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Effects of Spray Drying, Freeze Drying and Gamma Irradiation on the Antioxidant Activities of Camel and Cow Milk Fractions

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Abstract: This work aimed to establish an integrated approach to investigate the total phenolic content and antioxidant activities of dried skim camel and cow milk and their fractions. The milk fractions were obtained by acid or enzymatic coagulation followed by spray drying (inlet temperature/outlet temperature: 125 ± 2 °C/90 ± 2 °C) or freeze drying (−50 °C, 0.05 mbar) coupled or not to gamma irradiation (at 5, 11, 22 kGy). The results showed that the total phenolic content (measured in gallic acid equivalent, GAE) varied depending on the drying technique. The freeze-drying process corresponded to the highest values of total phenolic compounds, with 247.23 ± 2.08 mg GAE/100 g powder for the β-casein fraction of camel milk (βC CaM) and 621.13 ± 4.16 mg GAE/100 g powder for the β-casein fraction of cow milk (βC CoM). Compared to spray-dried fractions, freeze-dried fractions showed generally higher ferric reducing antioxidant power for both camel milk and cow milk. The highest values of free radical scavenging activity were seen in the spray-dried β-casein fractions of camel milk (βC CaM) and cow milk (βC CoM) and in the freeze-dried acid whey of cow and camel milk (AW CaM and AW CaM). Freeze-dried acid whey (AW CaM and AW CoM) appeared to be less sensitive to gamma irradiation at 5 and 11 kGy.

Keywords: milk fractions; freeze drying; spray drying; gamma irradiation; antioxidants

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1. Introduction

Milk is a good source of proteins, fats, carbohydrates and minerals which are essential for bone growth and development in young children. Milk is also advantageous for the elderly, particularly for menopausal women. [1]. With an estimated amount of 734 million tons produced in 2020, cow milk will continue to be the world's most consumed milk and occupy a vital strategic position in the global economy [2]. However, in harsh and semi-arid environments, other types of milk, such as camel milk, serve as the primary dairy source. There has been a lot of interest in processing camel milk over the past century in order to diversify dairy products and re-use camel milk in the form of functional ingredients and dietary supplements. Such ingredients that interesting levels of protein enhanced with antioxidants and minerals. According to the FAO, the world's annual camel milk production was about 3.15 million tons in 2020 [3]. There are significant distinctive protein and vitamin profiles in camel and cow milk [4] which may induce different techno-functional and biological properties such as antioxidant activities. Recent studies have

confirmed the therapeutic benefits of fresh and fermented camel milk, protein hydrolysates and whey fraction. These benefits include its relevant antidiabetic and antihypertensive properties as well as its capacity to reduce autism symptoms and to prevent cancer [4,5]. These medicinal properties have been attributed to the milk's high vitamin C and mineral content, distinctive protein composition and the potential release of bioactive peptides during the digestive process [5]. With an estimated worldwide production of 190 billion kg per year [6], second cheese whey, the main byproduct of the dairy industry, remains an important and valuable substance to be valorized. Indeed, it has been processed into an interesting source of active substances and particular nutrients, such as lactose, soluble proteins, water-soluble vitamins, fatty acids and mineral elements [6–8].

Milk and its derivatives are inherently susceptible to numerous microbiological, physical and biochemical degradations. They can be processed into an interesting source of active substances such as soluble proteins, water-soluble vitamins and mineral elements [6–8]. Such composition requires one or more physical, thermal or biological types of stabilization process such as pasteurization, drying or fermentation [9,10]. Whole camel milk is reported to be technically more difficult to process than milk from other domestic animals [11–13]. Milk powder is mainly produced by freeze drying and spray drying. These processes offer several advantages to consumers and producers, such as extending shelf life, reducing transportation costs for packaging, and ease of handling milk. They also allow the production of milk powder with acceptable nutritional and functional properties [14,15]. Several previous works studied the effects of conventional heat treatment (pasteurization and sterilization), freeze drying and spray drying on the nutritional properties, stability and protein fractions of milk [9,16–18] and their main techno-functional properties [19–21]. Spray drying is a dehydration method that rapidly removes water from small milk droplets exposed to a dry and hot air stream [22]. In recent studies, it has been reported that spray drying of milk at high inlet temperatures (230–250 °C) resulted in a higher extended Maillard reaction in comparison to lower temperatures (190–200 °C) and this was strongly correlated with improved antioxidant properties of the dried product [23,24]. Freeze drying is considered an excellent dehydration process for heat-sensitive products. This process minimizes degradation reactions and maintains adequate physical, chemical and biological stability of the product during long-term storage [25]. Freeze drying was also considered to be an effective method for producing dried powder from camel skim colostrum with the preservation of its nutritional and antioxidant properties [26]. It allows the preservation of vitamins and macroelements of reconstituted camel milk [27] and of the bioactive properties of human milk [28]. Salar et al. (2021) [17] reported that spray drying (inlet temperature of 123 °C) or freeze drying coupled with low-temperature, short-time pasteurization (57, 60, 63 °C) could be more effective than pasteurization at higher temperatures and for a longer time, and this combination allows the improvement of buffalo and cow colostrum shelf life and maintaining the bioactivity of colostrum samples. While milk and dairy products are rarely industrially irradiated, irradiation is still a safe and environmentally beneficial method for preserving foods [29,30]. To further reduce the risk of bacterial contamination during packaging and preserve the bioactive proteins, bovine colostrum treated with low-temperature, long-time pasteurization (63 °C, 30 min), followed by spray drying allows preserving the bioactive proteins of powdered bovine colostrum used as a dietary supplement for sensitive patients. ɤ-irradiation (0–10 kGy) was reported to be efficient in the inactivation of foodborne pathogens in infant formula [31–33]. Robichaud, 2020 [33] confirmed that gamma irradiation at 5 kGy positively affected the nutritional and antioxidant properties of powdered infant formula whereas the antioxidant properties of the liquid formulation were more sensitive to this treatment. To the best of our knowledge, a comparative investigation of the antioxidant properties of spray-dried, freeze-dried and irradiated freeze-dried camel and cow milk and their corresponding acid whey, sweet whey and casein fractions has not been previously performed. Milk fractionation into protein-rich fractions and their drying is a promising approach for upgrading camel milk and producing innovative functional milk derivatives, which is one novelty

of this work. Moreover, the novelty of this work is the investigation of the antioxidant properties of dried and irradiated freeze-dried camel milk compared to cow milk and its rich protein fractions. The objectives of this work were (i) to establish an easy-to-use and simple approach to milk fractionation into protein-rich fractions, allowing their further use as functional milk derivatives, (ii) to investigate the antioxidant activity of the extracts of freeze-dried and spray-dried milk protein fractions: sweet whey, acid whey, sodium caseinate and β-casein, and (iii) to assess the effect of gamma radiation (at 5, 11 and 22 kGy) on the antioxidant properties of selected freeze-dried milk fractions.

2. Materials and Methods

2.1. Raw Milk and Fractions Preparation

2.1.1. Milk Samples

Fresh Tunisian cow milk (CoM) and camel milk (CaM) were procured aseptically from southern Tunisian farms. Milk samples were then transported to the laboratory at 4°C within 30 min. For additional processing and examination, the milk was frozen at −20 °C. The milk samples were thawed at 4 °C for 48 h to perform separation into different protein-rich fractions. No aggregates were detected in the milk samples after thawing. The pH was systematically measured before proceeding with any further treatment. Following thawing, skimming was carried out as follows: for cow milk, centrifugation was carried out once at 2000× *g* for 15 min at 5 °C, while it was carried out three times at 2000× *g* for 15 min at 5 °C for camel milk [34]. To avoid the variability and the influence of fat content, fresh skim camel and cow milk were used to prepare sweet whey by enzymatic coagulation. Skim camel (SCaM) and skim cow milk (SCoM) were fractionated as summarized in Figure 1 to obtain four fractions for each type of milk: sweet whey (SW CoM and SW CaM), acid whey (AW CaM and AW CoM), sodium caseinate (SC CaM and SC CoM) and β-casein (βC CaM and βC CoM).

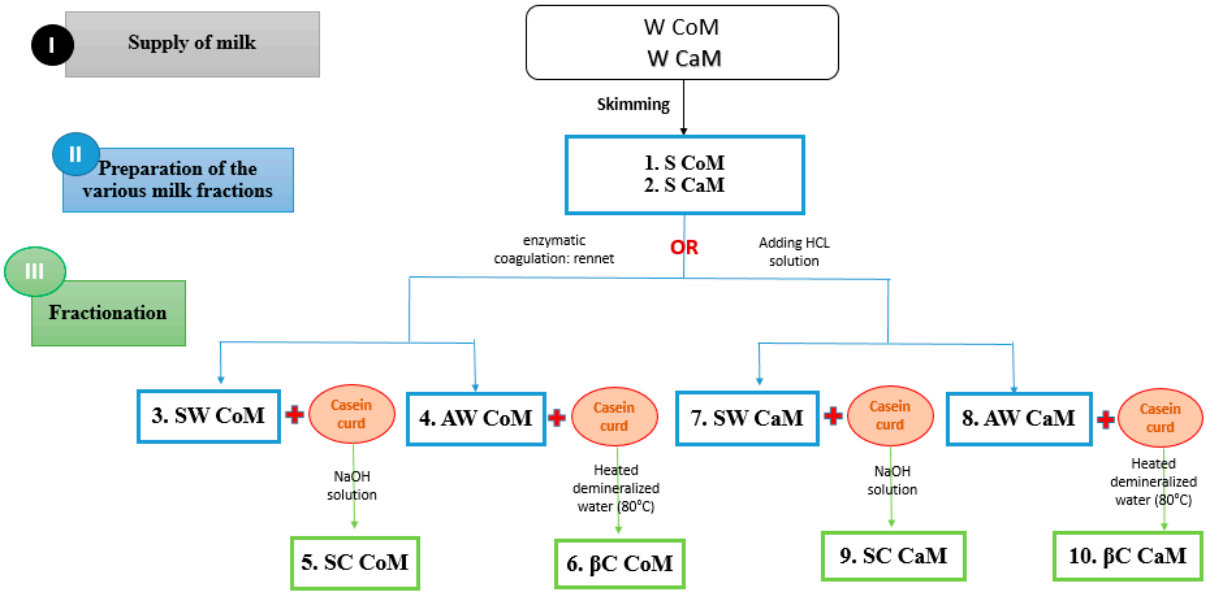


Figure 1. Scheme of milk fractionation by acid, and enzymatic coagulation and centrifugation. CoM: cow milk; W CoM: whole cow milk; S CoM: skim cow milk; AW CoM: acid whey from cow milk; SW CoM: sweet whey from cow milk; SC CoM: sodium caseinate, from cow milk; βC CoM: β-casein from cow milk; W CaM: 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.

2.1.2. Protein Fractions Preparation

- Acid whey

Skim camel milk and skim cow milk were acidified with a sufficient amount (10 mL/L milk) of 12 N HCl solution to lower the pH to 4.6 for cow milk and 4.3 for camel milk. At these pH values, casein micelles completely lose their micellar structure and form small casein aggregates dispersed in an aqueous phase. These aggregates are then separated from the aqueous phase by centrifugation at 5000× *g* for 15 min at 20 °C. The supernatant representing the acid whey was collected.

- Sodium caseinate

Once the caseins were recovered after separation of the acid whey, distilled water was added (the same volume as the recovered acid whey) and then equilibrated with a sufficient amount of NaOH solution (10 mL/L milk) to raise the pH to 6.7.

- Sweet whey

Fresh skim camel and cow milk were used to prepare sweet whey by enzymatic coagulation and under the action of rennet at 37 °C (fungal origin: *Mucor miehei*). The amount of rennet added to camel milk (1.4 mL/L) was four times higher than that added to cow milk. The obtained coagulum was manually divided into several fragments. Sweet whey was obtained after centrifugation of these fragments at 5000× *g* for 15 min at 20 °C.

- β-Casein

After separating the casein fraction of camel or cow milk from the sweet whey, a volume of distilled water equal to the volume of whey recovered was added to the curd. The mixture was kept in a water bath at 80 °C for 5 min to stop the action of the rennet, and then placed in a cooled incubator at 4 °C to allow extraction of the β-casein. After 24 h, the mixture was centrifuged at 5000× *g* for 15 min at 4 °C. The supernatant, containing β-casein, was then stored at −20 °C for further studies [35].

2.2. Freeze Drying, Spray Drying and Irradiation

The eight milk fractions obtained from camel milk and cow milk (SW CoM, SW CaM, AW CoM, AW CaM, SC CoM, SC CaM, βC CoM and βC CaM) and skim milk (S CoM, S CaM) were used for spray-drying and freeze-drying assays.

2.2.1. Spray Drying

Skim camel milk and skim cow milk as well as the corresponding milk fractions were turned into powder without any prior preparation. A Lab-Pilot spray dryer (B-190 Buchi Labortechnik AG, Flawil, Switzerland) with a two-fluid nozzle of 0.7 mm and an atomizing volume of 0.2–1 kg/h was used. For all trials, the inlet temperature was 125 ± 2°C. The outlet temperature was 90 ± 2°C. The absolute humidity of the inlet air was 5 g of water per kg of dry air. The airflow rate was 1 v.v.m. The pressure was set at 3 bar. The feed flow rate was 0.5 ± 0.1 L/h.

2.2.2. Freeze Drying

All milk fractions, skim camel milk and skim cow milk were put in glass bottles and cooled to −80 °C for 24 h in preparation for freeze drying. The milk in the glass bottles was subsequently freeze-dried, using a Lab Pilot freeze dryer (USCFROID, SMH-15, 290.94) of a total volume of 14 L, at −50 °C for 48 h under vacuum (0.05 mbar).

2.2.3. Irradiation Experiments

A cobalt-60 experimental chamber (Precisa 22 model, Graviner Lda, UK, 1971) with a total activity of 1.67 kCi (62 TBq, October 2021) was used for gamma radiation treatment [36]. Freeze-dried milk powder fraction samples in falcon tubes were irradiated in triplicate at the doses of 5.4 kGy, 11.3 kGy and 21.8 kGy at a dose rate of 0.8 kGy/h with a dose uniformity (DUR) of 1.05. The dose absorbed by the samples was estimated by calibrated Amber Perspex radiochromic dosimeters (Harwell Dosimeters, United Kingdom) for gamma radiation [37]. For simplicity, the absorbed doses will be referred to as 5, 11 and 22 kGy.

In order to analyze the effect of gamma radiation in skim milk and milk fraction powder, non-irradiated (0 kGy) samples submitted to the same experimental procedure were used as control.

2.3. Samples Preparation for Antioxidant Activities

The milk powder extracts were prepared by a solid–liquid extraction. The extraction procedure included the addition of 0.2 g of freeze-dried milk powder to 2 mL solvent (deionized water/95% ethanol (*v/v*, 15/85) and shaking for 1 h at 30 °C. The mixtures were then centrifuged at 7800× *g* (micro-centrifuge 5417R, Eppendorf) at 5 °C for 15 min. The supernatants were kept at −20 °C in the dark until further analyses.

2.4. Total Phenolic Content and Antioxidant Activities

Total phenolic content (TPC) was determined by the Folin–Ciocalteau method [36,38] using extracts concentrated at 1 mg/mL. The formation of the blue-colored complex between molybdenum and tungsten present in the Folin–Ciocalteu reagent upon reaction with reducing agents was monitored at 765 nm using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The standard curve was calculated using gallic acid (Sigma, St. Louis, MO, USA), and the results were expressed as mg of gallic acid equivalent (GAE) per 100 g of milk powder. The experiment was performed in triplicate.

The antioxidant activity was evaluated by two assays based on different mechanisms of action: DPPH radical scavenging activity (DPPH RSA) and ferric reducing antioxidant power (FRAP) as described by Barkaoui et al. (2020) [36] and Madureira et al. (2019) [38]. Both assays were performed in triplicate.

The DPPH method (free radical scavenging activity) was carried out using 96-well plates and an EZ Read 1200 Microplate Reader (Biochrom, Cambridge, UK). The reaction mixture in each well consisted of the sample extracts (30 µL) and a methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} M). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Aldrich (St. Louis, MO, USA). The mixture was left in the dark for 60 min. The reduction of DPPH radicals was determined by measuring the absorption at 515 nm.

The results were expressed as the percentage of inhibition of DPPH radicals according to the following equation:

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

where $\text{Abs}_{\text{blank}}$ is the absorbance of the blank and $\text{Abs}_{\text{Extract}}$ is the absorbance of the extract.

For the ferric reducing antioxidant power (FRAP) assay, the FRAP reagent was freshly prepared by mixing 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 $FeC_{l3}{\cdot}6H_2O$ in a 10:1:1 ratio at 37 °C. In a test tube, aliquots of 100 µL of the prepared milk extract solutions (1 mg/mL) were mixed with 3 mL of the reagent FRAP. After incubation at 37 °C for 15 min, the absorbance was measured at 593 nm in a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The results were expressed as mmol of ferrous sulfate equivalent (FSE) per 100 g of milk powder according to the following equation:

$$\text{FRAP}\ (\text{mmol FSE}/100\ \text{g powder}) = \frac{(\text{Abs}_{\text{Extract}} - \text{a})}{\text{b}} \times 100 \quad (2)$$

where $\text{Abs}_{\text{Extract}}$ is the absorbance of the extract; a = 0.0097 and b = 0.681, are the coefficients of the FSE calibration curve.

2.5. Data Analysis

All experimental analyses were performed in triplicate and expressed as the mean value ± standard deviation. ANOVA followed by a post hoc (Tukey HSD procedure) test

for comparing TPC, FRAP and DPPH RSA results in milk samples and corresponding fractions were carried out. A significance level of $p < 0.0001$ was set. Principal component analysis (PCA) on TPC and antioxidant activities (using DPPH- and FRAP assays) of camel milk, cow milk and their corresponding fractions were analyzed in order to select the samples to be irradiated at 5, 11 and 22 kGy. The number of dimensions considered for the PCA was chosen equal to 2 in order to allow meaningful interpretations and to guarantee their reliability. All statistical analyses were performed using Windows XLSTAT software version 2018.

3. Results and Discussion

3.1. Total Phenolic Content and Antioxidant Activities of Dried Milk Fractions

The results of total phenolic content (TPC), DPPH radical scavenging activity (DPPH RSA) and ferric reducing antioxidant power (FRAP) of freeze-dried and spray-dried cow and camel skim milk and the corresponding whey and casein fractions are shown in Figures 2–4, respectively. High variability of TPC was observed between skim milk and milk fractions (sweet whey, acid whey, sodium caseinate and β-casein) based on the type of milk (cow or camel). Skim milk, sodium caseinate, and β-casein obtained from camel milk had higher TPC than those obtained from cow milk. For a fixed milk fraction, low variation was recorded according to the drying process (freeze-dried or spray-dried) (Figure 2). Whatever the type of milk or the applied process, acid whey and β-casein milk fractions had higher TPC (239.23 ± 2.08 mg GAE /100 g powder and 247.23 ± 2.08 mg GAE /100 g powder, respectively, for cow milk and 155.9 ± 2.65 mg GAE /100 g powder and 621.13 ± 4.16 mg GAE /100 g powder for camel milk, respectively) than other fractions and higher than the corresponding skim milk (S CaM: 90.90 ± 1 mg GAE /100 g powder, S CoM: 70.90 ± 2.65 mg GAE /100 g powder). These results suggest that the fractionation of milk by acid and enzymatic coagulation allows obtaining milk fractions with higher TPC contents than the corresponding skim milk. The last observation could be explained by the fact that acid and enzymatic coagulation allow the release of active peptides with phenolic rings as reported in the literature [4,5], and/or a better concentration of phenolic compounds in sweet, acid whey and casein fractions and/or the different sensitivity of milk and milk fractions to drying. When comparing both drying methods, the highest TPC values (≥150 mg GAE /100 g powder) were generally found in freeze-dried fractions, including the acid whey of cow milk (SW CoM), the β-casein of cow milk (βC CoM) and camel milk (βC CaM), and sodium caseinate of camel milk (SC CaM). The total phenolic compounds of freeze-dried and spray-dried skim camel milk and sodium caseinate of cow milk were not significantly different ($p > 0.05$). This suggests that both processes retain the TPC of milk and freeze drying seems to be a more effective method. Because of the applied low processing temperatures and vacuum pressure, freeze drying usually results in less bioactive component breakdown [39]. To the best of our knowledge, the TPC of powdered camel milk fractions has rarely been assessed. Whereas several results are available on the phenolic content of pasteurized and UHT-treated liquid cow milk, which ranged from 505.46 ± 16.66 to 982.14 ± 168.42 mg gallic acid equivalent, GAE /L [40]. Figure 3 shows that the higher DPPH RSA were recorded for spray-dried fractions: skim camel milk (44.95 ± 1.85%), acid whey (AW CoM: 30.49 ± 0.54%; AW CaM: 49.08 ± 1.24 %) and β-casein fractions from camel milk (60.49 ± 2.69 %) and cow milk (58.33 ± 6.55 %). In addition, the freeze-dried acid whey of cow milk and camel milk (AW CoM: 40.33 ± 0.22%; AW CaM: 48.9 ± 0.38) showed a relatively high percentage of inhibition. However, low DPPH RSA was found for freeze-dried sweet whey fractions and no detectable DPPH RSA was found for freeze-dried βC CoM and βC CaM.

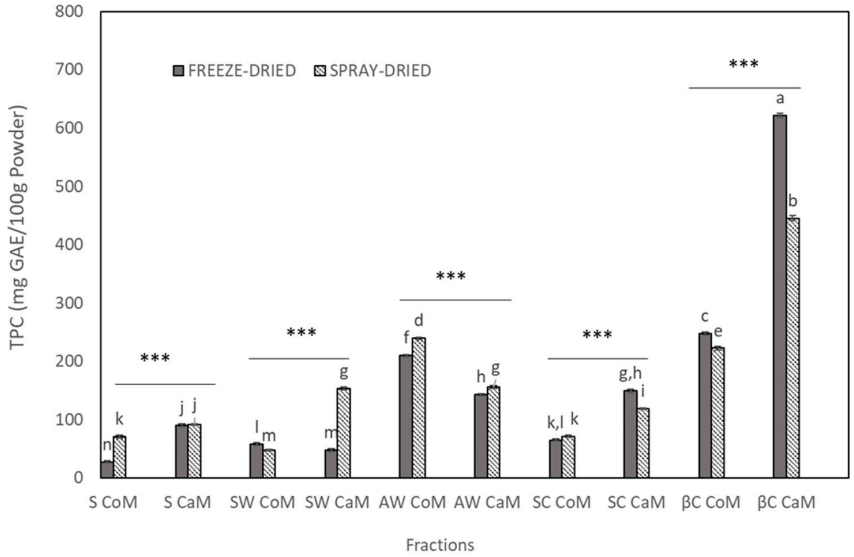


Figure 2. Total phenolic contents of non-irradiated freeze-dried and spray-dried cow and camel milk powder fractions. S CoM: skim cow milk; AWCoM: acid whey from cow milk; SW CoM: sweet whey from cow milk; SC CoM: sodium caseinate, from cow milk; βC CoM: β-casein from cow milk; S CaM: skim camel milk; 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. Error bars correspond to 95% confidence intervals about mean values (n = 3). In each bar, different letters mean significant differences between average values corresponding to process effect: spray drying and freeze drying ($p < 0.001$). *** indicates a significant difference between the type of milk of each fraction ($p < 0.001$).

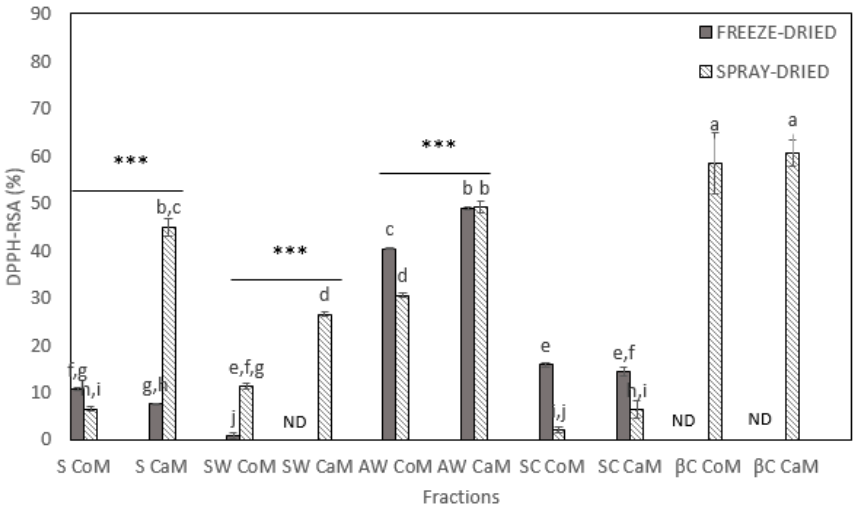


Figure 3. Antioxidant activity by radical scavenging activity (DPPH assay) of non-irradiated freeze-dried and spray-dried cow and camel milk powder fractions. S CoM: skim cow milk; AWCoM: acid whey from cow milk; SW CoM: sweet whey from cow milk; SC CoM: sodium caseinate, from cow milk; βC CoM: β-casein from cow milk; 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; ND: Not Detected. Error bars correspond to 95% confidence intervals about mean values (n = 3). In each bar, different letters mean significant differences between average values ($p < 0.001$). *** indicates a significant difference between the type of milk of each fraction ($p < 0.001$).

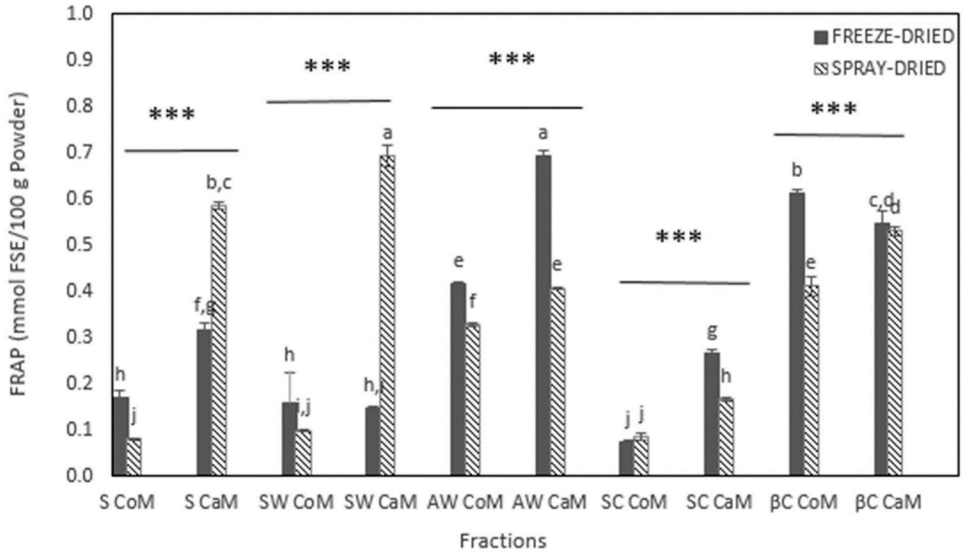


Figure 4. Ferric reducing antioxidant power (FRAP) of non-irradiated freeze-dried and spray-dried cow and camel milk powder fractions. S CoM: skim cow milk; AWCoM: acid whey from cow milk; SW CoM: sweet whey from cow milk; SC CoM: sodium caseinate, from cow milk; βC CoM: β-casein from cow milk; S CaM: 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. Error bars correspond to 95% confidence intervals about mean values (n = 3). In each bar, different letters mean significant differences between average values ($p < 0.001$). *** indicates a significant difference between the type of milk of each fraction ($p < 0.001$).

Figure 4 shows that freeze-dried extracts of skim milk and the corresponding fractions presented FRAP values varying between 0.07 and 0.62 mmol FSE/100 g powder and corresponding spray-dried fractions had FRAP values ranging between 0.08 and 0.69 mmol FSE/100 g powder. The fractions with the highest FRAP values for the freeze-drying process were the acid whey and the β-casein from both types of milk. Camel milk fractions generally exhibit higher ferric reducing antioxidant power than those corresponding to cow milk (except for the sweet whey fraction, SWCaM).

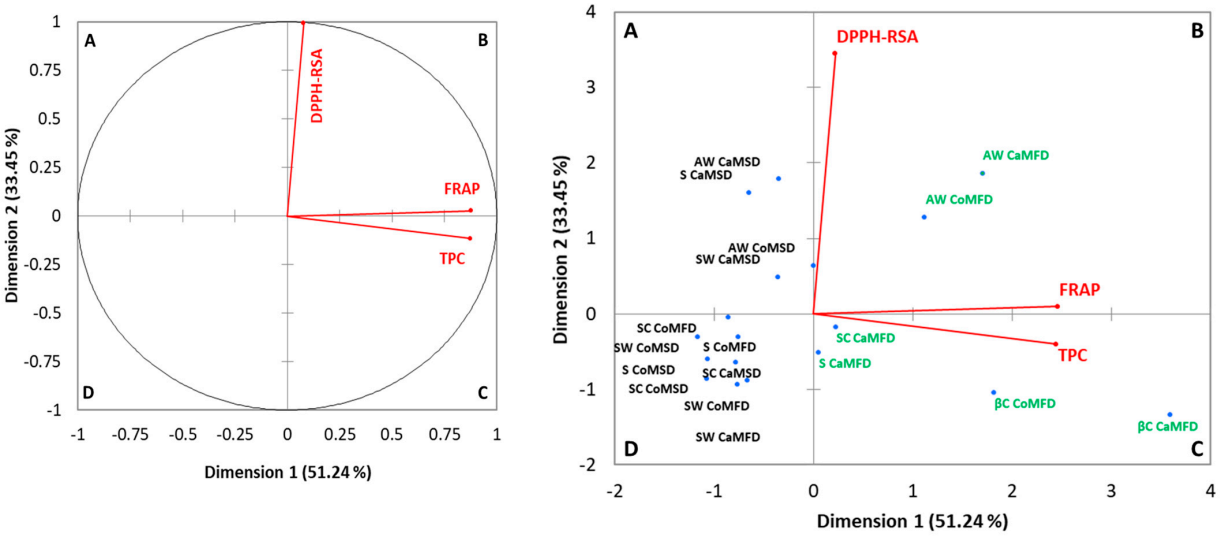
Cow milk contains bioactive secondary phenolic compounds formed by the bacterial gut microbiota of cattle from plant phenolic compounds. Equol, an isoflavandiol estrogen metabolized from daidzein, is considered the major phenolic compound of biological interest in milk. Equol has shown antioxidant activity by inhibiting reactive oxygen species (ROS) and increasing nitric oxide production in vitro [41]. In addition, as mentioned in previous publications [19,42], SDS-PAGE electrophoresis of liquid cow and camel milk and liquid chromatography coupled to mass spectrometry [43] have shown the presence of several antioxidant proteins such as β-lactoglobulin and α-lactalbumin (dominant proteins in the whey fractions of cow milk) and lactoferrin (major protein of the camel whey fractions).

Previous studies conducted on dairy products have focused on different types of milk (in the liquid state) such as raw milk [44], fermented milk (yogurt and kefir [45]), whole and skimmed ultra-high temperature (UHT) cow milk [46], whereas the antioxidant potential of dried whey and casein fraction have rarely been assessed. Our results indicate that skim dried camel and cow milk, as well as their acid whey and sodium caseinate fractions are endowed with DPPH RSA and that whey fractions had higher DPPH RSA than sodium caseinate fractions. This observation is consistent with the scarce available literature on freeze-dried whole camel milk and camel colostrum [26]. Le Tien et al. (2001) [47] studied the effects of milk protein-based edible coatings on the browning reaction of sliced apples and potatoes. The authors reported that whey protein powder had better antioxidant activity than calcium caseinate, and the difference in antioxidant activity between whey protein and caseinate was attributed to the amino acid profile. In recent studies [23,24],

it was reported that spray drying of camel milk at high inlet temperatures (230–250 °C) and outlet temperature (70–92 °C) resulted in a higher extended Maillard reaction, in comparison to lower temperatures (inlet temperature: 190 °C, outlet temperature: 70 °C) and this was strongly correlated with improved antioxidant properties and low casein solubility, whereas whey protein solubility was preserved. Generally, whey protein's antioxidant activity is attributed to lactoferrin and sulfur-containing amino acids such as cysteine, phosphate, vitamin A and carotenoids. On the other hand, due to differences in phosphate content, milk contains different casein fractions, and these phosphates govern the antioxidant activity of caseins [48]. Salar et al. (2021) [17] reported that spray drying (inlet temperature of 123 °C; outlet temperature of 48 °C) or freeze drying (temperature of—50 °C, low pressure) coupled to low-temperature, short-time pasteurization (57, 60, 63 °C) allows improvement of buffalo colostrum and cow colostrum shelf life and maintaining their bioactivity. The last explanation is also in agreement with the studies by Calligaris et al. (2004) [49] who reported that depending on the time–temperature combinations of heat treatments (at 80 °C, 90 °C and 120 °C) a potential depletion in the overall antioxidant properties of liquid cow milk can be observed. However, only the application of severe heat treatments, associated with the formation of brown melanoidins, allows a recovery and even a possible increase in milk antioxidant properties.

3.2. Principal Components Analysis

Data from the TPC, FRAP and DPPH assays of the dried protein fractions and skim milk were analyzed by principal component analysis (PCA) (Figure 5). 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 plot of component loadings shows that the first two dimensions' account for most of the variance of all quantified variables (51.24% and 33.45%, respectively). Axis 1 is positively influenced by the FRAP and TPC factors: 0.878 and 0.872, respectively. The second axis is positively represented by DPPH activity (0.078).



(**a**) (**b**)

Figure 5. PCA biplot of objects and component loads for the grouping of descriptors for bioactive contents (TPC, DPPH- and FRAP assays) (**a**) and groups of milk fraction samples (**b**). 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; 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. FD: freeze-dried; SD: spray-dried. A-D are the quadrants of the PCA biplot.

Quadrant B is represented by DPPH and FRAP activities with a component of (0.078, 0.995) and (0.878, 0.027), respectively. In this quadrant, it was found that the fractions of acid whey of freeze-dried camel and cow milk formed a cluster. In quadrant C, represented by the TPC, the fractions of freeze-dried skim camel milk, sodium caseinate of camel milk and β-casein of cow and camel milk formed a second cluster. The third and fourth clusters in quadrants A and D had lower antioxidant activities and/or total phenolic contents and generally correspond to the spray-dried fractions.

After PCA and considering the distribution of the different fractions, the skim milk and milk fractions with better antioxidant activities and total phenolic content were selected for gamma radiation treatment (samples in quadrant B and C). They correspond to the following freeze-dried fractions: acid whey of camel and cow milk, β-casein of camel and cow milk, sodium caseinate of camel milk and skim camel milk. Skim freeze-dried cow milk (SCoM) in quadrant D indicated low antioxidant activity and was also selected as a negative control for the irradiation experiments.

3.3. Effect of Gamma Radiation on the Antioxidant Activity of Milk Powder Fractions

The TPC and antioxidant activities of freeze-dried cow and camel milk samples treated with gamma irradiation, assessed using DPPH RSA and FRAP assays are shown in Tables 1 and 2.

Irradiation resulted in changes in the total phenolic content (TPC) of the skim cow milk fraction (S CoM), as the untreated samples had a value of 27.2 ± 2.1 mg GAE /100 g powder and the TPC values almost doubled after irradiation (44.9 ± 2.0 mg GAE /100 g powder at 22 kGy, Table 1). Alothman et al. (2009) [50] pointed out that irradiation (10 kGy) could break the chemical bonds that bind phenolic compounds to other molecules and therefore soluble phenolic compounds would be released. These authors also pointed out that degradation could occur at high doses of irradiation (20 kGy). This is in agreement with our findings. In fact, in the present study, no significant differences were observed in the TPC values of all irradiated cow milk fractions between 5–11 kGy, whereas the TPC value for the acid whey of cow milk (AW CoM) irradiated at 22 kGy decreased, to reach a value of 153.2 ± 2.1 against 209.6 ± 2.1 mg GAE /100 g milk powder for the non-irradiated sample. Similar trends were observed for the TPC of the irradiated camel milk fractions (Table 1). Based on the obtained results, it seems that irradiation has an incremental effect on total phenolic content for complex matrices such as skim milk relying on the phenolic compound release from matrix interactions and potential breakage of larger phenolic molecules into smaller ones. For milk fractions that could have specific phenolic compounds due to the fractionating process, the TPC decreases at higher doses due to compound degradation. Similar explanations were also reported by Madureira et al. (2022) [30] for gamma irradiation of other food matrices.

Table 1. Total phenolic contents and antioxidant activity measured by FRAP and DPPH assays, in extracts of irradiated freeze-dried cow milk fractions. The results are presented as the mean ± standard error.

	Skim Cow Milk (SCoM)				Acid Whey of Cow Milk (AWCoM)				β-Casein of Cow Milk (β CoM)			
	0 kGy	**5 kGy**	**11 kGy**	**22 kGy**	**0 kGy**	**5 kGy**	**11 kGy**	**22 kGy**	**0 kGy**	**5 kGy**	**11 kGy**	**22 kGy**
TPC (mg GAE/100 g powder)	27.2 ± 2.1^{c}	33.9 ± 2.6^{b}	$32.9 \pm 2.6^{b,c}$	44.9 ± 2^{a}	209.6 ± 2.1^{a}	184.9 ± 2^{b}	182.9 ± 2^{b}	153.2 ± 2.1^{c}	247.2 ± 2.1^{b}	244.6 ± 2.3^{b}	241.9 ± 2.7^{c}	259.9 ± 2.7^{a}
FRAP (mmol FSE/100 g powder)	0.17 ± 0.02^{a}	0.18 ± 0.04^{a}	0.19 ± 0.02^{a}	0.21 ± 0.02^{a}	0.42 ± 0.01^{a}	0.44 ± 0.03^{a}	0.42 ± 0.02^{a}	0.34 ± 0.01^{b}	0.61 ± 0.01^{b}	0.65 ± 0.01^{b}	0.69 ± 0.01^{a}	0.21 ± 0.01^{c}
DPPH RSA (%)	10.5 ± 0.3^{d}	14.2 ± 0.4^{c}	16.2 ± 0.1^{b}	20.6 ± 0.1^{a}	40.3 ± 0.2^{a}	39.6 ± 0.11^{b}	36.5 ± 0.3^{c}	33.6 ± 0.3^{d}	ND	ND	ND	ND

In each row, different letters mean significant differences between average values ($p < 0.0001$). TPC: total phenol content; ND: not detected; GAE: gallic acid equivalent; FSE: ferrous sulfate equivalent.

Table 2. Total phenolic contents and antioxidant activity measured by FRAP and DPPH assays, in extracts of irradiated freeze-dried camel milk fractions. The results are presented as the mean ± standard error.

	Skim Camel Milk (SCaM)				Acid Whey of Camel Milk (AW CaM)				Sodium Caseinate of Camel Milk (SC CaM)				β-Casein of Camel Milk (βC CaM)			
	0 kGy	**5 kGy**	**11 kGy**	**22 kGy**	**0 kGy**	**5 kGy**	**11 kGy**	**22 kGy**	**0 kGy**	**5 kGy**	**11 kGy**	**22 kGy**	**0 kGy**	**5 kGy**	**11 kGy**	**22 kGy**
TPC (mg GAE/100 g powder)	90.2 ± 2.5^{b}	94.2 ± 2.1^{b}	93.9 ± 2^{b}	$126.2 \pm 2.5_{a}$	$143 \pm 2^{a,b}$	147 ± 2^{a}	141 ± 2^{b}	$143 \pm 2^{a,b}$	149 ± 3^{a}	74 ± 3^{c}	74 ± 0.6^{c}	104 ± 2^{b}	621 ± 4^{a}	596 ± 4^{b}	592 ± 4^{b}	560 ± 1^{c}
FRAP (mmol FSE/100 g powder)	$0.32 \pm 0.02^{a,b}$	0.3 ± 0.01^{b}	0.26 ± 0.01^{c}	0.33 ± 0.02^{a}	0.69 ± 0.01^{a}	0.64 ± 0.02^{b}	0.58 ± 0.02^{c}	0.57 ± 0.02^{c}	0.16 ± 0.01^{c}	0.21 ± 0.01^{b}	0.09 ± 0.001^{d}	0.26 ± 0.003^{a}	0.55 ± 0.03^{a}	0.43 ± 0.02^{b}	0.42 ± 0.02^{b}	0.45 ± 0.01^{b}
DPPH RSA (%)	7.5 ± 0.1^{a}	7.2 ± 0.5^{a}	4.4 ± 0.3^{b}	ND	48.9 ± 0.4^{a}	45.2 ± 0.4^{b}	32.3 ± 0.2^{d}	38.3 ± 0.2^{c}	13.4 ± 1^{a}	ND	ND	ND	ND	ND	ND	ND

In each row, different letters mean significant differences between average values ($p < 0.0001$). TPC: total phenol content; ND: not detected; GAE: gallic acid equivalent; FSE: ferrous sulfate equivalent.

The fractions of cow and camel milk irradiated at 5 and 11 kGy (Tables 1 and 2) tended to show a decrease in antioxidant DDPH activity compared with the non-irradiated fractions, with the exception of the skim cow milk (S CoM), whose radical scavenging capacity appeared to increase at 5 and 11 kGy and doubled from 10.5 ± 0.3% to 20.6 ± 0.1% after irradiation at 22 kGy (Table 1). FRAP increased slightly from 0.17 ± 0.02 to 0.21 ± 0.2% in skim cow milk (S CoM) irradiated at 22 KGy and remained unchanged in camel skim milk fractions (S CaM). This observation correlated with an increase in the TPC of the corresponding skim milk.

The results presented in Tables 1 and 2 indicate that freeze-dried skim milk and acid whey fractions were the most resistant fractions to the applied irradiation doses (5, 11 and 22 KGy). Meanwhile, the significant increase in the TPC of camel skim milk and the preservation of the phenolic content of acid whey camel milk (AW CaM), β-casein of camel (βC CaM) and cow milk fractions (βC CoM) were not associated with an increase in their antioxidant activity at 22 kGy. On the other hand, irradiation at 5 and 11 kGy did not induce detectable changes in TPC and antioxidant activity if compared to the untreated fractions, and this is generally observed in all irradiated fractions and both types of milk. The differences in the effect of irradiation on TPC may be attributed to the milk type and corresponding composition, and the applied dose of gamma irradiation as reported above. According to the literature, milk is constituted by enzymatic and non-enzymatic antioxidants [51] that act in a multidirectional way. Indeed, these diverse antioxidants can capture ROS, chelate metals or modulate enzymes [52], which can respond differently to processing methods, radiation dose and antioxidant activity assays.

In general, foods exposed to gamma radiation endure direct and/or indirect irradiation effects depending on their state (liquid or powder) and this effect is more pronounced for moisture-rich products [53]. Indeed, it has been reported that a liquid solution such as milk, when exposed to gamma radiation at 25 kGy, produces hydrated electrons and hydroxyl radicals, which can then interact with molecules to create covalent bonds [54]. Gamma radiation may involve alterations to protein conformations, the stimulation of processes such as the oxidation of amino acids and peptide cleavage, and the formation of disulfide bonds by combining aromatic and heterocyclic residues [54]. Kuana et al. (2013) [55] indicated that depending on the dose applied, the exposure time and the type of food proteins, irradiation treatment has been demonstrated to either cross-link (polymerize) or break down (depolymerize) food proteins. This protein coagulation effect induced by gamma radiation could explain the low or the non-detection of DPPH scavenging activity of the casein fraction in our case. On the other hand, the breakdown of antioxidant proteins could justify the decrease in DPPH in the other milk fractions.

Chatterton et al. (2020) [31] demonstrated that gamma radiation at 14 kGy increased the denaturation of unpasteurized bovine colostrum powder, which may be due to protein oxidation promoting the formation of interprotein disulfides, protein homodimers, mixed dimers and aggregates. It has been reported that compared to unpasteurized liquid bovine colostrum, gentle spray drying and gamma radiation (14 kGy) increased protein denaturation by 6% and 11%, respectively, and by 19% and 27% after long-term low-temperature pasteurization and by 48% after short-term high-temperature pasteurization, while gamma radiation had no further effect. They reported that methionine, a protective amino acid against oxygen free radicals, was oxidized by short-term high-temperature pasteurization coupled to gamma irradiation. Robichaud et al. (2020) [33] demonstrated that radiation treatment at different doses (5 and 10 kGy) did not result in any appreciable alteration in the antioxidant properties of milk. Moreover, irradiated liquid formulation samples had lower antioxidant capacity, but there was no difference between samples treated with 5 and 10 kGy. According to the authors, the decrease in antioxidant activity may be related to the depletion of free radical scavengers such as vitamin C and oxidative damage [56]. Chawla et al. (2009) [57] also investigated the effect of radiation processing at different doses (0–100 kGy) on the DPPH radical scavenging activity of whey protein powder. Their results are in agreement with our finding for whey fractions. Indeed, the authors indicated

that DPPH free radical scavenging activity increased with the radiation dose until reaching a linear plateau at the highest dose of radiation. Similar trends were reported by Syed et al. (2021) [29].

The antioxidant activity results obtained for the milk fractions did not follow any specific trend with gamma radiation treatment. Generally, gamma irradiation at 5–11 kGy preserved or enhanced the antioxidant activities of milk fractions. Other antioxidant activity assays (e.g., TBARS, ABTS and cellular antioxidant activity) should be performed to understand and mechanistically characterize the behavior of milk antioxidant compounds and their radiologic products, and ultimately identify them by spectrometry methods.

4. Conclusions

The present work revealed that fractionation of milk by acid and enzymatic coagulation followed by freeze drying or spray drying allowed obtaining dried milk fractions with higher total phenolic content and antioxidant activities than corresponding skim milk. The high levels of total phenol content were generally recorded in freeze-dried milk fractions. Acid whey and β-casein of camel and cow milk showed the highest ferric reducing antioxidant power (FRAP assay) among all fractions and corresponding skim milk. Acid whey from both camel and cow milk seems to maintain potent antioxidant activity whatever the drying process and antioxidant assay used. Generally, irradiation at 5 and 11 kGy doses preserved the total phenolic compounds and antioxidant activity of all analyzed milk powder fractions except for sodium caseinate milk fraction, which initially had low antioxidant activity and was the most sensitive to irradiation. Considering this, gamma radiation in the range of 5–11 kGy could be used to enhance the preservation of powdered milk. Irradiated and non-irradiated freeze-dried skim cow milk, skim camel milk, and acid whey of cow and camel milk fractions seem to be the most interesting to be investigated for further evaluation by different antioxidant assays and for other biological activities (e.g., anti-inflammatory and anti-diabetic activities). To better understand the effect of freeze drying, spray drying and gamma irradiation on the antioxidant properties of camel and cow milk and their corresponding fractions, the main antioxidant compounds could be identified and quantified by a proteomic profiling approach using a liquid chromatography coupled to mass spectrometry.

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