk demonstrated anti-proliferative activity against A549 tumor cell line. Irradiated and non-irradiated whey fractions of camel and cow milk exhibited interesting inhibition percentage of α-amylase (varying from 73.24 ± 2.71% to 98.99 ± 0.84% for cow milk and from 64.68 ± 2.49% to 99.39 ± 0.17% for camel milk). Whey fractions seemed to be the most resistant to e-beam irradiation. Ebeam treatment (5–20 kGy) preserved or improved the DPPH radical scavenging activity of the majority of cow and camel milk fractions, ranging from 6.27 ± 0.74% to 59.75 ± 0.84%.

Industrial relevance: The present work highlighted the suitability of e-beam irradiation as safe and friendly treatment for the preservation of cow and camel milk and corresponding whey and casein fractions. *E*-beam treatment at 10 kGy seems to be effective treatment for whey of camel and cow milk, preserving or enhancing the antiproliferative, antioxidant and antidiabetic activities. Ebeam treatment at 5–10 kGy is suggested to be an emergent food preservation technology with the ability of preservation or promotion of milk bioactivity.

1. Introduction

Milk is considered as a complete food because it contains the main necessary elements (Zhang et al., 2021), including calcium, phosphorus, and vitamin D, which are especially important for bone health, as well as a variety of bioactive peptides (caseins, whey proteins), fatty acids (milk polar lipids, α-linolenic acid) and lactose (Bouglé & Bouhallab, 2017). Cow milk accounts for nearly 734 million tons of the world's total milk production in 2020 (Nagy, Skidmore, & Juhasz, 2022). It continues to be the principal source of milk on earth and has a vital strategic role in international trade. Unfortunately, in many arid and semi-arid regions, heat, water scarcity, and the lack of natural resources are unfavorable for milk production from dairy cows. In these arid conditions, camelids are crucial to the access of indigenous nomadic populations to milk (Medhammar et al., 2012). Camel milk production in 2020 was estimated to be about 3.15 million tons in the world (Polidori et al., 2021).

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With its abundance in proteins, phenolics, vitamins, and minerals, camel milk is also known for performing a variety of physiological functions. In fact, it has been reported that camel milk has antiproliferative activity against tumor cell, such as colon cancer cell lines (Caco2) and breast cancer cell line (MCF-7) (Ayyash, Al-Nuaimi, Al-Mahadin, & Liu, 2018). It also stops the development of human hepatoma cell line (HepG2) as well as the activation of cell-line-specific death receptors and oxidative stress-related processes (Kaskous, 2016). Furthermore, it has been demonstrated recently that casein micelles have the ability to carry polyphenols that significantly reduce colon cancer cells (Sabouri, Arranz, Guri, & Corredig, 2018). Further to its use in the treatment of diabetes, camel milk lowers blood sugar, reduces insulin resistance, and enhances blood lipid profile (Ayoub, Palakkott, Ashraf, & Iratni, 2018). Many studies showed that the antioxidant activity of camel milk was related to the presence of sulfur amino acids in proteins, the abundance of vitamins A, E, C, and β-carotene, and oligo-elements (Stobiecka, Król, & Brodziak, 2022). In fact, Salami et al. (2010) reported that compared to cow whey protein, camel whey protein showed 40% greater antioxidant activity by 2,2′-azinobis assay (3- ethylbenzothiazoline-6-sulphonic acid) (ABTS assay).

Food irradiation is a reliable method of food preservation that has been certified for usage in >60 countries (Ferreira, Antonio, & Cabo Verde, 2018). Syed et al. (2021) reported that at a high temperature / short time (HTST) treatment, protein denaturation increased with loss of secondary structure. Additionally, denaturation of whey proteins occurs at temperatures ranging from 60 to 100 °C. Casein micelles were also denaturated and aggregated by high-pressure processing (200–400 MPa). *E*-beam irradiation is a non-thermal technology that offers a clean and environmentally friendly food processing treatment. This method does not involve the use of chemicals or produce any chemical residues (Madureira et al., 2022). In e-beam treatment the food is exposed to a high-energy electron beam for a short time, allowing the preservation of the nutritional value (Pizarro-Oteíza et al., 2020). Processing milk by e-beam irradiation is primarily used for inactivating foodborne pathogenic microorganisms (Mediwaththe, Bogahawaththa, Grewal, Chandrapala, & Vasiljevic, 2018). This will further reduce the risk of bacterial contamination during packaging (Chatterton et al., 2020) and in powdered milk (Hong et al., 2008; Osaili et al., 2008). Hong et al. (2008) reported the effectiveness of electron-beam (2–16 kGy) against *Enterobacter sakazakii, Bacillus cereus,* and *Salmonella Typhimurium* in infant formula milk. Ward, van Schaik, Samuel, and Pillai (2019) reported that *E*-beam treatment of raw milk at 2 kGy eliminated *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli* from raw milk. In many European countries such as France and Czech Republic, the irradiation could be applied at 3 kGy to casein and caseinate and up to 30 kGy to dried milk products (IAEA, 2012). Despite of the availability of data dealing with the effectiveness of E-beam to control foodborne pathogens in milk and dairy products, no previous study reported the suitability of this treatment in terms of cytotoxicity and biological activities. Therefore, the main purpose of this work was to assess the suitability of e-beam irradiation (5–20 kGy) to treat cow and camel milk and corresponding whey and casein fractions. The cytotoxicity assays using normal and tumor cell lines were performed and the in vitro antioxidant and antidiabetic activities were also evaluated.

2. Materials and methods

2.1. Raw milk and milk fractions preparation

Camels and cows' fresh milk were aseptically collected from Tunisian farms and delivered to the laboratory at 4 °C. Before proceeding with any additional treatment, the pH was carefully measured. Skimming was performed once by centrifugation at 2000 ×*g* for 15 min at 5 °C for cow milk and three-time centrifugation for camel milk at the same condition (Zouari et al., 2018). Milk fractions were prepared from skim milk as reported by Lajnaf, Gharsallah, Attia, and Ayadi (2021) and Harizi et al. (2023). The acid whey fraction was made by adding HCl (12N) and the sodium caseinate is produced by neutralizing with NaOH (1 M). Rennet enzymes (Parachimic, Laboratories Arrazi, Sfax, Tunisia, strength = 1: 10,000) were used to coagulate sweet whey at 37 °C. After separation of whey and casein fractions, demineralized water was added to the curd. The β casein was then extracted at 5000 x *g* for 15 min at 5 °C after being kept at 4 °C for up to 24 h.

2.2. Irradiation experiments

Irradiation experiments were performed using a linear electron-beam accelerator (CIRCE-3, LINAC Technology) with an energy of 10 MeV and a maximum output of 5 kW at the National Center for Nuclear Science and Technology in Tunisia (Barkaoui et al., 2021). Different cow and camel milk fractions in singular graduated Bottle (10 g per bottle) were double-sided irradiated at room temperature at doses of 5, 10, and 20 kGy. The conveyor speed was 400 cm.min^{-1} for 5 kGy, 200 cm.min^{-1} for 10 kGy and 80 cm.min^{-1} for 20 kGy. Dosimetry was carried out by placing a B3 DoseStix dosimeter (GEX Corporation, USA) covering the height of each milk bottle, and estimating the absorbed doses with a Genesys 20 spectrophotometer (Thermo Scientific, USA). Non-irradiated samples (0 kGy) served as controls.

2.3. Cell viability: WST-1 proliferation assay

WST-1 is used to evaluate cell growth by cleavage of the tetrazolium salt WST-1 to formazan. Expansion in the number of viable cells increases the activity of mitochondrial dehydrogenases, leading to an increase in the amount of formazan dye formed. The WST-1 assay quantifies mitochondrial activity that can be used to determine cell viability (Barkaoui et al., 2020). Two human cell lines were used, HEK293T: human embryonic kidney, considered as non-tumor cells (Schuh et al., 2012) and A549: human alveolar basal epithelial cells, that are adenocarcinomic—lung cancer cells (Baek et al., 2018). HEK293T and A549 cells were cultured at 37 °C and 5% CO_2, in Dulbecco's modified Eagle medium (DMEM) fortified with L-glutamine (4 mmol.L^{-1}), 10% Fetal Bovine Serum (FBS), penicillin (1 U.mL^{-1}), streptomycin (100 μg mL^{-1}), sodium pyruvate (1 mmol.L^{-1}), non-essential amino acids (1 X) and HEPES buffer (10 mmol.L^{-1}). HEK293T and A549 cell lines were seeded into 96-well plates at a density of 1×10^5 cells/well twenty-four hours before the experiment. The next day, cells were rinsed twice with PBS, and to each well it was added 100 μL of 2× concentrated DMEM and 100 μL of camel and cow milk fractions. The cells were incubated for 24 h with non-irradiated and irradiated camel and cow milk fractions at 37 °C and 5% CO_2. The following day, the wells' inoculum was taken out and replaced with 100 μL of new medium (DMEM with 10% FBS) and 10 μL of the cell proliferation reagent WST-1 (Roche, Switzerland). Cells were incubated for 6 h at 37 °C. The absorbance at 450 nm was measured using a 620 nm reference wavelength using a microplate reader (EZ Read 800, Biochrom, Cambridge, UK). The cell viability was calculated as percentage of the average absorbance derived from triplicate runs of treated cells relative to untreated control cells.

2.4. Total phenolic content and antioxidant activities

Total phenolic content (TPC) and antioxidant activities (DPPH - RSA and FRAP assay) were assessed directly on milk samples as follows (Abd El-Fattah et al.,2020).

2.4.1. Total phenolic content determination

Total phenolic content (TPC) was determined by the Folin–Ciocalteau method (Bobo-García et al., 2015). Briefly, in a 96-well plates 20 μL of the sample and 100 μL of 1:4 diluted Folin–Ciocalteu reagent were mixed and agitated for one minute. After 4 min at room temperature, 75 μL of sodium carbonate solution (100 g.L^{-1}) were

added. After incubation for two hours at room temperature, the absorbance was measured at 765 nm using a microplate reader (Easy Read 1200, Biochrom, Cambridge, UK). Gallic Acid was use to establish the calibration curve and TPC is expressed as mg Gallic Acid Equivalent per L (mg GAE.L^{-1}).

2.4.2. DPPH-RSA

The 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA) was performed using 96-well plates according to Barkaoui et al. (2020). Each reaction mixture in a well included 30 μL of sample and 270 μL of a methanolic solution at 6×10^{-5} M of DPPH radicals. DPPH was purchased from Aldrich (St. Louis, MO, USA). The mixture was incubated 60 min in the dark at room temperature. The absorbance at 515 nm was measured using a microplate reader (Easy Read 1200, Biochrom, Cambridge, UK), to quantify the reduction of DPPH radicals. According to the following equation, the results were presented as the percentage of DPPH radical scavenging activity:

$$\text{DPPH} - \text{RSA}\ (\%) = \frac{(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{blank}}} \times 100 \quad (1)$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of the blank and $\text{Abs}_{\text{Sample}}$ is the absorbance of the sample.

2.4.3. FRAP assay

The ferric reducing antioxidant power (FRAP) test was performed using 96-well plates according to Al-Duais, Müller, Böhm, and Jetschke (2009). 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-Tris (2-pyridyl)-triazine (TPTZ; Fluka, Buchs, Switzerland), and 20 mM $FeCl_3.6H_2O$ were mixed to prepare the FRAP reagent at 10:1:10 ratio. Each well's reaction mixture included 10 μL of sample and 200 μL of FRAP solution. After incubation for 8 min, the absorbance was measured at 593 nm in the microplate reader (EZ Read 1200, Biochrom, Cambridge, UK). The results were presented as mmol of ferrous sulfate equivalent (FSE) per L of milk. The calibration curve was performed with Ferrous Sulphate Solution in the range of 0.016–0.789 mmol.L^{-1}.

2.5. In vitro antidiabetic activity

2.5.1. α-Amylase inhibition assay

The α-amylase assay was performed using the method described by Chen et al. (2020). Briefly, 20 μL of sample and 20 μL of α-amylase solution (1.0 U.mL^{-1}) were mixed and incubated at 37 °C for 10 min. After that, 40 μL of starch solution (0.5%, *w/v*) was added and further incubated for an additional 10 min at 37 °C. Following the addition of 80 μL of the 3,5-dinitrosalicylic acid (DNS) reagent, the reaction was stopped by incubating the mixture for 5 min in boiling water. Acarbose was used as the positive control, and the absorbance was measured at 540 nm using a microplate reader (EZ Read 1200, Biochrom, Cambridge, UK).

The following formula was used to determine the inhibition percentage of α-amylase:

$$\text{I}\ \alpha_{\text{amylase}}(\%) = \left[1 - \frac{(\text{A}_{\text{sample}} - \text{A}_{\text{blank}})}{\text{A}_{\text{control}}}\right] \times 100 \quad (2)$$

Where $\text{A}_{\text{control}}$ is the absorbance of mixture containing α-amylase and starch solution; A_{sample} is the absorbance of mixture containing α-amylase, starch solution and sample; and A_{blank} is the absorbance of mixture containing sample and starch solution.

2.5.2. α-Glucosidase inhibition assay

The capacity of samples to inhibit α-glucosidase was measured based on a method previously described by Silva et al. (2020). The test was carried out in a 96-well microplate using a reaction mixture composed of 50 μL of sample and 50 μL yeast α-glucosidase (2 U.mL^{-1} in phosphate buffered saline). Following a 10 min incubation period, 50 μL of the substrate *p*-nitrophenyl-α-D-glucopyranoside (5 mM in phosphate buffered saline) was added to the reaction mixture. The release of *p*-nitrophenol was quantified spectrophotometrically at 405 nm after 20 min incubation at 37 °C. The following formula was used to determine the inhibition percentage of α-glucosidase:

$$\text{I}\ \alpha_{\text{Glucosidase}}(\%) = \left[1 - \frac{(\text{A}_{\text{sample}} - \text{A}_{\text{blank}})}{\text{A}_{\text{control}}}\right] \times 100 \quad (3)$$

Where $\text{A}_{\text{control}}$ is the absorbance of mixture containing α-glucosidase, substrate and solution solvent; A_{sample} is the absorbance of mixture with α-glucosidase, substrate and sample and A_{blank} is the absorbance of mixture containing sample and substrate solution. Acarbose was used as positive control.

2.6. Data analysis

All analyses were performed in triplicate, and mean values were used for statistical analysis. XLSTAT 19 software was used to examine the statistical differences. To compare the samples, a one-way analysis of variance (ANOVA) of measurements was used with Tukey's test. Differences were considered significant at *p*-value <0.05. Principal component analysis (PCA) was performed on measured parameters showing significant differences: antioxidant activities (using DPPH-RSA and FRAP assays), α-amylase inhibition percentage (Iα-Amylase) and α-glucosidase inhibition percentage (Iα-Glucosidase).

3. Results and discussion

3.1. Cells viability – Antiproliferative assay

3.1.1. HEK293T cell line viability

The effect of e-beam irradiation on the cytotoxicity of cow and camel milk fractions was examined using the WST-1 cell viability assay (Fig. 1). No significant inhibitory activity on HEK293T cell viability was observed in the presence of non-irradiated cow milk fractions (Fig. 1a). This trend was maintained after irradiation for all cow milk fractions, except for irradiated βC CoM (at 10 and 20 kGy) which showed a significant decrease ($p < 0.05$) in the percentage of viability of HEK293T cells (from 97% at 0 kGy to 57% at 10 kGy). It was also noteworthy that only at the dose of 20 kGy, the sweet whey of cow milk fraction (SW CoM) exhibited a significant decrease in the percentage of HEK293T cell viability ($p < 0.05$). The e-beam treatment at 5 kGy did not demonstrate any cytotoxic effect of defatted milk and different milk fractions.

Cytotoxicity assay performed for the camel milk and its fractions (Fig. 1b) showed that the non-irradiated and irradiated AW CaM fraction exhibited the lowest percentage of HEK293T cell viability (53%). This could indicate that AW CaM could had a cytotoxic effect on HEK293T non-tumor cells, and with irradiation this effect was maintained. The observed cytotoxic effect may be attributed to the HCl residue remaining in samples after fractionation process.

3.1.2. A549 lung cancer cell line viability

The increase in e-beam radiation dose induced a significant inhibition of A549 lung cancer cells viability for βC CoM fraction treated at 10 and 20 kGy (Fig. 1c). Moreover, there was a significant decrease ($p < 0.05$) in the viability of A549 cells treated with the acid whey of camel milk fraction (AW CaM) at various radiation doses, while the sweet whey of camel milk (SW CaM) exhibited a significant decline ($p < 0.05$) in the viability of this tumor cells only after e-beam irradiation at 5 and 20 kGy (Fig. 1d). Overall, the irradiated β casein of cow milk, βC CoM, at 10 kGy and 20 kGy, the non-irradiated and irradiated acid whey of camel milk, AW CaM, at 5–20 kGy, and the irradiated sweet whey of camel milk, SW CaM at 5–20 kGy seem to be the most interesting fractions for further investigations. These results suggested that the viability of tumor cell line was inhibited by using milk fractions treated or not by e-beam. Several mammalian species' milk and dairy products have been

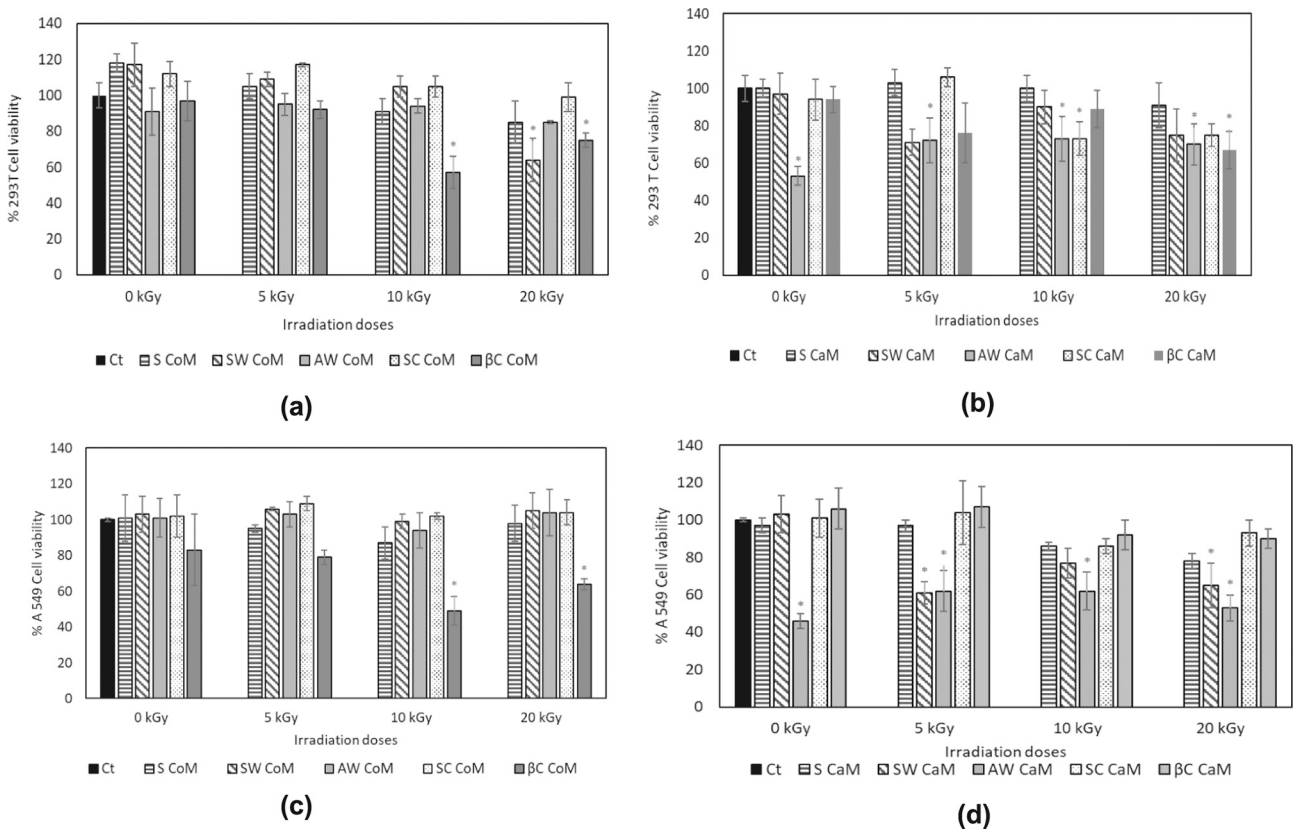

Fig. 1. Cellular viability of HEK293T (a,b) and A549 (c,d) cell lines in the presence of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (a, c) and camel (b,d) milk fractions. CoM: Whole cow milk, S CoM: Skimmed cow milk, AW CoM: Acid whey from cow milk, SW CoM: Sweet whey from cow milk, SC CoM: Sodium caseinate, from cow milk, βC CoM: β casein from cow milk, CaM: Whole camel milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk, SC CaM: Sodium caseinate, from camel milk, βC CaM: β- casein from camel milk. Error bars correspond to 95% confidence intervals about mean values ($n = 3$; $\alpha = 0.05$). For each cell line, bars with * indicate a statistically significant difference from control at $p < 0.05$.

shown to have anti-tumoral properties on various cell types. For instance, it has been demonstrated that some elements in cow milk have anti-cancer potential (Gill & Cross, 2000). Indeed, cow lactoferrin, which is a glycoprotein present in milk whey fraction, was shown to inhibit the growth of MCF-7, T-47D, MDA-MB-231 and Hs578T breast cancer cells (Zhang, Lima, & Rodrigues, 2015). Additionally, camel milk lactoferrin was shown to inhibit the proliferation of HCT-116 colon cancer cells (Habib, Ibrahim, Schneider-Stock, & Hassan, 2013) and to induce apoptosis in human B-lymphoma cells (Furlong, Mader, & Hoskin, 2010). Nevertheless, to the best of our knowledge, none of the used cell lines have been tested against cow and camel milk irradiated fractions. Our findings showed that e-beam treatment may preserve or improve anti-proliferative effects of milk and milk fractions. These effects could be attributed to the resistance or function improvement of active proteins and antioxidant compounds after e-beam treatment. Indeed, phenolic compounds, known for their anti-inflammatory and antioxidant properties, play a significant role in the anti-tumor effects of foods and their extracts (Mehanna et al., 2014). These compounds can promote apoptosis by halting the cell cycle, modulating carcinogen metabolism and ontogenesis expression, preventing DNA binding, cell adhesion, migration, proliferation, and differentiation, while also obstructing signaling pathways (Abotaleb, Liskova, Kubatka, & Büsselberg, 2020; Mehanna et al., 2014). Additionally, peptides originating from milk proteins have been associated with significant biological roles, including anticancer and antioxidant properties (Sah, Vasiljevic, McKechnie, & Donkor, 2015). These peptides could scavenge free radicals in vitro, suggesting their ability to enhance endogenous antioxidants in the human body (Elfahri, Vasiljevic, Yeager, & Donkor, 2015). Furthermore, they exhibit cytotoxic effects, specifically targeting cancer cells through apoptosis (Phelan, Aherne, FitzGerald, & O'Brien, 2009). For instance, specific peptides like those derived from bovine lactoferrin have shown antiproliferative effects on leukemia (HL −60) and neuroblastoma cell lines (Kelly, SK-N-DZ, and IMR-32). This effect is achieved by triggering apoptosis through the activation of caspases (caspase-6, caspase-7, and caspase-9), ultimately leading to cell death (Eliassen et al., 2006; Roy, Kuwabara, Hara, Watanabe, & Tamai, 2002).

3.2. Total phenolic content

The results of total phenolic content (TPC) of cow and camel milk and their corresponding whey and casein fractions, before and after irradiation are presented in Fig. 2. The amount of TPC varied in non-irradiated (0 kGy) fractions of cow milk (Fig. 2a) from 264.2 ± 9.4 mg GAE.L^{-1} (β casein of cow milk, βC CoM) to 757.1 ± 3.4 mg GAE.L^{-1} (skimmed cow milk fraction, S CoM). For non-treated camel milk samples (Fig. 2b), TPC varied from 321.1 ± 6.4 mg GAE.L^{-1} (skimmed camel milk, S CaM) to 1223.3 ± 15.1 mg GAE.L^{-1} (sodium caseinate of camel milk, SC CaM). The non-irradiated whole cow milk (CoM), the skimmed (S CoM), and whey fractions (SW CoM and AW CoM) had higher TPC than the corresponding fractions of non-irradiated camel milk (Fig. 2). Sodium caseinate camel milk (SC CaM) presented the highest TPC (1223.3 ± 15.1 mg GAE.L^{-1}). Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018) assessed the TPC of several milk types (ewe, cow and kefir milk), undergoing different treatments (raw, heated and fermented milk). According to the last study, TPC of raw and heated cow milk were 689.1 mg GAE.L^{-1} and 1521.4 mg GAE.L^{-1}, respectively. In the same way the TPC ranged between 667 and 1768.7 mg of GAE.L^{-1} for cow kefir. The total phenolic contents of UHT-treated and

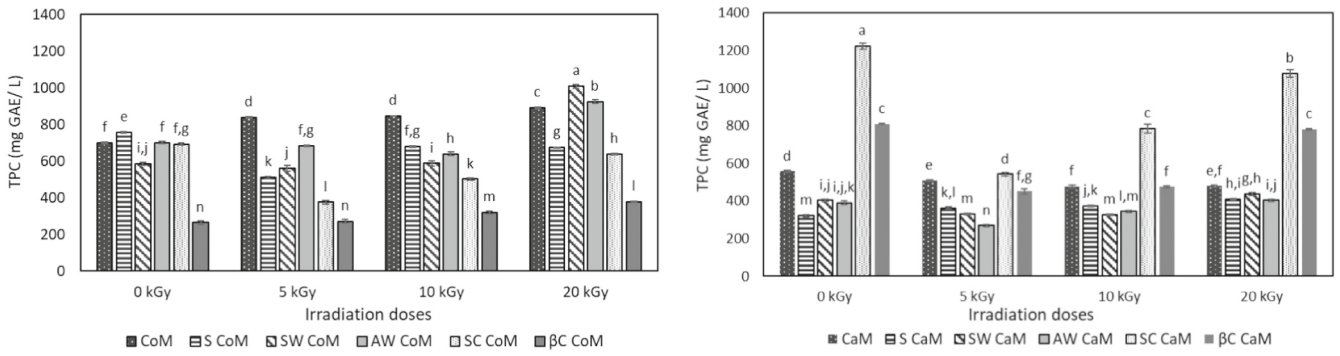

Fig. 2. Total phenolic compound content (mg GAE/L) of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (a) and camel milk (b) fractions. CoM: Whole cow milk, S CoM: Skimmed cow milk, Acid whey from cow milk: AW CoM, SW CoM: Sweet whey from cow milk, SC CoM: Sodium caseinate, from cow milk, βC CoM: β casein from cow milk, CaM: Whole camel milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk, SC CaM: Sodium caseinate, from camel milk, βC CaM: β-casein from camel milk. Error bars correspond to 95% confidence intervals about mean values (n = 3; α = 0.05). In each bar, different letters mean significant differences between average values ($p < 0.05$).

pasteurized milk samples were also determined by Ertan et al. (2017) and they ranged from 505.46 ± 16.66 to 982.14 ± 168.42 mg GAE.L^{-1}.

After irradiation, each fraction reacted differently depending on the dose applied (5, 10 and 20 kGy) and the type of milk. Our results presented in Fig. 2, showed that irradiation (at 20 kGy) improved the levels of TPC of the fractions of whole milk (CoM), sweet whey (SW CoM) and acid whey of cow milk (AW CoM) fractions. In fact, the untreated milk fractions presented TPC of 699.3 mg GAE.L^{-1} (whole cow milk fraction: W CoM), 584.2 mg GAE.L^{-1} (sweet whey cow milk fraction: SW CoM) and 700.3 mg GAE.L^{-1} (acid whey cow milk fraction: AW CoM), while after irradiation at 20 kGy, the TPC increased significantly to 890.9 mg GAE.L^{-1}, 1008.7 mg GAE.L^{-1}, and 925 mg GAE.L^{-1}, respectively (Fig. 2a). Similarly, the irradiation treatment at 10 kGy increased significantly ($p < 0.05$) the TPC of whole milk and the β casein of cow milk compared to the untreated fractions (0 kGy: 699.3 mg GAE.L^{-1} and 264.2 mg GAE.L^{-1}, respectively and 10 kGy: 846.5 mg GAE.L^{-1} and 319.3 mg GAE.L^{-1}, respectively). On the other hand, the sodium caseinate (SC CoM), acid whey (AW CoM), and skimmed cow's milk (S CoM), exhibit a significant decrease in the TPC after irradiation at 5 and/or 10 kGy. The camel milk fractions indicated a preservation of the TPC for AW CaM and βC CaM fractions at 20 kGy (Fig. 2b), an increase ($p < 0.05$) of this content at 20 kGy for the samples S CaM and SW CaM (Fig. 2b) and a significant decrease ($p < 0.05$) of total phenolics with irradiation for the fractions CaM and SC CaM.

3.3. Antioxidant activity

The results of antioxidant activities (DPPH and FRAP assays) of cow and camel milk and their corresponding whey and casein fractions, before and after irradiation are presented in supplementary Data, Table S1. The FRAP values ranged between 1.3 mmol FSE.L^{-1} (βC CoM at 0 kGy, 10 kGy and 20 kGy and βC CaM 5 kGy) and 8.2 mmol FSE.L^{-1} (CoM 10 kGy and 20 kGy). The whey fractions of camel milk (SW CaM and AW CaM) have significantly ($p < 0.05$) higher antioxidant activity than the whey fractions of cow milk (SW CoM and AW CoM). FRAP antioxidant activity after e-beam irradiation indicated a slight significant increase ($p < 0.05$) in the whole cow milk fraction (CoM: from 6.3 mmol FSE.L^{-1} at 0 kGy to 8.2 mmol FSE.L^{-1} at 10 and 20 kGy, Table S1a) and a significant decrease ($p < 0.05$) in the SC CoM fraction (from 7.1 mmol FSE.L^{-1} at 0 kGy to 5.3 mmol FSE.L^{-1} at 20 kGy, Table S 1a. and Table S 1b indicated that, the FRAP of different milk fractions was preserved or promoted by e-beam irradiation (except of the whole camel milk, CaM, the skimmed camel milk, S CaM, and the sodium caseinate cow milk fraction, SC CoM). The DPPH radical scavenging activities (DPPH-RSA) of the non-irradiated cow and camel milk fractions were lower than 20%. In fact, the values ranged from 7.82% to 17.45% for cow milk (Table S 1a) and from 7.54% to 16.03% for camel milk (Table S 1b). Similar scavenging activities were reported by Abd El-Fattah, Azzam, Elkashef, and Elhadydy (2020) for whole milk. These authors found that the whole cow and camel milk fractions exhibit DPPH-RSA of 18.89 ± 0.08% and 18.57 ± 1.88%, respectively. Similarly, and in line with these findings, DPPH-RSA of the skimmed milk was 9.05 ± 0.49% for the cow milk and 12.42 ± 1.25% for the camel milk. According to Chauveau-Duriot, Doreau, Noziere, and Graulet (2010) antioxidant activity of milk is influenced by water-soluble components such phenols, thiol groups, and ascorbate as well as casein, whey proteins, milk fat fraction containing tocopherols, retinol, and carotenoids. Moreover, Chen, Lindmark-Mansson, Gorton, and Akesson (2003) showed that the skimming may produce a milk soluble fraction with additional potent antioxidant components in a more concentrated defatted product. The fractions of cow and camel milk responded differently to e-beam irradiation (Table S 1). There was a significant increase of the DPPH-RSA with irradiation for all camel milk fractions and almost the majority of cow milk fractions (values varying from 6.27 ± 0.74% to 35.86 ± 1.21% for cow milk and from 12.82 ± 0.54% to 59.75 ± 0.84% for camel milk). Even though there have been numerous studies on the antioxidant properties of liquid raw milk (Grażyna, Hanna, Adam, & Magdalena, 2017), milk hydrolysates (Ecem, 2021), and heat-treated milk (Kuhnen et al., 2014), those corresponding to sweet, acid whey, or casein fractions were limited (Lajnaf et al., 2021). Irradiation processing has been extensively studied and is presently used for a range of food products (fruits, vegetables, cereals, etc.). However, no previous research has examined how e-beam irradiation affects the antioxidant activities of milk fractions. Generally, when food with a high-water content (such as milk) is submitted to ionizing radiation, some indirect effects of ionizing radiation can occur in food matrices through water radiolysis. A rise in enzyme activity linked to improved antioxidant activity post irradiation (e.g., phenylalanine or peroxidase) was also reported in literature (Ferreira et al., 2018; Madureira et al., 2022).

3.4. Antidiabetic activity

Several attempts have been made to find more potent and secure α-glucosidase and α-amylase inhibitors from natural sources for the treatment and management of diabetes.

Fig. 3 shows the in vitro antidiabetic activity of cow and camel milk fractions assessed using α-amylase (Iα-Amyl) (Fig. 3a, b), and α-glucosidase inhibition percentages (Iα Gluco) (Fig. 3c, d), as well as the effect of e-beam radiation treatment on these activities.

The results indicated that among the six fractions of each non-irradiated milk type (0 kGy), three cow milk fractions (SW CoM, AW CoM and SC CoM) and the majority of camel milk fractions (CaM, S CaM, SW CaM and AW CaM) exhibit a percentage of α-amylase inhibition higher than 50% (varying from 51.17 ± 1.05% to 97.89 ± 1.03%). Interestingly, these results revealed that both non-irradiated SW and AW fractions of cow and camel milk had the highest levels of α-amylase inhibition (Fig. 3a, b). The non-irradiated whey fractions of cow milk displayed values of 87.75 ± 0.57% for the sweet whey (SW CoM) and 97.89 ± 1.03% for the acid whey (AW CoM) (Fig. 3a). The whey fraction of camel milk showed α-amylase inhibition percentage of 97.43 ± 1.51% for the sweet whey (SW CaM) and of 93.89 ± 1.08% for the acid whey (AW CaM) (Fig. 3b). Regarding the effect of electron beam irradiation on the percentage of α-amylase inhibition, the same trend was maintained for the cow milk fractions initially showing a high percentage of inhibition for SW CoM, AW CoM (the values of inhibition varying from 73.24 ± 2.69% to 98.99 ± 0.84%). Nevertheless, for the whole cow milk and casein fractions (CoM, S CoM and βC CoM) the dose-effect relationship is different with the exception of whole cow milk (CoM), whose percentage of inhibition appeared to increase ($p < 0.05$) at 5 kGy, then decrease ($p < 0.05$) at 10 kGy and 20 kGy. The fractions of skimmed milk (S CoM) and β-casein of cow milk (βC CoM) tended to show an increase in α-amylase inhibition percentage with e-beam irradiation doses (from 4.41 ± 0.16% and 5.27 ± 1.05% at 0 kGy to 24.7 ± 1.6% and 28.5 ± 1.3% at 20 kGy, respectively). In the contrary to cow milk, the camel milk fractions appeared to be more reactive to electron beam irradiation. In fact, the trend found in the non-irradiated fractions is maintained but with a little variation. After treatment, the 5 kGy dose significantly decreased ($p < 0.05$) the percentage of inhibition of the majority of camel milk fractions in comparison with the non-irradiated fractions (CaM: 0 kGy 73.59 ± 2.5% vs 61.53 ± 1.24% at 5 kGy, S CaM: 0 kGy 76.41 ± 0.66% vs 37.74 ± 2.64% at 5 kGy, SW CaM: 0 kGy 97.43 ± 1.51% vs 70.93 ± 3.11% at 5 kGy and AW CaM: 0 kGy 93.89 ± 1.08% vs 77.5 ± 2.43% at 5 kGy). Nevertheless, this trend is reversed with high e-beam irradiation doses (10 kGy and 20 kGy). Indeed, the α-amylase inhibition was improved at these doses (Fig. 3b). Overall, it could be concluded that e-beam irradiation may promote or preserve the anti-diabetic potential in terms of α-amylase inhibition of some cow and camel milk fractions.

It is important to note that whatever the milk type, fraction or applied irradiation dose, the percentages of inhibition of α-glucosidase are substantially lower than those of α-amylase. In fact, they varied from non-detected to 50.32% for all milk fractions before irradiation and from 2.7% to 38.3% after the treatment with different doses.

The fractionation process resulted in changes in the α-glucosidase inhibition percentage between fractions of the same species (skim milk, sweet whey, acid whey, sodium caseinate, and β-casein). Four non-irradiated cow and camel milk fractions (CoM, S CoM, CaM and AW

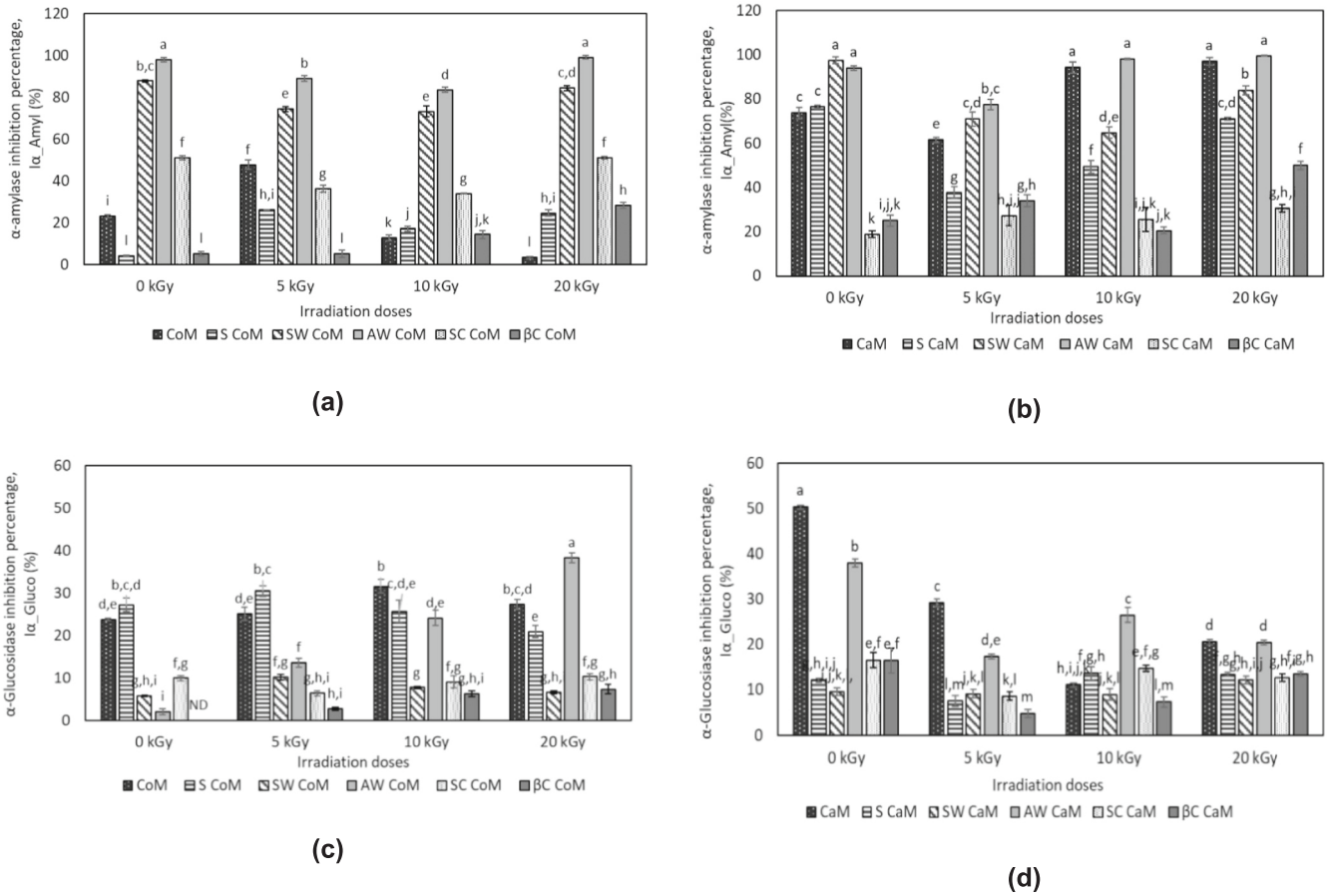

Fig. 3. Antidiabetic activities by α-amylase (a, b) and α-glucosidase inhibition assays percentage (c, d), Iα (%) of non-irradiated (0 kGy) and irradiated (5, 10 and 20 kGy) cow (a,c) and camel milk (b, d) fractions. CoM: Whole cow milk, S CoM: Skimmed cow milk, Acid whey from cow milk: AW CoM, SW CoM: Sweet whey from cow milk, SC CoM: Sodium caseinate, from cow milk, βC CoM: β casein from cow milk, CaM: Whole camel milk, S CaM: Skimmed camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk, SC CaM: Sodium caseinate, from camel milk, βC CaM: β- casein from camel milk. Error bars correspond to 95% confidence intervals about mean values (n = 3; α = 0.05). In each bar, different letters mean significant differences between average values ($p < 0.05$).

CaM) exhibited a percentage of α-glucosidase inhibition higher than 20%, with the highest values for the whole camel milk fraction (CaM) with 50.32% and for acid whey of camel milk fraction (AW CaM) with 37.95% (Fig. 3d). Also, after e-beam irradiation it was observed a significant increase ($p < 0.05$) of the percentage of the α-glucosidase inhibition for some fractions of cow and camel milk. Indeed, enzyme inhibition appeared to be dose-dependent as the nature and composition of the fraction, seemed to clearly affect the level of enzyme inhibited. Nevertheless, the majority of camel milk fractions (Fig. 3d) demonstrated to be sensitive to irradiation since there is a noticeably decrease ($p < 0.05$) at 20 kGy in the percentage of inhibition of α-glucosidase. In contrast to camel milk, the acid whey (AW CoM), and β-casein (βC CoM) had shown significant increase ($p < 0.05$) in the percentage of the α-glucosidase inhibition at 20 kGy (Fig. 3c). Antioxidant compounds of milk (phenolic compounds and vitamins) and active peptides are reported to have antidiabetic activity. These bioactive compounds including caseins (α, β-, γ- and κ-casein) and proteins (β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin) play a primary role in the inhibitory activity of enzymes, a pivotal factor in the antidiabetic potential of milk. Despite the lack of data on how electron beam radiation affects the enzymes inhibitory activity of milk fractions, many authors reported antidiabetic potential of non-irradiated milk, and available results varied according to the used antidiabetic assay (DPP-IV inhibitory activity, α-amylase, α-glucosidase and lipase inhibitory activities) and to the type of milk analyzed (raw milk, fermented, or hydrolysate milk). Mudgil, Kamal, Chee Yuen, and Maqsood (2018) demonstrated that camel milk proteins are a unique source of bioactive peptides that inhibit three key metabolic enzymes related to diabetes which are dipeptidyl peptidase-IV (DPP-IV), porcine pancreatic α-amylase (PPA), and pancreatic lipase (PPL). In another hand, whey protein isolate, α-lactalbumin and β-lactoglobulin from bovine milk showed both DPP-IV and α-glucosidase inhibitory actions, whereas those of lactoferrin and serum albumin could only inhibit DPP-IV (Lacroix & Li-Chan, 2013).

3.5. Correlation between antioxidant and antidiabetic activities

Principal component analysis (PCA) was used to evaluate data from the milk samples bioactivity corresponding to the antioxidant (DPPH -RSA and FRAP) and the antidiabetic (α-amylase and α-glucosidase inhibition) of the irradiated and non-irradiated fractions (Fig. 4). The PCA biplot consists of two axes, a horizontal axis 1 and a vertical axis 2. The intersection of these two axes gives four quadrants: A, B, C and D, and each quadrant has different components. The biplot reveals that PCA described 69.06% of data variation through the first two dimensions. Respectively, dimension 1 explains 41.79% of the variance, and dimension 2 accounts for an additional 27.26% of the variance (Fig. 4a). The first dimension was represented positively by the α-amylase inhibition activity (Iα-Amyl) (0.058), FRAP (0.779) and the α-glucosidase inhibition activity (Iα-Gluco) (0.865), whereas the negatively valued attributes are represented by DPPH-RSA (Fig. 4b). Four significant groups of different irradiated and non-irradiated camel and cow milk fractions were distinguished: the first group (quadrant A), represented by DPPH-RSA, is formed by all the irradiated sweet whey fractions of camel milk (SW CaM 5 kGy, SW CaM 10 kGy and SW CaM 20 kGy), the non-irradiated (0 kGy) and irradiated (10 and 20 kGy) sweet whey cow milk fractions, the irradiated fraction of acid whey of camel milk (AW CaM 5 kGy), the non-irradiated and irradiated at 5 kGy acid whey fraction of cow milk (AW CoM 0 kGy and AW CoM 5 kGy), the irradiated at 10 and 20 kGy skimmed camel milk fractions (S CaM 10 kGy and S CaM 20 kGy), both irradiated at 20 kGy sodium caseinate cow milk fraction (SC CoM 20 kGy) and β-casein of camel milk fraction (βC CaM 20 kGy). The second group (quadrant B) mainly formed by the non-irradiated and irradiated camel milk fractions (CaM), the acid whey fractions of camel and cow milk with the irradiation doses 10 and 20 kGy (AW CaM 10 kGy, AW CaM 20 kGy, AW CoM 10 kGy and AW CoM 20 kGy) and the non-irradiated sweet whey fraction of camel milk (SW CaM 0 kGy). All of these fractions present a positive correlation with Iα-Amyl, Iα-Gluco and FRAP variables. Following PCA and taking into account the distribution of the various fractions, the non-irradiated and irradiated (5, 10 and 20 kGy) whole camel milk fraction (CaM), the non-

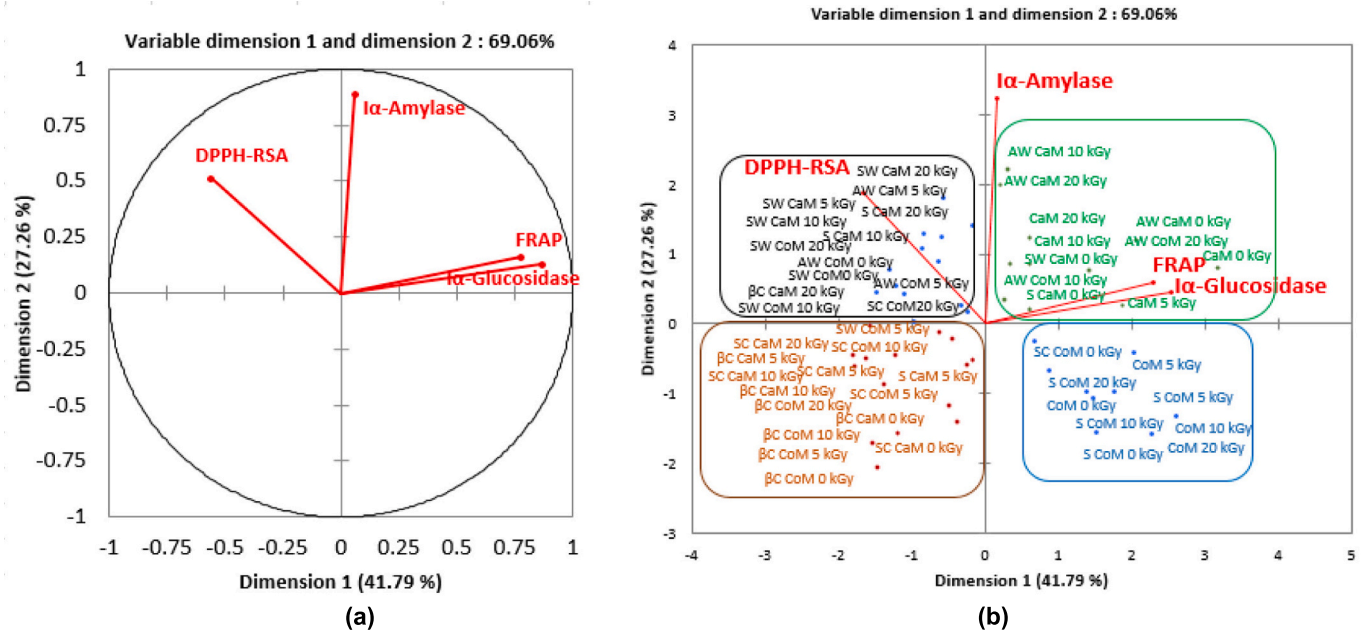

Fig. 4. PCA biplot of objects and component loads for grouping of descriptors for bioactive contents (DPPH-RSA, FRAP, α-amylase inhibition percentage and α-glucosidase inhibition percentage) (a) and groups of milk fractions samples (b). CoM: whole cow milk, S CoM: Skim cow milk, AWCoM: Acid whey from cow milk, SW CoM: Sweet whey from cow milk, SC CoM: Sodium caseinate, from cow milk, βC CoM: β casein from cow milk, CaM: whole camel milk, S CaM: Skim camel milk, AW CaM: Acid whey from camel milk, SW CaM: Sweet whey from camel milk, SC CaM: Sodium caseinate, from camel milk, βC CaM: β casein from camel milk. A-D are the quadrants of the PCA biplot.

irradiated skim camel milk (S CaM 0 kGy), the non-irradiated and irradiated (10 and 20 kGy) acid whey of camel milk (AW CaM) and the irradiated (10 and 20 kGy) acid whey of cow milk fraction (AW CoM) with stronger antioxidant and antidiabetic properties (quadrant B and C) appeared to be the most intriguing to be examined for future assessment for other biological activities (samples in quadrant B).

4. Conclusion

Antiproliferative, antidiabetic and antioxidant activities of defatted liquid camel and cow milk and their whey and casein fractions treated or untreated with e-beam (at 5, 10 and 20 kGy) were investigated. *E*-beam radiation treatment at 5 kGy of milk and different cow and camel milk fractions did not demonstrate any cytotoxic effect on non-tumor cell line. However, cytotoxic effects were observed in non-tumor cell line for irradiated β casein (at 10 kGy and 20 kGy) and for irradiated sweet whey of cow milk (at 20 kGy). A significant inhibitory activity of A549 lung cancer cells proliferation was observed for irradiated β casein of cow milk (at 10 and 20 kGy), the acid whey and the irradiated sweet whey of camel milk fractions milk (at 10 and 20 kGy). E-beam radiation treatment preserved or slightly increased the phenolic content in camel milk. It increased the TPC of the fractions of whole milk, sweet whey, and acid whey of cow milk. Acid whey and sodium caseinate of camel milk, as well as the skimmed cow milk and its β-casein fraction, were the most resistant to the applied e-beam irradiation doses. Furthermore, e-beam irradiation enhanced or preserved the antioxidant activity and α-amylase inhibition of cow and camel milk fractions. The irradiated and non-irradiated sweet whey of camel milk seem to be the most promising to be investigated for further bioactivity analyses (e.g. anti-inflammatory, anti-metastatic activities) and identification of bioactive compounds using liquid chromatography coupled to mass spectrometry.

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Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2023.103457.

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