IAFP Latino 2024
Simpósio Latinoamericano em Segurança dos Alimentos
Santos - SP - Brasil
11 a 14 Nov, 2024

Metabolomic Profiling of Canastra Cheese: Insights into the Maturation Dynamics

Luciano da Silva Pinto[1], Uelinton Manoel Pinto[1], Ariane Flavia do Nascimento[2], Bernadette Dora Gombossy de Melo Franco[1]

[1.] University of São Paulo, School of Pharmaceutical Sciences, São Paulo/SP, Brazil
[2.] Federal Institute of Sul de Minas, Campus Inconfidentes, Inconfidentes/MG, Brazil

The production of artisanal cheeses is experiencing growth in Brazil, with a significant diversity of cheeses produced across the country. Canastra cheese produced in the state of Minas Gerais in the Serra da Canastra region, is noteworthy. This cheese is produced using raw bovine milk and an endogenous starter culture known as “pingo,” which is derived from the whey collected from the previous day’s production. Maturation occurs at room temperature for a minimum of 22 days, and the manufacturing process does not include a heat treatment step. Throughout the maturation process, the microbiota contributes to the prolific production of metabolites derived from protein hydrolysis and lipid metabolism, and the generation of various compounds. These metabolites collectively play a significant role in determining the quality and identity of the cheeses.In this study, UHPLC-HRMS MS/MS was used for the differentiation of the metabolome of Canastra artisanal cheese for annotation through a free compounds database over the ripening period. Canastra cheeses were collected directly in the production sites, at various ripening times (0-5, 10, 14, 18, and 22 days), in triplicates. Samples (1,5g) of each cheese were extracted with methyl tert-butyl ether (MTBE) and methanol (MeOH), and the methanolic crude extracts (QAM) were subjected to protein precipitation using ice-cold methanol, resulting in QAMp extracts. The QAMp extracts were diluted to 200 ppm using MeOH-H_2O (1:1, HPLC grade). Then, samples were analyzed using an Ultra-High-Performance Liquid Chromatography-Diode Array Detector (UHPLC-DAD) system coupled with a Bruker Compact Mass Spectrometer, employing negative ion mode electrospray ionization to obtain MS and MS/MS spectra from selected samples. The acquired MS and MS/MS data were processed with MS-DIAL (version 4.9.221218) for metabolomic deconvolution. Compound annotation was performed using MS-FINDER (version 3.60), utilizing a free database. Statistical analysis of compound differentiation among the cheese samples during the 22-day ripening period was conducted using the MetaboAnalyst 6.0 online platform with MS and MS/MS raw data. The metabolomic analysis and statistical evaluation revealed distinct groupings in different quadrants for samples collected during the initial days of maturation (0-5 days) and the later days (10, 14, 18, and 22 days) in the Principal Component Analysis (PCA). Meanwhile, the Partial Least Squares Discriminant Analysis (PLS-DA) graphic, alongside Variable Importance in Projection (VIP) scores, identified the metabolites that exerted the greatest influence on the differentiation of samples across the maturation period. Several compounds were annotated based on both in silico predictions and experimental databases, including: 1-Oleoylglycerophosphoserine (**1**) LPS 18:0 (**2**), 2-amino-3-((hydroxy(2-hydroxy-3-((9E,12E)-octadeca-9,12-dienoyloxy)propoxy)phosphoryl)oxy)propanoic acid (**3**), LPI(16:1) (**4**), and 1-Arachidonoylglycerophosphoinositol (**5**). The metabolomic profiling follows the dynamic change in the microbiota in the Canastra cheeses along maturation, as results from the initial days of maturation (0-5 days) differed from those observed in the later days (10, 14, 18, and 22 days) ($p<0,005$). The detected compounds can be used as chemomarkers for quality control of these

cheeses, particularly in relation to ripening time.

Agradecimentos: Financial support: FAPESP processes 2013/07914-8 and 2022/15055-4