

POPULAR SCIENTIFIC SUMMARY OF THE PROJECT

The objective of this project is to determine the effect of selected strains of fungi on the transformations occurring in beef during its dry aging, in a comprehensive approach using metabolomic studies.

Literature data concerning the methods of beef aging by both wet and dry methods indicate that beef subjected to the dry aging process has a more favorable flavor profile and texture compared to wet-aged meat. This process involves a complex microbiota, which, in addition to numerous species of bacteria, also includes fungi. Their enzymatic activity may contribute to the development of, *inter alia*, a unique flavor and aroma profile of such beef and shape its tenderness. However, so far, there is a lack of comprehensive assessment of the individual influence of each fungus on changes occurring in aged beef. There is also a lack of data on the assessment of differences in their metabolome, *i.e.*, differences in the profile of metabolites produced, which may be reactants as well as intermediate and end products of metabolism. Also, the safety of dry-aged beef is not known, given that fungi can be a source of toxic secondary fungal metabolites (*e.g.*, mycotoxins), which is particularly important from a public health point of view. Some of the formed metabolites may also be important due to the inhibition of the development of competitive microbiota. In order to meet the expectations regarding the understanding of the role of individual fungi in developing the quality of dry-aged beef, this project proposes to determine their individual influence on the metabolomic profile. Thanks to the use of modern chromatographic techniques combined with a high-resolution mass spectrometer with compatible data analysis software, it will be possible to map the metabolic pathways of fungi involved in dry aging beef. It is assumed that only comprehensive research related to the understanding of the role of the metabolome characteristic for an individual microorganism during the dry aging of beef will allow an understanding of this complex process. This is a novelty on a national and global scale.

The first stage of the planned research will be to determine the metabolomic and metagenomic profiles, as well as the quality (including sensory) of commercially available dry-aged beef. In the next stage, two strains of fungi (strain A, strain B) correlated with the unique quality of such meat will be selected and isolated, and their full characterization will be carried out (morphological characteristics, growth conditions, extracellular (protein and lipid) enzyme activity). The inoculums (vaccine solutions) will be prepared from each strain of selected fungi, which will be used in the remaining stages of this project. In the third and fourth stages, it is planned to conduct two experiments in parallel, *i.e.*, a control experiment in which the dry aging of beef will be carried out without inoculation, and the test experiment, where the dry aging process of the raw material will be carried out with inoculation with the appropriate strain of fungus A or B. At these stages, the raw material for research will be the heel of the round (beef with low marbling, *i.e.*, intramuscular fat - so far, research has focused on the use of raw material with significant marbling). The raw material will be obtained from Holstein-Friesian young bulls, the most popular breed of cattle in Poland. The meat aging process will be carried out for 63 days in climate chambers, under controlled conditions, typical for beef dry aging, *i.e.*, temperature: 0-4°C, relative air humidity: 75-85%, air flow speed: 0.2- 2.5 m/s.

In stages three and four, analyses will be carried out to determine the role of metabolites (*e.g.*, biogenic amines, fatty acids, and volatile compounds) of selected fungi strains in the beef aging process. These will be investigated on several levels (*i.e.*, analytical, microbiological, physicochemical, and also sensory). The metabolome profiling will be performed at 7-day intervals, from the 1st to the 63rd day of the process. Metabolomic characteristics will be realized by comparing metabolite profiles in the meat before the dry aging process and during dry aging without inoculation (control) with profiles of the meat inoculated with the appropriate fungi strain at each stage of the drying process. In addition, every 7 days of the raw material aging process, tests will be carried out to assess the broadly understood quality of meat, including pH measurement, determination of weight changes during aging, chemical composition, including changes in fats; color, and texture measurement, and microbiological quality. At 14-day intervals, a sensory evaluation of the meat will also be carried out, and its metagenomic profile will be determined. The obtained results will lead to a better understanding of how the metabolic profiles of individual strains of fungi develop the quality, including the flavor profile and tenderness of dry-aged beef, and allow for assessing its safety.