Colegio de Postgraduados

Biochemical and functional characterization of milk from alpina and toggenburg goat breeds

Mejía-Palma; Miriam L.[1], Cruz-Monterrosa; Rosy G.[1], Arce-Vazquez; María B.[1], García-Garibay Mariano[1,2]; Jiménez-Guzmán, Judith[1*]

[1] Universidad Autónoma Metropolitana, Unidad Lerma. Departamento de Ciencias de la Alimentación, Av. De las Garzas #10, Col. El Panteón, C. P. 52005, Lerma de Villada, Estado de México.
[2] Universidad Autónoma Metropolitana, Unidad Iztapalapa. Departamento de Biotecnología, San Rafael Atlixco #186, Col. Vicentina, C. P. 09340, Iztapalapa, CDMX
* Correspondence: j.jimenez@correo.ler.uam.mx

ABSTRACT

Objective: This work aimed to evaluate the biochemical and functional properties of milk from two goat breeds (Alpina and Toggenburg) which could give goat's milk a higher added value and market, making it an attractive option for milk producers.

Design/methodology/approach: Several biochemical analyses were performed to both breed milks: Total Fat (Gerber); Total Protein (Lowry); Lactose (reducing sugars); Fatty acid composition (Mass-mass coupled gas chromatography) and antihypertensive activity (angiotensin-converting enzyme inhibition). To determine possible applications, functional characteristics of yogurt and cheese were also evaluated. Statistical analyses were performed using NCSS software.

Results: Fat content of Alpina breed was higher than Toggenburg's (4.76% *vs.* 2.96%, α=0.00013), as was lactose (8.26% *vs.* 5.37%, α=0.003), while Toggenburg presented higher protein content (5.53% *vs.* 4.77%, α=0.00016). Potential biologically active fatty acids were found in both milks in similar concentrations. Toggenburg milk sowed higher antihypertensive activity than Alpina (100% *vs.* 77.27%), which was maintained and, in some cases, increased, when fermented to obtain different derivatives such as cheese and yogurt.

Limitations on study/implications: Further study is still needed to determine the entire biofunctionality of goat's milk and provide milk producers with options to increase market and added value of their products.

Findings/conclusions: Both, Alpina and Toggenburg goat's milk showed a high Biofunctional potential due to their fat and protein fractions; The fact that biofunctionality can be transferred to derivatives such as yogurt or cheese may increase producers' interest in producing and commercializing it, since the products can be marketed as "functional foods".

Keywords: goat milk, antihypertensive activity, fatty acids, oligosaccharides, functional foods.

Citation: Mejía-Palma; M. L., Cruz-Monterrsa; R. G., Arce-Vazquez; M. B., García-Garibay M., & Jiménez-Guzmán, J. (2023). Biochemical and fnctional characterization of milk frm alpina and toggenburg goat breeds. *Agro Productividad*.https://doi.org/10.32854/agrop.v16i10.2705

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: June 23, 2023.
Accepted: October 16, 2023.
Published on-line: January 03, 2024.

Agro Productividad, 16(12). December. 2023. pp: 127-134.

INTRODUCTION

Human beings consume milk from birth to adulthood due to the fact that it is a nutritionally complete food containing water (85-87%), carbohydrates (4.8-5%), and fats (3.8-5%); 5.5% of which comprises short-chain fatty acids, high biological value proteins (2.9-3.5%) as well as vitamins A, D, K, B6 and B12, and mineral salts such as K and Ca (Foroutan *et al.*, 2019). Dairy products emerged over 8,000 years ago and are currently

consumed by more than 6 billion people worldwide (Guha *et al.*, 2021). The increase in world milk consumption has forced consumers and researchers to look for different milk sources to help satisfy the demand. In this respect, goat milk in addition to having similar nutritional characteristics as cow's milk, can provide more essential health benefits production and has shown several bioclimatic and economic advantages (Wadhwani *et al.*, 2023). Currently in Mexico, goat milk (GM) is the second most popular milk with a share of only 1.28% of national production (SIAP 2020). Likewise, some components of goat milk have been attributed to promoting health benefits, as shown in Figure 1. This work aimed to evaluate the biochemical and functional properties of milk from two goat breeds (Alpina and Toggenburg) that could give goat's milk a higher added value and market, making it an attractive option for milk producers.

MATERIALS AND METHODS

Materials: Milks were collected from two different goat farms: Alpine from the herd of La Cabrita ranch, located at Rancho "El Arenal" SN, Tecoyuca, 73306 Chignahuapan, Puebla; and Toggenburg from Santa Irene, Ranch Ejidos, San Luis Huexotla, Texcoco, Méx. After collection milks were separated in sterile jars into 125 mL batches; They were labeled, frozen and stored for further analysis.

Physical-chemical analysis

Protein determination: Protein content was determined according to Lowry (1951).

Lactose determination: Lactose content was determined according to Müller reducing sugars test (DNS, Miller, 1959).

Fat determination: The Gerber method was used. Ten mL of 80% sulfuric acid was added to the Gerber butyrometer, then 1 mL of isoamyl acid was added. Finally, the 10 mL of milk to be analyzed was added and mixed by inversion until the casein was dissolved. It was centrifuged at 1000 rpm for 10 minutes and placed in a 65 °C hot water bath for 5 minutes. Finally, the reading was made on the butyrometer scale.

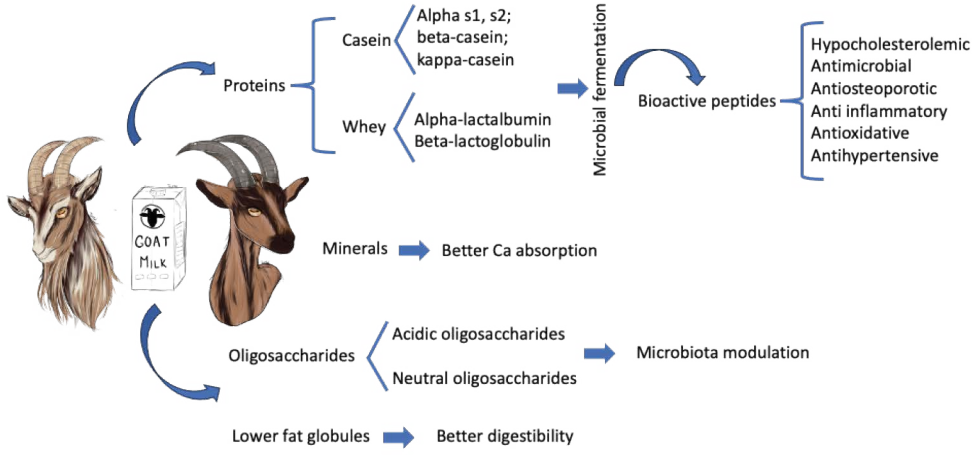


Figure 1. Molecules with beneficial effects of goat milk.

Fatty acid analysis: Fat was extracted by Moubry Technique according to Frank *et al.* (1975). Esterification was performed as established by Piccioli *et al.*, 2019, mixing a 400 mg fat sample sample with 4 mL of hexane; followed by the addition of 200 μL of saturated 2M KOH solution (in methanol). The mixture was allowed to stand for 30 min at 4 °C. It was then centrifuged at 5000 rpm for 5 min, and the upper phase was recovered. It was then injected into a gas chromatograph equipped with self-contained capillary ionization detector (Agilent GC SYSTEM Model 78900B, Santa Clara, U.S.A). An HP-88 column, 100 m×0.250 mm×0.20 μm film thickness (Agilent, Santa Clara, U.S.A) and a temperature ramp (50 °C initial temperature, 1 °C/min to 160 °C, 20 min at 198 °C, 1 °C/min to 230 °C, 15 °C/min, with 250 °C interface) were used. Helium was used as carrier gas and the injection was done in split mode (1:50 ratio). A standard fatty acid mix (Supelco 37 component FAME Mix, Inc., Bellefonte, PA, USA) was used to identify fatty acid methyl esters.

Dairy derivatives processing: Panela Cheese and yogurt were made from Alpina and Toggenburg goat milk. Panela cheese was made inoculating milk with 2% mesophilic lactic starter culture (freeze-dried cultures, DEM3, Centro Sperimentale del Latte) and fermented for 3 hours at 42 °C. Milk was then clotted with natural rennet (1:10000 strength); the curd was cut into 1 cm^3 cubes. It was then drained and molded in a 20 cm diameter circular container. The product was stored at 4 °C until analysis. Yogurt was prepared, pasteurizing milk at 90 °C for 5 minutes, then 5% Yogurt starter culture (freeze-dried cultures, YSC, Centro Sperimentale delLatte) was added as inoculum and fermentation was carried out at 42 °C until a pH of 4.5 was reached. Yogurt was then stored at 4 °C until analysis.

Antihypertensive activity: Alpine and Toggenburg goat milk and cheese and yogurt samples were assayed for antihypertensive activity with a slight modification of the method proposed by Cushman and Cheung (1971). A reaction tube was prepared mixing 80 μL product extract sample with 200 μL borate buffer (0.1 M, pH 8.3); 5 mM Hypuryl-Histidyl-Leucine (HHL, SIGMA Aldrich, USA) solution diluted in 0.3 mM NaCl (JT Baker, Xalostoc, Mexico) was used as substrate. Then 20 μL of rabbit Angiotensin Converting Enzyme (ACE, SIGMA Aldrich, USA) were added to the substrate mixture and incubated for 60 minutes at 37 °C. Reaction was stopped with 250 μL of HACL and 1.7 mL of ethyl acetate (JT Baker, Xalostoc, Mexico). Solution was centrifuged at 4500 rpm for 5 minutes. After which 800 μL of supernatant were evaporated at 95 °C for 30 minutes and resuspended in distilled water. Absorbance was recorded at 230 nm. To determine the degree of ACE inhibition, two standards were prepared: one with ACE enzyme without inhibitor (A, 100% ACE activity) and the other without ACE (C, 0% Activity). The assay was performed in triplicate for each sample. The degree of inhibition was calculated using the following equation:

$$\%\,inhibition\ ACE = \frac{A-B}{A-C} * 100$$

Where: A is the absorbance of the standard with 100% *ACE* activity; B is the absorbance obtained with the hydrolysates; C is the absorbance with 0% *ACE* activity.

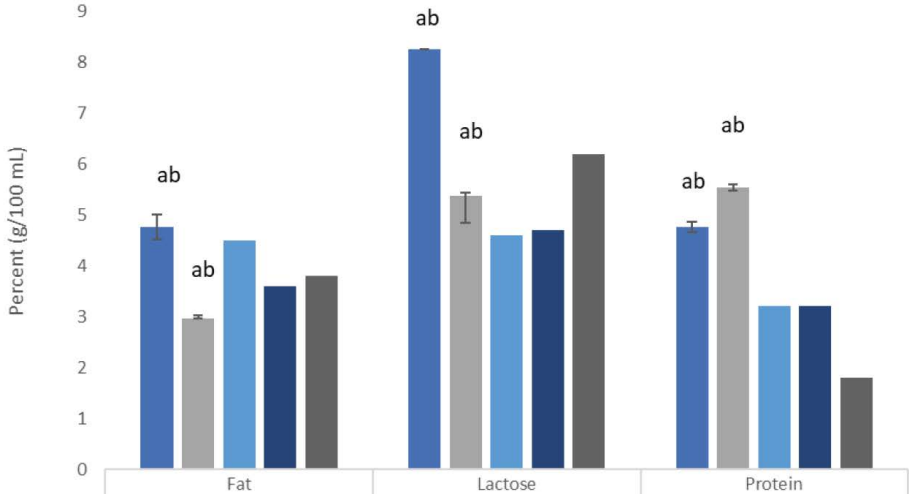
Statistical analysis: All tests were performed in triplicate and statistical analyses were done using NCSS software.

RESULTS AND DISCUSSION

Physico-Chemical composition

Physico-chemical composition of Alpine (AGM) and Toggenburg (TGM) goat milk are shown in Figure 2. AGM contained approximately twice the fat concentration of TGM (4.76±0.25% *vs.* 2.96±0.057%, respectively) and similar to goat milk from stall cattle (SCGM) reported by Kumar *et al.*, (2016) while containing approximately 20% more fat than cow (CM) and human (HM) milk (Wadhwani *et al.*, 2023). Nayik *et al.*, (2021) indicates that a diet rich in natural pastures generates fat and micronutrients rich milk, further suggesting that goats fed and grazed in the mountains with access to green pastures can produce milk in smaller quantities but with high protein and fat content. The fat content may also vary according to the mammal and the lactation cycle since, as the process progresses, the amount of fat decreases, which will have repercussions on the fatty acid content of each species.

Concerning lactose content (Figure 2), it was observed that AGM content is 35% higher compared to TGM (8.26±0.25% *vs.* 5.27±0.52%, respectively) and twice as much compared to SCGM and CM. In comparison, it is only 25% higher than HG; this can be attributed to the fact that AGM milk was obtained from recently parturient goats, and lactose is always higher at the beginning of lactation as, among other functions, it is important to modulate intestinal microbiota during the first stages of life.



	Fat	Lactose	Protein
AGM **	4.76	8.26	4.77
TGM**	2.96	5.37	5.53
SCGM (Kumar et al., 2016)	4.5	4.6	3.2
CM (Wadhwani et al., 2023).	3.6	4.7	3.2
HM (Wadhwani et al., 2023).	3.8	6.2	1.8

Figure 1. Physico-Chemical composition of milk in different mammals. Different letters indicate statistically significant differences at $p<0.05$.

Protein content (Figure 2) in TGM reaches up to 5.53±0.06%; significantly higher (α=0.00) than that observed for AGM SCGM and CM (4.77%, 3.2% and 3.2%) Due to the fact that goats milk undergo a high range of postransductional modifications and rearrangements, and to the high protein concentration, TGM and AGM would be expected to contain a higher concentration of bioactive peptides, increasing their biofunctional potential. The increase in protein concentration in TGM and AGM with respect to SCGM implies an improvement in the nutritional value of milk obtained through free grazing with night supplementation.

Marletta *et al.* (2007) reported that casein fraction of GM undergoes post-translational modifications that derive into changes at the structural level of these fractions, facilitating the release of peptides and their corresponding bioactivity as opposed to CM. This finding increases the added value of the milk produced by this method and opens the door to determining bioactivities such as antihypertensive activity.

Fatty acids profile

GM is a high source of medium-chain fatty acids, highlighted for their functionality because they are present in small fat globules, making them more digestible than the fat globules present in MC (Wadhwani *et al.*, 2023). Table 1 shows the results obtained for fatty acid content in AGM and TGM. When comparing with the data obtained by Verruck *et al.*, (2019), a slight decrease is observed in some short chain fatty acids for example, 33%↓↓ butyric (C4:0), 23%↓↓ caproic (C6:0), and 19.7%↓↓ caprylic (C8:0), which are responsible for some sensory properties of goat milk such as odour and taste; on the other hand, long chain fatty acids show an increase of 17-18.15%↓↑ fatty acids in both breeds studied such as stearic (C18:0), Oleic (C18:1) with respect to CM. Some isomers of Conjugated Linoleic

Table 1. Fatty acid content in the milk of different mammals.

Fatty acid	AGM (%)	TGM (%)	GM* (%)	CM* (%)
Butyric ($C_{4:0}$)	1.45	1.38	2.18	3.70
Caproic ($C_{6:0}$)	1.83	1.74	2.39	2.40
Caprylic ($C_{8:0}$)	2.19	2.19	2.73	1.50
Capric ($C_{10:0}$)	6.57	6.57	9.97	3.20
Laurico ($C_{12:0}$)	4.0	4.00	4.99	3.60
Myristic ($C_{14:0}$)	9.25	9.25	9.81	11.10
Myristoleic ($C_{14:1}$)	0.14	0.14	0.18	0.90
Pentadecanoic ($C_{15:0}$)	0.85	0.85	0.71	1.20
Palmitic ($C_{16:0}$)	28.80	28.80	28.0	28.30
Palmitoleic ($C_{16:1}$)	0.85	0.85	1.59	160
Stearic ($C_{18:0}$)	10.83	10.83	8.88	11.80
Oleic ($C_{18:1}$)	23.25	23.25	19.3	18.80
Linoleic ($C_{18:2}$)	2.83	2.83	3.19	1.40
Linolenic ($C_{18:3}$)	0.19	0.04	0.42	0.90
Conjugated Linoleic ($C_{18:2}$)	0.04	0.08	0.70	1.10

* Source: Verruck *et al.*, 2019.

Acid (CLA, C18:2) were observed in AGM and TGM in concentrations higher than those of CM, which would increase milk functionality since CLA has has been reported to decrease risks for cardiovascular diseases and atherosclerosis or inhibit some types of cancer (Verruck *et al.*, 2019).

Likewise, in the AGM and TGM milks analyzed, the presence of vaccenic, α-linoleic and arachidonic, which are considered functional compounds, was observed. Fatty acid composition of milk depends both on the microorganisms found in the rumen and the feeding, so if both goats herds are managed under the same conditions and nearby, it is possible to explain the similarity in fatty acids by these factors.

Antihypertensive activity

Since antihypertensive activity is mostly related to peptides rather than native proteins, milk was hydrolyzed with porcine chymotrypsin emulating the digestive process that would naturally occur in the human digestive tract. ACE-Inhibiting activity was then determined in the hydrolysates using porcine kidney angiotensin converting enzyme (ACE) and hypuryl-histidyl-leucine (HHL) as substrate. In order to give milk producers several options for milk and dairy products commercialization, ACE-Inhibiting activity was also determined in dairy products such as cheese and yogurt to study if the antihypertensive effect could be affected by the fermentation process.

ACE-Inhibition results are shown in Figure 3. Antihipertensive activity observed in both AGM and TGM was high (77.27%±6.74 y 100.00%±3.47 y 69.05, for AGM, TGM and CM respectively. Statistical analysis showed a significant difference between both breeds and species (α=0.0001).

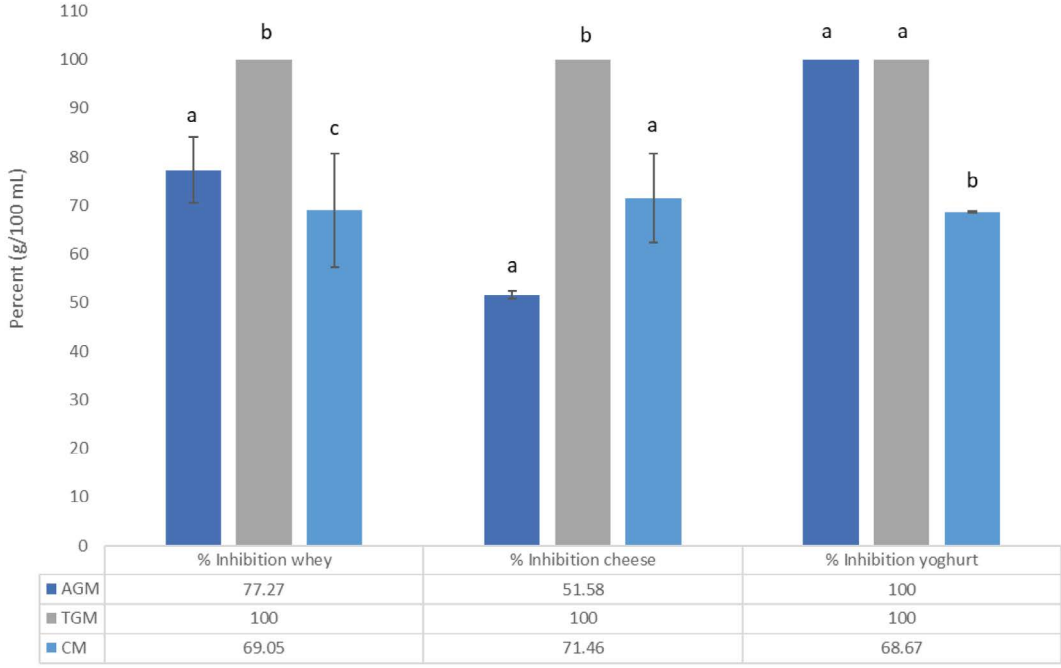


Figure 3. Antihypertensive activity of AGM, TGM and CM derived products. Different letters indicate statistically significant differences at $p<0.05$. Bars with no common letters are significantly different ($p<0.05$).

A slight, but significant diminution in ACE-I activity was observed when analyzing cheeses made with Alpina breed milk, which diminished to reach 51.58%±0.75 (α=0.00). Toggenburg milk cheese maintained a 100% ACE-I activity, suggesting that it was not significantly affected by fermentation with the mesophilic mixed culture (α=0.12). On the other hand, ACE-I of cows milk cheese was determined to be 71.46%±9.1, which is not significantly different from that found for cows milk (α=0.395). Duarte (2021) reported that ACE-I activity of cheese depends on the culture used for fermentation as well as its ripening stage,this is due to the fact that bioactive peptides are produced by enzymatic hydrolysis of proteins during fermentation and/or digestion. The results obtained could be due to the fact that during fermentation the proteolytic system of the microorganisms contained in the culture may have hydrolysed some of the peptidic chains ranging from 300 to 1000 Da, which contain residues of Tyr, Pro y Phe, which have been identified to be of substantial importance for ACE-I activity (Parmar *et al*., 2020).

Lactococcus lactis proteases are highly specific in their cutting sites; hence, slight genetic variations (as between breeds) or post-transductional changes in the primary protein structure may change the affinity of the enzyme for the protein, explaining the different ACE-I activity observed in the cheeses made from both breeds in which proteolysis may have been affected by the primary structure of the proteins (genetic variations) or the steric hindrance given by rearrangements caused by postransductional modifications.

On the other hand, yogurts made with both Alpina and Toggenburg milk showed an ACE-I activity close to 100%, while cows milk yogurt showed am ACE-I activity of 68%±0.29, significantly lower than both goat milks (α=0.0000) but not statistically different from that of cows milk or cows milk cheese, and also comparable to that reported by Vera (2017) who reported a 70% ACE-I activity for cows milk yogurt. Comparing the results obtained for goats milk vs cows milk it is observed that both species have a high potential for commercialization as functional dairy products.

CONCLUSIONS

Biofunctionality of fatty acids, as well as antihypertensive activity in goat milk has been little studied, therefore, these kinds of analyses are of great interest and a beginning for exploration, especially for goats bred in Mexico.

The bio-functional potential of goat's milk is clear considering the functionality of the most abundant milk biomolecules such as fat, and protein fractions. Its high functional fatty acid content, as well as the antihypertensive activity observed which is higher than that of cow's milk, may increase the added value of milk, mostly in the case of Toggenburg breed. The fact that this potential may be transferred to derivative products such as yogurt or cheese may also increase the interest of producers to use welfare care breeding conditions in their herds, since the products may be commercialized as "functional foods."

ACKNOWLEDGEMENTS

COMECYT supported this project through the Funding for the Research of Women Scientists derived from the EDOMÉX-FICDTEM-2022-01 Call for Proposals.

REFERENCES

Cushman, D.W. y Cheung, H.S. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology. 20*(7): 1637-164

Duarte, E (2021) Evaluación de péptidos bioactivos con actividad antihipertensiva en quesos madurados y sus efectos sobre la salud del consumidor. Facultad de Química. Universidad

Foroutan, A.; Guo, A. C.; Vazquez-Fresno, R.; Lipfert, M.; Zhang, L.; Zheng, J.; Wishart, D. S. (2019). Chemical composition of commercial cow's milk. *Journal of agricultural and food chemistry, 67*(17), 4897-4914. Doi 10.1021/acs.jafc.9b00204.

Frank R, Smith EH, Braun HE, Holdrinet M, McWade JW (1975) Organochlorine insecticides and industrial pollutants in the milk supply of the southern region of Ontario, Canada. *J Milk Food Technol.* 38: 65-72

Guha, S., Sharma, H., Deshwal, G. K., & Rao, P. S. (2021). A comprehensive review on bioactive peptides derived from milk and milk products of minor dairy species. *Food production, processing and nutrition, 3*(1), 1-21.

Kumar A, Singh J & Kumar S (2016). Composition, nutritional and therapeutic values of goat milk: A review. *Asian J. Dairy & Food Res., 35* (2), 96-102.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of biological chemistry, 193*(1), 265–275.

Marletta, D., Criscione, A., Bordonaro, S., Guastella, A. M., & Urso, G. D. (2007). Casein polymorphism in goat's milk. *Lait, 87*(1), 491–504.

Miller, G.L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry. 31*(1), 426-428. https://doi.org/10.1021/ac60147a030

Nayik GA, Jagdale YD, Gaikwad SA, Devkatte AN, Dar AH, Dezmirean DS, Bobis O, Ranjha MMAN, Ansari MJ, Hemeg HA and Alotaibi SS (2021) Recent Insights Into Processing Approaches and Potential Health Benefits of Goat Milk and Its Products: A Review. *Front. Nutr. 8*(789117), 1-16. doi: 10.3389/fnut.2021.789117

Parmar, H., Hati, S., Panchal, G., & Sakure, A. A. (2020). Purification and production of novel angiotensin I-converting enzyme (ACE) inhibitory bioactive peptides derived from fermented goat milk. *International Journal of Peptide Research and Therapeutics*, 26, 997-1011.

Piccioli, A. F., Santos, P. D., Silveira, R. D., Bonafé, E., Visentainer, J. V., & Santos, O. O. (2019). Fatty acid determination in fermented milk samples employing direct esterification and gas chromatography. *Journal of the Brazilian Chemical Society, 30*(1), 1350-1357.

Servicio de Información Agroalimentaria y Pesquera (SIAP), Secretaría de agricultura y Desarrollo Rural. 2023. https://www.gob.mx/siap

Vera, A (2017) Desarrollo de un yogur bebible enriquecido con péptidos bioactivos obtenidos de la hidrólisis enzimática de proteínas de suero lácteo utilizando papaína. Facultad de Química. Universidad autónoma de Querétaro.

Verruck, S.; Dantas, A.; Schwinden, E. P. (2019) Functionality of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *Journal of Functional Foods* 243-257, ISSN 1756-4646, https://doi.org/10.1016/j.jff.2018.11.017..

Wadhwani, K. N.; Bhavsar, M.; Islam, M. M.; Patel, J. H.; Lunagariya, P. M. (2023). Modern health concept of non-bovine milk: A review. *Indian Journal of Animal Production and Management, 37*(1), 12-22. Doi 10.48165/ijapm.2023.37.1.8