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ORIGINAL PAPER

Functional nanoemulsions from colostrum and camel milk whey proteins with antifungal properties

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ABSTRACT. Camel colostrum and milk are rich sources of bioactive proteins with diverse biological and antimicrobial properties. The purpose of this study was to develop nanoemulsions from enzymatically hydrolysed whey protein concentrates (WPCs) derived from camel colostrum and milk and to evaluate their antifungal activity against multiple *Candida species*. WPCs were obtained by acid precipitation and hydrolysed using Alcalase and pepsin. The hydrolysates were subsequently incorporated into nanoemulsions using high-shear homogenisation followed by ultrasonication. The degree of hydrolysis was determined using the O-phthaldialdehyde (OPA) method. Antifungal activity was assessed using broth microdilution and biofilm inhibition assays against several *Candida* strains. Nanoemulsions derived from colostrum WPC hydrolysates had significantly lower minimum inhibitory concentrations (MICs) and stronger inhibition of biofilm formation compared to formulations prepared from camel milk WPC ($P < 0.05$). The enhanced antifungal activity was attributed to a higher degree of protein hydrolysis, the generation of low molecular-weight peptides, and improved nanoscale dispersion of active components. These findings demonstrate that nanoemulsions formulated based on camel colostrum whey protein hydrolysates represent promising natural antifungal systems and indicate the potential of camel dairy proteins as functional bioactive ingredients for applications related to animal health and sustainable utilisation of dairy resources.

Introduction

The rising prevalence of mucosal fungal infections, particularly those caused by *Candida* species (Schille et al., 2025), and the emergence of antifungal resistance (Chaudhary and Thakur, 2025; van Rhijn and Rhodes, 2025) has intensified the demand for alternative antimicrobial agents derived from natural sources. *Candida* infections are associated with biofilm formation and high resistance to conventional antifungal therapies in both human and veterinary medicine (Bolotnikov et al., 2026; Hizlisoy et al., 2025), indicating the need for new antimicrobial compounds. Milk proteins and peptides have recently gained attention as potential agents due to their natural origin, safety, and multifunctional biological properties (Minj and Anand, 2020; Quintieri et al., 2025).

Milk from various dairy species (bovine, goat, and sheep) contains biologically active components such as lactoferrin, immunoglobulins, lysozyme, lactoperoxidase, and other peptides (Dyrda-Terniuk and Pomastowski, 2023; Kuniyal et al., 2024; Li et al., 2025), which confer antimicrobial, antioxidant and immunomodulatory properties.

Enzymatic hydrolysis of milk proteins can generate bioactive peptides with antibacterial and antifungal activity, which has increased interest in their use as natural preservatives in food, nutraceuticals and veterinary health products (Ali et al., 2022; Murtaza et al., 2022; Soutelino et al., 2024). Several studies have demonstrated antimicrobial activity of hydrolysed whey proteins derived from bovine and small ruminant milk (Alsaloom, 2024; Mudgil et al., 2022), indicating their potential as natural alternatives to standard pathogen-suppressing agents.

Camel milk has attracted increasing scientific interest due to its unique biochemical composition and high levels of defence proteins compared to conventional dairy species (Hasi, 2025). Camel milk contains relatively high concentrations of lactoferrin, immunoglobulins, and other antimicrobial proteins that contribute to its recognised medicinal and therapeutic effects (Abdelazez et al., 2024; Arain et al., 2023; Muthukumaran et al., 2023).These characteristics have positioned camel milk as a promising source of bioactive compounds for the development of functional foods, and animal health products (Abdelazez et al., 2024; Arain et al., 2023).

Colostrum, the first secretion of the mammary gland after parturition, is rich in immune-related proteins, as well as growth factors and antimicrobial components (Alnadari et al., 2025; Eker et al., 2024; Poonia and Shiva, 2022). It contains higher concentrations of immunoglobulins, lactoferrin, lactoperoxidase and other bioactive molecules than mature milk, which are important for passive immunity and neonate protection (Alnadari et al., 2025; Anaya, 2026). Regarding the production of biologically active compounds, whey proteins derived from colostrum are considered a particularly valuable source of antimicrobial activity, as they can serve as precursors of potent antimicrobial peptides following enzymatic hydrolysis (Kumar et al., 2016).

In addition to the generation of bioactive peptides, the delivery system used to introduce these molecules significantly influences their effectiveness (Wang et al., 2025). Nanoemulsion-based systems have gained attention as promising delivery vehicles for bioactive peptides because they improve solubility, stability and interaction with bacteria (Adjonu et al., 2014; He et al., 2023; Madhavi et al., 2025). Nanoemulsions, typically composed of nanodroplets ranging from 20 to 200 nm, can improve surface contact with microbial membranes, penetration into biofilms, and dispersion of antimicrobial compounds (Shukla et al., 2025). These properties make nanoemulsion technology particularly attractive for enhancing the biological performance of protein hydrolysates.

Although camel milk proteins have been investigated for their antimicrobial activities, information on the antifungal potential of hydrolysed camel whey proteins formulated as nanoemulsions remains limited. Moreover, the functional differences between hydrolysates derived from camel milk proteins and those from camel colostrum proteins in antifungal applications have not yet been comprehensively compared.

The aim of the current study was to prepare nanoemulsions from enzymatically hydrolysed camel colostrum and milk whey protein concentrates (WPCs) and to evaluate their antifungal and antibiofilm activities against several clinically significant *Candida* species. This study sought to broaden the functional applications of camel dairy proteins, elucidate their bioactivity for value-added products in animal health and food security, and provide guidance for the sustainable utilisation of renewable animal-derived resources.

Material and methods

Sample collection and preparation

All procedures involving animals were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of King Faisal University.

Camel colostrum and milk samples were obtained from healthy female dromedary camels (*Camelus dromedarius*) maintained under standard farm conditions in the Qatif region, Saudi Arabia. Colostrum was collected within the first 24 h postpartum, while mature milk was obtained from the same animals during the established lactation period (approximately 30–45 days after parturition).

A total of five clinically healthy camels, aged 5–8 years, were included in the study. Milk and colostrum were collected once from each animal using sterile collection procedures. Directly after collection, the samples were transported to the laboratory in insulated containers with ice and processed within 2 h. Upon arrival, the samples were defatted by centrifugation at 4,000 × g for 15 min at 4 °C to remove the cream layer. The resulting skimmed fractions were collected and used for subsequent whey protein extraction and preparation of whey protein concentrates (WPCs).

Preparation of WPCs

WPCs were prepared from skimmed camel milk and colostrum using an acid precipitation method. The pH of the skimmed samples was adjusted to

4.6 using 1 M HCl at 37 °C to precipitate casein proteins. The mixtures were then centrifuged at 4,000 × g for 20 min, and the resulting supernatants containing whey proteins were collected. The whey fractions were sequentially filtered through 0.45 µm and 0.22 µm membrane filters to remove residual particles. The filtrates were subsequently concentrated using a 10 kDa ultrafiltration membrane (Sigma-Aldrich, St. Louis, MO, USA), and the retained whey protein fractions were freeze-dried (lyophilised) to obtain WPC powders derived from camel milk and colostrum.

Enzymatic hydrolysis of WPCs

Enzymatic hydrolysis of the WPCs was performed to generate bioactive peptides. WPC powders were dissolved in appropriate buffer solutions to obtain 5% (w/v) protein suspensions. Two proteolytic enzymes were used: Alcalase (pH 8.0, 50 °C) (Sigma-Aldrich, St. Louis, MO, USA) and pepsin (pH 2.0, 37 °C) (Sigma-Aldrich, St. Louis, MO, USA). Enzymes were added at enzyme-to-substrate ratios of 1, 2, and 4% (w/w). Hydrolysis reactions were carried out for 1, 3, and 6 h with continuous stirring. The reactions were terminated by heating the mixtures at 95 °C for 10 min to inactivate the enzymes. The hydrolysates were then dialysed using a 3 kDa molecular weight cut-off membrane (Sigma-Aldrich, St. Louis, MO, USA) to remove small molecules and salts, followed by lyophilisation to obtain dry hydrolysed whey protein samples for subsequent analysis.

Determination of degree of hydrolysis (DH)

DH of the whey protein hydrolysates was determined using the OPA spectrophotometric method, as described previously (Nielsen et al., 2001). This method quantifies the free amino groups released during enzymatic hydrolysis. Briefly, hydrolysate samples were reacted with OPA reagent, and absorbance was measured at 340 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Serine was used as the calibration standard. The degree of hydrolysis was calculated as the percentage of cleaved peptide bonds relative to the total number of peptide bonds in the protein substrate.

Densitometric analysis

Densitometry was performed to evaluate the relative distribution of peptide fragments generated during enzymatic hydrolysis of camel milk and colostrum whey protein concentrates. SDS–PAGE electrophoretic profiles of the hydrolysates were analysed using ImageJ software (Fiji, NIH, USA). Band intensities corresponding to different molecular weight regions were quantified using the Plot Lanes and Wand Tool functions in ImageJ. Detected bands were grouped into four molecular weight ranges (> 75 kDa, 37–75 kDa, 15–37 kDa, and < 15 kDa) to assess the distribution of peptide fragments after enzymatic hydrolysis. The relative intensity of each band was normalised to the total intensity within each lane and expressed as the percentage contribution of each molecular weight fraction. These densitometric profiles were used to compare the extent of peptide fragmentation patterns under different hydrolysis conditions and between camel milk and colostrum protein hydrolysates.

Preparation of nanoemulsions

Nanoemulsions were prepared by dispersing hydrolysed WPC (1% w/v) in deionised water containing 2% (v/v) Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) as surfactant, following the method of Yang et al. (2023). The mixture was pre-homogenised using a T25 digital ULTRA-TURRAX high-speed homogeniser (IKA, Staufen, Germany) at 12,000 rpm for 5 min, and then ultrasonicated (20 kHz, 400 W, 5 min, 40% amplitude) in an ice bath. Particle size and polydispersity index (PDI) were determined by dynamic light scattering (DLS). Nanoemulsions were stored at 4 °C until further use.

Candida strains and culture conditions

Standard strains were obtained from the American Type Culture Collection (ATCC): *C. albicans* (10231, 14053, 90028), *C. glabrata* (2001), *C. krusei* (14243), *C. kefyr* (2512), *C. rugosa* (10571), and *C. parapsilosis* (90028). Cultures were maintained on Sabouraud Dextrose Agar (SDA) at 4 °C and subcultured before testing.

Antifungal susceptibility testing

Antifungal activity was evaluated using the Clinical and Laboratory Standards Institute (CLSI) M27-A3 broth microdilution method. Two-fold serial dilutions of each nanoemulsion formulation (0.0156–8 mg/ml) were prepared in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) buffered with 3-(N-morpholino)propanesulphonic acid (MOPS).

Standard antifungal agents were included as positive controls: fluconazole (0.125–64 µg/ml) and amphotericin B (0.031–2 µg/ml). Fungal inocula were prepared and adjusted to 2×10^3 CFU/ml. Microtitre plates were incubated at 35 °C for 48 h, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration pro-

ducing ≥ 90% inhibition of visible fungal growth. Appropriate growth, sterility, and drug controls were included in all assays.

Biofilm inhibition assay

Biofilm inhibition was assessed using the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay. *Candida* suspensions (10^6 CFU/ml) were incubated with nanoemulsions at sub-minimum inhibitory concentration (MIC) in 96-well microplates for 24 h to allow biofilm formation.

Following incubation, wells were gently rinsed to remove non-adherent cells. Subsequently, 100 µl of XTT-menadione solution was added to each well and incubated for 2 h at 37 °C. The metabolic activity of the biofilm cells was quantified by measuring absorbance at 490 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA).

Biofilm inhibition (%) was calculated relative to untreated control wells.

Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 10).

Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A P-value < 0.05 was considered statistically significant.

Results

Degree of hydrolysis of camel milk and colostrum WPCs

Enzymatic treatment of camel milk and colostrum WPCs with Alcalase and pepsin resulted in a measurable increase in the DH% at all time points. As shown in Table 1, colostrum WPC showed a significantly higher degree of hydrolysis compared to milk WPC for both Alcalase and pepsin treatments ($P < 0.05$), with maximum DH values of 42% and 38%, respectively, after 6 h of hydrolysis. For Alcalase treatment, DH values ranged from 18% to 42%, while pepsin hydrolysis resulted in DH values of 15–38%, depending on incubation time. The higher DH observed in colostrum samples reflected their higher content of lactoferrin, immunoglobulins, and other proteins susceptible to hydrolysis.

Table 1. Degree of hydrolysis (DH%) of camel milk and colostrum whey protein concentrates (WPCs) following enzymatic hydrolysis

Enzyme	Sample	1 h DH (%)	3 h DH (%)	6 h DH (%)
Alcalase	Camel milk WPC	18^b	28^b	32^b
Alcalase	Camel colostrum WPC	25^a	35^a	42^a
Pepsin	Camel milk WPC	15^c	24^c	30^c
Pepsin	Camel colostrum WPC	20^b	32^b	38^b

Data are presented as mean values (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test within each hydrolysis time point. Comparisons were made between enzyme treatments (Alcalase vs. Pepsin) and sample types (camel milk vs. camel colostrum WPC). Means with different superscript letters within the same column ($^{a–c}$) are significantly different ($P < 0.05$). DH, degree of hydrolysis

Densitometry profiles

Densitometric quantification revealed clear differences in peptide distribution between milk and colostrum-derived hydrolysates. Colostrum samples, particularly those treated with Alcalase, had a higher proportion of lowmolecular-weight peptides, consistent with more extensive proteolysis. In contrast, milk hydrolysates retained a larger fraction of midrange molecular weight peptides, indicating comparatively lower hydrolysis efficiency. These trends, presented in Figure 1, demonstrate that the degree of peptide fragmentation increased in the following order: Milk-Pepsin < Milk-Alcalase < Colostrum-Pepsin < Colostrum-Alcalase, which aligns with the measured DH% values. Although the original SDS–PAGE gel image could not be reproduced, the quantitative densitometric data are sufficient to distinguish hydrolysis patterns across treatments.

Physicochemical properties of nanoemulsions

Nanoemulsions were successfully prepared from all hydrolysed WPC samples using high-shear homogenisation and ultrasonication. Dynamic light scattering analysis showed that mean droplet sizes was 95–130 nm with PDI values < 0.30, indicating uniform nanoscale distribution and good colloidal stability (Figure 2). No significant differences in droplet size were observed between milk- and colostrum-derived nanoemulsions; however, colostrum hydrolysates produced slightly lower PDIs, suggesting improved dispersibility of peptide components. All formulations remained physically stable during refrigerated storage for the duration of the study. Table 2 summarises the droplet size and PDI values for all nanoemulsion formulations.

Antifungal activityagainst *Candida* species

Standard antifungal controls showed expected activity, confirming assay reliability. Fluconazole treatment resulted in MIC values of 0.5–2 µg/ml for fluconazole-susceptible *C. albicans* and

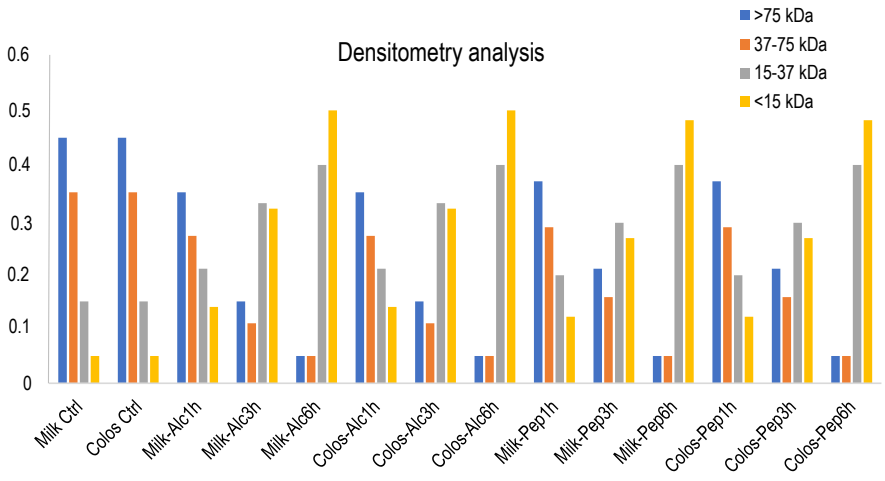

Figure 1. Densitometric quantification of SDS–PAGE band intensities of camel milk and colostrum WPC hydrolysates. Stacked bar plots represent the relative band intensity (arbitrary units) within defined molecular-weight regions: >75 kDa, 37–75 kDa, 15–37 kDa, and <15 kDa.

SDS–PAGE – sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WPC – whey protein concentrate; kDa, kilodalton, Alc – alcalase; Pep – pepsin; Colos – colostrum, Ctrl – control.

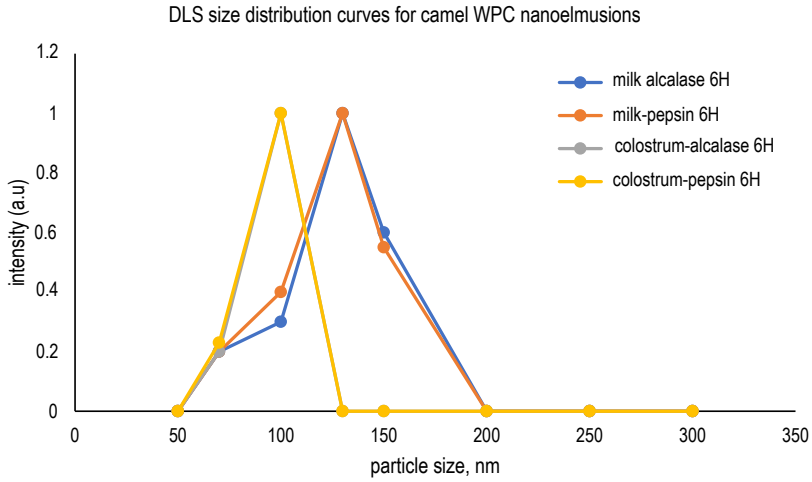

Figure 2. Dynamic light scattering (DLS) size distribution curves for nanoemulsions prepared from hydrolyzed camel milk and colostrum whey protein concentrates. Nanoemulsions derived from camel colostrum whey protein concentrates (WPC) (Alcalase 6 h and Pepsin 6 h) exhibited narrower and left-shifted size distribution profiles (peak intensities at ~95–105 nm), indicating smaller droplet sizes and improved uniformity compared to milk-derived nanoemulsions (peaks at ~120–135 nm). All formulations displayed unimodal distributions with consistent nano-range particle sizes, confirming successful formation of stable nanoemulsion systems. No statistically significant differences were observed ($P > 0.05$).

Table 2. Droplet size and polydispersity index (pdi) of nanoemulsions prepared from hydrolyzed camel milk and colostrum whey protein concentrates (WPCs)

Hydrolysate type	Enzyme	Hydrolysis time, h	Droplet size (nm) ± SD	PDI ± SD
Camel milk WPC	Alcalase	6	125 ± 4.8^{c}	0.27 ± 0.01^{c}
Camel milk WPC	Pepsin	6	130 ± 5.1^{d}	0.28 ± 0.02^{d}
Camel colostrum WPC	Alcalase	6	102 ± 3.5^{b}	0.22 ± 0.01^{b}
Camel colostrum WPC	Pepsin	6	95 ± 3.1^{a}	0.20 ± 0.01^{a}

Data are presented as mean ± standard deviation (SD) (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Comparisons were made among nanoemulsions prepared from different sample types (camel milk vs. camel colostrum) and enzyme treatments (Alcalase vs. Pepsin). Means with different superscript letters within the same column ($^{a–d}$) are significantly different at $P < 0.05$. PDI – polydispersity index.

C. parapsilosis, while *C. glabrata* demonstrated reduced susceptibility (16–32 µg/ml) and *C. krusei* showed intrinsic resistance (MIC > 64 µg/ml). Amphotericin B showed consistent broad-spectrum activity against all strains (MIC 0.25–1 µg/ml), confirming appropriate assay performance. Nanoemulsions prepared from hydrolysed camel colostrum WPC reached significantly lower MIC values compared to those from camel milk WPC ($P < 0.05$). MIC values for colostrum nanoemulsions were in the range of 0.25–1.0 mg/ml, whereas milk nanoemulsions ranged from 1.0 to 4.0 mg/ml. Among all strains tested, *C. albicans* ATCC 90028, *C. albicans* ATCC 14053, and *C. parapsilosis* ATCC 90028 were the most susceptible. Colostrum hydrolysate nanoemulsions demonstrated antifungal activity comparable to

or exceeding that of fluconazole against certain *C. albicans* strains, indicating the therapeutic potential of colostrum-derived peptides. Amphotericin B showed uniform activity for all species (MIC 0.25–1.00 µg/ml), serving as a suitable fungicidal reference (Table 3). Figure 3 presents a comparison of MIC values (mg/ml) for nanoemulsions prepared from hydrolysed camel milk WPC and camel colostrum WPC. Colostrum nanoemulsions consistently demonstrated lower MIC values, indicating higher antifungal activity ($P < 0.01$).

Inhibition of *Candida* biofilm formation

All nanoemulsions demonstrated concentration-dependent inhibition of biofilm formation, as measured by the XTT reduction assay. Biofilm inhibition was significantly higher for colostrum WPC nanoemulsions, reaching 70–86% inhibition at 0.5 × MIC for several strains, including *C. albicans* ATCC 14053, 90028 and *C. parapsilosis* ATCC 90028 (Table 4). In contrast, milk-derived nanoemulsion treatments resulted in 40–60% inhibition under the same conditions ($P < 0.005$).

Table 3. Minimum inhibitory concentrations (MIC, mg/ml or µg/ml) of nanoemulsions and standard antifungal agents against *Candida* species

Candida species	Camel milk WPC nanoemulsion, mg/ml	Camel colostrum WPC nanoemulsion, mg/ml	Fluconazole, µg/ml	Amphotericin B, µg/ml
C. albicans ATCC 10231	2.00 ± 0.20^{b}	0.50 ± 0.10^{a}	1–2	0.25–0.5
C. albicans ATCC 14053	1.50 ± 0.15^{b}	0.38 ± 0.08^{a}	0.5–1	0.25–0.5
C. albicans ATCC 90028	1.00 ± 0.10^{b}	0.25 ± 0.05^{a}	0.5–1	0.25
C. glabrata ATCC 2001	3.00 ± 0.25^{b}	1.00 ± 0.15^{a}	16–32 (reduced susceptibility)	0.5–1
C. krusei ATCC 14243	2.50 ± 0.20^{b}	0.50 ± 0.10^{a}	>64 (intrinsic resistance)	0.5–1
C. kefyr ATCC 2512	2.00 ± 0.18^{b}	0.75 ± 0.12^{a}	1–2	0.25–0.5
C. rugosa ATCC 10571	4.00 ± 0.30^{b}	1.50 ± 0.20^{a}	2–4	0.5–1
C. parapsilosis ATCC 90028	1.50 ± 0.10^{b}	0.38 ± 0.05^{a}	1–2	0.25–0.5

WPC – whey protein concentrates. Data are presented as mean ± standard deviation (SD). $^{ab\neg}$ – means within a row with different superscripts are significantly different at $P < 0.05$. n = 3 independent experiments. Fluconazole and amphotericin B values are presented as reference ranges based on standard susceptibility profiles. Statistical analysis: one-way ANOVA followed by Tukey's post hoc test comparing camel milk vs. colostrum WPC nanoemulsions. Lower MIC values indicate stronger antifungal activity.

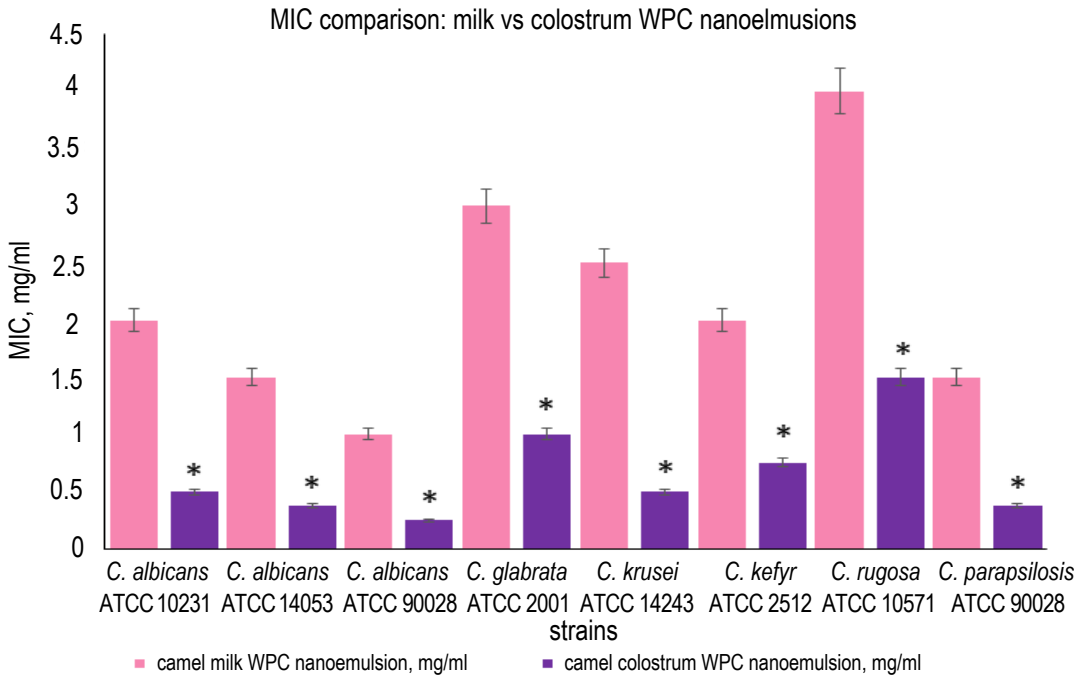

Figure 3. Minimum inhibitory concentration (MIC) comparison between camel milk and camel colostrum whey protein concentrates (WPC) nanoemulsions against eight Candida species. The results presented are averages of three independent experiments each done in triplicate and expressed as the mean + SD. SD – standard deviation. *$P < 0.01$, one way ANOVA with Bonferroni/Dunnett's test compared to camel milk WPC nanoelmusions.

Table 4. Biofilm inhibition (%) of nanoemulsions from hydrolyzed wpc of camel milk and camel colostrum against *Candida* species compared with standard antifungal agents, %

Candida species	Milk WPC nanoemulsion	Colostrum WPC nanoemulsion	Fluconazole	Amphotericin B
C. albicans 10231	55.8 ± 2.5[b]	78.3 ± 3.1[a]	40–60	70–85
C. albicans 14053	48.6 ± 2.1[b]	82.5 ± 2.8[a]	35–55	70–90
C. albicans 90028	60.4 ± 3.0[b]	86.2 ± 2.5[a]	45–65	75–90
C. glabrata 2001	42.7 ± 2.8[b]	69.1 ± 3.2[a]	25–40	65–80
C. krusei 14243	49.9 ± 2.6[b]	81.0 ± 3.0[a]	<20	60–75
C.kefyr 2512	45.5 ± 2.3[b]	70.8 ± 2.9[a]	35–50	70–85
C..rugosa 10571	40.3 ± 2.1[b]	66.7 ± 2.7[a]	20–30	55–70
C..parapsilosis 90028	52.4 ± 2.9[b]	84.5 ± 2.3[a]	35–50	70–85

WPC – whey protein concentrates. Data are presented as mean ± standard deviation (SD). [ab] – means within a row with different superscripts are significantly different at $P < 0.05$., n = 3 biological replicates. Fluconazole and amphotericin B values are presented as reference ranges based on standard susceptibility profiles. Measured by XTT metabolic assay at sub-MIC concentrations (0.5 × MIC). Higher inhibition (%) indicates stronger suppression of biofilm formation

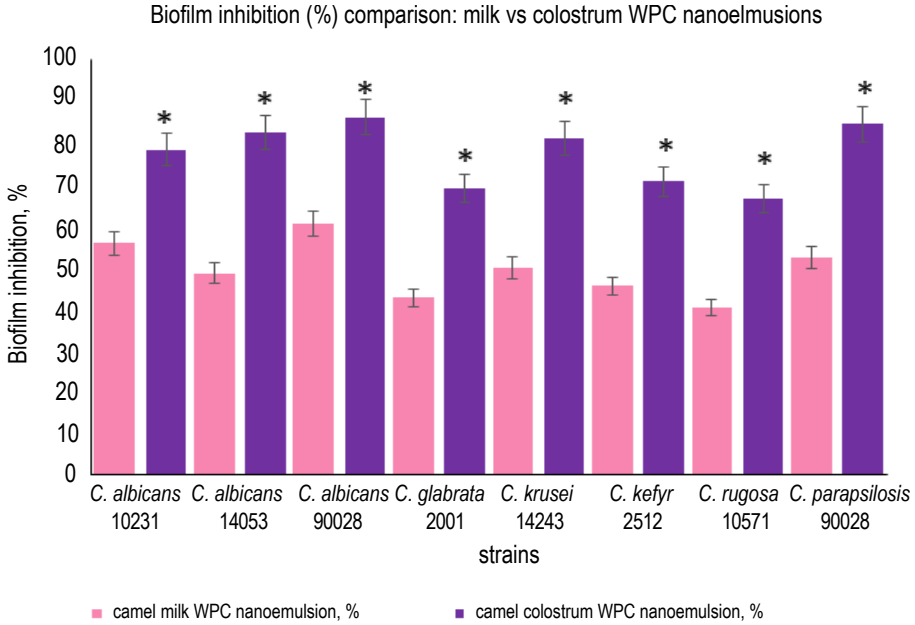

Figure 4. Biofilm inhibition comparison of camel milk and camel colostrum whey protein concentrates (WPC) nanoemulsions against eight Candida species. The results presented are averages of three independent experiments each done in triplicate and expressed as the mean + SD, SD – standard deviation; * $P < 0.01$, one way ANOVA with Bonferroni/Dunnett's test compared to camel milk WPC nanoelmusions.

These results indicate stronger antibiofilm activity of colostrum-derived peptides, consistent with their higher degree of hydrolysis and richer bioactive composition. Figure 4 compares biofilm inhibitory effects (% inhibition) between camel milk and camel colostrum WPC nanoemulsions. Colostrum-derived nanoemulsions showed significantly higher antibiofilm activity against all species, nearly matching the activity of amphotericin B for several strains.

Correlation between degree of hydrolysis and antifungal efficacy

A clear trend was observed, where samples showing higher DH% displayed stronger antifungal activity. For example, Colostrum–Alcalase (6 h), which had the highest DH value (42%), produced the lowest MIC (0.25–0.38 mg/ml) against multiple *Candida* strains and the highest biofilm inhibition (86%). In contrast, lightly hydrolysed samples such as Milk–Pepsin (1 h), with the lowest DH value (15%), demonstrated weaker activity (MIC = 2–3 mg/ml, biofilm inhibition 42%) (Figure 5A and B).

Pearson correlation analysis confirmed a strong negative correlation between DH% and MIC ($r = –0.94$, $P < 0.05$) and a strong positive correlation between DH% and biofilm inhibition ($r = 0.94$, $P < 0.05$). These findings support the interpretation that extensive proteolysis generates low–molecular-weight peptide fractions with enhanced antifungal and antibiofilm activity (Figure 5C).

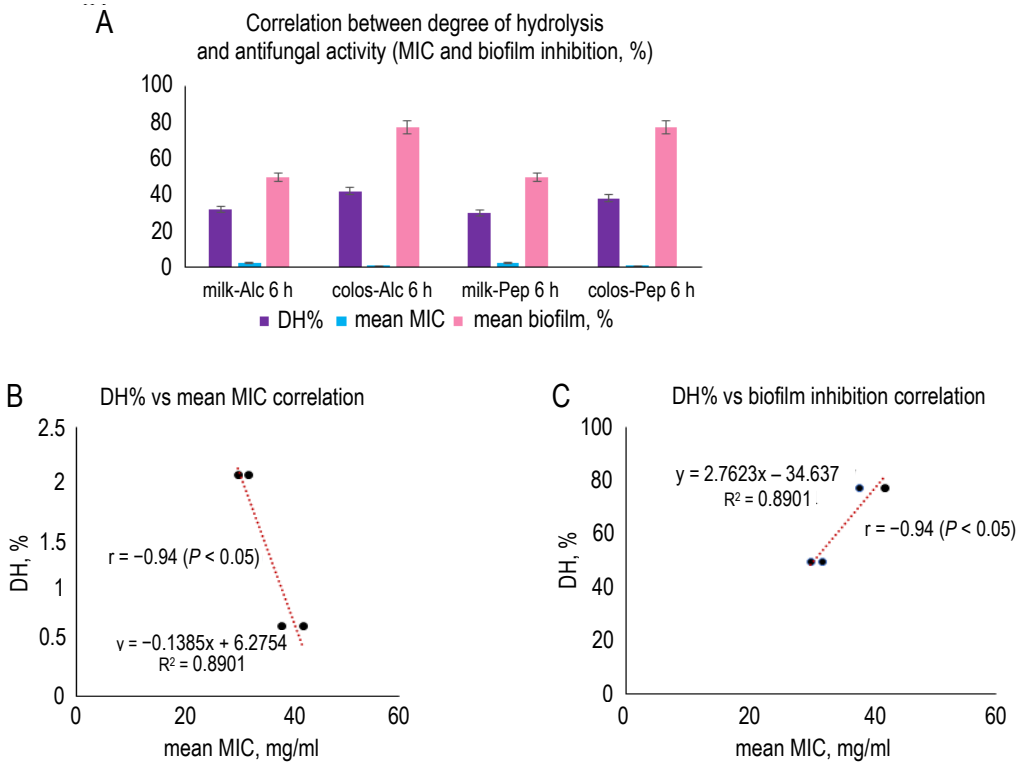

Figure 5. Correlation between degree of hydrolysis and antifungal activity.

(A) Relationship between degree of hydrolysis (DH%) and antifungal activity, expressed as minimum inhibitory concentration (MIC) and biofilm inhibition (%), for milk- and colostrum-derived samples hydrolyzed using Alcalase (Alc) and pepsin (Pep); (B) Correlation between DH% and mean MIC values. (C) Correlation between DH% and biofilm inhibition (%). Pearson correlation coefficients (r) and significance levels are indicated ($P < 0.05$).

Discussion

The present study demonstrates that nanoemulsions derived from enzymatically hydrolysed camel whey protein concentrates exert significant antifungal and antibiofilm activity against various *Candida* species. Interestingly, nanoemulsions derived from camel colostrum whey protein hydrolysates consistently showed greater antifungal potency than those prepared from mature camel milk proteins. This increased activity can be attributed to the higher content of bioactive proteins found in colostrum and low-molecular-weight peptide formation through enzymatic hydrolysis.

The results indicate that enzymatic hydrolysis plays a critical role in activating the antifungal potential of camel whey proteins. Proteolytic enzymes such as Alcalase and pepsin cleave intact milk proteins into smaller peptides that may possess antimicrobial properties (Freitas and Felipe, 2023). Similar findings have been reported previously, demonstrating that hydrolysates of camel and bovine milk proteins are more active against microbes than intact proteins. For example, Mudgil et al. (2022) reported that enzymatically hydrolysed camel milk proteins inhibited the activity of several *Candida* species, indicating that peptides released during hydrolysis contributed significantly to antifungal effects.

In addition to antifungal peptides, certain fatty acids naturally present in whey can also influence *Candida* growth and morphology (Clément et al., 2007, 2008; Utama et al., 2024). Free fatty acids in bovine whey are known to inhibit the germination of *C. albicans*, indicating that these small lipid molecules may contribute to antifungal activity, especially when efficiently dispersed in a nanoemulsion (Clément et al., 2007). However, the substantially higher activity observed in hydrolysed samples compared to the non-hydrolysed controls suggests that bioactive peptides are the main factors responsible for the antifungal effects under the present experimental conditions.

A key finding of this study is that nanoemulsions prepared from colostrum hydrolysates showed stronger antifungal activity than those from milk hydrolysates. Colostrum contains substantially higher levels of immune-related proteins such as lactoferrin, immunoglobulins, and antimicrobial enzymes (Loushigam et al., 2026; Rasheed, 2017; Yu and Satyaraj, 2025). These proteins can generate potent antifungal peptides upon enzymatic hydrolysis (Dadashi et al., 2026; Usha and Aparna, 2026).

Previous research has demonstrated that camel lactoferrin has strong antimicrobial and antifungal properties, partly due to its capacity to disrupt microbial membranes and limit iron availability required for microbial growth (Alkhulaifi et al., 2024; Mohamed and Emam, 2017; Niaz et al., 2019). The greater abundance of such bioactive proteins in colostrum likely explains the higher antifungal efficacy observed in the present study.

In addition to peptide generation, the nanoemulsion delivery system likely contributed to the enhanced biological activity. Nanoemulsions with particle sizes of approximately 100 nm improve dispersion and increase surface contact with microbial cells (Marena et al., 2023). This nanoscale distribution may facilitate interaction between bioactive peptides and fungal cell membranes, thereby strengthening their antifungal action (Jayathilaka et al., 2022). Previous studies have shown that nanoemulsion-based delivery systems can significantly improve the antimicrobial effectiveness of bioactive compounds by increasing solubility, stability, and penetration into microbial biofilms (Marena et al., 2023). The strong inhibition of *Candida* biofilm formation observed in this study supports the potential of nanoemulsion-based formulations as effective delivery platforms for antimicrobial peptides.

The tested *Candida* species showed varying susceptibility, with *C. albicans* and *C. parapsilosis* generally being more sensitive to the nanoemulsion formulations than species such as *C. glabrata* or *C. rugosa*. Similar patterns of species-specific susceptibility have been reported in earlier antifungal research and are likely related to differences in cell wall composition, membrane structure, and intrinsic resistance mechanisms between *Candida* species (Mudgil et al., 2022). The capacity of colostrum-derived nanoemulsions to inhibit multiple species, including strains with reduced susceptibility to conventional antifungal drugs, indicates their potential as broad-spectrum antifungal agents.

The findings of this study highlight the potential of camel colostrum whey proteins as valuable sources of natural antifungal peptides. The combination of enzymatic hydrolysis and nanoemulsion technology improves both the biological activity and delivery efficiency of dairy-derived bioactive compounds. From an animal science perspective, these results support the development of functional formulations derived from camel dairy proteins that may improve animal health, farm hygiene, and sustainable utilisation of animal-derived bioresources. Such natural bioactive systems may also find applications in veterinary medicine, food preservation, and the alternative antimicrobial strategies. In addition, these results are consistent with the One Health concept, as they promote the development of natural antimicrobial agents from animal-derived materials that may reduce reliance on synthetic antifungal drugs.

Limitations and future perspectives

Although the results demonstrate promising antifungal activity *in vitro*, several limitations should be considered. The study did not evaluate the long-term stability or detailed physicochemical properties of the nanoemulsions beyond initial characterisation, and biological activity was assessed exclusively using *in vitro* assays. Future research should therefore investigate the stability, safety, and efficacy of these formulations in more complex biological systems. Further identification of the specific peptide sequences responsible for antifungal activity would also provide valuable insight into the mechanisms underlying the observed effects.

Conclusions

Nanoemulsions formulated from hydrolysed whey protein concentrates of camel milk and colostrum demonstrated clear antifungal activity against multiple *Candida* species, with colostrum-derived formulations showing higher potency and stronger biofilm inhibition. These findings indicate that camel colostrum whey proteins are a useful source of bioactive dairy components with improved biological activity when delivered in nanoemulsion systems. The study extends the application of camel-derived proteins beyond conventional nutritional roles and supports their application in functional formulations related to animal-derived products and more efficient use of dairy resources.

Statement of novelty

This study presents a novel functional application of camel milk and colostrum whey proteins through the development of nanoemulsions based on enzymatically hydrolysed whey protein concentrates. While camel milk bioactive components have been widely studied for their nutritional and antimicrobial properties, their formulation into peptide-based nanoemulsions with antifungal activity has not been reported. The combination of controlled protein hydrolysis with nanoscale delivery represents an innovative strategy to improve the biological activity and stability of dairy-derived peptides, particularly against biofilm-forming *Candida* species.

From an animal science perspective, this work highlights the potential of camel whey proteins as multifunctional bioactive components with applications other than nutrition. The strong antifungal and antibiofilm activity demonstrated by colostrum-derived nanoemulsions suggest potential use in livestock health, farm hygiene, and functional animal products. These findings expand the functional scope of camel milk bioactives and support their development as natural alternatives aligned with sustainable animal production and One Health principles.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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