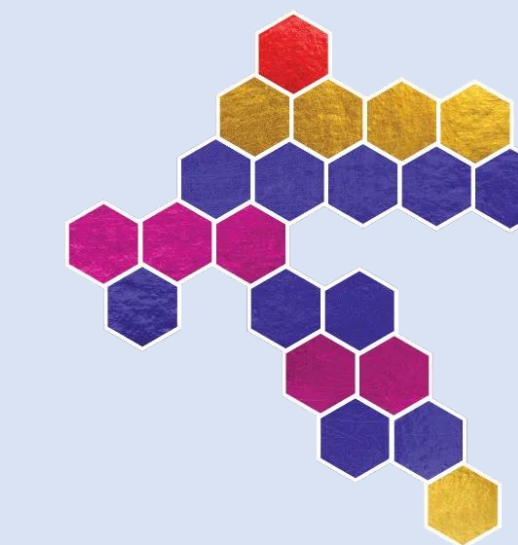


MALDI TOF mass spectrometry for identification aerobic mesophilic bacteria in raw and preserved milk

Snježana Kazazić¹, Nina Lovrić², Monica Darrer², Nataša Mikulec²

¹ Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

² University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia



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INTRODUCTION

The number of aerobic mesophilic bacteria in milk is one of the indicators of the hygienic quality of milk. Their quantity greater than prescribed indicates inadequate hygiene during production and processing, and their contamination represents a potential health hazard and negatively affects the quality of milk and milk products. Control of aerobic mesophilic bacteria's presence and identification is of great importance for the modern dairy industry. With MALDI-TOF mass spectrometry, it is possible to quickly and easily identify bacteria at the species and genus level and get a better insight into the hygienic quality of milk and the presence of certain bacterial species. The work aims to determine aerobic mesophilic bacteria and their number in raw unpreserved milk and milk preserved with azidiol, which is now ubiquitous in the dairy analysis as a bacteriostatic preservative.

MATERIALS AND METHODS

In 40 samples of raw and preserved milk with azidiol, the total number of aerobic mesophilic bacteria was determined using the flow cytometry method and the classic method of counting colonies on a nutrient medium according to the international standard HRN EN ISO 4833-1:2013.

Identification of bacteria was carried out by MALDI-TOF mass spectrometry.

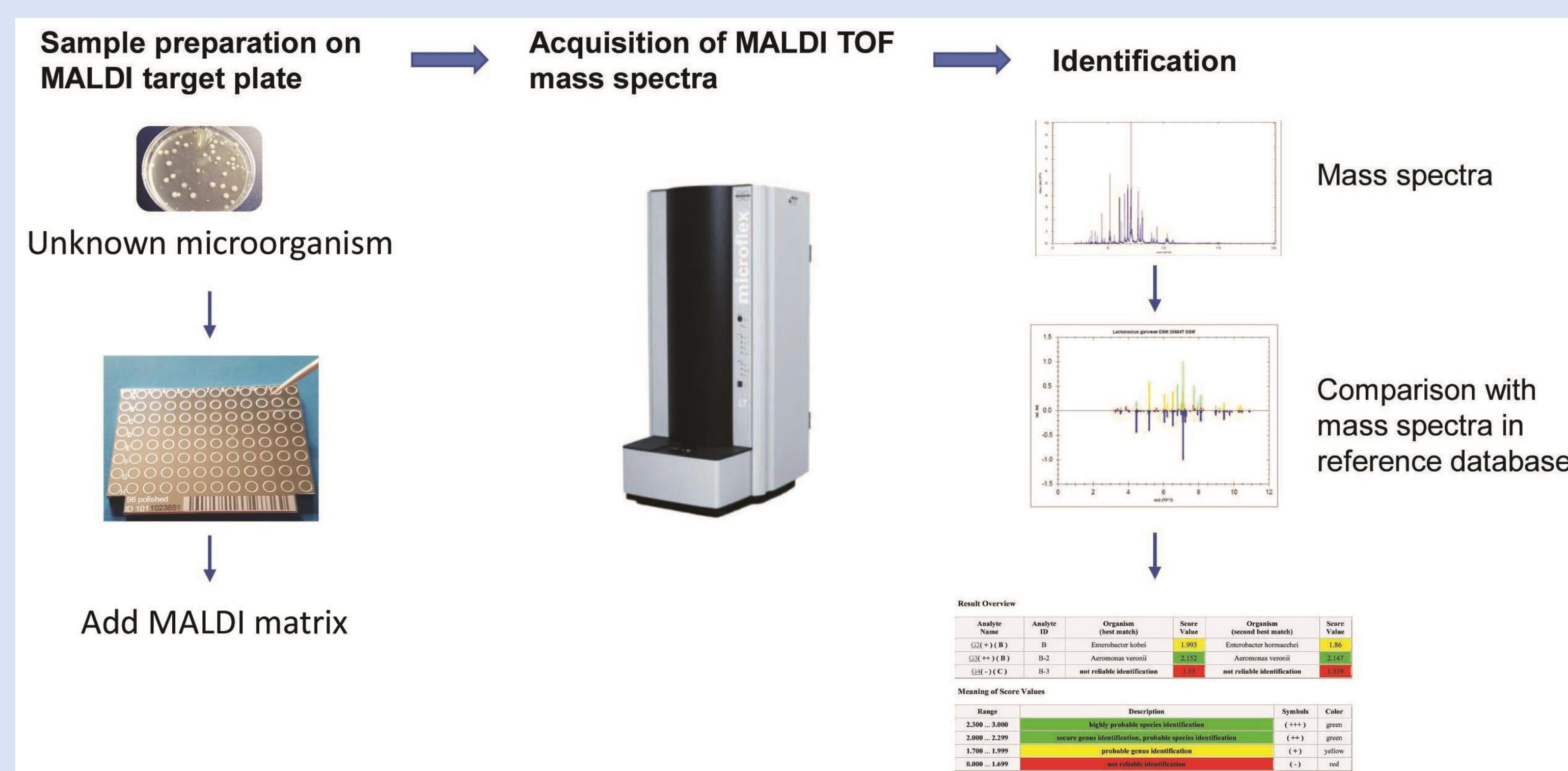


Figure 1 General workflow of identification with MALDI TOF mass spectrometry.

RESULTS

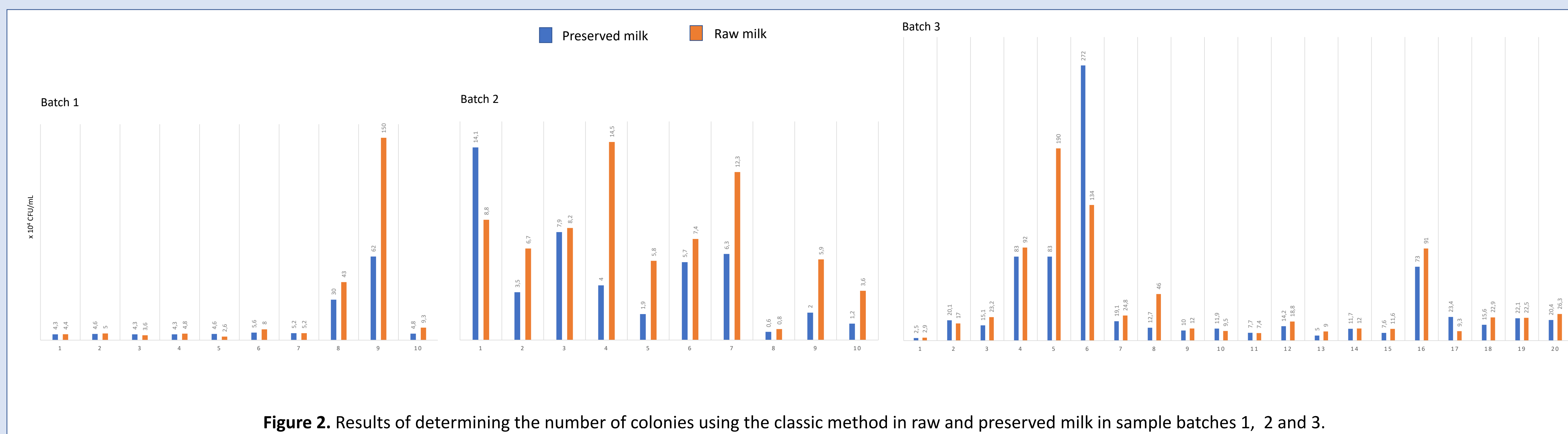


Figure 2. Results of determining the number of colonies using the classic method in raw and preserved milk in sample batches 1, 2 and 3.

	Batch 1 raw milk	Batch 1 preserved milk	Batch 2 raw milk	Batch 2 preserved milk	Batch 3 raw milk	Batch 3 preserved milk
Result (log score)						
2.000– 3.000	55	53	30	26	69	56
	44,00%	46,90%	41,10%	41,27%	35,38%	40,00%
1.7000– 1.999	39	32	27	22	78	53
	31,20%	28,32%	36,99%	34,92%	40,00%	37,86%
< 1.699	31	28	16	15	48	31
	24,80%	24,78%	21,92%	23,81%	24,62%	22,14%
TOTAL	125	118	73	63	195	140

Table 1. MALDI Biotyper results for sample batches 1, 2 and 3 for raw and preserved milk

Using the MALDI-TOF mass spectrometry, 29 genera and 45 bacterial species were identified in raw milk, and 25 genera and 36 bacterial species in preserved milk with azidiol.

The following influence of preservatives on the presence of certain bacterial species in raw and preserved milk was determined: the bacterial species that prevailed in raw milk samples were *Lactococcus lactis* (18.46%), *Acinetobacter johnsonii* (10.07%), *Lactococcus garvieae* (8.72%), *Enterococcus faecalis* (7.72%), *Serratia liquefaciens* (6.04%), *Enterobacter cloacae* (5.70%), *Rhodococcus erythropolis* (4.36%), while in preserved milk samples the following prevailed: *Lactococcus lactis* (28.51%), *Lactococcus garvieae* (9.09%), *Enterococcus faecalis* (8.26%) and *Serratia liquefaciens* (6.61%). Likewise, 16 bacterial species previously found in raw milk samples were not found in preserved milk samples, namely: *Acinetobacter baumannii*, *Acinetobacter lwofii*, *Brachybacterium nesterenkovi*, *Chryseobacterium gleum*, *Corynebacterium xerosis*, *Enterobacter asburiae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Masillia timonae*, *Moraxella osloensis*, *Ochrobactrum gallinifacis*, *Pseudoclavibacter helvolus*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Staphylococcus haemolyticus* and *Staphylococcus hominis*.

CONCLUSION

- The results showed a trend of decreasing the number of grown colonies in milk preserved with azidiol.
- The number of samples is too small to conclude the negative influence of azidiol on the total number of aerobic mesophilic bacteria in milk.
- More detailed insight into milk's hygienic quality and preservative's influence on the presence of certain species before and after being preserved with azidiol was obtained.

ACKNOWLEDGMENT

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