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Review

Alkaline Phosphatase (ALP) in Non-Cow Milk and Dairy Products: A Review of Current Evidence and Future Trends

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Abstract: Alkaline phosphatase is used as the main marker in the evaluation of successful milk pasteurization, although there is concern about whether this method is appropriate for non-cow milk. We systematically reviewed articles related to ALP in non-cow milk and dairy products. From a total of 183 studies retrieved, 31 articles were included in the review. Our study shows that most research is focused on goat and sheep milk, while other species are rather neglected as far as the use of ALP as a milk safety marker is concerned. More evidence on ALP kinetics is required for non-cow milk and its products and on alternative pasteurization-efficiency markers, such as other enzymes, as these issues are crucial for consumer health.

Keywords: alkaline phosphatase; ALP; non-cow milk; safety

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1. Introduction

Traditionally, humans consume milk and dairy products as part of a balanced diet, aiming to acquire a wide range of nutritional substances. Cow milk represents 85% of worldwide milk production, while buffalo milk represents 11%, followed by goat milk at 2.3%, sheep milk at 1.4% and camel milk at 0.2% [1]. For other animal species, such as horses and donkeys, their contributions to global milk production are less than 0.1% [2]. In order for milk to be safely consumed, pasteurization is required. The European Regulation EC 2074/2005 [3] specifies the temperature and time conditions for the proper pasteurization of milk: (i) high temperature for a short time (at least 72 °C for 15 s), (ii) low temperature for a long time (at least 63 °C for 30 min) or (iii) any other temperature–time combination sufficient to achieve an equivalent effect, so that the end product shows a negative reaction to alkaline phosphatase after heat treatment.

The analytical methods developed for estimating heat load are based on the estimation of changes in the physicochemical states of milk components after heat treatment. Thermal modifications cause various modifications to the chemical properties of milk. The main changes concern specific protein fractions (enzymes, whey proteins) and the formation of Maillard reaction products [4]. In particular, alkaline phosphatase (ALP) has become the most widely used indicator of milk pasteurization effectiveness. Alkaline phosphatase is slightly more resistant to thermal inactivation than the target bacterial pathogens *Coxiella burnetii* and *Mycobacterium tuberculosis* that are present in milk [5]. Therefore, if ALP activity is reduced after pasteurization, milk can be considered safe from a microbiological point of view.

Alkaline phosphatase is a protein that was first described by Suzuki et al. [6]. It is a non-specific monoesterase that catalyzes the hydrolysis of various phosphates and phosphoric acid anhydrides under alkaline conditions. It belongs to the non-specific phosphatases, and it is abundantly present in nature and in many human body tissues, in the kidneys and in bone and blood cells. It is also found in milk and other fluids from many organisms at varying levels [7]. Alkaline phosphatase (ALP) is present in the milk of all mammals in different amounts, and its activity depends on the species, the milk fat percentage and the possibility of reactivation, as well as the conditions of pasteurization [8]. ALP in milk is reported to be concentrated in the fat layer [9]. Products with elevated milk fat levels can have higher initial ALP values. Claeys et al. [10] reported that although whole milk exhibits higher initial ALP activities than non-fat milk, fat content might not be the sole factor that influences the results of pasteurized milk ALP tests. The phosphatase test was originally designed and applied to cow milk, which is mainly consumed as drinking milk. Purified bovine ALP was found to have a molecular mass of 187 k Da and an isoelectric point ranging from pH 5.4 to 6.0 [11]. It has maximal activity in the pH range 9.65 to 10.1 at 37 °C. The thermal inactivation of ALP has been found to follow first-order kinetics, and the denaturation midpoint for milk ALP has been determined at 56 °C for a 30 min heating. Indigenous milk ALP can also be inactivated by high-pressure processing in the range of 400 to 800 MPa and at temperatures ranging from 5 to 40 °C [12].

Regarding the methodological approaches for ALP detection, there are various data on their suitability and reliability. The methods that are used to detect ALP activity can be classified into four types: photometric, fluorimetric, chemiluminescent and immunochemical. These methods have been applied for many years, but only the photometric, fluorimetric and chemiluminescent methods have been recognized as validated methods for pasteurization verification of dairy products [5]. Nowadays, the dairy industry widely uses rapid ALP tests based on the abovementioned technologies. Thus, these tests are also used to control pasteurization in non-cow milk, without sufficient data on their suitability. Due to the differences in ALP activities (lower in goat milk and 35 to 350 times lower in horse milk but higher in sheep milk compared to bovine milk) and in thermal inactivation rates (slower in goat and sheep milk and faster in horse milk compared to bovine milk), a negative ALP test does not seem to be sufficient to determine the efficacy of pasteurization for all types of milk [13–17]. Most studies on ALP detection so far have been conducted on cow milk, while very few have focused on non-cow milk and dairy products [18,19]. Although non-cow dairy foods still represent a relatively small market, there is a need to validate non-cow milk and dairy product pasteurization and understand the associated ALP activities.

In addition to these considerations, recently, the EFSA, in a report published in 2021 [8], highlighted that an in-depth thermal inactivation kinetics study for ALP in different milk types is needed, while a special focus should be placed on camel and equid milk in relation to the assessment of their efficient pasteurization.

The aim of the present study was to assess the current research data in relation to ALP in non-cow milk and dairy products, to highlight gaps in ALP use as an efficient pasteurization indicator and to indicate the research that needs to be undertaken in future in this field.

2. Methodology

2.1. Search Strategy and Data Sources

Three reviewers (EM, AM, SF) searched electronic databases (SCOPUS and PUBMED) using the following keywords: alkaline phosphatase OR ALP AND milk AND pasteurization. Studies identified from the search of the electronic databases were combined, duplicates were removed, along with articles published before the year 2000, and the remaining papers were screened for relevance to the review based on the information contained in their titles and abstracts. Abstracts were screened by another reviewer (TT) and potentially eligible papers were identified.

2.2. Inclusion/Exclusion Criteria

Studies were included if (A) ALP testing for the verification of pasteurization was used for non-cow milk and non-cow dairy products (namely, sheep, goat, camel, equid, buffalo milk and dairy products), (B) the studies contained numerical results and (C) they were published in English.

2.3. Study Selection and Data Extraction

The full texts of the references identified as potentially relevant were obtained and papers were included if they met the inclusion criteria. Review papers were not included. The number of papers obtained by the search strategy were 104. Of these, 73 papers were excluded based on the criteria used. The total number of studies included in this review was 31 (Figure 1).

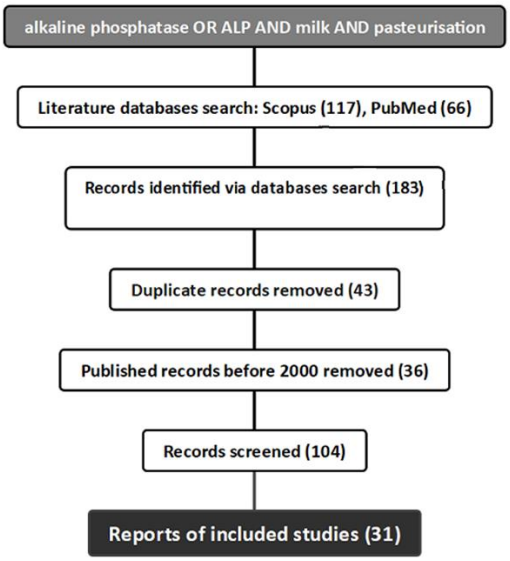

Figure 1. Systematic literature review and study selection strategy.

3. Results and Discussion

A total of 31 research articles were included in this systematic review dealing with non-cow milk, either solely or in combination with the milk of other species (Table 1; Figure 2). More specifically, according to our results, a significant interest in ALP in goat milk was identified worldwide (in the USA, Canada, Venezuela, India, the UK and the EU). Especially after 2000, goat milk was the milk which was studied the most after cow milk (17 published research papers). Twelve (12) papers have been published on sheep milk and ALP, with most of the studies performed in European countries. Eight (8) papers have been published on buffalo milk and ALP, mainly from India, while only four (4) published research papers from Belgium, the USA and Italy have reported on ALP in horse and donkey milk. Finally, there was also a limited number of studies reported on the detection of ALP in camel milk (4 papers).

Table 1. List of selected references on the activity of ALP in different types of milk and dairy products.

Year	Title	Country	Reference
	Sheep milk		
2000	Evaluation of spectrophotometric and fluorometric methods for alkaline phosphatase activity determination in ewe's milk	Italy	[20]
2005	Collaborative evaluation of a fluorometric method for measuring alkaline phosphatase activity in cow's, sheep's, and goat's milk	UK, USA	[18]
2006	Evaluation of a chemiluminescence method for measuring alkaline phosphatase activity in whole milk of multiple species and bovine dairy drinks: Interlaboratory study	USA	[21]
2006	Residual alkaline phosphatase activity after heat treatment of ovine and caprine milk	Greece	[17]
2007	Heat stability and enzymatic modifications of goat and sheep milk	France, USA	[15]
2008	Assessment of the colorimetric and fluorometric assays for alkaline phosphatase activity in cow's, goat's, and sheep's milk	Canada	[22]
2010	Inactivation kinetics of food enzymes during ohmic heating	Slovak	[23]
2010	Activities of alkaline phosphatase, γ-glutamyltransferase and lactoperoxidase in cow, sheep and goat's milk in relation to heat treatment	Germany	[13]
2011	Evaluation of Alkaline Phosphatase Detection in Dairy Products Using a Modified Rapid Chemiluminescent Method and Official Methods	USA	[24]
2014	Inactivation kinetics of alkaline phosphatase from different species of milk using quinolyl phosphate as a substrate	Romania	[25]
2020	Mix-and-read method for assessment of milk pasteurization using a smartphone or a common digital camera	Greece	[26]
2021	Changes in Native Whey Protein Content, Gel Formation, and Endogenous Enzyme Activities Induced by Flow-Through Heat Treatments of Goat and Sheep Milk	Greece	[27]
	Goat milk		
2000	Efficiency of Pasteurization the Goat Milk in Cheesemaking Miniplant	Venezouela	[28]
2003	Dry-reagent strips for testing milk pasteurization	India	[29]
2005	Collaborative evaluation of a fluorometric method for measuring alkaline phosphatase activity in cow's, sheep's, and goat's milk	UK, USA	[18]
2006	Evaluation of a chemiluminescence method for measuring alkaline phosphatase activity in whole milk of multiple species and bovine dairy drinks: Interlaboratory study	USA	[21]
2006	Residual alkaline phosphatase activity after heat treatment of ovine and caprine milk	Greece	[17]
2007	Heat stability and enzymatic modifications of goat and sheep milk	France, USA	[15]
2008	Assessment of the colorimetric and fluorometric assays for alkaline phosphatase activity in cow's, goat's, and sheep's milk	Canada	[22]
2009	Activity and thermal stability of indigenous enzymes in cow, buffalo and goat milk	India	[30]
2010	Inactivation kinetics of food enzymes during ohmic heating	Slovak	[23]
2010	Activities of alkaline phosphatase, γ-glutamyltransferase and lactoperoxidase in cow, sheep and goat's milk in relation to heat treatment	Germany	[13]
2011	Evaluation of alkaline phosphatase detection in dairy products using a modified rapid chemiluminescent method and official methods	USA	[24]
2011	Determination of alkaline phosphatase activity in goat milk and cheese by fluorimetric method as a verification of efficacy of pasteurisation process	Poland	[31]
2013	Fluorometric detection of active alkaline phosphatase and gamma-glutamyl transferase in fluid dairy products from multiple species	USA	[32]
2014	Inactivation kinetics of alkaline phosphatase from different species of milk using quinolyl phosphate as a substrate	Romania	[25]
2016	Alkaline phosphatase activity and microbiological quality of heat-treated goat milk and cheeses	Poland	[33]
2021	The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products	EU	[8]
2021	Changes in Native Whey Protein Content, Gel Formation, and Endogenous Enzyme Activities Induced by Flow-Through Heat Treatments of Goat and Sheep Milk	Greece	[27]

Table 1. *Cont.*

Year	Title	Country	Reference
	Buffalo milk		
2000	Buffalo-milk enzyme levels, their sensitivity to heat inactivation, and their possible use as markers for pasteurization	Italy, Israel	[34]
2003	Dry-reagent strips for testing milk pasteurization	India	[29]
2006	Evaluation of a chemiluminescence method for measuring alkaline phosphatase activity in whole milk of multiple species and bovine dairy drinks: Interlaboratory study	USA	[21]
2009	Activity and thermal stability of indigenous enzymes in cow, buffalo and goat milk	India	[30]
2015	A three step approach for the purification of alkaline phosphatase from non-pasteurized milk	India	[35]
2016	Development of a Chromatographic Method for the Determination of Alkaline Phosphatase Activity in Pasteurized Milk	India	[36]
2021	The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products	EU	[8]
2021	Development of a HPLC Fluorescence Method for Determining Efficacy of Milk Pasteurization	India	[37]
Equid milk			
2009	Thermal inactivation kinetics of alkaline phosphatase in equine milk	Belgium	[14]
2013	Fluorometric detection of active alkaline phosphatase and gamma-glutamyl transferase in fluid dairy products from multiple species	USA	[32]
2016	Shelf life of donkey milk subjected to different treatment and storage conditions	Italy	[38]
2021	The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products	EU	[8]
	Camel milk		
2006	Comparative study on different enzymes evaluating heat treatment of dromedary milk	n/a	[39]
2008	Evaluation of alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) and lactoperoxidase (LPO) activities for their suitability as markers of camel milk heat inactivation	n/a	[40]
2011	Evaluation of indigenous enzymes in raw and pasteurised camel milk	Germany	[41]
2021	The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products	EU	[8]
	Non-cow dairy products		
2000	Efficiency of Pasteurization the Goat Milk in Cheesemaking Miniplant	Venezouela	[28]
2004	Alkaline Phosphatase Activity in Cheeses Measured by Fluorometry	USA	[42]
2006	Evaluation of a chemiluminescence method for measuring alkaline phosphatase activity in whole milk of multiple species and bovine dairy drinks: Interlaboratory study	USA	[21]
2009	Thermal inactivation kinetics of alkaline phosphatase in equine milk	Belgium	[14]
2011	Evaluation of alkaline phosphatase detection in dairy products using a modified rapid chemiluminescent method and official methods	USA	[24]
2011	Determination of alkaline phosphatase activity in goat milk and cheese by fluorimetric method as a verification of efficacy of pasteurisation process	Poland	[31]
2012	Alkaline phosphatase activity in Slovenian cheese made from pasteurized, thermized or raw milk	Slovenia	[43]
2013	Fluorometric detection of active alkaline phosphatase and gamma-glutamyl transferase in fluid dairy products from multiple species	USA	[32]
2016	Alkaline phosphatase activity and microbiological quality of heat-treated goat milk and cheeses	Poland	[33]
2021	The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products	EU	[8]
2021	Changes in Native Whey Protein Content, Gel Formation, and Endogenous Enzyme Activities Induced by Flow-Through Heat Treatments of Goat and Sheep Milk	Greece	[27]
2021	Alkaline Phosphatase Survey in Pecorino Siciliano PDO Cheese	Italy	[44]

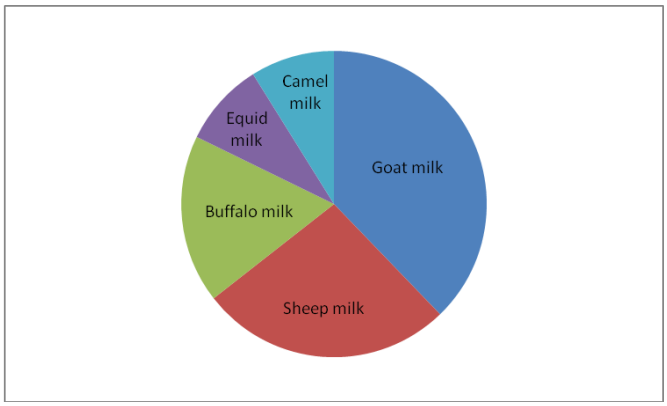

Figure 2. Research on non-cow milk and ALP from the last 20 years.

In order to compare and contrast the available data on ALP in non-cow milk, the abovementioned studies were further grouped according to the following research areas: ALP activity in raw and processed non-cow milk; methods applied for ALP determination in non-cow milk; thermal stability and kinetics of ALP in non-cow milk. Finally, another group was created for studies on ALP in non-cow-milk dairy products. Based on an in-depth and comprehensive review of the data published, following a comparative approach, the following results can be highlighted:

ALP activity in raw and processed non-cow milk

Based on the available data on ALP activity in raw and pasteurized non-cow milk, Figures 3 and 4, respectively, present the differences noted.

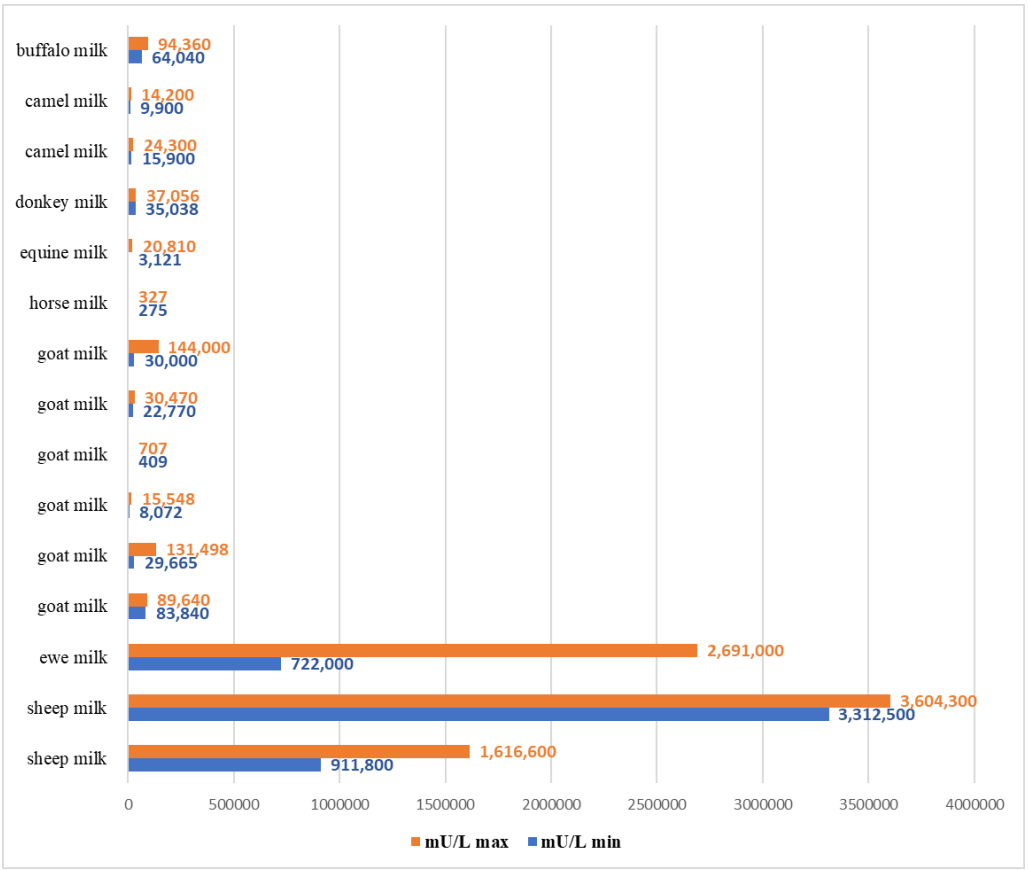

Figure 3. ALP activities in non-cow raw milks [13,14,22,25,27,30,32,38,39,41].

The available data on ALP in raw and pasteurized sheep milk vary between studies. According to Lorenzen et al. [13], the ALP average was 1413 U/L, while Jakob et al. [23] reported that ALP activity in raw sheep milk was three times higher than in bovine milk. According to Klotz et al. [22], the average ALP level in raw sheep milk was 3512 μg/mL phenol. The activity of ALP in whole goat milk was found to be 86.74 ± 2.9 U/L and to be 52.4 ± 2.1 U/L in skimmed goat milk [25], while, according to Rola and Sosnowski [31], it was about 20,000 mU/L. According to the European Food Safety Authority [8], there is a 95–99% probability (extremely likely) that pasteurized goat milk has ALP activity below a limit of 300 mU/L. ALP activity in buffalo milk presents notable differences from that in cow milk [34], which may be related to the different milk compositions (i.e., higher fat content). With regard to equid milk (horse and donkey), there is a rather common finding in the current literature, also highlighted by the EFSA (2021), that the currently available ALP tests are not sensitive enough due to the very low basal ALP activities in this type of milk. More specifically, the results of Giacometti et al. [38] showed that ALP activity in donkey milk is similar to that reported in equine milk [14],, while raw equine milk has approximately 35–350 times less enzyme activity than bovine milk [14]. Overall, there are extremely limited data on ALP activity in equid milk. The available data show that equine and donkey milk present very low levels of ALP, calling into question the appropriateness of ALP testing for pasteurization control, as previously stated by Marchand et al. [14], Malissiova et al. [45] and Tsiamita et al. [46], mainly due to the sensitivity levels of available tests but also due to the ALP profile in equid milk. With regard to camel milk, according to the EFSA [8], similar to equid milk, the current ALP tests are not sensitive enough. Lorenzen et al. [41] determined ALP activities in raw and pasteurized camel milk and concluded that ALP is not suitable as a marker for effective pasteurization of camel milk. ALP activity in pasteurized milk is below the detection limit, but the enzyme activity in raw camel milk is too low and the variation in the measured data too high for it to serve as a marker.

Methods applied in non-cow milk for ALP determination

In relation to the methods used for ALP determination in sheep milk, the fluorescence method showed that this control test is one of the most satisfactory techniques [25]. Moreover, chemiluminescence and spectrophotometry proved to be applicable for ALP determination for this milk type also [21,22]. Regarding the methods used for ALP determination in goat milk, the Fluorophos assay was approved as the most suitable method, followed by the colometric method [18]. According to the available data, some novel assays could be applied to assess ALP activity in buffalo milk. High-Performance Liquid Chromatography combined with Ultraviolet (HPLC-UV) or Fluorimetric Detection (HPLC-FD) showed especially good linearity, accuracy and precision, with low limits of detection and quantification [36]. In relation to equid milk, Marchand et al. [14] concluded that equine ALP would not be useful as an indicator with the universally accepted reference method for the determination of ALP in milk. Moreover, based on the findings of Ziobro and McElroy [32], the gamma-glutamyl transferase (GGT) content of equine milk is 4 to 20 times greater than the ALP content, such that GGT is possibly a more suitable marker in the measurement of the successful pasteurization of donkey and horse milk. In this regard, two new methods have been developed using fluorogenic substrates for two marker enzymes, alkaline phosphatase and gamma-glutamyl transferase. These two new methods for detecting ALP and GGT in dairy products are rapid, accurate and repeatable, and the results are positively correlated [32]. GGT testing seems to be a promising method for assessing pasteurization efficiency in non-cow milk, such as horse and donkey milk. Wernery et al. [39] concluded that ALP is not a suitable indicative endogenous marker enzyme for the confirmation of camel milk pasteurization using four different test systems (fluorimetric, photometric and two colorimetric methods). Recently, Tsiamita et al. [46], after evaluating the efficiency of several qualitative and quantitative commercially available ALP tests, concluded that ALP does not appear to be fully reliable as an indicator for the pasteurization of some types of non-cow milk, such as camel and donkey milk, or even

goat and sheep milk, with respect to the EFSA's proposed limits. ALP commercial kits may not be suitable as pasteurization indicators for various types of non-cow milk, and alternatives should be investigated.

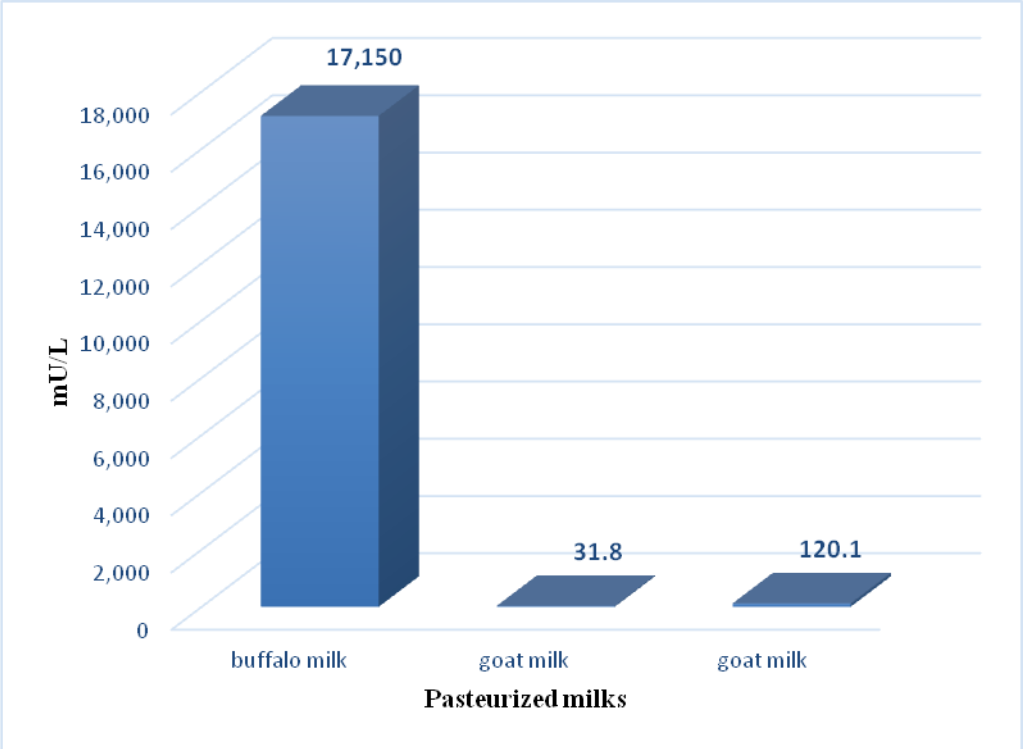

Figure 4. Means of ALP activities in non-cow pasteurized milks [22,29,33].

Thermal stability and kinetics of ALP in non-cow milk

There has been only limited research on ALP kinetics in non-cow milk, even though there is a recognized gap in the literature. The currently available data can be summarized as follows: at 72.5 °C, K values for the stable fraction of ALP in sheep milk are 2.86 ± 0.34 L/min, demonstrating that the stable fraction of bovine ALP is more slowly inactivated than the sheep ALP varieties [25]. Overall, raw sheep milk contains substantially higher ALP levels than raw cow milk (three times higher) [17,22,27], while the rate of thermal inactivation is lower in sheep milk than in cow milk. ALP levels are influenced in sheep milk, as in other species, by several factors, such as breed, season, lactation stage, fat content and presence of subclinical mastitis [47–50]. In terms of goat milk ALP kinetics, as Dumitrascu et al. [25] have reported, ALP inactivation behavior is not influenced by fat content at high temperatures, while large decreases in inactivation rates occur as temperature increases. ALP activity in raw goat milk is lower compared to bovine milk, while goat milk presents the lowest ALP activity levels before and after heat treatment [17]. Since it is the absolute value of ALP activity that serves as an index for proper pasteurization and not the loss of initial enzyme activity, it would be helpful to have limits for ovine and caprine milk defined [17]. Overall, both ALP activity and its thermal inactivation rate in goat milk are lower compared to cow milk [17,22,27]. More specifically, ALP activity is five times lower in goat milk, being influenced by several factors, as was above mentioned for sheep milk. The performances of the classical techniques for ALP assessment in goat milk present similarities to those for sheep and cow milk and therefore are considered adequate. Buffalo milk demonstrates less ALP activity than cow milk, while the reactivation rates are similar [30,51]. Nevertheless, there is no current research on buffalo milk to allow the review of more up-to-date data. As in other species, the presence of subclinical mastitis is reported to influence ALP activity [52]. Interestingly, Lombardi et al. [34] demonstrated that, in addition to ALP, GGT would be suitable as a potential marker for heat denaturation

in buffalo milk, GGT having the advantage given that its concentrations are higher. From a kinetic point of view, equine ALP has potential as an indicator for proper pasteurization, though this potential cannot be exploited because of the low intrinsic enzyme levels present in equine milk and the lack of sensitivity of the fluorimetric (reference) method. As camel milk contains low levels of and extremely heat-stable ALP, ALP testing is not considered suitable for pasteurization control, even though the rates of inactivation of ALP due to pasteurization were found to be comparable for both analytical (fluorimetric and colorimetric) methods [41]. It is recommended to evaluate the use of other endogenous enzyme markers, such as lactoperoxidase (LPO).

Studies evaluating the inactivation of ALP, GGT and LPO activities in sheep, goat and cow milks by isochrone heating of milk (35–85 °C for 90 s) have shown that the heat stability of the indigenous enzymes increased in the following order: ALP < GGT< LPO [13]. Additionally, according to Sharma et al. [30], the thermal inactivation patterns of individual enzymes (lactoperoxidase (LPO), N-acetyl-β-glucosaminidase (NAGase), γ-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP) and xanthine oxidase (XO)) in bovine, buffalo and goat milks were found to be similar. The thermal stabilities of these five enzymes are presented in Figure 5. The authors reported that the activities of these enzymes in goat milk were significantly lower than in bovine or buffalo milk.

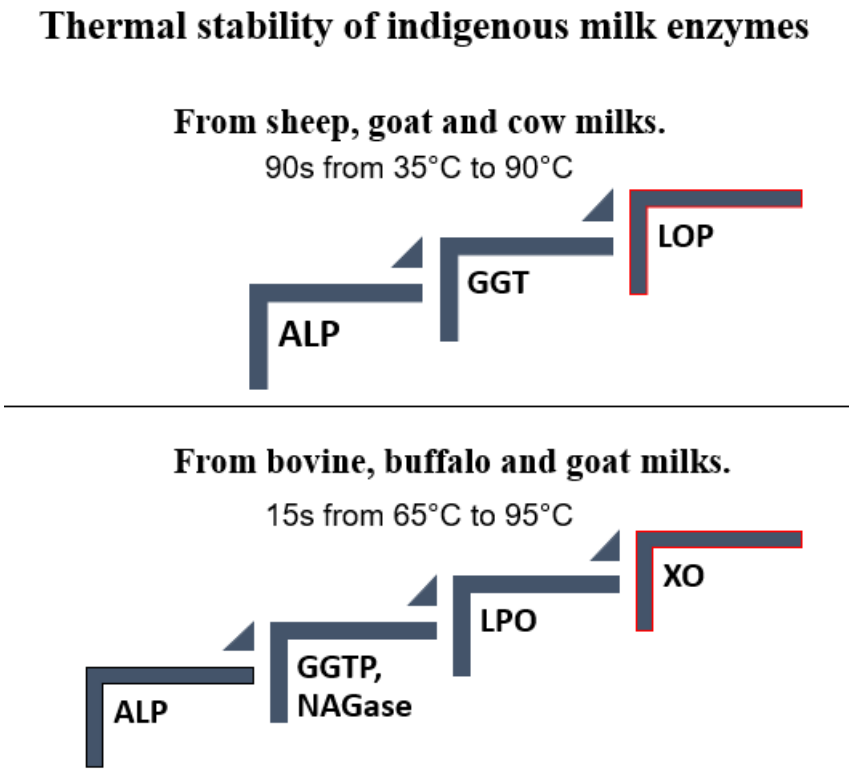

Figure 5. Thermal stability of indigenous milk enzymes in different types of milk [13,30]. *ALP: alkaline phospahtase, GGT: γ-glutamyl transpeptidase, LPO: lactoperoxidase, NAGase: N-acetyl-β-glucosaminidase, XO: xanthine oxidase.*

ALP in non-cow-milk dairy products

A total of 12 research papers have been published on non-cow-milk dairy products and ALP (Table 1). Overall, there are relatively limited data for non-cow milk products, considering the large variety and quantity of different dairy products available globally. Figure 6 presents residual ALP activities in different non-cow-milk cheeses, highlighting the differences noted. Data available for cheese do not allow ALP limits to be evaluated, while no data are available for other dairy products, such as yoghurt, ice cream, milk powder, cream or fermented milk. Interestingly, Todaro et al. [44] reported that ALP values

in the PDO Pecorino Siciliano cheese from raw milk fell within the range of ALP values detected in pasteurized milk cheeses. This highlights the caution needed when applying ALP as a control tool for raw or pasteurized milk cheeses. Alternative indicators should be used for dairy products, as ALP activity is influenced by several factors before and after heat treatment. In cultured milk and yoghurt, ALP can undergo an irreversible loss of activity at acidic pH [31], presenting a false-negative ALP test.

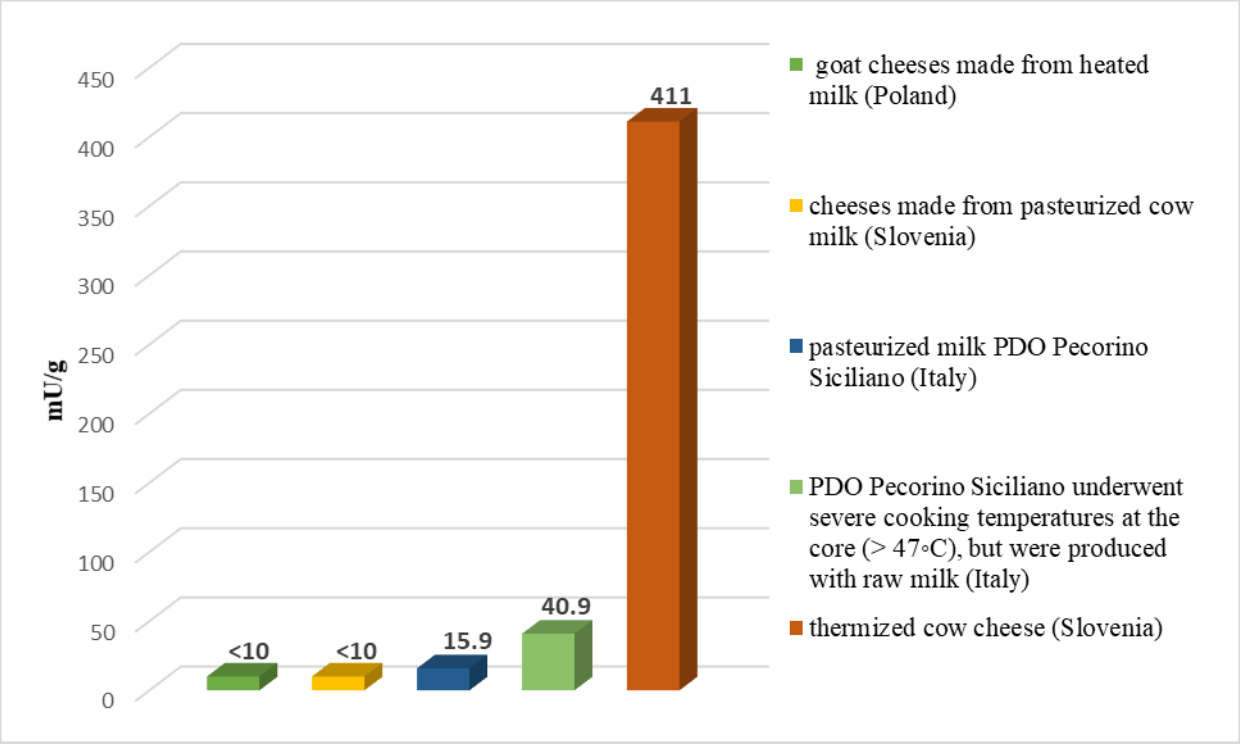

Figure 6. ALP in thermized and pasteurized milk cheeses [33,43,44].

The ISO 11816-2:2016 standard does not specify whether the method is applicable to cheese made from cow, goat or sheep milk, even though this is considered the official method for controlling cheeses with a protected designation of origin (PDO) that use raw milk [44], which may lead to false-negative results in ALP testing, as the methods of production for each type of cheese may include several different heat treatments. The fluorimetric method is applicable to milk-based drinks derived from cow, goat and sheep milk and also to milk powder after reconstitution [8]. The study of Rola and Sosnowski [31] confirmed the efficiency of the fluorimetric method for the determination of ALP activities in milk and cheese, according to the standard EN ISO 11816-1 and the new standard EN ISO 11816-2, under laboratory conditions.

According to a survey on ALP activity in pasteurized cheeses that was performed in France, Italy and Switzerland, a tentative target value of 10 mU/g of cheese was proposed [8]. According to Marchand et al. [14], equine ALP will not be useful as an indicator with respect to the universally accepted reference method for the determination of ALP in milk-based products.

One possible limitation of the present study is that articles not listed in the databases used may not have been included. Nevertheless, to the best of our knowledge, this is the first systematic literature review to focus on ALP activity in non-cow milk and its products, and it can definitely serve as a starting point for future research in this specific area. For the non-cow-milk dairy sector, based on the results of the current systematic review, it is apparent that special caution should be taken when applying the ALP test in evaluating the efficiency of pasteurization. The EFSA [8] has reported the following as possible proposed limits for the milk from various species: buffalo milk $\leq$380 mU/L, goat milk $\leq$ 330 mU/L,

sheep milk $\leq$ 530 mU/L. As the milks of these three species have similarities to cow milk in terms of ALP kinetics, on which there are some relevant studies in the literature, it is considered essential to focus on camel and equid milk but also on non-cow-milk dairy products in general. Both camel and equid milk definitely represent a small proportion of the global dairy industry, but as they are "characterized" as bio-functional [53,54] they have some fanatic consumers, including children. Therefore, with regard to their safety, special attention should be given to them, either in terms of documenting the efficiency of ALP as a pasteurization indicator or by proposing alternative ways of evidencing their safety. With regard to dairy products, several lapses have been identified in the literature in relation to ALP assessment; therefore, it is proposed to focus on evaluating ALP activities in different products or even to introduce a new approach for heat-treatment evaluation, as ALP is influenced by a series of factors and most probably cannot serve as a safety marker. As there is no legal limit set to date, the use of other enzymes should be investigated to ensure the efficient pasteurization of milk used to produce dairy products, especially cheeses.

4. Conclusions

Research on non-cow-milk ALP activity is relatively limited, and further attention from the research and academic communities is required. ALP kinetics, safety limits and alternative enzymes for the evaluation of successful pasteurization need to be further investigated. Special attention needs to be given to camel and equid milks, as there seems to be an issue with the current methods used to evaluate their safety.

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References

1. Gerosa, S.; Skoet, J. *Milk Availability: Trends in Production and Demand and Medium-Term Outlook*; ESA Working Papers; 289000; Food and Agriculture Organization of the United Nations, Agricultural Development Economics Division (ESA): Rome, Italy, 2012.
2. Faye, B.; Konuspayeva, G. The Sustainability Challenge to the Dairy Sector–The Growing Importance of Non-Cattle Milk Production Worldwide. *Int. Dairy J.* **2012**, *24*, 50–56. [CrossRef]
3. FAO.org. Available online: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC087031/ (accessed on 23 July 2022).
4. Ritota, M.; Di Costanzo, M.G.; Mattera, M.; Manzi, P. New Trends for the Evaluation of Heat Treatments of Milk. *J. Anal. Methods Chem.* **2017**, *2017*, e1864832. [CrossRef]
5. Rankin, S.A.; Christiansen, A.; Lee, W.; Banavara, D.S.; Lopez-Hernandez, A. Invited Review: The Application of Alkaline Phosphatase Assays for the Validation of Milk Product Pasteurization. *J. Dairy Sci.* **2010**, *93*, 5538–5551. [CrossRef]
6. Suzuki, U.; Yoshimura, K.; Takaishi, M. About the Enzyme "Phytase", Which Splits "Anhydro-Oxy-Methylene Diphosphoric Acid". *Bull. Coll. Agric. Tokyo Imp. Univ.* **1907**, *7*, 503–512.
7. Shakeel-ur-Rehman; Fleming, C.M.; Farkye, N.Y.; Fox, P.F. Indigenous Phosphatases in Milk. In *Advanced Dairy Chemistry—1 Proteins: Part A/Part B*; Fox, P.F., McSweeney, P.L.H., Eds.; Springer: Boston, MA, USA, 2003; pp. 523–543. [CrossRef]
8. European Food Safety Authority (EFSA); Clawin-Rädecker, I.; De Block, J.; Egger, L.; Willis, C.; Da Silva Felicio, M.T.; Messens, W. The Use of Alkaline Phosphatase and Possible Alternative Testing to Verify Pasteurisation of Raw Milk, Colostrum, Dairy and Colostrum-Based Products. *EFSA J. Eur. Food Saf. Auth.* **2021**, *19*, e06576. [CrossRef]
9. Kosikowski, F.V. Enzyme Behavior and Utilization in Dairy Technology. *J. Dairy Sci.* **1988**, *71*, 557–573. [CrossRef]
10. Claeys, W.L.; Van Loey, A.M.; Hendrickx, M.E. Kinetics of Alkaline Phosphatase and Lactoperoxidase Inactivation, and of Beta-Lactoglobulin Denaturation in Milk with Different Fat Content. *J. Dairy Res.* **2002**, *69*, 541–553. [CrossRef]
11. Vega-Warner, A.V.; Wang, C.-H.; Smith, D.M.; Ustunol, Z. Milk Alkaline Phosphatase Purification and Production of Polyclonal Antibodies. *J. Food Sci.* **1999**, *64*, 601–605. [CrossRef]
12. Rademacher, B.; Hinrichs, J. Effects of High Pressure Treatment on Indigenous Enzymes in Bovine Milk: Reaction Kinetics, Inactivation and Potential Application. *Int. Dairy J.* **2006**, *16*, 655–661. [CrossRef]

13. Lorenzen, P.C.; Martin, D.; Clawin-Rädecker, I.; Barth, K.; Knappstein, K. Activities of Alkaline Phosphatase, γ-Glutamyltransferase and Lactoperoxidase in Cow, Sheep and Goat's Milk in Relation to Heat Treatment. *Small Rumin. Res.* **2010**, *89*, 18–23. [CrossRef]
14. Marchand, S.; Merchiers, M.; Messens, W.; Coudijzer, K.; Block, J. Thermal Inactivation Kinetics of Alkaline Phosphatase in Equine Milk. *Int. Dairy J.* **2009**, *19*, 763–767. [CrossRef]
15. Raynal-Ljutovac, K.; Park, Y.W.; Gaucheron, F.; Bouhallab, S. Heat Stability and Enzymatic Modifications of Goat and Sheep Milk. *Small Rumin. Res.* **2007**, *68*, 207–220. [CrossRef]
16. Tziboula-Clarke, A. Goat Milk. *Encycl. Dairy Sci.* **2003**, *2*, 1270–1279.
17. Vamvakaki, A.-N.; Zoidou, E.; Moatsou, G.; Bokari, M.; Anifantakis, E. Residual Alkaline Phosphatase Activity after Heat Treatment of Ovine and Caprine Milk. *Small Rumin. Res.* **2006**, *65*, 237–241. [CrossRef]
18. Harding, F.; Garry, E. Collaborative Evaluation of a Fluorometric Method for Measuring Alkaline Phosphatase Activity in Cow's, Sheep's, and Goat's Milk. *J. Food Prot.* **2005**, *68*, 1047–1053. [CrossRef]
19. Moatsou, G. Indigenous Enzymatic Activities in Ovine and Caprine Milks. *Int. J. Dairy Technol.* **2010**, *63*, 16–31. [CrossRef]
20. Scintu, M.F.; Daga, E.; Ledda, A. Evaluation of Spectrophotometric and Fluorometric Methods for Alkaline Phosphatase Activity Determination in Ewe's Milk. *J. Food Prot.* **2000**, *63*, 1258–1261. [CrossRef]
21. Salter, R.S.; Fitchen, J. Evaluation of a Chemiluminescence Method for Measuring Alkaline Phosphatase Activity in Whole Milk of Multiple Species and Bovine Dairy Drinks: Interlaboratory Study. *J. AOAC Int.* **2006**, *89*, 1061–1070. [CrossRef]
22. Klotz, V.; Hill, A.; Warriner, K.; Griffiths, M.; Odumeru, J. Assessment of the Colorimetric and Fluorometric Assays for Alkaline Phosphatase Activity in Cow's, Goat's, and Sheep's Milk. *J. Food Prot.* **2008**, *71*, 1884–1888. [CrossRef]
23. Jakób, A.; Bryjak, J.; Wojtowicz, H.; Illeová, V.; Annus, J.; Polakovič, M. Inactivation Kinetics of Food Enzymes during Ohmic Heating. *Food Chem.* **2010**, *123*, 369–376. [CrossRef]
24. Albillos, S.M.; Reddy, R.; Salter, R. Evaluation of Alkaline Phosphatase Detection in Dairy Products Using a Modified Rapid Chemiluminescent Method and Official Methods. *J. Food Prot.* **2011**, *74*, 1144–1154. [CrossRef] [PubMed]
25. Dumitrascu, L.; Nicoleta, S.; Stanciu, S.; Rapeanu, G. Inactivation Kinetics of Alkaline Phosphatase from Different Species of Milk Using Quinolyl Phosphate as a Substrate. *Food Sci. Biotechnol.* **2014**, *23*, 1773–1778. [CrossRef]
26. Sevastou, A.; Tragoulias, S.S.; Kalogianni, D.P.; Christopoulos, T.K. Mix-and-Read Method for Assessment of Milk Pasteurization Using a Smartphone or a Common Digital Camera. *Anal. Bioanal. Chem.* **2020**, *412*, 5663–5669. [CrossRef]
27. Moatsou, G.; Moschopoulou, E.; Zoidou, E.; Kamvysi, A.; Liaskou, D.; Tsigkou, V.; Sakkas, L. Changes in Native Whey Protein Content, Gel Formation, and Endogenous Enzyme Activities Induced by Flow-Through Heat Treatments of Goat and Sheep Milk. *Dairy* **2021**, *2*, 410–421. [CrossRef]
28. Faría, R.J.F.; García, U.; García, A.; Tovar, V. A Efficiency of Pasteurization the Goat Milk in Cheesemaking Miniplant. *Rev. Cient. Fac. Cienc. Vet. Univ. Zulia* **2000**, *10*, 119–123.
29. Sharma, S.K.; Sehgal, N.; Kumar, A. Dry-Reagent Strips for Testing Milk Pasteurization. *LWT -Food Sci. Technol.* **2003**, *36*, 567–571. [CrossRef]
30. Sharma, R.; Kaur, S.; Rajput, Y.; Kumar, R. Activity and Thermal Stability of Indigenous Enzymes in Cow, Buffalo and Goat Milk. *Milchwissenschaft* **2009**, *64*, 173–175.
31. Rola, J.; Sosnowski, M. Determination of Alkaline Phosphatase Activity in Goat Milk and Cheese by Fluorimetric Method as a Verification of Efficacy of Pasteurisation Process. *Bull. Vet. Inst. Pulawy* **2011**, *55*, 705–708.
32. Ziobro, G.C.; McElroy, K.M. Fluorometric Detection of Active Alkaline Phosphatase and Gamma-Glutamyl Transferase in Fluid Dairy Products from Multiple Species. *J. Food Prot.* **2013**, *76*, 892–898. [CrossRef]
33. Sosnowski, M.; Rola, J.G.; Osek, J. Alkaline Phosphatase Activity and Microbiological Quality of Heat-Treated Goat Milk and Cheeses. *Small Rumin. Res.* **2016**, *136*, 132–136. [CrossRef]
34. Lombardi, P.; Avallone, L.; d'Angelo, A.; Mor, T.; Bogin, E. Buffalo-Milk Enzyme Levels, Their Sensitivity to Heat Inactivation, and Their Possible Use as Markers for Pasteurization. *J. Food Prot.* **2000**, *63*, 970–973. [CrossRef] [PubMed]
35. Upadhyay, L.S.B.; Verma, N. A Three Step Approach for the Purification of Alkaline Phosphatase from Non-Pasteurized Milk. *J. Food Sci. Technol.* **2015**, *52*, 3140–3146. [CrossRef] [PubMed]
36. Lakra, S.; Jadhav, V.J.; Garg, S.R. Development of a Chromatographic Method for the Determination of Alkaline Phosphatase Activity in Pasteurized Milk. *Food Anal. Methods* **2016**, *9*, 2002–2009. [CrossRef]
37. Jadhav, V.J.; Badgujar, P.C. Development of a HPLC Fluorescence Method for Determining Efficacy of Milk Pasteurization. *Food Anal. Methods* **2021**, *14*, 260–267. [CrossRef]
38. Giacometti, F.; Bardasi, L.; Merialdi, G.; Morbarigazzi, M.; Federici, S.; Piva, S.; Serraino, A. Shelf Life of Donkey Milk Subjected to Different Treatment and Storage Conditions. *J. Dairy Sci.* **2016**, *99*, 4291–4299. [CrossRef] [PubMed]
39. Wernery, U.; Maier, U.; Johnson, B.; George, R.M.; Braun, F. Comparative Study on Different Enzymes Evaluating Heat Treatment of Dromedary Milk. *Milchwissenschaft* **2006**, *61*, 281–285.
40. Wernery, U.; Fischbach, S.; Johnson, B.; Jose, S. Evaluation of Alkaline Phosphatase (ALP), γ-Glutamyl Transferase (GGT) and Lactoperoxidase (LPO) Activities for Their Suitability as Markers of Camel Milk Heat Inactivation. *Milchwissenschaft* **2008**, *63*, 265–267.
41. Lorenzen, P.; Wernery, R.; Johnson, B.; Jose, S.; Wernery, U. Evaluation of Indigenous Enzymes in Raw and Pasteurised Camel Milk. *Small Rumin. Res.* **2011**, *97*, 79–82. [CrossRef]

42. Yoshitomi, K. Alkaline Phosphatase Activity in Cheeses Measured by Fluorometry. *Int. J. Food Sci. Technol.* **2004**, *39*, 349–353. [CrossRef]
43. Hodošček, L.; Rupnik, S.; Ahčin, K.; Biasizzo, M. Alkaline Phosphatase Activity in Slovenian Cheese Made from Pasteurized, Thermized or Raw Milk. *Acta Agric. Slov.* **2012**, *100*, 265–268.
44. Todaro, M.; Lo Presti, V.; Macaluso, A.; Alleri, M.; Licitra, G.; Chiofalo, V. Alkaline Phosphatase Survey in Pecorino Siciliano PDO Cheese. *Foods Basel Switz.* **2021**, *10*, 1648. [CrossRef] [PubMed]
45. Malissiova, E.; Alexandraki, M.; Manouras, A. Preliminary Data on the Suitability of Alkaline Phosphatase Use as Pasteurization Indicator for Donkey Milk. *Acta Vet. Eurasia* **2022**. [CrossRef]
46. Tsiamita, A.; Valiakos, G.; Natsaridis, N.; Fotiadou, S.; Manouras, A.; Malissiova, E. Preliminary Results on the Comparative Evaluation of Alkaline Phosphatase Commercial Tests Efficiency in Non-Cow Milk Pasteurization. *BioTech* **2022**, *11*, 39. [CrossRef] [PubMed]
47. Ying, C.; Wang, H.-T.; Hsu, J.-T. Relationship of Somatic Cell Count, Physical, Chemical and Enzymatic Properties to the Bacterial Standard Plate Count in Dairy Goat Milk. *Livest. Prod. Sci.* **2002**, *74*, 63–77. [CrossRef]
48. Persson, Y.; Larsen, T.; Nyman, A.-K. Variation in Udder Health Indicators at Different Stages of Lactation in Goats with No Udder Infection. *Small Rumin. Res.* **2014**, *116*, 51–56. [CrossRef]
49. Katsoulos, P.D.; Christodoulopoulos, G.; Minas, A.; Karatzia, M.A.; Pourliotis, K.; Kritas, S.K. The Role of Lactate Dehydrogenase, Alkaline Phosphatase and Aspartate Aminotransferase in the Diagnosis of Subclinical Intramammary Infections in Dairy Sheep and Goats. *J. Dairy Res.* **2010**, *77*, 107–111. [CrossRef]
50. Narenji Sani, R.; Hajigolikhani, B.; Ahmadi-Hamedani, M.; Kafshdouzan, K. Diagnostic Evaluation of Milk Lactate Dehydrogenase and Alkaline Phosphatase Activities by Receiver Operating Characteristic Analysis Curve in Early Lactation of Ewes with Subclinical Mastitis. *Vet. Res. Forum* **2018**, *9*, 343–348. [CrossRef]
51. Sharma, R.S.; Ganguli, N.C. Purification and Properties of Reactivated Alkaline Phosphatase from Buffalo Milk. *Milchwissenschaft* **1974**, *29*, 79–84.
52. Patil, M.P.; Nagvekar, A.S.; Ingole, S.D.; Bharucha, S.V.; Palve, V.T. Somatic Cell Count and Alkaline Phosphatase Activity in Milk for Evaluation of Mastitis in Buffalo. *Vet. World* **2015**, *8*, 363–366. [CrossRef]
53. Izadi, A.; Khedmat, L.; Mojtahedi, S.Y. Nutritional and Therapeutic Perspectives of Camel Milk and Its Protein Hydrolysates: A Review on Versatile Biofunctional Properties. *J. Funct. Foods* **2019**, *60*, 103441. [CrossRef]
54. Aspri, M.; Economou, N.; Papademas, P. Donkey Milk: An Overview on Functionality, Technology, and Future Prospects. *Food Rev. Int.* **2017**, *33*, 316–333. [CrossRef]