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MALDI-TOF MS: Applications in Dairy and Related Sectors

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Abstract

MALDI-TOF MS is a soft ionization technique suitable for analysis of peptides, proteins, glycoproteins, oligosaccharides and oligonucleotides etc. In dairy sector, it can be used to analyze milk proteins, to identify adulteration in milk, isolation of phospholipids, characterization of proteins and enzymes of the microorganisms used in manufacture of cheese and other fermented products. In food industry, it can be used for evaluation and determination of fructo-oligosaccharides, inulins, certain pigments and flavor compounds present in different foods. This technique has found applications in a variety of disciplines including proteomics, glycomics, dairy, food, chemistry, biochemistry, microbiology, and physics. The purpose of this review is to summarize the contribution of the studies already performed with MALDI-TOF MS concerning dairy and related sectors.

Keywords: *MALDI-TOF, detector, mass analyser, laser, reflectron, spectrum, ionisation*

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INTRODUCTION

Mass spectrometry is an analytical tool which is used to determine the molecular mass of molecules depending on their m/z ratio. Matrix-assisted laser desorption ionization is a recent soft ionization technique (MALDI) commonly used in mass spectrometry for analysis of peptides, proteins, glycoproeins, oligosaccharides and oligonucleotides etc. It was first described by Karas and Hillenkamp [1]. MALDI is commonly used in conjunction with time of flight (TOF) mass analyser to achieve higher accuracy and better resolution. MALDI-TOF MS is a boon to the field of proteomics, genomics and glycomics.

MALDI-TOF MS can be used in characterization of milk proteins and determining post-translational modifications in them. This technique is also used for analysis of low abundance proteins in milk and proteolytic products produced during cheese manufacture [2].Other applications of MALDI-TOF MS include identifying protein markers and biomarkers for diagnosis of different diseases, identification of adulteration in milk [3], structure elucidation of glycosides and bacteriocin detection from whole bacterial cells [41] etc.

General Structure of Mass Spectrometer

Generally, a typical Mass Spectrometer consists of three parts: an ion source, a mass analyzer and a detector. The function of the ion source is to produce ions from the sample. The function of the Mass Analyzer is to separate ions with different mass-to-charge ratios. Then the numbers of different ions are detected by the detector. Finally, the mass spectrum is generated after all the data have been collected. Figure 1 is a scheme graph of the Mass Spectrometer [4].

PRINCIPLES OF MALDI-TOFF

First, the polymer needs to be dissolved in a solvent. Proteins are usually dissolved in water, sometimes acetonitrile is added. For other biomolecules, other solvents may need to be found. Also, some MALDI specs do not need to have the sample in solution, it can be in a solid state. Next, after the compound is in solution, a matrix needs to be added. Similar to AA, different matrices work better with different compounds. However, all matrices need absorb UV radiation. Some common matrices are trans-cinnamic acid or 2,5-dihydroxybenzoic acid, 2-(4-Hydroxyphenylazo) benzoic acid,3,5-Dimethoxy-4-hydroxy cinnamic acid, α-cyano-4-hydroxy cinnamic acid.

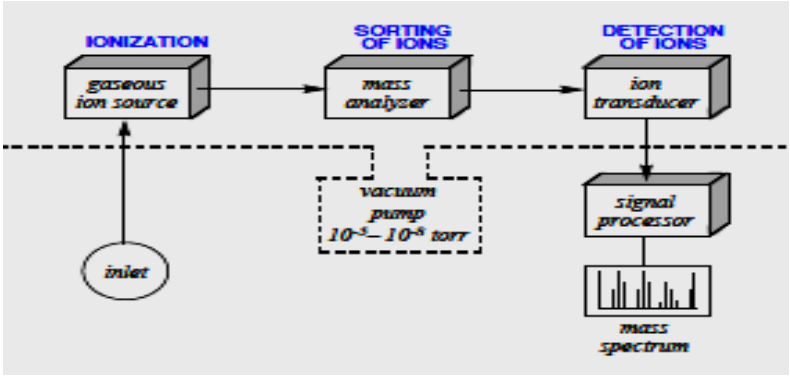

Fig. 1: *Scheme graph of MS.*

Functions of matrix is to reduce intramolecular interactions between sample molecules, protects sample molecules from laser decomposition, transfers energy to sample molecules to induce ionization. Matrix is generally added in an amount that is greater than 10^4 times the sample, ensuring that the matrix absorbs a majority of the radiation rather than the sample. This will prevent unwanted sample fragmentation. Another important aspect of the matrix is that it serves to isolate polymers from one another. Finally, the matrix serves as a source of protons for the sample to ionize.

After the sample has been prepared, the solution is loaded into the sample chamber, which is then vacuum pumped to evacuate all of the air in the chamber. At this time, the solvent evaporates, leaving the sample in a dispersed compound containing both the matrix and the sample (Figure 2).

Sample
⇩
2D Electrophoresis
⇩
Excise individual spots
⇩
Digestion of individual spot with trypsin
⇩
Mix the peptide mixture with a suitable volatile solvent, usually traces of trifluoroacetic acid (if +ve ionization) with concentration of sample = 10pmol/L
⇩
Mixing 1-2 µL aliquot of sample solution with a solution containing vast excess of matrix
⇩
Loading of the matrix-analyte mixture on metal slide/plate
⇩
Co-crystallization in oven & formation of matrix-analyte lattice
⇩
Loading of plate in the instrument

Fig. 2: *Sampling for MALDI-TOF.*

At this point, the laser shoots short pulses of light, in the 330–360 nm range, at the sample, causing it to essentially explode (Figure 3). The matrix is vaporized, and the sample is ionized into the +1 or –1 state. No one is really sure of the mechanism of this ionization, but for some reason it occurs, and the +/– 1 state is found.

It is also at this point that the polymers do something unusual. They evaporate. Usually, the polymers are too big and heavy to evaporate, but at these high temperatures and low pressures, evaporation or desorption occurs. Thus, we are left with ionized polymers in the gaseous phase.

Ions are accelerated by electrodes at opposite end of tunnel of a known length. If it is a negative ion, it accelerates to the cathode; positive ions accelerate towards the anode. The charge depends on the type of molecule being analyzed as well as the matrix used. This electrical force is used to accelerate the particles down tunnel towards the detector at the far end (Figure 4). Since all of the ions have the same charge, their acceleration, and thus the time it takes to reach the detector are completely dependent upon the mass of the fragment.

Once the molecules reach the detector, a peak is registered. These peaks are recorded via a high speed recording mechanism (Figure 5). The size of the peak is proportional to the number of molecules that reach the detector at a given time interval. Schematics of MALDI-TOF MS are shown in Figure 6.

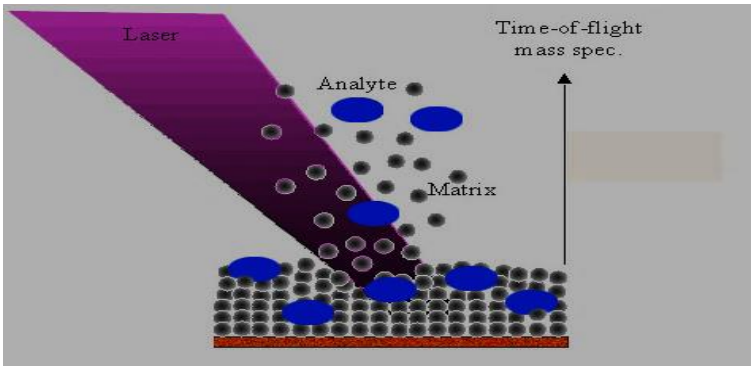

Fig. 3: *Principle of MALDI.*

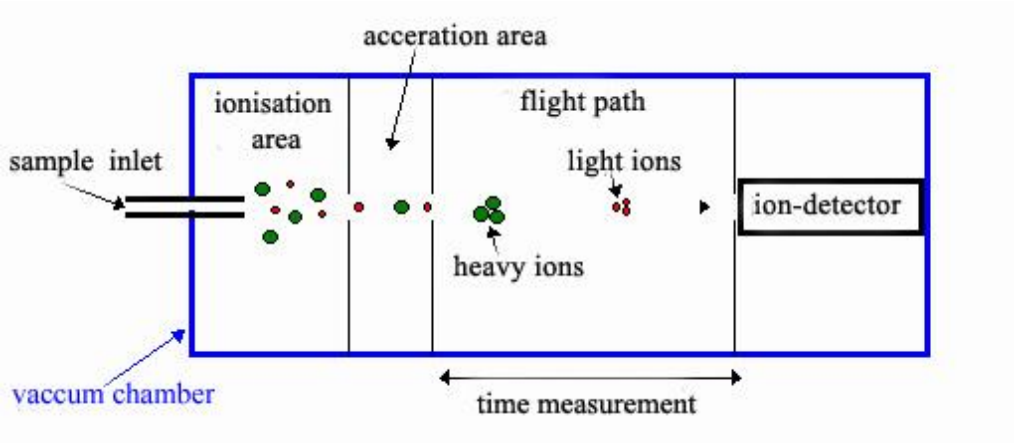

Fig. 4: *Principle of TOF.*

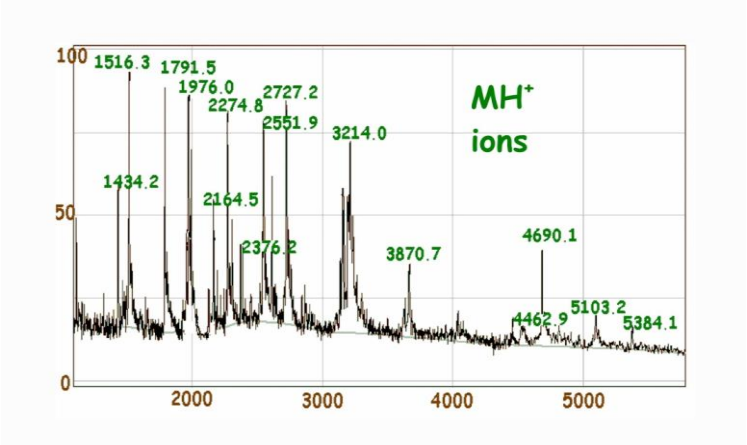

Fig. 5: *Positive Ionisation Maldi M/Z Spectrum of a Peptide Mixture Using Alpha- Cyano-4-Hydroxycinnamic Acid as Matrix.*

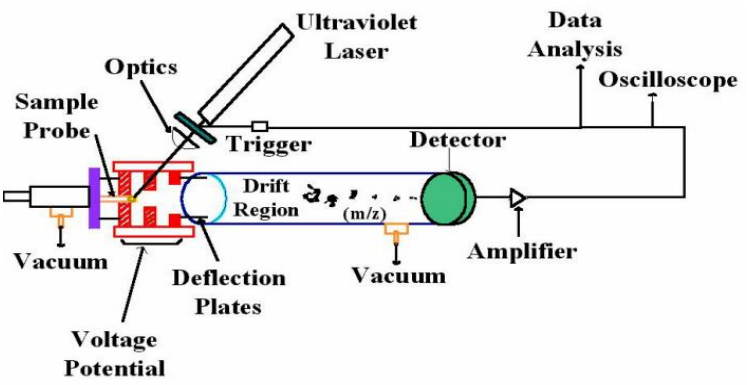

Fig. 6: *Schematics of MALDI-TOF MS.*

Analyzer of MALDI-TOF

The TOF mass analyzer measures the time it takes for the ions to fly from one end of the analyzer to the other and strike the detector. The flying speeds of ions are proportional to their mass-to-charge ratio. There are two kinds of mass analyzer, one works in linear mode, and the other works in reflectron mode.

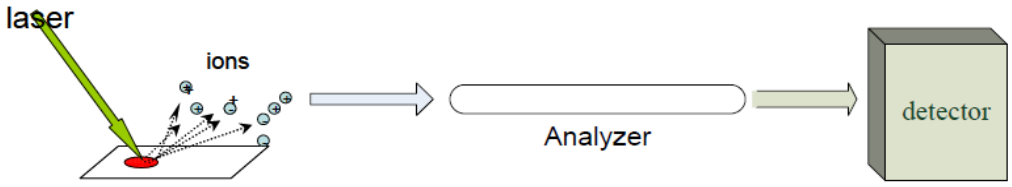

Fig. 7: *MALDI-TOF Analyzer in Linear Mode.*

Figure 7 is the scheme structure of MALDI-TOF MS in linear mode. In linear mode, MALDI-TOF analyzer works in a simple way. It just measure the time for flight for a ion to fly from one end to the other.

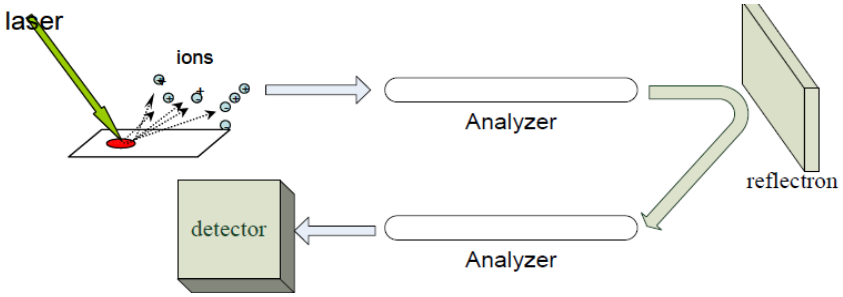

Fig. 8: *MALDI-TOF Analyzer in Reflectron Mode.*

Figure 8 is a scheme graph of MALDI-TOF MS in reflectron mode. Comparing Figure 7 and Figure 8 we can easily find that there is a reflectron in Figure 7, which brings us much advantage. The workflow in reflectron mode is much the same as that in linear mode. The only difference is that when the ion hit the reflectron, it will reflect and fly towards the detector. The reflectron focuses ions with the same m/z values, and makes them reach the detector at the same time, which results in more accurate detection [5].

Comparison of the Two Modes

The resolution of the linear mode with continuous extraction of ions was poor. The resolution means the ability to distinguish ions with similar m/z values. The resolution of the Mass Spectrometer is like the eyesight of a man. So the MS in linear mode is 'near sighted. However, with the help of the reflectron, Mass Spectrometer in reflectron mode enjoys a high resolution. So the reflectron acts like a set of lens which adjust the Mass Spectrometer's 'eyesight'.

Figure 9 gives a comparison of the mass spectrum of linear mode and reflectron mode [5]. This two mass spectrum are derived from the same sample, with Mass Spectrometer working in linear mode and reflectron mode, respectively. The x-axis is the m/z value, and the y-axle is the intensity. In reflectron mode, we can see four peaks clearly from the mass spectrum, while in linear mode, those four peaks merge into one, which makes it impossible for us to distinguish them (Figure 9) [5].

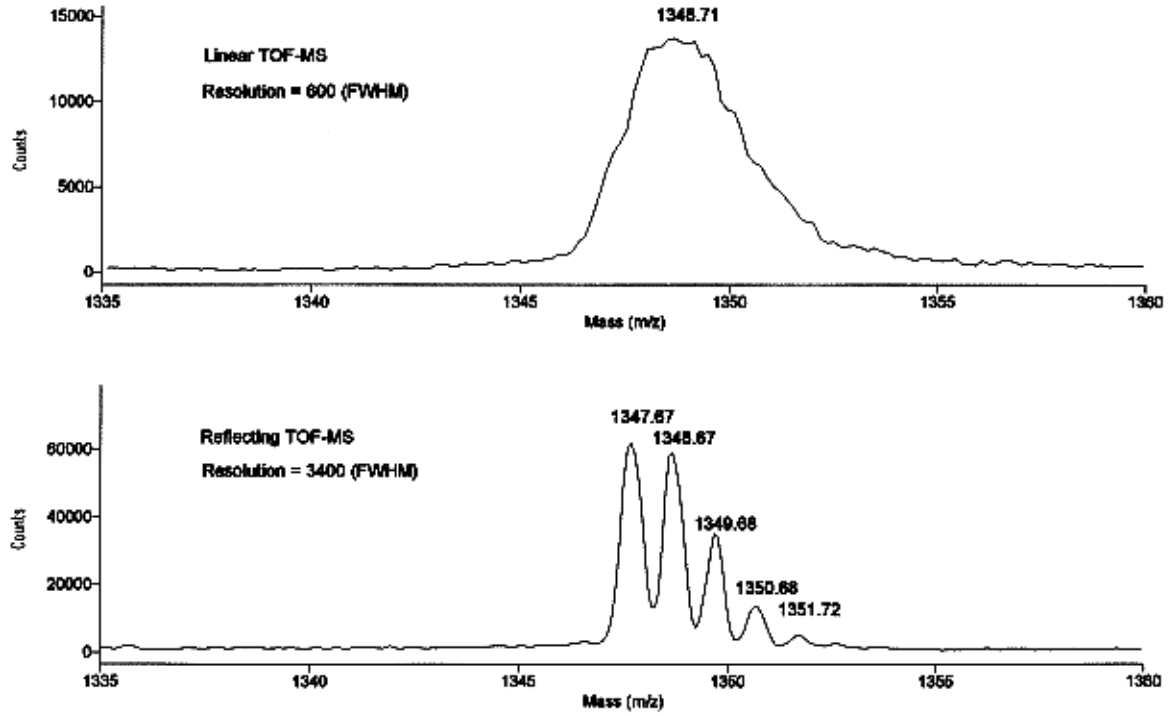

Fig. 9: *Mass Spectrum Given by Both Modes for the Same Material [5].*

ADVANTAGES

- Soft ionization technique.
- Easy sample preparation.
- High speed, very small sample loading, clean and simple spectra.
- Highly reproducible results.
- Fast data acquisition.
- No water or gas hook ups required.
- Good tolerance to non-volatile buffers and salts.
- Can give direct information on comparatively dirty samples.
- Produces large masses with high sensitivity.
- Little/no fragmentation, so valuable for heterogeneous mixtures.
- Practical mass limit ≈ 300 kDa.
- Low femtomole or low picomole sensitivity.
- Accuracy = 0.01.

DISADVANTAGES

- Low resolution.
- Matrix background can be a problem.
- MS/MS capability is minimum.
- Possibility of photo-degradation by laser.
- No possible to study non-covalent interactions.
- Low tolerance to phosphate buffers.

APPLICATIONS OF MALDI-TOF MS

Analysis of Milk Proteins

Characterization

- Polymorphism of goat α_{s1} casein was confirmed using MALDI-TOF MS [6].
- Mamone, *et al*., identified 34 casein components of ovine caseins using combined approach [7].

Post-translational Modifications

- Identification of phosphoaminoacyl residue in a peptide sequence, hence detection of phosphoserines and K-casein [8].

Low Abundance Proteins

- Separation and identification of bovine serum albumin, serotransferrrin and lactoferrin of bovine and human colostrums and mature milk [9].
- Identification of isoforms of lipocalin type prostaglandlin D synthase from whey fraction obtained from inflamed quarters [10].
- Wide coverage of growth factors in milk [11].

Proteolysis

- C-terminal truncated forms of β-lb in whey from Romegnola cow's milk [12].
- Determination of changes in milk protein profiles due to action of microbes during production [13].
- Characterization and determination of specificity of proteinases from LAB [14].

Complex Dairy Matrices

- Determination of different proteolytic enzymes and their specificity in different cheese types [15].
- Identification of peptides in Emmental cheese [16].

Some Common Examples of MALDI-TOF MS Applications of MALDI-TOF in Proteomics Glycomics

- MALDI-TOF MS in milk composition [17].
- Bovine milk proteomics and glycomics [18].
- Species-specific variations in casein phosphorylation [19].
- Identification of peptides in cheese or milk protein hydrolyzates, characterization of milk protein variants of glycoforms [20].
- Characterization of plasmin primary products from bovine and water buffalo milk using combined capillary IEF & MS [21].
- Quantification of β-casein fragments (f193-209) [22].
- Addition of probiotic microorganisms to improve proteolysis in cheddar cheese ripened at 4 and 8°C.
- Effect of minor milk proteins in chymosin separated whey and casein fractions on cheese yield as determined by proteomics and multivariate data analysis [23].
- Profiling of naturally occurring caseinophosphopeptides in human milk by TiO_2 columns & MS [24].
- Expression and characterization of bioactive recombinant human α-lactalbumin in milk of transgenic cloned cows [25].
- Profiling of N-glycoproteins in human milk [26].

Four steps included are:

- Isolation of milk protein with 12% TCA, dephosphorylation with alkaline phosphatise and tryptic digestion of protein.
- Selective glycopeptides enrichment from a complex peptide mixture via hydrophilic interaction.
- Deglycosylation of isolated glycopeptides by peptide N-glycosidase F (PNGase F).
- Sequencing of native and deglycosylated peptide by complementary MS technique (MALDI TOF) and identification of parent glycoprotein by correlation of tandem MS data with sequence database. On the whole, 30 sites of N-glycosylation were identified at Asn residues of consensus triplets Asn-X-Ser/Thr (X=amino acid).
- Analysis of insoluble proteins [27].
- Peptide sequencing of BSA (66kDa) determined by ISD [28].
- Peptide sequencing of oxytocin with PSD with intact disulphide bond and after its reductive cleavage to study post-translational modifications. Matrix used was α-cyano-4-hydroxy cinnamic acid [28].
- MALDI-TOF MS analyse peptides and proteins in wine and wine haze.
- Analysis of neutral and acidic carbohydrates in foods like milk and beer.
- MALDI-TOF MS in structure elucidation [29].

Lipid Extraction and Analysis using MALDI-TOF MS

- Selective extraction of phospholipids from dairy products by micro-solid phase extraction based on TiO_2 micro-columns and MALDI-TOF MS [30].
- New Ultraflectreme MALDI-TOF MS from Bruker Daltroniks Germany is a proprietary solution of samples from TLC-

MALDI detection like lipid identification directly from MALDI image [31].

Applications of MALDI-TOF MS in Genomics

- MALDI MS detection of oligonucleotides.
- Analysis of PCR products like short tandem repeats (STR) markers using MALDI-TOF MS [32].

Applications of MALDI-TOF MS in Disease Diagnosis

- Comparison of molecular weight based patterns in unhealthy versus normal tissue, help identifying potential biomarkers in lesions and in various stages of disease progress [33].
- MALDI-TOF MS in cancer diagnosis [34].
- To identify biomarkers of illegal treatment of cattle with growth promoting agents (GPA) [35].
- Detection of glycan markers for detection of hepatocellular carcinoma and chronic liver disease [36].
- Characterization of advanced glycation end products (AGEs)- mass changes in correlation to side chain modification [37].

MALDI-TOF MS for Identification of Adulteration

- Identification of adulteration in milk by MALDI-TOF MS [3].
- Identification of adulteration in water buffalo mozzarella and in ewe cheese by using whey protein as biomarkers and MALDI-TOF MS [3].

Detection of Chemical Substances from Whole Bacterial Cells using MALDI-TOF MS

- Bacteriocin detection from whole bacteria by MALDI-TOF MS [38].

Applications of MALDI-TOF MS in food Chemistry

- Evaluation of fructo-oligosaccharides (FAQs) and inulins as potentially health benefitting ingredients by HPAEC-PED & MALDI-TOF MS [39].
- MALDI-PSD TOF MS to identify and determine carotenoids and the esters in citrus juices [40,41]. They were able to determine 38 different carotenoids – oxygenated hydrocarbons as well as mono and di-esters.
- Identification and qualification of anthocyanins in foodstuffs like blueberries, red wine and fruit juice flavanoids, cerebrosides and saponins can be ionized and detected by MALDI-TOF MS.

CONCLUSION

MALDI-MS is a vital tool in mass analysis of biomolecules and organic macromolecules. Detection limits is of femtomole to attomole and the analysis is highly reproducible. In dairy sector, it can be used to analyze milk proteins, to identify adulteration in milk, isolation of phospholipids, characterization of proteins and enzymes of the micro-organisms used in manufacture of cheese and other fermented products. In food industry, it can be used for evaluation and determination of fructo-oligosaccharides, inulins, certain pigments and flavor compounds present in different foods. So, this technique has found applications in a variety of disciplines including proteomics, glycomics, dairy science, food, chemistry, biochemistry, microbiology, and physics. In future, it may help revolutionizing the medical world and lead to treatments for many diseases and be useful for DNA sequencing, thus can be useful for forensic investigations.

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