# Metabolomic approach to support food safety toward innovative milk processes

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Milk is considered a staple food and raw milk placed on the market for human consumption must be heat treated before packaging. The heat treatments that guarantee safety are mainly the pasteurization and sterilization. Considering the nutritional and organoleptic variations after these treatments, innovative nonthermal technology as Infrared (IR) technology can offer a promising solution for reducing microbiological loads while preserving quality traits, such as flavour [1]. The advantages of IR application are also energy efficiency, effective process control, shortening process time, uniform product temperature, high heat transfer coefficient, and to be environmentally friendly. Considering that milk processing is one of the most water-intensive industries in the agro-food system these peculiarities meet the needs of Sustainable Development Goals of the 2030 agenda in finding new technologies to reduce the environmental impact while maintaining high levels of safety and environmentally friendly food systems. However, the use of infrared radiation for milk and dairy products is still limited. Thermal processes are currently assessed by controls on the thermal inactivation kinetics of the endogenous milk enzyme, alkaline phosphatase, which is not applicable to IR treatment because it remains active despite being subjected to sufficient treatment to inactivate the pathogens. So, metabolomics could become a potential support for controls as well as secondarily investigating changes in milk. Metabolomics is a golden standard to identify chemical composition and quantify metabolites from products driven by various biochemical or treatment processes. Given the absence in literature of metabolomic studies on infrared radiation on milk, the aim of this work was to compare, through a metabolomic approach, the milk treated with IR radiation at 2 different energies with the starting raw milk, to identify possible treatment markers that can potentially be adopted at an inspection level. The milk of 3 different batches was sampled directly from the plant tank (100.000 L capacity, refrigerated at 4 °C), transported to the laboratory at 4 °C until the IR treatment at 2 energies (IR80 and IR85) on the basis of our recent preliminary study [1]. Then 2mL of raw milk, IR 80 and IR 85 milk samples were extracted with 4 mL of acetonitrile with 3% formic acid and then analysed by LC-HRMS analyses to investigate the metabolomic profile with subsequent data processing using Compound Discoverer software. Overall, 48 of 3005 items were confirmed in the negative electrospray ionization mode and 49 of 5240 in the positive one. IR-treated milk samples showed only one up-regulated compound (P2) compared to raw milk: hypoxanthine. In the presence of oxygen, xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine, further oxidized to uric acid, which has been shown, in literature, to display antioxidative activity in milk [2]. Moreover, from the differential analysis we noted a statistically significant increase of oleic acid, linoleic acid, mesaconic acid, arachidonic acid, dihomo-γ-linolenic acid, uridine, uric acid, uracil, vitamin B2, vitamin B4, carnitine, betaine, acetylcholine, cytidine, 7-Methylguanosine, L-tyrosine, β-alanine, L-glutamic acid, proline and a decrease of adenosine, citric acid, decanoylcarnitine, glucose, lactose, pyruvic acid, (±)9(10)-DiHOME, (±)12(13)-DiHOME. Currently, hypoxanthine could be a potential marker but further omic studies (e.g. lipidomic) will be conducted to discover other potential markers on this regard.

[1] Danesi et al. Preliminary Investigation towards the Use of Infrared Technology for Raw Milk Treatment. Foods, 13(7):1117, 2024
[2] Steffensen et al. Aldehyde-induced xanthine oxidase activity in raw milk. Journal of agricultural and food chemistry, 50(25):7392-7395, 2002.