

Impact of probiotics on the metabolome of cow and goat milk yogurt

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Introduction: Probiotics are micro-organisms that are beneficial for the human gut. The addition of probiotics to fermented dairy products such as yogurt produces health potentiating bioactive compounds. The formation of bioactive compounds depends on the bacterial culture and the food matrices. Metabolomics platform utilizes high-throughput technology to assess and predict quality of yogurt. The role of metabolites in yogurt quality during storage might also reflect starter cultures' metabolic behavior. Thus, metabolomics is one of the most promising approaches for investigating metabolite changes in fermented dairy products and allows the determination of metabolite (<1500 daltons) present at very low concentrations.

Purpose: The overall goal was to utilize a gas chromatography spectrometry-based metabolomics approach to study the differences in probiotic culture behavior in the milk of two different species and decipher the association of post-acidification changes with the metabolite formation during storage of probiotic yogurt.

Methods: Pasteurized cow milk and goat milk were inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis* cultures. Inoculated milk samples were incubated at 42 °C until a pH of 4.5-4.6 was achieved. The samples were analyzed using a GC-MS based untargeted metabolomics on day 0, 14, and 28 of refrigerated storage. The metabolites were identified using NISTv 17 library, and the mass spectral data were analyzed using R-programming and MetaboAnalyst.

Results: A total of 192 metabolites were identified in cow and goat milk probiotic yogurt. The principal component analysis indicated different clusters of metabolites present between goat and cow milk probiotic yogurt on day 14. Among these metabolites, 24, 18, and 19 metabolites were significantly different ($p < 0.05$) between the cow and goat milk probiotic yogurt on day 0, 14, and 28. The relative ion abundance of octanoic acid, L-kynurenic acid, 2- amino adipic acid, and hexadecenoic acid had ($p < 0.05$) a 10-fold change in goat milk probiotic yogurt. In contrast, amino acids and peptides predominated the cow milk probiotic yogurt ($p < 0.05$) on day 14. The volcano plot showed a distinction between significant and non-significant metabolites. Impact pathway analysis depicted the predominance of amino-acyl t-RNA biosynthesis, fatty acid metabolism, and glycine, serine, and threonine metabolism.

Significance: These findings provide practical information regarding the probiotic impact on species-specific differences in metabolite profile and potential application for developing consumer demand-based fermented dairy products.

Keywords: Cow milk, Fermented dairy product, GC-MS, Goat milk, Metabolomics, Probiotics