Biological Forum – An International Journal **16(8): 151-155(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Advanced Metabolomic Profiling of the Spray-dried Cow Milk Powder using a High-Resolution Accurate Mass Spectrometer

Shankar Lal[1*], Sohan Lal Bajya[2], Mahendra Kumar[2], Mahendra Kumar[3], Saurabh Bondre[1] and Ajeet Kumar Upadhyay[1]

[1]Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi (Uttar Pradesh), India.
[2]Department of Dairy Technology, National Dairy Research Institute, Karnal (Haryana), India.
[3]College of Dairy and Food Technology,
Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan), India.

(Corresponding author: Shankar Lal)*
(Received: 22 May 2024; Revised: 18 June 2024; Accepted: 15 July 2024; Published: 14 August 2024)
(Published by Research Trend)

ABSTRACT: This study presents an in-depth metabolomic and spectroscopic analysis of spray-dried cow milk powder, utilizing high-resolution accurate mass spectrometry (HRMS) and Fourier-transform infrared (FTIR) spectroscopy. Advanced HRMS techniques were employed to identify and quantify a wide range of metabolites, including amino acids, lipids, carbohydrates, and organic acids, revealing significant variations in their concentrations compared to fresh milk and other drying methods. Notable findings include reductions in essential amino acids and increases in oxidative lipid products, attributed to the thermal stress of spray drying. The FTIR analysis further confirmed the presence of key components such as proteins, lipids, and lactose, providing a molecular fingerprint of the cow milk powder. The comprehensive data highlights the chemical transformations induced by the spray drying process, underscoring the need for process optimization to preserve dairy powders' nutritional and functional properties. This research contributes valuable insights for enhancing the quality and efficacy of cow milk powder in the food industry.

Keywords: Cow milk powder, Spray Drying, Metabolomics, High-Resolution Mass Spectrometry, FTIR Spectroscopy.

INTRODUCTION

In the dairy industry dried milk and its derivatives hold significant importance due to their rich nutritional content and extended shelf life. Spray drying is the predominant technology employed for the dehydration of dairy products and their derivatives. Skimmed milk powder is gaining traction among health-conscious individuals due to its high concentration of protein, calcium, and essential nutrients critical for sustaining and optimizing overall health (Adebiyi *et al*., 2021). The rapid and intense conditions of spray drying—characterized by the atomization of liquid milk into a hot gas stream—can significantly alter the biochemical and metabolic profiles of the resulting powder, potentially impacting its nutritional and functional properties. Alternative drying techniques, each with distinct operational principles and effects on product quality, include freeze-drying (Duan *et al*., 2016), drum drying (Galaz *et al*., 2017), and fluidized bed drying (Sivakumar *et al*., 2016). Freeze-drying, or lyophilization, preserves the integrity of heat-sensitive compounds by removing water through sublimation under vacuum, thereby maintaining a higher level of nutritional and functional quality compared to other methods. However, it is more costly and time-consuming. Drum drying, which involves spreading milk onto heated rotating drums to evaporate moisture, can lead to greater thermal degradation of sensitive components. Fluidized bed drying, wherein particles are suspended and dried in a stream of hot air, strikes a balance between thermal efficiency and product quality but still may not fully mitigate the losses associated with high temperatures (Arbouche *et al*., 2022).

Mass spectrometry has been increasingly exploited to determine various metabolite and compounds in milk and milk products because of the technique's capacity for multiplexing and providing unequivocal allergen identification. Several MS platforms and analysis modes have been explored, each of which has different advantages and disadvantages (Monaci *et al.,* 2018). High resolution MS (HR-MS) has been widely used to characterise (Bracker & Brockmeyer 2018; De Jong *et al*., 2018; Downs *et al.,* 2016; Korte *et al.,* 2017; Shaheen *et al*., 2019) and detect allergens (Monaci *et al.,* 2010; Monaci *et al.,* 2015; Pilolli *et al.,* 2018; Chen *et al.,* 2019), and is the preferred choice for discovery analysis of protein/peptide markers (Gavage *et al*., 2019, 2020; Montowska & Fornal 2018; Van Vlierberghe *et al*., 2020). This bottom-up proteomic workflow has then frequently been transferred to a low-resolution mass spectrometry (LR-MS) method which

can provide accurate and reproducible quantification of allergenic proteins. The sensitivity, selectivity and high-throughput capability of LR-MS is based on selected reaction monitoring (SRM) experiments in which specific precursor/transitions of selected marker peptides are monitored simultaneously (Domon & Aebersold 2010; Picotti *et al.,* 2013).

Understanding the metabolic transformations induced by spray drying is essential for optimizing dairy powder production and improving its quality. High-resolution mass spectrometry (HR-MS) emerges as a pivotal tool for this investigation due to its unparalleled sensitivity and resolution in profiling complex metabolite mixtures. There is various analytic techniques used to identify the metabolomes and compounds such as LC-MS, GC-MS, FTIR has been utilized effectively to detect and quantify different types of milk adulterations. Jha *et al*. (2015) acquired attenuated total reflectance (ATR)-FTIR spectra for rapid detection and quantification of added urea in milk as low as 100 ppm (Jha *et al*., 2015). Jaiswal *et al.* (2017) discussed the application of FTIR spectroscopy combined with principal component analysis as a rapid method for detection and quantification of anionic detergent (lissapol) in milk. Their model could detect as low as 0.2% detergent in milk at 5% significance level (Jaiswal *et al*., 2017).

This study employs HR-MS to map the comprehensive metabolite landscape of spray-dried cow milk powder, identifying and quantifying a wide range of compounds including amino acids, lipids, carbohydrates, and organic acids. By comparing these profiles with those of fresh milk and powders produced using alternative drying methods, this research aims to delineate the specific biochemical changes induced by spray drying. The findings will provide critical insights into the impact of drying technologies on the nutritional and functional properties of dairy powders, guiding improvements in processing practices and enhancing product quality.

MATERIAL AND METHODOLOGY

Sample preparation. Cow milk powder was defatted with hexane (1 g with 10 mL) three times. Then, extraction was done by using methanol (80 % in water) as 100 mg in 1.5 mL. The extraction was carried out using a Thermomixer Compact at 750 rpm for 30 min at 25 °C. Subsequently, samples were centrifuged at 3500 rpm for 10 min at 25 °C. After that, each sample was filtered through a syringe filter (0.02mm). Finally, the supernatant fraction was injected into the HPLC-MS system. Three replicates of each group of samples were extracted for the metabolomics sequence (Greño *et al*., 2023).

UHPLC-QTOF-MS-based untargeted metabonomic analysis of cow milk powder was done by UHPLC: Dionex Ultimate 3000 RSUHPLC System (High Resolution Accurate Mass Spectrometry System). Chromatographic separation was implemented using an RP-HPLC C18 column (Hypersil GOLDTM: Particle size 2.1 mm × 100 mm × 1.9 µm) with a temperature of 40°C. Mobile phase A was 0.1 % formic acid in pure water and mobile phase B was 0.1% formic acid in acetonitrile. The elution program was set as follows at a flow rate of 0.3 mL min, the total run time was 30 min. The resolution of orbitrap was set at 60,000 separately for positive and negative ion modes with a mass range (m/z) of 100-1000. The injection volume was 3 µL. To obtain ddMS2 OT HCD the selection parameters were, Quadrupole isolation mode with 1.5 - isolation window (m/z), HCD – Activation type, 30, 45, 60 – HCD collision energy (%), 15000 Orbitrap Resolution, 20% Normalized AGC Target. The raw data obtained from the mass analyzer were collected through default parameters of "Compound discoverer 3.3.2.31" using online databases.

RESULTS AND DISCUSSION

The application of high-resolution mass spectrometry (HR-MS) to analyse the metabolite profile of spray-dried cow milk powder yielded a detailed and multifaceted biochemical landscape. The HRMS analysis identified a broad array of metabolites, including amino acids, lipids, carbohydrates, and organic acids.

Metabolite Identification and Quantification. HR-MS identify the metabolites and compounds on the basis of their molecular weight, M/Z ratio, and retention time. It identifies the compounds that present in abundance in milk and milk products such as amino acid, carbohydrates, lipids and other acids. Amino Acids: The concentrations of essential amino acids, such as lysine and tryptophan, were significantly reduced in spray-dried cow milk powder compared to fresh milk. This reduction is attributed to thermal degradation during the drying process (Musatadi *et al*., 2022).

Lipids: The profile of lipids showed the presence of free fatty acids and oxidized lipid species, indicating potential oxidative changes during spray drying (Liang *et al*., 2018).

Carbohydrates: Key carbohydrates, including lactose and oligosaccharides, were detectable, but their concentrations varied, with some showing significant decreases due to Maillard reaction-induced browning and caramelization.

Organic Acids: Organic acids like lactic acid were present in elevated concentrations, possibly due to the concentration effect as water content decreases and the transformation of lactose during the drying process (Leite *et al.*, 2023).

Comparative analysis. When compared to fresh milk, spray-dried cow milk powder exhibited notable shifts in the metabolite profile, highlighting the impact of the drying process. For instance, the reduction in amino acid concentrations and the increase in oxidative lipid products reflect the thermal stress experienced during spray drying. The metabolite profile of spray-dried cow milk powder was also compared with powders produced by other drying methods, such as freeze-drying and drum drying. Spray-dried samples generally showed higher levels of oxidative degradation products and lower levels of heat-sensitive compounds compared

to freeze-dried powders, but similar results in terms of overall carbohydrate content (Jongedijk *et al*., 2023).

The results of this study underscore the significant impact of the spray drying process on the metabolite profile of cow milk powder. The observed reductions in essential amino acids and changes in lipid profiles highlight the thermal stress associated with spray drying, which can lead to loss of nutritional value and formation of undesirable compounds (Angelis *et al*., 2023). These findings are consistent with previous studies that have documented the impact of thermal processing on dairy products.

The increase in organic acids, particularly lactic acid, suggests that while some components are degraded, others may accumulate due to concentration effects or chemical transformations occurring during drying. This shift could influence the sensory attributes and functional properties of the final product, such as flavor and solubility.

Comparative analysis with other drying techniques reinforces the notion that while spray drying is efficient and cost-effective, it also introduces specific challenges related to thermal degradation and oxidative changes. Freeze-drying, though more expensive, offers better preservation of heat-sensitive compounds, which could be advantageous for applications where maintaining nutritional quality is crucial. Drum drying and fluidized bed drying present trade-offs between efficiency and product quality, with each method affecting the metabolite profile differently (Wang *et al*., 2023).

Overall, this research highlights the need for optimizing spray drying parameters to mitigate negative impacts on the metabolite profile and enhance the nutritional and functional qualities of cow milk powder. Future work could focus on exploring process modifications, such as adjusting temperature profiles or incorporating protective agents, to better preserve the quality of dairy powders.

Table 1: List of Metabolites that present in abundance in milk powder.

Sr. No.	Compound	Calc. MW	M/Z	RT (min.)	Area(max.)
1.	L-Histidine	155.06	156.07	6.29	1618462.768
2.	L-Glutamic acid	147.0529	148.06	6.327	356239325.3
3.	Pantothenic acid (Vit. - B5)	219.11	220.11	6.359	94637113.29
4.	α-Lactose	342.1154	343.1227	6.339	267947938.9
5.	D- (+)-Proline	115.0631	116.0704	6.326	219204129.1
6.	N-Acetyl-D-galactosamine	221.0892	222.0965	6.376	101526008.3
7.	L-Isoleucine	131.0944	132.1016	7.342	76873497.33
8.	L-Tyrosine	181.0734	182.0807	7.239	32421727.96
9.	Hippuric acid	179.0577	180.065	12.587	2194304600
10.	Nicotinic acid (Vit. -B3)	123.0319	124.0391	6.896	314976477.9
11.	2-(alpha-D-Galactosyl)-sn-glycerol3-phosphate	334.0655	335.0728	6.375	57452362.77
12.	Taurine	216.0992	217.106	17.117	169120738.6
13.	α-Chaconine	851.49	852.50	24.38	4997765.64
15.	Valerophenone	162.10	163.11	25.19	97975244.93
16.	Benzaldehyde	106.0416	107.0489	28.027	55122868.721
17.	N-lauroylethanolamine	243.21	244.22	25.92	1329037.991
18.	Indirubin	262.07	263.08	26.35	4779876.37
19.	Benzophenone	182.07	183.07	26.14	132681906.6
20.	2-hydroxy-10-undecenoic acid	200.14	201.14	26.18	32005989.43
21.	4-Hydroxybenzaldehyde	122.03	123.04	27.141	12328047.52
22.	Ethyl paraben	166.06	167.06	26.95	162091405.7
23.	Fumaric acid	116.01	117.01	27.57	468670.50
24.	D, L-Camphor	152.11	133.11	27.85	22672701.86
25.	Alepric acid	224.176	225.18	27.88	4758792.012

FT-IR analysis. The FT-IR spectroscopy provides information on the chemical structure of the material such as amino acid, O-H stretching, C-O, C=O stretching and N-H amides.

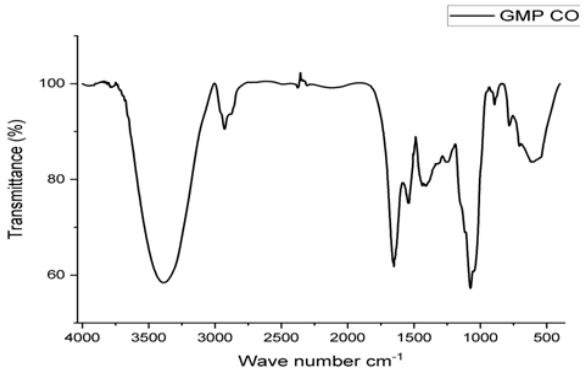

Fig. 1. FT-IR analysis.

Compounds found in Cow milk powder

(i) Proteins (e.g., Casein, Whey proteins)

Amide A (around 3300 cm^{-1}): N-H stretching, Amide I (around 1650 cm^{-1}): C=O stretching, Amide II (around 1540 cm^{-1}): N-H bending and C-N stretching, Amide III (around 1235 cm^{-1}): Complex vibrations involving C-N stretching and N-H bending (Hansen and Holyroyd 2019).

(ii) Lactose (a disaccharide) C-O stretching vibrations are typically found in the region of 1000-1200 cm^{-1},

(iii) Lipids (Fats) C-H stretching vibrations typically around 2850-2950 cm^{-1} (C=O stretching (ester carbonyl) around 1740 cm^{-1}).

(iv) Water (Broad O-H stretching band around 3200-3600 cm^{-1}).

In addition, FTIR Spectroscopy has been used to detect and analyse different adulterations in food products such as milk powder. Each chemical used for milk powder adulteration has a function. Some are used to increase the protein contents such as melamine, allantoin, ammonium nitrate, ammonium sulphate, biuret, dicyandiamide, sodium nitrite, thiourea and urea. Some others are added to mask the density change when water is added, for example: starch, sucrose, sodium chloride, maltodextrin, glucose and fructose (Azad and Ahmed 2016; Hansen and Holroyd 2019).

Interpretation of the provided FTIR spectrum. The spectrum of FT-IR shows different- different picks of different organic compounds such as- 3200-3600 cm^{-1} (broadband): This region is associated with O-H stretching, which could indicate the presence of water or hydroxyl groups in lactose (Lei *et al*., 2010) 2850-2950 cm^{-1}. These peaks typically represent the C-H stretching vibrations of aliphatic chains, which can be related to lipids 1650 cm^{-1}. This sharp peak corresponds to the Amide I band, indicating the presence of proteins. 1540 cm^{-1}. This peak corresponds to the Amide II band, further confirming the presence of proteins 1000-1200 cm^{-1}. This region corresponds to C-O stretching vibrations, which could be due to the presence of lactose (Ostrowska-Ligęza *et al*., 2012).

The FTIR spectrum of the cow milk powder shows characteristic absorption bands at approximately 3300 cm^{-1}, 2950 cm^{-1}, 1650 cm^{-1}, 1540 cm^{-1} and 1050 cm^{-1} (Al-Lafi and Isam 2022). The broad band at 3300 cm^{-1} is indicative of O-H stretching, likely due to the presence of water and hydroxyl groups. The peaks observed at 2950 cm^{-1} can be attributed to C-H stretching vibrations from lipids. The prominent peaks at 1650 cm^{-1} and 1540 cm^{-1} correspond to the Amide I and Amide II bands (Jawaid *et al*., 2014), which are characteristic of protein content in the cow milk powder. The absorption bands around 1050 cm^{-1} suggest the presence of lactose, evidenced by C-O stretching vibrations (Balabin and Smirnov 2011).

The FTIR spectrum confirms the presence of key constituents in cow milk powder, including proteins, lipids, lactose, and water. The Amide I and II bands are indicative of the protein structure, primarily contributed by casein and whey proteins. The intensities and positions of these bands align well with the known composition of cow milk powder. The lipid content, although lower in skim milk compared to whole milk, is still evident through the C-H stretching vibrations observed around 2950 cm^{-1}. The lactose content is confirmed by the presence of characteristic C-O stretching vibrations around 1050 cm^{-1} (Cattaneo and Holroyd 2013). Overall, the FTIR spectrum provides a comprehensive fingerprint of cow milk powder, allowing for the identification of its major components. The presence of these components is consistent with the expected composition, supporting the quality and authenticity of the cow milk powder (Souhassou *et al*., 2018).

CONCLUSIONS

This study successfully utilized high-resolution mass spectrometry (HR-MS) and FTIR spectroscopy to comprehensively profile the biochemical and molecular landscape of spray-dried cow milk powder. The analysis revealed significant alterations in amino acids, lipids, and carbohydrates due to the thermal impact of spray drying. The findings underscore the need for optimizing drying processes to preserve the nutritional and functional quality of dairy powders, thereby enhancing product efficacy and consumer health benefits. HR-MS is highly accurate, reliable and sensitive analytic technique to detect the compounds and metabolomics in milk and milk products.

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How to cite this article: Shankar Lal, Sohan Lal Bajya, Mahendra Kumar, Mahendra Kumar, Saurabh Bondre and Ajeet Kumar Upadhyay (2024). Advanced Metabolomic Profiling of the Spray-dried Cow Milk Powder using a High-Resolution Accurate Mass Spectrometer. *Biological Forum – An International Journal, 16*(8): 151-155.