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ADVANCED ANALYTICAL TECHNIQUES APPLIED IN COW MILK METABOLOMICS

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Abstract: Milk metabolomics provides a comprehensive analysis of the biochemical content of cow's milk, revealing both the animal's physiological condition and the quality of the end product. Through the identification and quantification of low-molecular-weight metabolites, such as amino acids, fatty acids, sugars, and organic acids, metabolomics enables the detection of biomarkers associated with mastitis, nutritional imbalances, and milk authenticity. The establishment of reliable metabolic profiles relies on advanced analytical techniques, including nuclear magnetic resonance spectroscopy and liquid or gas chromatography coupled with mass spectrometry. This study synthesizes and critically reviews recent research on bovine milk metabolomics employing these state-of-the-art methodologies, aiming to elucidate the biochemical diversity and metabolic complexity underlying milk composition.

• Introduction

- Milk is a complex biological fluid, the chemical composition of which reflects the physiological, nutritional and health status of cattle. In recent years, metabolomics has become a reference approach in milk analysis, providing a detailed insight into low molecular weight metabolites involved in essential biological processes.
- Modern techniques, such as nuclear magnetic resonance (NMR) and liquid or gas chromatography with mass spectrometry (LC-MS, GC-MS), can identify and quantify amino acids, fatty acids, sugars, organic acids and other bioactive compounds.
- Thus, metabolomics is emerging as an interdisciplinary field, integrating biology, analytical chemistry and bioinformatics to obtain a holistic assessment of milk quality and cattle health.

Table 1. Summary of metabolomic approaches applied to bovine milk, highlighting analytical techniques, target metabolites, and main research applications reported in the scientific literature

Application area	Purpose of analysis	Analytical techniques	Examples of relevant metabolites	Reference
Animal health	Identification of biomarkers for mastitis and other disorders	NMR, LC-MS	Lactate, citrate, creatine, volatile fatty acids	Sundekilde UK, Larsen LB & Bertram HC. <i>NMR-Based Milk Metabolomics</i> , Metabolites, 3(2):204-222, 2013.
Nutrition and diet	Evaluation of diet impact on milk composition	GC-MS, LC-MS	Urea, short-chain fatty acids, choline	Xu W et al. <i>Metabolomics of Milk Reflects a Negative Energy Balance in Dairy Cows</i> . J Proteome Res., 2020.
Authenticity & traceability	Detection of milk adulteration or origin verification	LC-MS, NMR	Lactose, orotate, amino acid profiles	Sen C. <i>A critical review on metabolomic analysis of milk and dairy products</i> , Food Chem., 2021.
Quality & processing	Monitoring chemical changes during processing and storage	NMR	Glycerol, citrate, carbonyl compounds	Sundekilde UK et al. <i>Association between the bovine milk metabolome and dairy technology traits</i> . J Dairy Sci., 97(8):4804-4817, 2014.

▪ Results and discussions

- Metabolomic analysis of cow's milk provides a complex insight into the biochemical dynamics that reflect the health of the animal and the quality of the final product.
- The integration of NMR, LC-MS and GC-MS techniques allows the simultaneous identification of a large number of metabolites, including amino acids, fatty acids, sugars and organic acids, with high sensitivity and specificity.

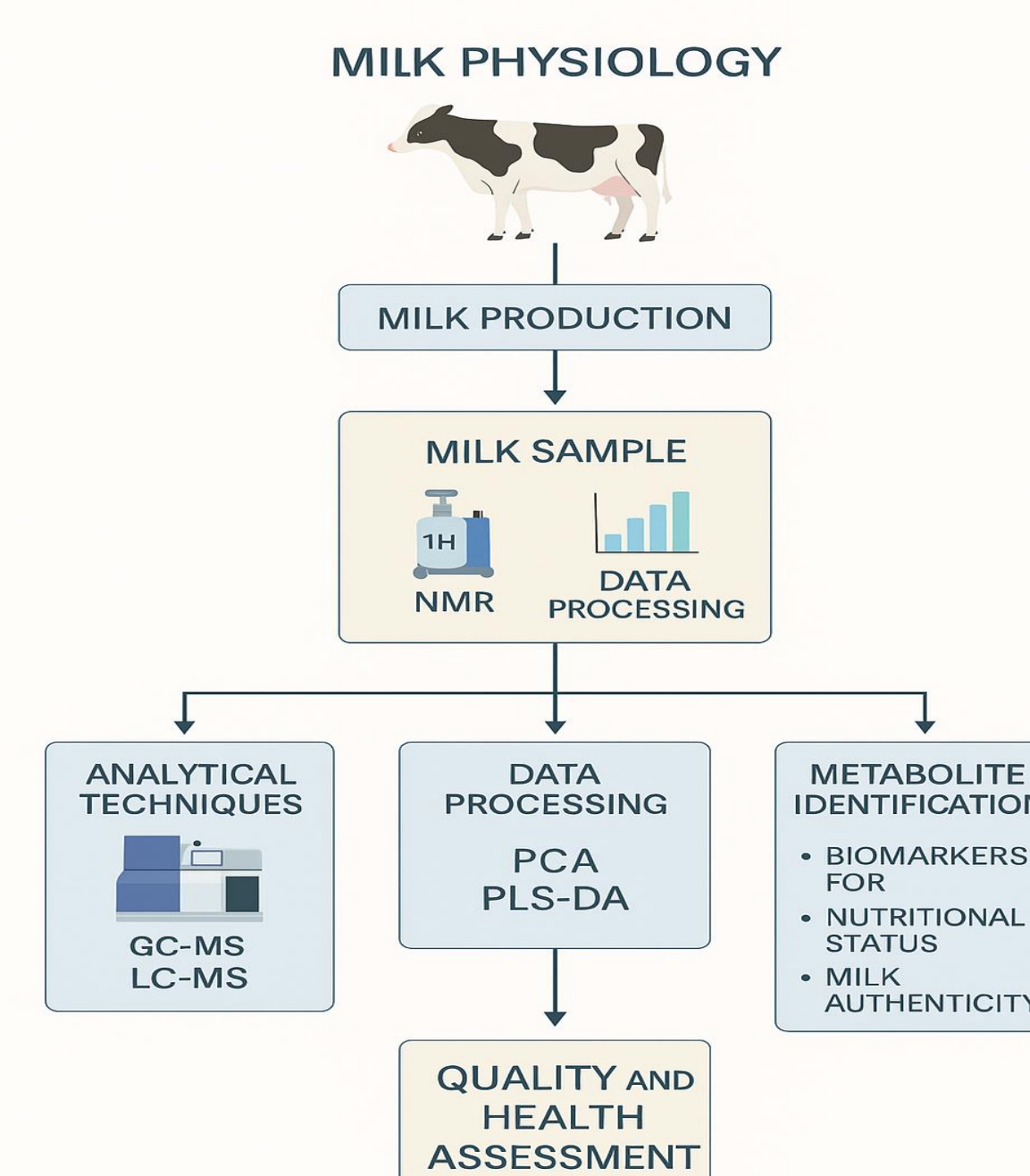


Figura 1. Schematic representation of the metabolomic workflow applied to bovine milk

- ✓ Figure 1 summarizes the workflow of metabolomics studies in the literature, from biological sample collection and processing to instrumental analysis and statistical interpretation of data.
- ✓ Metabolomics is emerging as an integrative field, capable of connecting chemical and biological data into a coherent system for assessing animal health and the nutritional value of milk.
- **Conclusions**
- ✓ The application of modern techniques (NMR, LC-MS, GC-MS) allows the identification of biomarkers associated with physiological state, diet and possible diseases, such as mastitis.
- ✓ Metabolic profiles can be used to assess the quality and authenticity of dairy products, contributing to traceability control in the food industry.
- ✓ Studies in the specialized literature confirm the role of metabolomics as an interdisciplinary field, capable of correlating chemical and biological data for a holistic evaluation of cow's milk.