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Nanostructure-Assisted Laser Desorption/Ionization (NALDI) for Analysis of Peptides in Milk and Colostrum

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Abstract. Several bioactive proteins have been identified in colostrum and milk. However there are needs for development of technologies to identify and purify low molecular weight (LMW) peptides with bioactivity. The most used method is Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS), but matrix suppression often prevents detection of LMW components. Our approach was to work out a suitable method for analysing small peptides in bovine milk and colostrum without extensive sample pre-treatment. Nanostructure-Assisted Laser Desorption/Ionization (NALDI) is a matrix-free method to identify such LMW components. We also made a comparison between MALDI and NALDI for detection of peptides from colostrum samples. Our results show that NALDI provides better intensity compared with MALDI. It allows us to sequence small peptides and to identify a fragment of β-casein from the colostrum sample. Further studies are needed for comprehensive identification and characterization of LMW bioactive peptides from colostrum and milk.

Key words: colostrum, low molecular weight peptides, MALDI, NALDI.

INTRODUCTION

Colostrum and milk are rich sources of nutrients and also assure optimal immune defence in the newborn due to their high content of biologically active compounds protecting against pathogens and diseases. Until now, several bioactive proteins have been identified in colostrum and milk: immunoglobulins, lactoferrin, cytokines, and antimicrobial proteins and peptides, such as defensins and cathelicidins (reviewed by Park, 2009; Stelwagen et al., 2009). Some of the bioactive components may be applicable as food formulations or pharmaceuticals (reviewed by Wheeler et al., 2007). However, a comprehensive description of and knowledge about function of LMW components in milk and colostrums is limited. More detailed information about individual bioactive components may allow more widespread use and add value to the dairy industry.

To increase knowledge about properties of individual bioactive components there are needs for technologies to identify these and to obtain the components in purified form. One of the possibilities is application of sophisticated proteomics technologies.

The most used techniques for analysis of proteins and peptides in different food matrices are electro spray ionization (ESI) and MALDI-TOF MS (Shevchenko et al., 2001; Trauger et al., 2002; Coon et al., 2005; Jørgensen et al., 2010). However, there are also reports available that analysis of low molecular weight compounds with MALDI can be complicated because of intense chemical noise from the matrix (Cohen & Chait, 1996; Knochenmuss et al., 1996; Glish & Vachet, 2003; Go et al., 2003).

These problems can be reduced by using a matrix-free setup like NALDI which is reported to show good performance for molecules up to 3000 Da (Thomas et al., 2001; Lewis, 2003; Daniels et al., 2008; Dikler & Kowalski, 2009).

Currently there are no scientific reports available about methods for analysis of small peptides from colostrum and milk by NALDI. Thus we started to test suitability of NALDI for identification of bioactive proteins and peptides from colostrum and milk for later application in the dairy industry.

MATERIALS AND METHODS

Preparation of colostrum samples

Spray-dried colostrum powder (Biofiber Damino, Denmark) was used as a source of colostrum for identification of low molecular weight proteins and peptides. The colostrum sample was prepared as described previously by Jørgensen et al., (2010). Briefly, colostrum powder was mixed with ultrapure (MilliQ) water at 200 g per L. Dialysis bags (cut off 12–14 kDa) filled with water were placed in the colostrum mix overnight on a rotor at 4^{o}C. The content of the dialysis bags was subsequently purified through C_{18} Sep–Pak (Waters) columns for further analyses.

Analysis of colostrum samples by MALDI and NALDI

Colostrum samples were analysed as described by Daniels et al., (2008). Angiotensin I and II (Sigma-Aldrich Co) were used as standards for testing the plate and calibrating. The peptides were diluted in ultrapure water to produce a dilution series containing 100 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg angiotensin I or II.

1 µl of Sep–Pak eluates from the colostrum samples were diluted with 9 µl of HPLC grade water (Baxter, IL, USA) directly deposited on the plates. The samples were serially diluted up to one million times. The dots of the sample were dried overnight. The MALDI samples received 1 µl of a saturated solution of α–cyano–4–hydroxycinnamic acid (Bruker Daltonics, Germany) in 70% of acetonitrile. All samples were analyzed with an Ultraflex IV TOF/TOF, (Bruker Daltonics, Germany) equipped with a 337 nm nitrogen laser in positive mode. The detection was set to 10–3510 Da. The total amount of shots was 200 at 5 different places on the sample dot. Spectra were analyzed with software's from Bruker Daltonics (Flex-Control and Flex-Analysis version 2.4).

RESULTS AND DISCUSSION

Prior to analysis of colostrum samples, the NALDI plate was tested with the angiotensin I and II. Fig. 1 shows their characteristic ions at m/z 1296.374 and 1046.226.

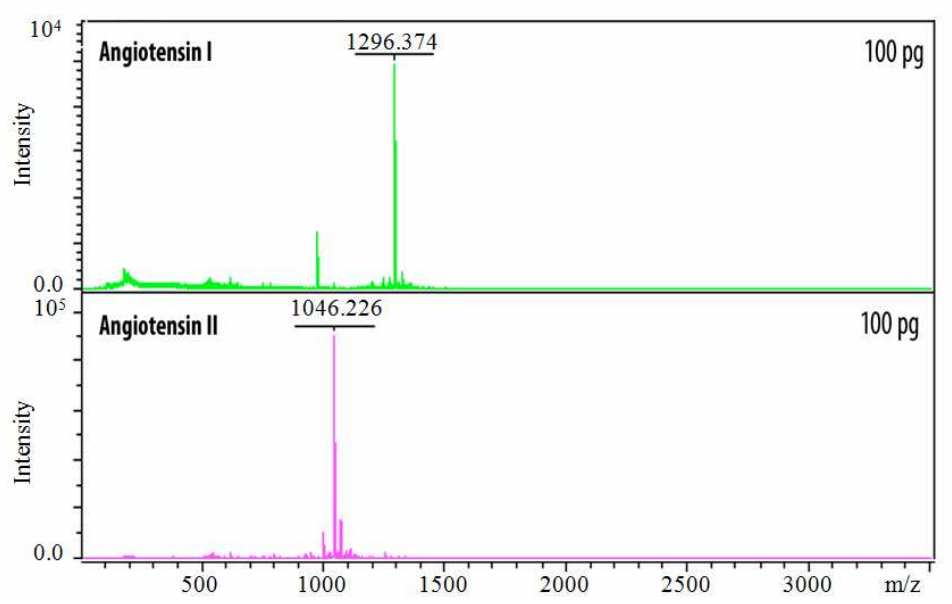

Figure 1. NALDI MS of Angiotensin I and II.

Fig. 2 shows dilution series of colostrum samples analysed by MALDI and NALDI. The results show that even a million fold dilution gives enough signal to detect peptides with NALDI. In contrast, MALDI gives spectra with intensity less than 300, so we are not able to fragment any of these ions. The intensity of the signal from the *β*-casein fragment is at least 10 times higher with NALDI than with MALDI.

The colostrum sample on NALDI can be diluted even more than a million fold. However it cannot be done with MALDI because of the increasing intensity of the matrix suppressing the signal from the colostrum sample. Fig. 2 shows that the spectra of NALDI and MALDI are not identical. We assume that this is probably due to differences in ionization between the two methods. Therefore both methods may be used for analysis of peptides in colostrum.

The most intense peak from the NALDI analysis was 1377.570 Da (Fig. 3) and was fragmented with tandem mass spectrometry.

The fragments were analyzed by *de novo* sequencing and Mascot search. The sequence was EPVLGPVRGPFPI (Fig. 4), which is identical to *β*-casein (210–222).

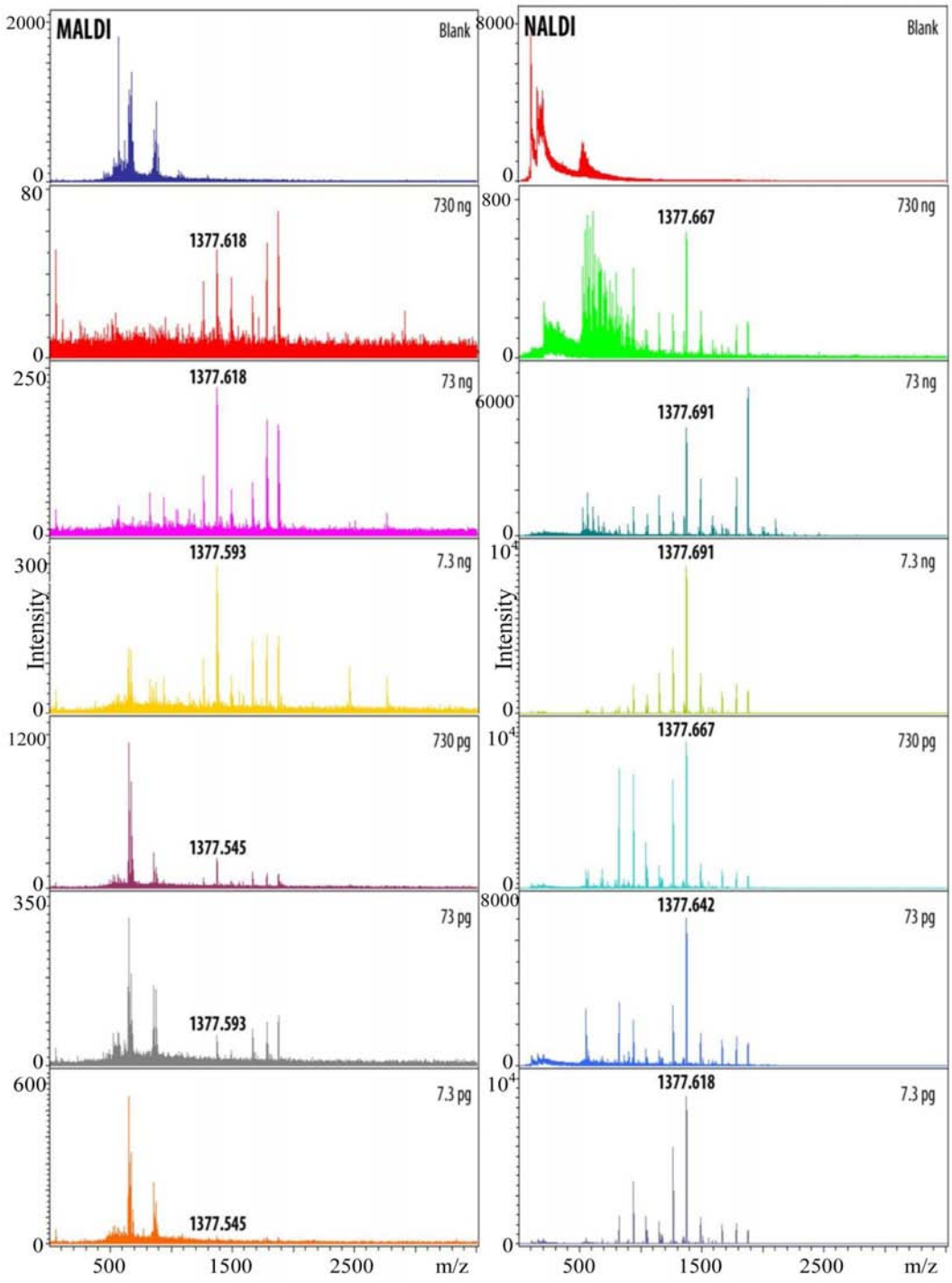

Figure 2. MS spectra of colostrum sample compared by MALDI and NALDI.

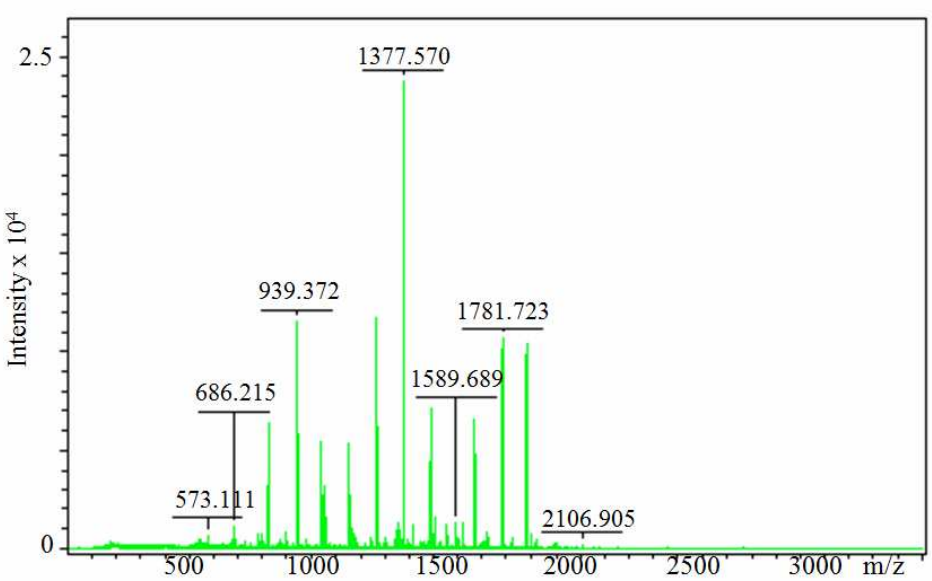

Figure 3. NALDI MS of colostrum sample.

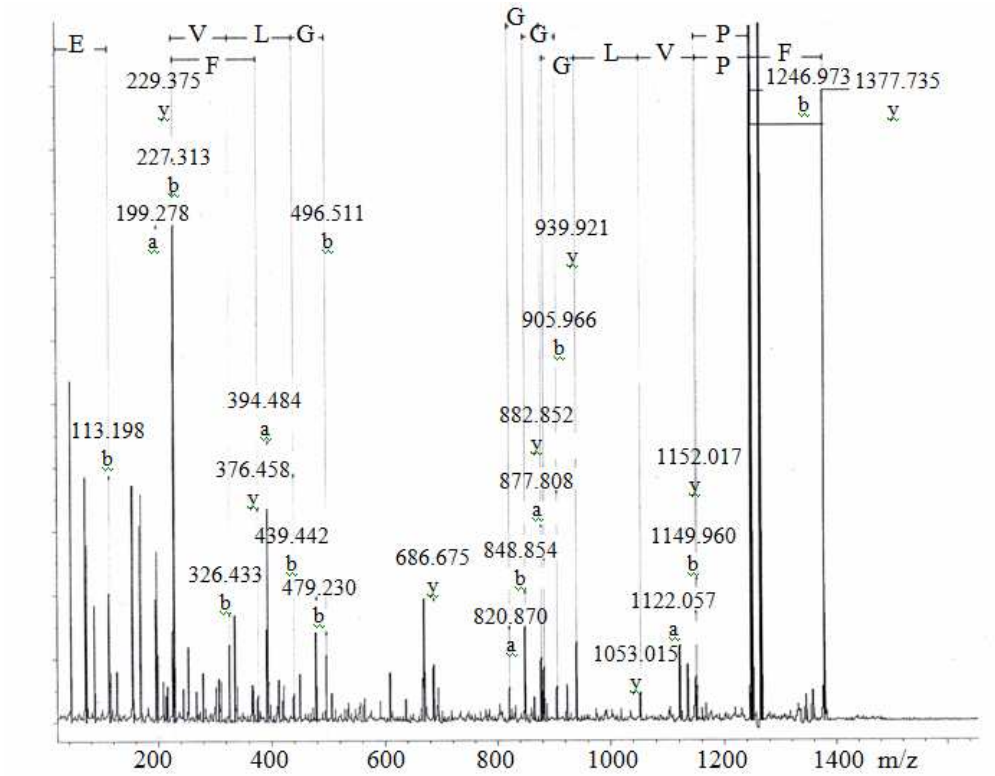

Figure 4. MS/MS spectrum of ion 1377.570. The figure shows detection of a, b and y ions after fragmentation and the fragment sizes.

CONCLUSIONS

This preliminary study was carried out to identify peptides from colostrum with molecular weight under 3000 Da. Our results show that NALDI is useful for analysis of low molecular weight peptides. NALDI has high sensitivity and is easy to use. Moreover, comparison between NALDI and MALDI shows that peak intensities with NALDI were at least ten times higher than those obtained with MALDI. In this study we were able to detect a β-casein fragment from colostrum. However further research is needed for a comprehensive identification and characterization of low molecular weight bioactive peptides from colostrum and milk.

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