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THE APPLICATION OF METABOLICS APPROACH IN THE INVESTIGATION OF SAFETY AND QUALITY OF FOOD PRODUCTS – REVIEW

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Abstract: This review concentrates on the application of the chemometric methods in the investigation of metabolomics in food science, particularly on the analysis of data produced from mass spectrometric techniques. Its purpose is to discuss the essential developments in metabolomics due to the combination of modern chemical analysis techniques with statistical and chemometric data analytical strategies. It investigates the potential of the chemometric methods in the analysis of a variety of different food products. Spectrometry (MS)-based methods including gas chromatography (GC) and liquid chromatography (LC) enable the precise quantification and qualification of metabolite profiles because of their sensitivity and good resolution. Such data results are subjected to the metabolomic interpretation using various multivariate tools such as principal component analysis (PCA), partial least squares (PLS), partial least squares discriminant analysis (PLS-DA) or hierarchical clustering. They offer huge potential in the analysis of food products in terms of guaranteeing safety for human consumption.

Key words: metabolomics, chemometric methods, biomarkers, identification of metabolites, liquid chromatography, gas chromatography.

Introduction

Metabolomics is becoming popular as an advanced analytical tool which makes it possible to monitor the quantitative and qualitative changes in the amounts of chemical substances in food products during their storage. Metabolomics approach combines the analytical methods such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS)-based methods including gas chromatography (GC) and liquid chromatography (LC) together with the chemometric methods such as principal component analysis (PCA), partial least squares (PLS), partial least squares discriminant analysis (PLS-DA) and hierarchical clustering. The metabolomics of agricultural products is used in food safety for analyzing the microbial infections, genetically modifying food, studying the effects of technological processes on the quality of final products, and studying the originality and authenticity of food products. There is huge application potential for metabolomics in microbiological food examination including the differentiation of types of bacteria and fungi in the search for natural food preservatives. There is an immense variety of food products which can be examined by metabolomics tools. They include different agricultural products and food products such as fruit and vegetables, cereals, milk, cheese, honey, bread, meat, meat products, fruit juices and alcohol drinks.

Metabolomics has appeared as a promising approach in a lot of disciplines including human diseases, nutrition, drug discoveries, and plant physiology. In food science, metabolomics is regarded as a tool guaranteeing the quality and safety of both raw materials and the final products. Metabolomics is called the study of „as-many-small-metabolites-as-possible" in a system. Metabolomic analyses have been categorized as targeted or untargeted. Targeted analyses concentrate on a particular group of intended metabolites which need to be identified and quantified [1]. The targeted analysis means the examination of concrete, identified chemical compounds which can be the markers of different biochemical processes and are the indicators of the suitability of food for human consumption. The targeted analyses are essential for the investigation of the relations of compounds belonging to a specific group present in the matrix under determined conditions. The untargeted metabolomics concentrates on the detection of as many groups of metabolites as possible to achieve patterns or fingerprints without the necessity of identification or quantification of a specific compound or compounds [2]. The untargeted analysis also called the profile analysis is a complex approach examining the whole profile of all low molecular weight compounds (MW<1500 Da) present in the food sample including amino acids, fatty acids, sugars, amines, nucleotides, organic acids, and phenols. It is a holistic approach describing the characteristics of the product.

The metabolomic studies are also defined as discriminative, informative, and predictive. Discriminative studies examine the differences between sample populations without developing the statistical models or analysing possible pathways which might contribute to these differences [3]. Discrimination is realized by the application of multivariate data analysis (MVDA) and principal components analysis (PCA) [4, 5]. The informative metabolomic analyses have concentrated on the identification as well as quantification of targeted or untargeted metabolites to achieve reliable information about the sample. It is applied in the creation of metabolite databases of the human metabolome [6]. It analyses pathways of the appearance of novel bioactive compounds and biomarkers. Metabolomics studies enable researchers to predict the statistical models which are based on metabolite profiles.

In food science, metabolomics enables researchers to solve a variety of problems concerning quality, nutrition, and food components analysis. Metabolomics is a new approach to the food analysis. Its purpose is the identification and quantification of all the metabolites present in the matrix. Metabolomics is focused either on specific compounds and metabolic pathways or on global chemical profiles called fingerprints. Its aims include achieving the knowledge of the metabolites present within the matrix, classifying samples between two or more conditions, and identifying new biomarkers. Metabolic fingerprinting is possible due to the technologies of small molecule separation and identification. The analysis of metabolite profiles is possible to carry out applying Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS)-based methods including gas chromatography (GC) and liquid chromatography (LC). GC detects thermostable compounds and compounds characterised by the proper volatility, and LC detects small and macro-molecules, ionic compounds and thermolabile compounds. It is known that metabolite profiling called metabolomics involves the identification of biomarkers in biological samples. It enables the qualification and quantification of metabolites in biological samples applying chemometric methods. Such methods facilitate the detection of biomarkers for drug effects and toxicity. They find their application in recognizing various diseases.

Discussion

Sadecka et al. [7] examined microbial diversity and volatile odour-active compounds accumulated in barrelled ewes' cheese. This is a long-ripened intermediate product created in the production of winter bryndza cheese which is Slovakian bryndza cheese made of unpasteurized ewes' milk. They analyzed a share of different microorganisms in this product. They proved that lactococci, lactobacilli and Geotrichum spp. were prevailing while coliforms and coagulase-positive staphylococci were at relatively acceptably levels. The dominant species involved *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus* and species belonging to Geotrichum group. The profiles of odour-active compounds were examined by gas chromatography supported by mass spectrometry. Such a method led to the identification of thirty-nine odour-active compounds. The main ones involved butanoic acid, ethyl butanoate, isovaleric acid, hexanoic acid, octanoic acid, decanoic acid, methyl octanoate, ethyl hexanoate, ethyl octanoate, p-cresol, and d-decalactone. The results showed that barrelled ewes' cheese contains specific microflora and a very specific rich profile of odour-active volatile compounds. Cheese is produced from ewes' milk directly after milking by renneting using chymosin without any starter cultures. The curd is drained for 24 h, and then left to ripen for 3 days at 18-20°C. Then individual lumps are pressed, milled and loaded in layers mixed with salt in barrels. Then the cheese is left to ripen at 2-10°C for 2 months. Then the barrelled milled cheese is mixed with cheese produced from pasteurized cows' milk, at a ratio of 51:49 %, making winter bryndza which is an aromatic cheese with a spreadable texture.

The aim of this study was to achieve information about a portion of microorganisms and a variety of main odour-active compounds in barrelled ewes' cheese being an intermediate product in the production of winter bryndza cheese. They used culture-based and culture-independent techniques to examine the microflora and gas chromatography with a mass spectrometry to examine the odour-active compounds.

Lactobacilli were incubated on de Man–Rogosa–Sharpe agar (Merck, Darmstadt, Germany), lactococci on M17 agar (Merck), total mesophilic aerobic counts on glucosetryptone-yeast extract agar (Merck), coliforms and *E. coli* on Chromocult C medium (Merck), Staphylococcus sp. on Bairde Parker agar (Merck). Coagulase activity of staphylococci was examined by a rabbit plasma tube coagulase test (Bio-Rad, Marnes-la-Coquette, France). Yeasts, fungi and Geotrichum spp. were incubated on yeast extract-glucose-chloramphenicol agar (Merck). Samples of cheese were analyzed by gas chromatography with a mass spectrometry (GCeMS) using the gas chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, California, USA) coupled with the mass spectrometric detector 5973 inert (Agilent Technologies) equipped with fused silica capillary column Ultra 1 (50 m x 0.32 mm x 0.52 mm; Agilent Technologies) operating with a temperature programme 40°C (1 min), 5°C (1 min), 250°C (1 min). The linear velocity of carrier gas helium was 35 cm/s (measured at 143°C). Pulse splitless injection was used at an injector temperature of 250°C. Ionization voltage (EI) was 70 eV. Volatile odour-

active compounds were identified on the base of their linear retention indices, mass spectra, GC analysis of standards, and by comparison of data on occurrence and odour description with literature [8–10]. Identification of compounds by comparison of mass spectra was done using Wiley and NIST MS libraries (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

The microbiological results showed high levels of total mesophilic aerobes (10^6 – 10^7 CFU/g) and relatively low levels of coliforms and *E. coli* (from <50 CFU g/1 to <500 CFU/g). The levels of total staphylococci were pretty high (10^5 – 10^6 CFU/g), but levels of coagulase-positive staphylococci were appreciably low (<50 CFU/g). To sum up, the long ripening of barrelled ewes' cheese causes changes in the share of microorganisms leading to a decrease in a number of potentially pathogenic Gram-negative bacteria and coagulase-positive staphylococci [11–13]. The levels of yeasts were always lower in comparison to those previously determined for short-ripened ewes' curd cheese and May bryndza cheese. Levels of fungi and Geotrichum spp. were relatively low. Lactoccocci and lactobacilli were present at high levels (10^5 – 10^7 CFU/g) and constituted prevailing microorganisms in barrelled ewes' cheese.

The odour profile of barrelled ewes' cheese consisted of different chemical compounds such as free fatty acids, esters, alcohols, ketones, aldehydes, hydrocarbons, lactones and sulfurous compounds. GC-MS technique identified butanoic acid, ethyl butanoate, isovaleric acid, hexanoic acid, octanoic acid, decanoic acid, methyl octanoate, ethyl hexanoate, ethyl octanoate, p-cresol, and d-decalactone as predominant compounds responsible for the overall odour of all the investigated cheeses. The following compounds appeared in the highest amounts: butanoic acid and ethyl butanoate (responsible for unpleasant fermented, rotten cheese-like, fruity, sweet odour); isovaleric acid (resembling rancid blue cheese, sweaty, faecal, putrid odour); ethyl hexanoate (wine-like, brandy, valeriana-like undertow); hexanoic acid (sweaty, rancid, savoury cheesy, fatty, sharp pungent, goat-like smell); methyl octanoate (balsamic, exotic flower-like, caramel-like, valeriana-like odour), and d-decalactone (coconut, creamy, buttery). Other compounds were identified at lower intensities and they included namely, p-cresol (plastic, rubber-like odour), octanoic acid (unpleasant, fatty, waxy, rubber-like, smoky smell), ethyl octanoate (pleasant, fruity, floral, fresh, sweet, soap odour) and decanoic acid (fatty, unpleasant rancid odour, with weak terpenic citrus-like note). In cows' lump cheese, the following compounds were identified in relatively high amounts: ethyl acetate, 3-methylbutanal, acetoin, 3-methylbutanol, 2-methylbutanol, isovaleric acid, isoamyl acetate and 3-methylthiopropanal. To sum up, cows' lump cheese possesses volatile odour-active compounds which complete aroma of barrelled ewes' cheese making the complex aroma profile of winter bryndza.

Le Boucher et al. [14] applied the mass spectrometry metabolic fingerprinting to investigate bacterial metabolism in a model cheese. They used LC- and GC-MS-based methods to characterise the cheese metabolome called „cheeseome" under specific conditions including various ripening conditions and various starter cultures. Their aims involved obtaining MS metabolic fingerprints from a solid and fermented food matrix, detecting cheese metabolic fingerprints during the bacterial multiplication and identifiifying achieved metabolites. The model cheese was produced from the ultrafiltrated milk concentrate inoculated with *Lactococcus lactis* LD61. Metabolic compounds were examined after 0,8 and 48 h. They were classified into the water-soluble fraction applying the liquid chromatography – high resolution – MS and the volatile fraction applying the gas chromatography – MS. The huge variety in the amounts of metabolic compounds was observed over time. They detected forty-five metabolites covering 12 amino acids, 25 volatile metabolites, four vitamins, uric acid, creatine and L-carnitine. Cheese is a fermented product which undergoes the fermentation process releasing metabolites. Such metabolites are the effect of the activity of the microorganisms which are in food, and their share undergoes changes over time. The cheese production involves the ripening period which leads to achieving different textures and flavours. They are dependent on the starter culture, the endogenous microflora and the ripening parameters. There is still a strong need to obtain more knowledge about the activity of microorganisms during cheese ripening.

Metabolites were assessed by comparing the mass spectra and retention times with those of authentic standards. The lactococci population increased by 4 log10 (cfu/ml) within 48 h from 2.4 x 10^5 cfu/ml to 2.2 x 10^9 cfu/ml. The acidification reached a final value of 4.83. Free amino acids constituted most of the detected metabolites. Twelve free amino acids were detected and the concentrations of nine of them significantly increased from 0 to 48 h. Isoleucine and valine at first diminished in their contents from 0 to 8 h and then significantly increased from 8 to 48 h. Some vitamins and organic acids were also detected. The contents of riboflavin, nicotinamide and pyridoxal, part of the B-vitamin group decreased significantly from 0 to 48 h. The concentration of citric acid decreased significantly from 0 to 8 h and then was stable. The concentration of uric acid significantly increased from 8 to 48 h.

The share of metabolites significantly changed depending on the incubation time (from 0 to 48 h). The main differences were found between 0 or 8 h and 48 h of incubation.

Le Boucher et al. [14] proved that there were variations of the metabolome found during the fermentation of *L. lac-*

tis subsp. *lactis* in a model cheese. The kinds of detected metabolites identified before fermentation and their evolution during the fermentation corresponded to the current knowledge on milk composition and on the metabolism of *L. lactis* subsp. *lactis biovar diacetylactis* in dairy products. There was a huge variety of metabolites in the cheese model after inoculation. They involved some amino acids or volatile metabolites, vitamins, uric acid, and L-carnitine. Free amino acids are present in milk in very small amounts. *L. lactis* requires a lot of nutrients including amino acids such as Glu, Ile, Leu, His, Met and Val [15]. During the first hours of fermentation, *L. lactis* subsp. *lactis* metabolised the free amino acids present in milk to sustain its growth [16], which means that the contents of Ile and Val decreased significantly from 0 to 8 h. During the first exponential growth phase of *L. lactis* the share of free amino acids in model cheese was relatively low. During the second exponential growth phase there was a process of utilizing proteases hydrolyzing milk proteins, essentially casein, into peptides and free amino acids. It delivers *L. lactis* amino acids for its for growth, contributing to a global increase in free amino acids in the medium. They detected a huge increase in amounts of 11 free amino acids (Pro, Tyr, His, Lys, Val, Phe, Trp, Met, Ile, Cyt, cis-hydroxyproline) coming from casein hydrolysis between 8 and 48 h. Such amino acids are responsible for the flavour of cheese and play the role of precursors in further enzymatic or chemical reactions contributing to the appearance of volatile flavour metabolites.

They detected a high concentration of citrulline between 8 and 48 h. This amino acid is not an effect of the casein hydrolysis, but it is produced as a result of bacterial metabolism. Some strains of *L. Lactis* produce ATP from arginine are a result of the arginine deiminase (ADI) pathway, by transforming arginine to citrulline and ornithine, followed by further transformation to CO_2 and NH_3. All volatile metabolites detected in this study increased in their contents during the fermentation. Diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), and acetic acid showed the highest increases in their contents (>100 times between 8 and 48 h). Diacetyl, acetoin and acetic acid are generated on the way of the lactose metabolism, and the citric acid metabolism carried out by *L. lactis* biovar *diacetylactis* strains.

The decrease of citric acid, detected by LC?MS, remains in proportion with the increase of volatile metabolites coming from the citric acid metabolism. The volatiles which were detected were the products of *L. lactis* metabolism in milk or cheese. Ethanol is a by-product of the mixed fermentation by homofermentative lactic acid bacteria and might be released lactococci in low concentrations. Most of the other volatiles detected in this study are usually found in cheese and come from the catabolism of amino acids or fat. Dimethyl disulphide and methional come from methionine, 2-methylpropanal from valine, and benzaldehyde and phenylacetaldehyde from phenylalanine. Methylketones and straight-chain aldehydes come from the degradation of fat [17,18]. They contribute to the creation of flavour of many cheeses [17].

Some vitamins were also identified in this study. They are very important for the growth of *L. lactis* susbp. *Lactis*, and they included riboflavin (vitamin B2), nicotinamide and pyridoxal (vitamin B6) [19]. They were metabolised by *L. lactis* for its growth, which contributed to a decrease in concentration of these three vitamins from 0 to 48 h of fermentation. The metabolomic approach applied in this study enabled researchers to detect the changes in concentration of metabolites for which the metabolism of bacteria is not well recognized in milk or in cheese. These metabolites involved creatine, uric acid and L-carnitine. Creatine is defined as a non-protein nitrogenous metabolite present in milk. It is produced as a result of the protein metabolism of the cow, and it is released into the udder. The decrease in amount of creatine found between 8 and 48 h may result from the activity of creatinase.

To sum up, the LC- and GC-MS based methods are found as a proper and reliable techniques to examine the cheeseome. It enable researchers to examine the presence of metabolites which appear either at low concentration or are present only transiently in dairy products. This metabolomic approach is a powerful tool which gives information on little-known metabolites present in fermented dairy matrices. MS metabolic fingerprinting is recognized as a reliable approach. It brings the possibility of detection of even slight quantitative differences. It might be used to examine different queries connected with cheese quality, including technological conditions and the biodiversity of cheese ecosystem.

Castejon et al. [20] examined metabolomics of meat exudates and its potential to assess beef meat conservation and aging. They analyzed the exudate of beef using nuclear magnetic resonance (NMR). Exudate is the natural juice from raw meat which may be easy to get from a matrix. Meat exudate can give the metabolic information about the quality of meat. They examined 48 beef samples coming from different breeds, cattle and storage times. The data obtained using H NMR spectroscopy were compared with the ones received using High Resolution Magic Angle Spinning (HRMAS) for the original meat pieces. They observed a huge correlation between both spectra (>95% of coincident peaks in both records). Such a result proved that the meat exudate is a very good alternative analytical matrix to examine meat metabolomics. These chemometric tools showed metabolite changes connected with meat aging. NMR technique classified meat samples according to the storage time using Principal Component Analysis (PCA), and made it

possible to predict such storage time applying Partial Least Squares (PLS) regression.

Beef has got a high nutritional value. Hence, suitable analytical methods are needed to examine meat metabolome and the chemical composition regarding low molecular weight compounds including nucleotides, amino acids, dipeptides and sugars which contribute to the meat aging processes. Different methods are used to analyze the content of meat compounds. High resolution Nuclear Magnetic Resonance (NMR) spectroscopy is a very good technique used for examining the complex mixtures, making it possible to detect different low molecular weight components. NMR spectroscopy gives reliable information on the chemical composition of the food matrix without extensive manipulation. Castejon et al. applied NMR spectroscopy to examine metabolite profiles of meat samples coming from different breeds. The NMR spectroscopy modality known as High Resolution Magic Angle Spinning (HRMAS-NMR) has been found to be a very reliable method for examining the metabolic profile (metabolome) of intact muscles [21]. Meat exudate mainly contains water soluble sarcoplasmic proteins and their degradation by-products including nucleotides, amino acids, peptides, proteins, and many soluble enzymes. The aims of their study were to examine the quality of beef exudates using NMR analysis, to define the major metabolites, to estimate the usefulness of H NMR to record the chemical changes in beef exudate during storage and to use NMR based chemometric techniques to characterise beef samples according to postmortem time (aging) [22].

To sum up, the analysis of exudates revealed that different processes happen simultaneously while meat ages. The exudates is regarded to be a good matrix which gives reliable information about the composition of a meat sample during storage. Metabolic profile changes connected with meat aging were observed by recording exudate spectra. The results obtained proved that the application of NMR with multivariate analysis techniques may contribute to a reliable classification of meat samples based on storage time and predicting their aging time. H NMR profiling of meat exudates makes it possible to monitor the quality and to control the safety of meat.

Argyri et al. [23] investigated the potential of HPLC method to identify the spoilage status of minced beef which was stored at different temperatures and with different packaging systems. They checked the shelf life of minced beef which was packed aerobically, under modified atmosphere packaging (MAP), and under MAP with oregano essential oil (MAP/OEO) and stored at 0, 5, 10, and 15°C. The microbial status and the temporal biochemical changes were examined. The quality of meat was checked by the assessment of total viable counts (TVC), Pseudomonas spp., Brochothrix thermosphacta, lactic acid bacteria, Enterobacteriaceae, and yeasts/moulds. The organic acid profile was assessed by HPLC analysis and pH measurement. Data achieved by HPLC method were the subject of statistical analysis such as principal component analysis (PCA) and factorial discriminant analysis (FDA). Partial least squares regression (PLS-R) was applied to assess quantitative predictions of TVC, Pseudomonas spp., Br. thermosphacta, lactic acid bacteria, Enterobacteriaceae, and yeasts/moulds. The HPLC method of the profile of organic acids made it possible to assess the spoilage and microbial quality of minced beef. This method is valuable in controlling the quality of meat during storage and monitoring freshness and safety. They proved that the relationship between microbial growth and chemical changes in meat revealed during storage is valuable in assessing the meat quality and freshness [24,25]. There are various factors such as packaging, temperature, preservatives, pH, glucose concentration which are responsible for the qualitative production of potential indicators [25]. The qualitative and quantitative analyses of metabolic compounds being the result of microbial activity made it possible to develop biomarkers which might have practical applications to guarantee the quality and safety of meat and meat products. There is a huge variety of volatile and non-volatile microbial metabolites in naturally contaminated samples of meat which can be detected with GC, GC-MS or HPLC [24–29]. The most frequently met end-products of the glycolytic pathway included lactic, citric, formic, acetic, gluconic, propionic, and succinic acids [24,25]. The application of high performance liquid chromatography (HPLC) to control changes in the profile of organic acids in meat during storage is gaining popularity [30,31]. Argyri et al. [23] monitored the chemical profile of meat on the basis of data achieved from HPLC analysis combined with chemometrical methods including factorial discriminant analysis (FDA) and partial least squares regression (PLS-R) and proved their efficiency in assessing beef quality.

They proved that the growth rate of each member of the microbial association increased with the increase in storage temperature of meat. The storage under MAP and MAP/EO contributed to the predominance of lactic acid bacteria while pseudomonas occurred to be dominant under aerobic conditions. On the base of the sensory analysis, the shelf life of the minced beef was shorter when the storage temperature was higher. The combination of MAP and oregano essential oil contributed to the elongation of shelf life of minced beef in comparison to aerobic and MAP packaging. The HPLC analysis revealed 17 discrete peaks (with purity greater than 99%). The information appearing in all peaks is very important for defining the microbial spoilage. The data showed changes in the chromatographic areas un-

der the peaks of the eluted acids which were connected with storage conditions. The total amount of acetic acid revealed an increase at all storage temperature and under all packaging conditions. Such an increase can result from the fact that acetic acid is produced by Pseudomonas spp., Br. thermosphacta, Enterobacteriaceae, lactic acid bacteria, and these bacteria were present in meat stored under aerobic or modified atmosphere conditions [18, 32–34]. The values of lactic acid peaks decreased in all samples which were stored aerobically. MAP stored meat revealed the total amount of lactic acid which stayed constant and showed an increase at the end of shelf life, whereas a significant increase was found in meat stored under MAP/OEO. Such a phenomenon is connected with changes in the metabolic pathways of bacteria such as the homofermentative or heterofermentative pathways of glucose metabolism by lactic acid bacteria [25]. It can be concluded that HPLC analysis is a very valuable tool for the quantification and prediction of the remaining shelf life of meat and its products. The analysis of the profile of organic acids can be used as a very useful technique for the qualitative classification and prediction of the microbial growth regardless of storage conditions.

Surowiec et al. [35] examined the adulteration of food products with mechanically recovered meat using GC-MS. Mechanically recovered meat (MRM) is produced on the mechanical treatment of remnants following hand deboning. Such meat is not defined by EU regulations as meat, so it must be detected and differentiated from hand-deboned meat (HDM) and desinewed meat. The detection of MRM was carried out on the base of metabolite profiles with GC-MS; then they were subjected to OPLS-DA for the precise classification of MRM, HDM and desinewed pork and chicken samples. Their aims included the separation of three classes of products and the selection of compounds which are potential markers for MRM detection. Mechanically recovered meat (MRM) has got a definition of „residual material, off bones, obtained by machines operating on auger, hydraulic or other pressure principles in such a manner that the structure of the material is broken down sufficiently for it to flow in puree form from the bone" [36]. This product is cheap and results in a cost reduction in the production of meat pies, sausages and so-called ‚economy burgers'. Consumers are afraid of the safety of MRM consumption, and its addition to food products must be labelled clearly. The chemical composition including the contents of free fat, moisture, nitrogen, ash, collagen, calcium, iron and total purines in different classes of meat have been examined in order to find potential chemical markers to detect MRM. However, the results of such investigations revealed the variations depending on the raw material and the technical conditions applied during meat recovery [37, 38]. Surowiec et al. [35] applied GC-MS followed by PLS analysis to detect MRM on the base of metabolite profiles in meat samples. Eighty compounds achieved from GC chromatograms were used for the development of the chemometric model. They chose the compounds manually from TIC chromatograms, and their peak areas were then calculated automatically for characteristic ions and retention times applying the MSD Chemstation software (Agilent, Atlanta, USA). The evaluation of natural variation was carried out by PCA following the orthogonal partial least squares discriminant analysis (OPLS-DA). The GC-MS method made it possible to achieve the metabolite profiling of methanolic extracts from meat material. PCA and OPLS-DA were applied to gain knowledge about the meat sample. They proved that the three-class OPLS-DA models gave the proper separation of all classes of products (MRM, HDM and desinewed).

Jang-Eun Leea et al. [39] studied the influence of grape vintage on the production of metabolites as well as the relationship between wine metabolites and meteorological data. There are different factors such as grape variety, climate, soil and bacterial strains which influence the wine quality. They used H NMR analysis coupled with multivariate statistical data sets and principal component analysis (PCA) which indicated a profile change between Meoru wines that were vinified with the same yeast strain and Meoru grapes collected from the same vineyard but with a different vintage. They identified the following metabolites such as 2,3-butandiol, lactic acid, alanine, proline, γ-aminobutyric acid (GABA), choline and polyphenolsoading plot. Significantly bigger amounts of proline, lactic acid and polyphenols were found in the 2006 vintage wines compared to the wines from 2007 vintage. It stayed in accordance with the meteorological changes. The amount of sunlight and rainfall in 2006 were two times more and four times less, respectively, than in 2007.

It proved that climate changes had a huge influence on the chemical compositions of the grape. Jang-Eun Leea et al. [39] proved that NMR technique gives reliable metabolomic data defining the chemical composition of wine and grape. The Korean wild grape Meoru contains a significant amount of polyphenolic compounds such as anthocyanin and resveratrol contributing to achieving attractive flavour of grape juice and wine. They proved that proline and polyphenol levels in wine are dependent not on yeast strains but on grape vintage. The qualities of vintages of grapes and wines depend on the amount of sunlight and rainfall rather than temperature and relative humidity. They underlined the significance of multivariate statistical data of global metabolites of wine and the meteorological data in the vineyard provides more reliable characterization of wine or grape.

Conclusion

The chemometric methods in the analysis of food products are gaining a lot of interest. They make it possible to analyse metabolomics which is treated as an important tool in the development of food science areas such as the compliance with regulations, processing, quality, safety, and microbiology. The potential of metabolomics in food science can be applied to detect the compounds contributing to consumers' taste preferences. Metabolomics is an interdisciplinary field of science belonging to the system biology, which can be used on the large scale starting from medicine, through the analysis of the food, and finishing with environmental analysis. In the analysis of agricultural products and food products it can be practically applied to obtain various information that can influence the final product including information on the technological processes, authenticity, origin, impact on health, quality and microbiological risk. Metabolomics might be successfully connected with other analytical areas involving genomics. It enables researchers to detect the selected biomarkers essential to examine levels of bacterial contamination, accompanying flora, and biomarker response in food responsible for the health and safety of food products. The rapid development of metabolomics in the food science delivers a lot of information about the possible chemical, physical and microbiological contamination of food products and is considered to be an effective tool in solving problems in the food industry.

Literature

[1] Jong G., Ramautar R., Demirci A. Capillary electrophoresis in metabolomics. *Trends in Analytical Chemistry*, 25(5):12, 2006.

[2] Soga T., Monton M.R.N. Metabolome analysis by capillary electrophoresisemass spectrometry. *Journal of Chromatography*, 1168:1–2, 2007.

[3] Van den Berg F., Hwang G.S., Park W.M., Lee C.H. Son H.S., Kim K.M. H-1 nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. *Journal of Agricultural and Food Chemistry*, 56(17):8007–8016, 2008.

[4] Dainty J.R., Watson A.D., Harvey L.J., Tapp H.S., Kemsley E.K., Le Gall G et al. Multivariate techniques and their application in nutrition: a metabolomics case study. *British Journal of Nutrition*, 98(1):1–14, 2007.

[5] Hankemeier T., Van der Werf M. J., Jellema R.H. Microbial metabolomics: replacing trial-and-error by the unbiased selection and ranking of targets. *Journal of Industrial Microbiology & Biotechnology*, 32(6):234–252, 2005.

[6] Knox C., Eisner R., Guo A.C., Young N., Wishart D.S., Tzur D. HMDB: the human metabolome database. *Nucleic Acids Research*, 35:521–526, 2007.

[7] Pangallo D., Korenova J., Kolek E., Puskarov A., Sadecka J., Sakova N. et al. Microbial diversity and volatile odour-active compounds of barrelled ewes' cheese as an intermediate product that determines the quality of winter bryndza cheese. *LWT - Food Science and Technology*, 70:237–244, 2016.

[8] Mallatou H., Massouras T., Pappa E.C. Headspace analysis of volatile flavour compounds of teleme cheese made from sheep and goat milk. *International Journal of Dairy Technology*, 59:250–256, 2006.

[9] De Luca S., Scifo G.O., Caggia C., Randazzo C.L., Pitino I. Effect of wild strains used as starter cultures and adjuct cultures on the volatile compounds of the pecorino siciliano cheese. *International Journal of Food Microbiology*, 122:269–278, 2008.

[10] Ribbera A., Caggia C., Randazzo C.L., Pitino I. Pecorino crotonese cheese: study of bacterial population and flavour compounds. *Food Microbiology*, 27:363–374, 2010.

[11] Valík L., Gorner F. *Aplikovana mikrobiologia pozívatín*, chapter Mikrobiologia syrov., pages 273–338. Male centrum, Bratislava, 2004.

[12] Madec M.N., Pediliggieri C., Tuminello L., Tanguy G., Lortal S., Di Blasi A. Tina wooden vat biofilm: a safe and highly efficient lactic acid bacteria delivering system in pdo ragusano cheese making. *Internantional Journal of Food Microbiology*, 132:1–8, 2009.

[13] Valík L., Medvedova A. *Structure and function of food engineering*, chapter Staphylococcus aureus: characterisation and quantitative growth description in milk and artisanal raw milk cheese production., pages 71–102. InTech, Rijeka, 2012.

[14] Jeanson S., Chereau S., Maillard M., Royer A., Thierry A., Dervilly-Pinel G. et al. First mass spectrometry metabolic fingerprinting of bacterial metabolism in a model cheese. *Food Chemistry*, 14:1032–1040, 2013.

[15] Mulholland F., Niven G.W., Knight D.J. Changes in the concentrations of free amino acids in milk during growth of Lactococcus lactis indicate biphasic nitrogen metabolism. *Journal of Dairy Research*, 65:101–107, 1998.

[16] Kunji E.R.S., Konings W.N., Gripon J.C., Richard J., Juillard V., Lebars D. Oligopeptides are the main source of nitrogen for Lactococcus lactis during growth in milk. *Applied and Environmental Microbiology*, 61:3024–3030, 1995.

[17] Bosset J.O., Curioni P.M.G. Key odorants in various cheese types as determined by gas chromatography–olfactometry. *International Dairy Journal*, 12:959–984, 2002.

[18] Spinnler H.E., Molimard P. Review: Compounds involved in the flavour of surface mold-ripened cheeses: Origins and properties. *Journal of Dairy Science*, 79:169–184, 1996.

[19] Law B.A., Marshall V.M.E. Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier Applied Science Publishers., 1984.

[20] Escudero R., Herrera A., Cambero M.I., Castejon D., García-Segura J.M. Metabolomics of meat exudate: Its potential to evaluate beef meat conservation and aging. *Analytica Chimica Acta*, 901:1–11, 2015.

[21] Failla M., Ritota L., Casciani S. HRMAS-NMR spectroscopy and multivariate analysis meat characterization. *Meat Sci.*, 92:754–761, 2012.

[22] Ye Y., Cao J., Liu C., Pan D. 1h NMR and multivariate data analysis of the relationship between the age and quality of duck meat. *Food Chem.*, 141:1281–1286, 2013.

[23] Blana V., Panagou E., Nychas G., Argyri A., Doulgeraki A. Potential of a simple HPLC-based approach for the identification of the spoilage status of minced beef stored at various temperatures and packaging systems. *Int. J. Food Microbiol.*, 150:25–33, 2011.

[24] Dainty R.H. Chemical/biochemical detection of spoilage. *Int. J. Food Microbiol.*, 33:19–34, 1996.

[25] Tassou C.C., Koutsoumanis K.P., Nychas G., Skandamis P.N. Meat spoilage during distribution. *Meat Sci.*, 78:77–89, 2008.

[26] Blixt Y., Borch E., Kant-Muermans M.L. Bacterial spoilage of meat products and cured meat. *Int. J. Food Microbiol.*, 33:103–120, 1996.

[27] Torrieri E., Masi P., Villani F., Ercolini D., Russo F. Changes in the spoilage related microbiota of beef during refrigerated storage under different packaging conditions. *Appl. Environ. Microbiol.*, 72:4663–4671, 2006.

[28] Nasi A., Ferranti P., Villani F., Ercolini D., Russo F. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. *Appl. Environ. Microbiol.*, 75:1990–2001, 2009.

[29] Skandamis P.N., Nychas G. Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol.*, 79:35–45, 2002.

[30] Nychas G., Drosinos E.H., Mataragas M., Skandamis P. Modeling and predicting spoilage of cooked, cured meat products by multivariate analysis. *Meat Sci.*, 77:348–356, 2007.

[31] Nychas G., Tsigarida E., Boziaris I.S. Bacterial synergism or antagonism in a gel cassette system. *Appl. Environ. Microbiol.*, 69:7204–7209, 2003.

[32] Otero A., Garcia-Lopez M.L., Prieto M. *The Microbiology of Meat and Poultry.*, chapter The physiological attributes of Gramnegative bacteria associated with spoilage of meat and meat products., page 1–34. Blackie Academic and Professional, London, 1998.

[33] Richter H., Unden G., Zaunmüller T., Eichert M. Variations in the energy metabolism of biotechnologically relevant heterofermentative Lactic acid bacteria during growth on sugars and organic acids. *Appl. Microbiol. Biotechnol.*, 72:421–429, 2006.

[34] Kagkli D.M., Nychas G.J.E., Doulgeraki A.I., Paramithiotis S. Lactic acid bacteria population dynamics during minced beef storage under aerobic or modified atmosphere packaging conditions. *Food Microb.*, 27:1028–1034, 2010.

[35] Patel R., Halket J., Bramley P.M. Surowiec I., Fraser P.D. Metabolomic approach for the detection of mechanically recovered meat in food products. *Food Chemistry*, 125:1468–1475, 2011.

[36] British meat manufacturers association. BMMA Standard for the Preparation of Mechanically Separated Meat (MSM) Otherwise Commonly Known as Mechanically Recovered Meat, BMMA, London, 1991.

[37] Higman R.C., Stewart C.A., Hargin K.D., Crosland A.R., Patterson R.L.S. Investigation of methods to detect mechanically recovered meat in meat products–I: Chemical composition. *Meat Science*, 40:289–302, 1995.

[38] Field R.A. Bone marrow measurements for mechanically recovered products from machines that press bones. *Meat Science*, 51:205–214, 1999.

[39] Van Den Bergc F., Leea Ch.H., Young-Shick H., Leea J.E., Hwangb G.S. Evidence of vintage effects on grape wines using 1h NMR-based metabolomic study. *Analytica Chimica Acta*, 648:71–76, 2009.

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