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Biochemical Investigation of Goat Milk Casein and Whey Protein Crude Methanol Extract

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Abstract

Milk and milk products is implicated in many human health benefits. Several animals milk source is used for that porous such as cow, cheap, camel and goat. Goat milk has a unique composition compared to other animals. In this study, we investigate the antioxidant, antibacterial and anticancer potencies of casein and whey proteins of goat milk fractions crude methanol extract. As a result, whey protein extract exhibited high antioxidant activity than casein against stable radical DPPH and ABTS with IC_{50} value 0.12 and 0.237 µg/ml, respectively compared to casein fraction extract. The anticancer properties were investigated against two cancerous cell lines, including: breast (MCF-7) and liver (HepG2). Results of the MTT assay were in agreement of the antioxidants activity, when whey fraction methanol extract exhibited specific anticancer activities against MCF-7 cell line compared to casein and doxorubicin positive control. However, no cytotoxic activities of extract were observed against two non-cancerous control cell lines (BJ-1and MCF-12). Casein and whey proteins extract could prevent DNA damage induced by oxidative stress. The antimicrobial activity of goat milk methanol extract was tested against five bacterial strains including gram positive (*Bacillus cereu* EMCC 1080 and *Staphylococcus ureuse* ATCC 13565), gram negative (*Escherichia coli* O157-H7 ATCC 51659, *Salmonella typhi* ATCC 15566 and *Pseudomonas aeruginosa* NRRL B-272. Among them, *Bacillus cereu* and *Escherichia coli* were extensively affected, when the inhibition zone was 4.3 and 5 cm, respectively. The fact that goat milk is enriched in features belonging to elements, vitamins and short peptide derivatives. In addition, goat milk has specific fat composition of the medium chain fatty acids led to increase the antioxidant and antibacterial and anticancer properties.

Keywords: *Goat milk, Breast cancer, Antioxidant, Antibacterial, whey proteins*

1. Introduction

Milk is one of the most important physiological fluid which contains high nutritional elements, including energy, proteins, vitamins and minerals. Milk derived proteins are a unique component that has been reported to reduce human health risk [1]. Several reports indicated that milk components exhibit different biological properties due to the milk-derived peptides and proteins [2]. Milk proteins are divided between whey protein and casein which contain approximately 20% and 80%, respectively. Five major proteins are identified in whey, including α-lactalbumin, glycomacropeptide, β-lactoglobulin, immunoglobulins and serum albumin. While, milk casein has four different subunits αs1, αs2, β and κ casein [3-5]. However, it has been reported that milk fat also enriched in several potential components, including conjugated linoleic acid, sphingomyelin, butyric acid and ether lipids [6]. Conjugated linoleic acid exhibited anti-proliferation potency against wide range of human cancer cell lines, such as colorectal, breast and lung cancer cell lines [7-9].

Goat milk has been used as human nutrient especially for people who have lactose intolerance problem and sensitive to other animals' milk. Goat milk contains a mixture of nutrients which are important for human health such as fat, protein, lactose, vitamins, enzymes and mineral salts. The levels of these nutrients are higher than other milk producing animals. For instance, goat's milk contains 25% more vitamin B6, 47% more vitamin A and 13% more calcium compared to cow's milk [10].

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However, goat milk is preferred to cow's milk in many countries in the world, specially Gulf area because of its high contents of inherent antibodies [11-13]. In addition, goat milk fat contains high percentage of (15%) medium chain fatty acids caproic 6:0, caprylic 8:0 and capric acid 10:0. These three fatty acids give improperly handled goat milk its characteristic off-taste and smell [14-15]. Moreover, goat milk is also used as therapy against different problems including gastrointestinal disturbances, vomiting, colic, diarrhea, constipation and respiratory problems [16].

2. Materials and methods:

2.1. Chemicals and supplies

All organic solvents were analytical grade reagents (AR) procured from Merck Chemical Inc. (Darmstadt, Germany). MTT (3-[4,5-dimethylthiazol-2yl]-2.5-diphenylterazolium bromide), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was obtained from Sigma Aldrich company (St Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM)/high glucose, L-glutamine and penicillin/streptomycin were purchased from Gibco Inc. (NY, USA).

2.2. Preparation of caseins and whey proteins

Goat milk used in study was obtained from the herd of Faculty of Agriculture, Cairo University, Giza, Egypt. caseins and whey fractions were separated according to the method by (Chiang and Chang [17] with slightly modfications. In brief, one litter of goat milk was adjusted to pH 4.0 using 1.0N HCl while stirring, the mixture was centrifugation at 5000 rpm for 30 min. the pellet was collected was considered as casein fraction. The supernatant was transferred into a clean tube and the pH was adjusted to 7.0 with 1.0N NaOH, followed by centrifugation again at 5000 rpm for 30 min. The final supernatant collected was considered as whey proteins fraction. Casein and whey proteins were stored at −20°C until use. Casein and whey protein fractions were extracted using 200 ml of methanol 80% in an ultrasonic bath for 3 h at 40℃. The mixture was filtered through whatman filter paper 0.45 µm (cellulose acetate, Sigma, USA). After cooling, the mixture was evaporated by rotary evaporator and lyophilization to produce the dried methanol extracts.

2.3. Antioxidant activity

2.3.1. DPPH assay.

Total free radical scavenging capacity of the casein and whey proteins extract were evaluated by using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical Ye et al., [18] with slightly modification. In DPPH assay, different concentration (0.05, 0.1, 0.25, 0.5 and 1 µg/ml) of extract in final volume 100µl were added to 900 µl of 0.1mM DPPH solution. The mixture was vortexed and kept at room temperature for 30 min in dark. The absorbance of the reaction mixture was measured at 517 nm spectrophotometrically.

Antioxidant % = $(A_{control}–A_{sample})/A_{control})\times100$; where, $A_{control}$ is the absorbance of the DPPH radical without extract; A_{sample} is the absorbance of the extract at t= 30 min. A calibration curve was plotted with % DPPH scavenged versus concentration of the standard antioxidant butylated hydroxytoluene (BHT).

2.3.2. ABTS radical scavenging activity

The potential of ABTS radical scavenging of casein and whey proteins extract were also measured using a modified method described by Floegel *et al*., [19]. Briefly, 980 µL of ABTS solution was added to a mixture of 20 µL containing different concentration (0.05, 0.1, 0.25, 0.5 and 1 µg/ml) of each sample. The mixture was then set to react at 37 °C for 10 min in dark. The absorbance was measured at 734 nm and the ABTS radical scavenging percent is calculated using the equation:

% Inhibition = $[(A_0 – A_1) /A_0] X 100$ (A_0 is the $ABTS^{\bullet+}$ absorbance of the control reaction and A_1: is the ABTS absorbance of the sample).

2.4. DNA damage protection.

In this assay we investigate the potential protection activity of casein and whey protein fractions extract against DNA damage induced by oxidative stress using Fenton's reagents was investigated as it has been previously reported by Leba *et al*., [20]. Three different concentration of *casein and whey protein methanol extract* (0.25, 0.5 and 1 µg/ml) were added to a mixture containing 2µl of Ribonuclease Inhibitor (RNH1) plasmid DNA (90µg/µl), 5mM of H_2O_2, 0.35mM of $FeSO_4$, 0.60mM of EDTA and the final volume was completed to 25µl with phosphate buffer 8.3mM, pH 7.4. After 20 min of mixture incubated for at 37°C, the mixture was loaded into agarose gel 1.5% and separated bands were analyzed. RNH1 plasmid DNA (3µl + 23µl of phosphate buffer) were used as DNA protection control and RNH1 plasmid DNA (3µl + Fenton's reagent) were used as DNA damage control.

2.5. Antibacterial activity

Two different concentrations casein and whey proteins extract (0.05, 0.1, 0.25, 0.5 and 1 µg/ml) were tested for their antibacterial activity against different strains of bacteria, including gram positive (*Bacillus cereu* EMCC 1080**)**, gram negative (*Escherichia coli* O157-H7 ATCC 51659) according to (Sah et al. 2016 [21]). Antibacterial activity was

conducted using well diffusion assay on nutrient agar medium. The inhibition zones were measured in millimeters (mm) using the disk diffusion assay method.

2.6. In vitro cytotoxicity

All cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Two human cancer cell lines were used in this study, mammary adenocarcinoma (MCF-7, ATCC® HTB-22™) and hepatocellular carcinoma (HepG2, ATCC® HB-8065™), as well as two normal cell lines, skin fibroblast BJ-1 (ATCC® CRL-2522™) and epithelial breast MCF-12 (ATCC® CRL-10782™) were used. Cell lines were cultured in DMEM/high glucose media supplemented with 2 mM L-glutamine, 10% FBS and 1% penicillin/streptomycin kept in Corning® 75cm² U-Shaped canted neck cell culture flask with vent cap (Corning, New York, USA). Then, sub-confluent cultures (70–80%) were trypsinized (Trypsin 0.05%/0.53 mM EDTA) and spilt depending on the seeding ratio [22, 24].

2.6.1. MTT assay

Cells (1×10^5/well) were plated into 100 µl of medium/well in 96-well plates (Hi media). After 48 h incubation the cell reaches the confluence. Then, the media was replaced with RPMI-1640 media containing different concentration of casein and whey protein fractions extract. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-- tetrazolium bromide cells (MTT) phosphate- buffered saline solution were added. After 4h incubation, 130 µl 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm with reference at 655nm. The concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of cancer and normal cell lines (mentioned above) were expressed as cytotoxicity %, using the following formula**:**

Cytotoxicity % = 100- A_{570} of treated cells / A_{570} of control cells × 100% [25].

*2.7. **Statistical analysis:***

Statistical analyses were conducted by use of CoStat for Windows version 6.45. All data were represented as mean ± standard deviation (SD) with 3 individual experiments each in duplicate. Treatments were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Antioxidants activity on DPPH and ABTS.

Previous studies have reported that milk and milk products displayed antioxidant activities by different mechanisms [26]. The antioxidant activity of casein and whey protein extracts were measured by the potential scavenging of two artificial radicals ABTS and DPPH. As a result, whey protein fraction extract exhibited higher antioxidant activity compared to casein fraction extract (Table 1 and 2). The results also indicated that the antioxidants activity values were increased in response to higher concentrations. The concentration that induce 50% of the radicals scavenging IC_{50} was calculated as observed in Table 1 and 2. The IC_{50} data indicated that whey protein fraction extract was lower compared to casein fraction extract, with IC_{50} 0.12 and 0.237 µg/ml for DPPH and ABTS, respectively. The results indicated that whey protein fraction extract might contain valuable components, that have the potential radical scavenging activity. In this context, milk and milk products have been shown high antioxidant activity for their unique mixture of substances including vitamins and short peptides increasing the antioxidants activity [27]. It has been reported that goat whey protein displays antioxidant activity against numerous free radicals, such as $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ in vitro as well as $OH^{\bullet}$ and $O_2^{\bullet-}$ that normally exist in the organisms [28]. Furthermore, fermented goat milk also released great amount of antioxidant peptides. During fermentation process, yeasts and lactic acid bacteria are produce antioxidant peptides [29]. Other strains such as *L. casei* L61, produces antioxidant peptides with high scavenging capacity of fermented goat milk [30,31].

Table 1. Antioxidant activity and IC_{50} of goat milk casein methanol extract.

Extract (µg/ml)	Antioxidant activity%		IC_{50} (µg/ml)	
	DPPH	ABTS	DPPH	ABTS
0.05	22.14	19.21		
0.1	36.11	31.16	0.281 [a] ± 0.0010	0.423[b] ± 0.0012
0.25	49.24	47.36		
0.5	57.78	54.32		
1	73.23	69.65		

All experiments were performed in triplicate; all data are expressed as the mean ± SD.
Means with different letters are significantly different at $p \leq 0.05$.

Table 2. Antioxidant activity and IC_{50} of goat milk whey protein methanol extract.

Extract (µg/ml)	Antioxidant activity %		IC_{50} (µg/ml)	
	DPPH	ABTS	DPPH	ABTS
0.05	35.43	28.54		
0.1	46.65	39.98	0.121[a] ± 0.0010	0.2375[b] ± 0.0012
0.25	58.542	53.76		
0.5	75.32	66.36		
1	87.96	78.43		

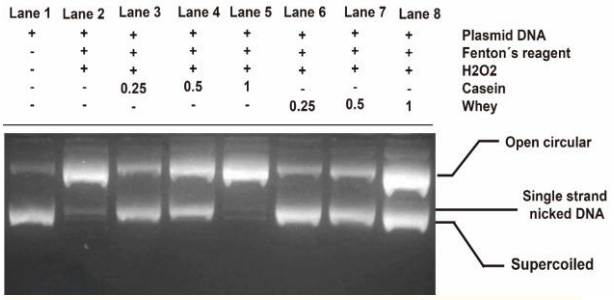

Figure 1. DNA damage protection capacity of casein and whey protein of goat milk fractions extract. Lane 1: *RHN1* DNA Plasmid, Lane 2: *RHN1* DNA Plasmid treated with Fenton's reagent, Lane 3-5: casein fraction, Lane 6-8: whey fraction at three concentrations (0.25, 0.5 and 1 µg/ml), respectively. All the reaction mixtures were incubated for 20 min at 37°C.

3.2. DNA damage protection.

The DNA damage protection induced by Fenton's reagent using different concentrations (0, 0.25 and 0.5 and 1 µg/ml) of casein and whey extracts were analyzed. In agreements with our antioxidant obtained data, *RHN1* DNA supercoiled circular band was intense compared to the linear band in both extracts. These data indicated that casein and whey proteins of goat milk fraction has a potency to protect DNA from degradation using Fenton's reagent (Figure 1; lane 3-8). Surprisingly, casein showed DNA damage at 1 µg/ml of that can be due to an increase of some minerals such as bivalent cations that converted to pro-oxidants and therefore induces DNA damage (Figure 1; lane 5). Altogether, the results indicated that whey protein of goat milk extract enhances the protection capacity of DNA damage induced by Fenton's reagent compared to respective controls.

3.3. Antimicrobial activity of casein and whey proteins fraction extract

The antimicrobial activity of casein and whey proteins goat milk fractions was expressed in Table 3. Different concentrations were tested in this assay (0.1, 0.25, 0.5 and 1 µg/ml) for each fraction extrat. The obtained data showed that whey protein fraction extract showed antibacterial activity against *Bacillus cereus* and *E. coli* (Table 3 and Figure 2). *E. coli* recorded the highest inhibition zones among ranged from 1.3 to 4.64 cm in response to higher concentrations Table 3 and Figure 2. While the inhibition zone of *B. cereus* was ranged from 0.003 to 2.8 cm, these data indicated that whey fraction methanol extract including several nutrients that implicated in bacterial treatment. For casein fraction extract no significant differences were observed compared to whey protein fraction extract and miconazole antibacterial positive control Table 3.

Table 3. Antibacterial activity of whey fraction extract.

	Zone Inhibition (cm)					
Conc µg	Whey fraction		Casein fraction			
	B. cereus	*E.coli*	*B. cereus*	*E.coli*	Miconazole	LSD
0.1	0.0031± 0.57[a]	1.3 ±1.00[a]	0.0031± 0.001[a]	0.0015 ±0.0001[a]	1.3 ±0.02[a]	1.21
0.25	1.7±0.56[b]	2.1±1.15[a]	0.047± 0.006[b]	0.0021±0.0003[a]	2.6±0.15[a]	1.33
0.5	2.2±0.0[b]	3.2±2.00[a]	0.062±0.00[b]	0.0027±0.00[a]	4.43±1.00[a]	1.95
1	2.8±0.58[b]	4.6±1.53[a]	0.071±0.001[b]	0.0036±0.003[a]	6.34±1.53[a]	1.63

All experiments were performed in triplicate; all data are expressed as the mean ± SD.; Means with different letters (at the same row) are significantly different at $p \leq 0.05$.; *LSD* = Least Significant Difference.

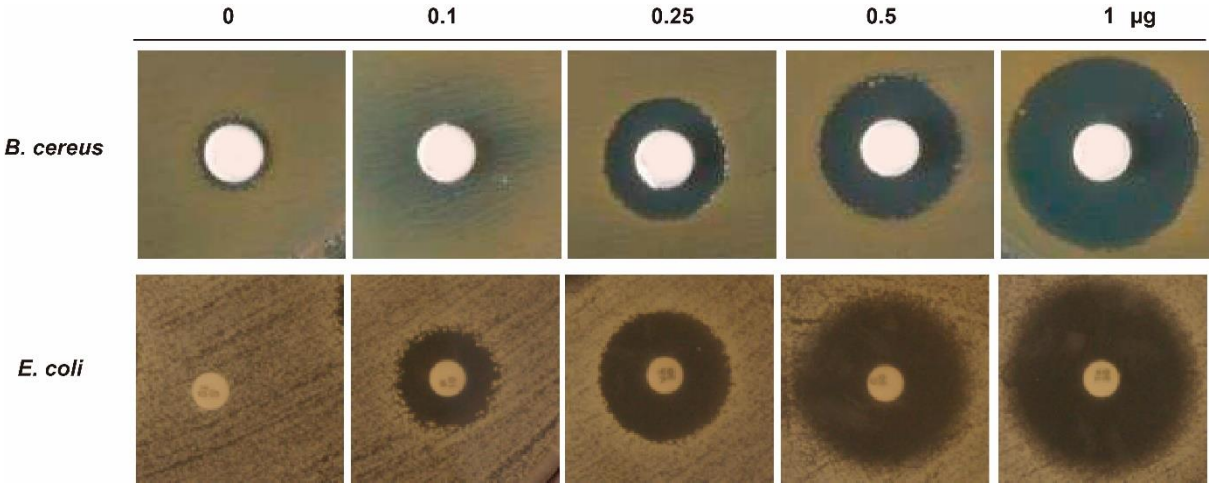

Figure2. Antibacterial activity of whey protein goat milk fraction methanol extract. Five different concentrations (0, 0.1, 0.25.0.5 and 1 µg extract) were tested against two bacterial strains *B. cereus* (gram positive) and *E. coli* (gram negative) The inhibition zone was measured using imagej software.

3.4. Anticancer activity of casein and whey goat milk fractions

Cancer therapy is a global health challenge worldwide [34]. In this study, casein and whey goat milk fractions extract were tested for their anti-proliferation activity against two cancerous and two normal human cell lines as showed in Table 4. Results showed that, whey protein extract at 2 µg/ml had the highest potential cytotoxic activity against MCF- and 7 HepG-2 with 78.03 and 57.06 %, respectively (Table 4). Whereas no significant cytotoxic activity was observed against MCF12F and Bj-1 normal cell line with 1.35 and 1.18%, respectively. The cytotoxic effects of casein fraction extract against HepG-2 and MCF-7 were 57.07 and 22.5 % cell death, respectively. Our results indicated that, whey protein fractionated from goat milk reduces the cytotoxic effect on MCF-12F and Bj-1 compared to doxorubicin control.

It has been previously reported that goat fermented milk display anticancer properties through cell cycle arrest, modulation of immune system, and apoptosis induction [35]. In addition, goat milk and its fermented products kill the cancer cell via apoptosis induction and therefore inhibition of proliferation [36]. Our results revealed that, whey protein of goat milk has high pharmaceutical properties, including the antioxidants, antibacterial and anticancer activities compared to casein fraction.

Table 4. Anticancer activity and IC_{50} of casein and whey protein goat milk methanol extract.

	Cytotoxicity % at 2 µg/ml			IC_{50} µg/ml		
Cell line	Casein	Whey	Doxorubicin	Casein	Whey	Doxorubicin
MCF-7	57.5^{a}± 1.4	78.03^{b}±0.4	89.2^{c}±0.05	1.73^{c}±0.46	1.27^{b}±0.68	1.12^{a}±0.9
MCF12F	1.6^{a}±0.40	1.35^{b}±0.03	18.05^{c}±0.01	62.5^{c}±2.7	74.07^{b}±3.4	5.54^{b}±1.4
HepG2	22.5^{a}±0.87	57.06^{b}±0.67	78.06^{c}±0.02	4.4^{c}±0.6	1.75^{b}±0.7	1.75^{b}±0.7
BJ-1	1.4^{b}±0.023	1.18^{c}±0.05	13.76^{a}±0.06	71.42^{a}±5.9	84.74±13.2	7.26^{b}±1.3

All experiments were performed in triplicate; all data are expressed as the mean ± SD.; Means with different letters (at the same row) are significantly different at $p \leq 0.05$.

4. Conclusions

The fact that milk and milk products considered very rich fluids in its nutritional values. Our results indicated that, goat milk whey protein fraction was much higher in its pharmaceutical poteincies as antioxidants, anticancer and antimicrobial. Thus, the present study could distinguish between two important protein fractions of goat milk for human diseases risk reduction. Also, it had antimicrobial activity against tested bacteria.

Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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References

1. Da Costa, W. K. A., De Souza, E. L., Beltrão-Filho, E. M., Vasconcelos, G. K. V., & Santi-Gadelha, T. (2014). Comparative protein composition analysis of goat milk produced by the Alpine and Saanen breeds in Northeastern Brazil and related antibacterial activities. *PLoS ONE*, 9(3), e93361.

2. Baig, H., Ahmed, D., Zara, S., Auzla, M. I., & Asghar, M. N. (2011). *In vitro* evaluation of antioxidant properties of different solvent extracts of *Rumex acetosella* leaves. *Oriental Journal of Chemistry*, 27, 1509– 1516.

3. Kapadiya, D. B., Prajapati, D. B., Jain, A. K., Mehta, B. M., Darji, V. B., & Aparnathi, K. D. (2016). Comparison of Surti goat milk with cow and buffalo milk for gross composition, nitrogen distribution, and selected minerals content. *Veterinary World*, 9, 710– 716.

4. Genene, A., E. B. Hansen, M. Eshetu, Y. Hailu, and R. Ipsen. 2019. Effect of heat treatment on denaturation of whey protein and resultant rennetability of camel milk.

5. Ahmed, A. S., El-Bassiony, T., Elmalt, L. M., & Ibrahim, H. R. (2015). Identification of potent antioxidant bioactive peptides from goat milk proteins. *Food Research International*, 74, 80– 88.

6. Park, Y. (2006). Goat milk chemistry and nutrition. In Y. W. Park & G. F. W. Hanlein (Eds.), *Handbook of milk of non-bovine mammals* (pp. 34– 58). London, UK: Blackwell Publishing

7. Hassan, Z. M., El Din, H. M., Ali, A. A., Mehanna, N., & El-Messery, T. M. (2013). Interaction of some low molecular weight phenolics with milk proteins. *World Applied Science Journal*, 23, 182– 187.

8. Alyaqoubi, S., Abdullah, A., Samudi, M., Abdullah, N., Addai, Z. R., & Al-Ghazali, M. (2014). Effect of different factors on goat milk antioxidant activity. *International Journal of ChemTech Research*, 6, 3191– 3196.

9. Bučević-Popović, V., Delaš, I., Međugorac, S., Pavela-Vrančić, M., & Kulišić-Bilušić, T. (2014). Oxidative stability and antioxidant activity of bovine, caprine, ovine and asinine milk. *International Journal of Dairy Technology*, 67, 394– 401.

10. Amati, L., Marzulli, G., Martulli, M., Tafaro, A., Jirillo, F., Pugliese, V., Jirillo, E. (2010). Donkey and goat milk intake and modulation of the human aged immune response. *Current Pharmaceutical Design*, 16, 864– 869.

11. Attaie, R., & Richter, R. L. (2000). Size distribution of fat globules in goat milk. *Journal of Dairy Science*, 83, 940– 944.

12. Jirillo, F., Jirillo, E., & Magrone, T. (2010). Donkey's and goat's milk consumption and benefits to human health with special reference to the inflammatory status. *Current Pharmaceutical Design*, 16, 859– 863.

13. Kerasioti, E., Stagos, D., Tzimi, A., & Kouretas, D. (2016). Increase in antioxidant activity by sheep/goat whey protein through nuclear factor-like 2 (Nrf2) is cell type dependent. *Food and Chemical Toxicology*, 97, 47– 56.

14. Singh, B., Mal, G., & Marotta, F. (2017). Designer probiotics: Paving the way to living therapeutics. *Trends in Biotechnoloy*, 35, 679– 682.

15. Wang, X., Zhao, X., Huang, D., Pan, X., Qi, Y., Yang, Y., … Cheng, G. (2017). Proteomic analysis and cross species comparison of casein fractions from the milk of dairy animals. *Scientific Reports*, 7, 43020.

16. Haenlein G.F.W. (2004). Goat milk in human nutrition. *Small Rumin. Res.*

17. Chiang SH, and Chang CY, (2005). Antioxidant properties of caseins and whey proteins from colostrums. *J Food Drug Anal*, 13, 57-63

18. Ye M., Ren L., Wu Y., Wang Y., Liu Y. (2013) Quality characteristics and antioxidant activity of hickory-black soybean yogurt. *LWT-Food Sci. Technol*. 51:314–318.

19. Floegel, Anna, et al. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity

in popular antioxidant-rich US foods. *Journal of food composition and analysis*. 1043-1048.
20. Leba, Louis-Jérôme, et al. (2014). Optimization of a DNA nicking assay to evaluate Oenocarpus bataua and Camellia sinensis antioxidant capacity. *International journal of molecular sciences* 15(10): 18023-18039.
21. Sah, B.N.P., Vasiljevic, T., McKechnie, S., Donkor, O.N. (2016). Antibacterial and antiproliferative peptides in synbiotic yogurt—Release and stability during refrigerated storage,Journal of Dairy Science, 99 (6), 4233-4242
22. Aboul-Soud, Mourad AM, et al. (2020). Biochemical and Molecular Investigation of In Vitro Antioxidant and Anticancer Activity Spectrum of Crude Extracts of Willow Leaves Salix safsaf." *Plants* 9(10): 1295.
23. El-Hallouty, Salwa M., et al. (2020). Crude Methanol Extract of Rosin Gum Exhibits Specific Cytotoxicity against Human Breast Cancer Cells via Apoptosis Induction. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* 20(8): 1028-1036.
24. Abo-Salem HM, Nassrallah A, Soliman AAF, Ebied MS, Elawady ME, Abdelhamid SA, El- Sawy ER, Al-Sheikh YA, Aboul-Soud MAM. (2020). Synthesis and Bioactivity Assessment of Novel Spiro Pyrazole-Oxindole Congeners Exhibiting Potent and Selective *in vitro* Anticancer Effects. Molecules. 25(5):1124.
25. Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J.Immunol.Methods*65: 55-63.
26. Osuntoki A and Korie I, (2010). Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *Food Technol Biotechnol* 48: 505–511.
27. Haenlein, G. F. W. 2004. Goat milk in human nutrition. Small Ru-min. Res. 51:155–163.
28. Kalyankar, S. D., C. D. Khedkar, and A. M. Patil. 2016. Goat: Milk. Pages 256–260 in Encyclopedia of Food & Health. Elsevier Inc., Amsterdam, the Netherlands.
29. Qi, P. X., D. Ren, Y. Xiao, and P. M. Tomasula. 2015. Effect of ho-mogenization and pasteurization on the structure and stability of whey protein in milk. Dairy Sci. 98:2884–2897.
30. Joyce, A. M., A. Brodkorb, A. L. Kelly, and J. A. O'Mahony. 2017. Separation of the effects of denaturation and aggregation on whey-casein protein interactions during the manufacture of a model in-fant formula. Dairy Sci. 96:787–806.
31. Chen, D., X. Li, X. Zhao, Y. Qin, C. Wang, and C. Wang. 2019. Com-parative proteomics of goat milk during heated processing. Food Chemistry. 275:504–514.
32. Ruffin, E., T. Schmit, G. Lafitte, J.-M. Dollat, and O. Chambin. 2014. The impact of whey protein preheating on the properties of emul-sion gel bead. Food Chem. 151:324–332.
33. Giroux, H., N. Veillette, and M. Britten. 2018. Use of denatured whey protein in the production of artisanal cheeses from cow, goat and sheep milk. Small Rumin Resreach. 161:34–42.
34. Costa, W. K. A., E. L. Souza, E. M. Beltrão-Filho, G. K. V. Vascon-celos, T. Santi-Gadelha, C. A. de Almeida Gadelha, O. L. Franco, and M. Magnani. 2014. Comparative protein composition analy-sis of goat milk produced by the Alpine and Saanen breeds in northeastern Brazil and related antibacterial activities. PLoS One 9:e93361.
35. Ceballos, L. S., E. R. Morales, G. de la Torre Adarve, J. D. Castro, L. P. Martínez, and M. R. S. Sampelayo. 2009. Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. Food Composition Analysis. 22:322–329.
36. Felipe, X., M. Capellas, and A. J. R. Law. 1997. Comparison of the effects of high-pressure treatments and heat pasteurization on the whey proteins in goat's milk. Agriculture Food Chemistry. 45:627–631.