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Uroš Andjelković, Djuro Josić

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Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety

Uroš Andjelković [a, b, *] and Djuro Josić [b, c, *]

[a] Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Serbia
[b] Department of Biotechnology, University of Rijeka 51000 Rijeka, Croatia
[c] Warren Alpert Medical School, Brown University, Rhode Island, Providence RI, USA

* Corresponding authors:
Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia.
E-mail: djuro.josic@uniri.hr, djuro_josic@brown.edu (D. Josić);
Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia. E-mail: uros@chem.bg.ac.rs (U. Andjelković)

ABSTRACT

Background: As a comprehensive discipline that studies food and nutrition, foodomics requires reliable qualitative and quantitative information about the food proteome component in order to extract new, integrative information from the complex multivariable space of omics. This information is necessary to achieve a higher level of understanding of processes in food science and technology, consequently new functions of food and improved markers of food quality and safety and transform the concept of food safety.

Scope and Approach: We are presenting mass spectrometry (MS) based proteomic approaches that are being utilized in different proteomic studies, not necessarily only in the field of foodomics. Current analytical capabilities of MS-based proteomics together with sample preparation procedures and quantification strategies, and recent technical developments were presented.

Key Findings and Conclusions: MS-based proteomics enables the analysis of different aspects of proteins and provides a variety of approaches for reliable quantification of individual proteins and/or food proteome. This is a complex field and its successful implementation requires a

dedicated analyst, thorough design of sample preparation procedure, proper selection of an MS technique and approach, adequate type of mass spectrometer, and both thorough data analysis and validation. Improvements in the technology of mass spectrometery are continuously expanding capabilities of MS-based proteomics.

Keywords: foodomics, proteomics, mass spectrometry, food quality, food safety

List of abbreviations: AQUA, absolute quantification; CID, collision-induced dissociation; CV, coefficient of variation; DDA, data dependent acquisition; DIA, data independent acquisition; ESI, electrospray ionization; HCD, high energy collision dissociation; HRMS, high-resolution mass spectrometry; ECD, electron capture dissociation; ETD, electron transfer dissociation; iTRAQ, isobaric tag for relative and absolute quantification; LC, liquid chromatography; LFQ, label free quantification; LIT (LTQ), linear ion trap; MALDI, matrix assisted laser desorption ionization; MRM, multiple reaction monitoring; NMR, nuclear magnetic resonance spectroscopy; OT, orbitrap; PPI, protein – protein interactions; PRM, parallel reaction monitoring; PTM, posttranslational modification; QqQ, triple-quadrupole; SILAC, stable isotope labelling with amino acids in cell culture; SRM, selected reaction monitoring; TMT, tandem mass tag; TOF, time of flight;

Highlights:

- Mass spectrometry based proteomics, as one of the main technologies in foodomics, is presented.
- MS-based proteomic approaches in food research, quality and safety control are introduced.
- Improvements in sample preparation for mass spectrometry analyses are described.
- Critical points for application of MS-based proteomics in food analysis are analysed.
- Future directions of MS-based proteomics are discussed.

1. Introduction

Contemporary food research provides evidence that food serves not just to fulfil basic dietary needs, but to actively influence a healthy life, playing a pivotal role in both the improvement of its quality, and as natural prevention against a wide range of diseases (D'Alessandro, 2012). Researchers are increasingly aware of the differences among individuals at the genome, proteome and microbiome levels, and the existence of an individual's optimal metabolic space is also becoming evident, which in combination with environmental factors result in an individual's phenotype. Advances in understanding of the molecular basis of disease susceptibility and food quality together with interaction of food with the individual's metabolic space, introduced the concept of personalized nutrition as a part of personalized medicine (Noecker, 2016; Vimaleswaran, 2015).

Analytical approaches based on MS are one of the fastest growing methodologies in food analysis. The application of proteomics in food research, quality control (sensory characteristics, nutritional value, product traceability), authenticity assessment (adulterations, geographic origin, presence of GMO) and safety control (toxins formed from proteins during food processing, bacterial and fungal toxins, allergens, antinutritients, foodborne pathogens, prions, biopesticides, GMO food) of food of animal and plant origin has been extensively reviewed (Agrawal, 2013; Andjelković, 2017; Chassy, 2010; Colgrave, 2017; Cunsolo, 2014; D'Alessandro, 2012; Fasoli, 2015; Martinović, 2016; Piras, 2016; Sauer, 2015; Tedesco, 2014).

Over twenty year old field of MS-based proteomics became one of the main pillars of the group of technologies with the common name "omics". Proteomics methods complement their genomic and transcriptomic counterparts, but also provide additional biological information that is not accessible by genomics and transcriptomics (Mann, 2013; Zubarev, 2013). Moreover, proteomics

also provide information that is necessary for the understanding metabolome. Proteomics is of special importance for foodomics since from the moment when the genome has lost its active influence, and when food proteins are subjected to different factors that are possibly not genetically regulated, as well as to the food processing conditions. All above listed factors can significantly alternate protein properties and interactions with components in the food matrix. Research of the food proteome and its alterations influenced by plant and animal strains (genetics), different conditions of plant and animal growth, pests, food processing and storage conditions, enable establishment of correlations between the food proteome and quality parameters (nutritional value, sensory characteristics, suitability for processing, safety, sustainable growth, etc.). These correlations will enable tailoring of sensory, nutritional and technological properties of food, personalized diet, and identification of quality and safety control parameters (individual protein or protein networks as quality markers).

Future research will explore interactions between many different layers (genome, transcriptome, proteome, peptidome, metabolome, microbiome) of both, food and consumer. Out of this complex picture a way to extract meaningful information from a multilayer interaction network should be paved. It is very important to minimize the increasing risk to become flooded with wrong or biased information. A way of systematic organization of data blocs coming from different sources was presented in order to assess the analytical performance, to improve the interpretability, prevent systematic errors, and finally, unreliable results (Chassy, 2010; Skov, 2014).

The MS-based proteomics generate a vast amount of data. In order to support research efforts to understand the complexity of food MS-based proteomic data should be collected and submitted to data repositories according to guidelines. This strategy will enable the use of data in a more

efficient manner, in order to provide the quality of interpretation and the comparability of analysis. A draft of guidelines was already published for the largest proteomic attempt – The Human Proteome Project (HPP) (Deutsch, 2016). In a broader sense, research of food quality and safety at the level of proteins is the investigation of the interactions between two proteomes: the food proteome and the proteome of a particular consumer. Thus, HPP is the systematic and comprehensive project that provides basis for future complementary proteomic projects or expanding of the HPP to include the topic of food. When combined with the current complexity of MS-based proteomic approaches and in order to be systematically addressed, the impact of the microbiome on the complex food proteome and vice versa, as well as the interactions between the proteomes of the food-microbiome-consumer requires the use of enormous resources in order to be systematically addressed. Hence, the construction of a human proteome and food proteome resource must be a long-term process. The creation of large-scale proteomic methods already paved the way to new types of questions concerning both protein expression and modification profiling. These methods are now poised to address how protein expressions or modifications will change as a function of disease and further in regard to foodomics as a function of factors that influence food quality and safety. Sequencing the genome was perhaps the easiest part, and making sense of the constantly moving and changing picture of the proteome (and later, metabolome) will require a lot of time, effort and creativity (Nilsson, 2010a).

In this review available MS-based proteomic technologies, approaches and critical points important for MS-based proteomic experimental design are summarized.

2. MS-based proteomics

Various analytical methods can be used for the investigation of biological systems at the protein level. High degree of proteome complexity and low abundance of many important proteins in the

investigated sample require the use of highly specific and sensitive analytical techniques. Availability of genome sequence databases, technical and conceptual advances, as well as advances in bioinformatics, made MS a method of choice for proteome studying (Aebersold, 2016). The definition of the proteome changed in line with technical and methodological developments (Ahrens, 2010; Mann, 2013). The complete proteome comprises all expressed proteins in a sample (cells, tissue, or a whole organism), their proteoforms, modification states and organization in macromolecular assemblies, in a given time and space. At the moment it is still not possible to achieve such a vast and in-depth view into the proteome, and it is questionable whether we can achieve it at all, at least with the currently available technologies (Ahrens, 2010). Further technical advances and developments of new bioinformatic tools will certainly open new opportunities to work toward this goal in the near future (Aebersold, 2016). Nevertheless, depending on the question posed, it may not be always necessary to have such a vast and deep view for a given experiment (Michalski, 2011).

Mass spectrometry based proteomic techniques are applied to obtain data important for understanding both the structure and function of proteins (Fig.1). Examples of application of MS-based proteomics in research and assurance of food quality and safety are provided in Table 1.

2.1. MS-based proteomics in analysis of PTMs

More than 300 already known post-translational modifications (www.abrf.org/delta-mass) are one of the sources of proteome complexity (Walsh, 2005). Glycosylation is a highly abundant PTM and about 50% of all proteins are glycosylated. Large numbers of glycan structures (www. glycobase.nibrt.ie) are formed by a combination of relatively small numbers of monosaccharide units. Different physicochemical properties of proteins and glycan components of glycoproteins

172 requires different technical and methodological approaches for their analysis by MS (Scott,
173 2011). Glycoproteomics is a sub-group of MS-based proteomic approaches specialized for the
174 analysis of glycoproteins, Fig.1. At the current level of technology, the high complexity of the
175 glycoproteome is hard to elucidate solely by use of glycoproteomic approaches. Consequently, a
176 special group of approaches called glycomics was established, Fig.1-2. Glycomics technologies
177 attempt to define the structure and quantify the complete set of glycans of one cell, tissue or
178 organism (Mechref, 2013). The use of glycomics technologies gives an insight into the enormous
179 capacity of glycans, and the information coding leads us towards understanding of the sugar code
180 in living systems (Gabius, 2015). The growing importance of glycomics in foodomics research
181 also supports the fact that the most important allergens in food belong to the group of
182 glycoproteins (Andjelković, 2017; Leonard, 2005; Li, 2016).

183 The next widely investigated PTM is phosphorylation of hydroxyl amino acids of proteins.
184 Phosphorylated proteins are directed links in signalling networks between upstream kinases and
185 downstream transcription factors altering DNA expression profiles and many other biochemical
186 regulatory mechanisms. Phosphorylation is a dynamic PTM arising in a very short time period,
187 within minutes, as a result of activity of kinases. Moreover, it may also disappear within minutes
188 due to activity of the phosphatases. Physiological importance of this PTM, its vulnerability and
189 available technical level of methods for analysis of protein phosphorylation, especially MS
190 inspired a special proteomic discipline – phosphoproteomics. It strategically uses and further
191 develops selective approaches and bioinformatic tools for the investigation of phosphorylated
192 proteins, their position in interaction networks and the flow of biological signals throughout
193 these networks (Riley, 2016). The response of cells to different stimuli is one of key information
194 about signalling and it can be quantified using phosphoproteomics.

MS-based proteomic approaches allow for the identification and quantitation of thousands of PTM sites in a single experiment (Doll, 2015). Currently, sensitive and dedicated MS-based proteomic strategies are available only for a few different types of PTM (Doll, 2015; Venne, 2014). A comprehensive and simultaneous view of PTMs and PTM sites is important to get insight into the mechanisms of regulation of protein function by multi-PTM interplay (Pejaver, 2014; Venne, 2014). Protein conformation can be modulated by PTMs, hence protein turnover, localization, PPI and enzyme activity can be affected.

As presented in Table 2, the complexity of the food proteome is additionally increased by the number of non-enzymatic and enzymatic PTMs. Also, PTMs can arise as a result of reactions with food matrix components, additives, microbial enzymes and toxins, or other components emerged during food processing and storage/transport. These modifications are usually not stoichiometric, and are often in low abundance. However, they can have a significant physiological role, whether it is in triggering of food allergies, or other harmful processes such as food poisoning or carcinogenesis. Moreover, they are important for technological properties, as well as for nutritional and sensory food properties. Investigation of enzymatic and non-enzymatic PTMs that have occurred after food processing or storage, showed that PTMs can be used as parameters for food quality control (Agrawal, 2013; Arena, 2017; Paredi, 2012). Consequently, there is a need for the design of enrichment and data analysis strategies for the detection and assessment of these modifications. This fact is very important for experimental design when MS-based proteomics or other high-throughput approaches are used. If not perfectly designed, such kind of investigation can be a source of significant analytical problems and systematic errors. A particular problem in discovery proteomics is data analysis. A large number of possible PTMs of food proteins cannot be easily included in search engines since that requires

strong computers and significantly increases analysis time. Simplified strategies cover a few predicted PTMs included as variable modifications, and error-tolerant searches are allowed during a sequence database search. However, once identified and validated as a marker of food quality and safety, a particular PTM can be routinely quantified using a targeted proteomic approach (*vide infra*).

2.2. Conformational proteomics

Conformational changes are essential for biological functions of proteins, the investigation of structural dynamics is necessary to understand their physiological role. A group of proteins known as "intrinsically disordered" are so dynamic that under physiological conditions they are characterized by a complete or an almost complete lack of an ordered structure (Dunker, 2013). Information obtained by MS can significantly complement *in vitro* 3D structure elucidation with X-ray crystallography, electron microscopy, NMR and other spectroscopic methods (Leney, 2017; Vandermarliere, 2013). MS-based methods need only a small amount of sample enabling the investigation of naturally occurring structures and PTMs which are complicated for purification and/or expression. Moreover, MS enables the probing of structural transitions of proteins in a complex biological environment on a large scale (Feng, 2014). Mass spectrometry combined with methods such as hydrogen-deuterium exchange (Rand, 2014), limited proteolysis (Feng, 2014), cross-linking (Leitner, 2016; Sinz, 2014) and MS footprinting (chemical surface labelling) can also provide information about surface accessibility of amino acids (Vandermarliere, 2016). This information can be used to characterize protein conformation, as well as the 3D structure of macromolecular protein assemblies and PPI. Consequently, the MS can give an additional contribution to the investigation of macromolecular protein complexes

240 including their composition, stoichiometry, copy number, topology and dynamics (Wohlgemuth,
241 2015).

242 **2.3. Analysis of protein-protein interactions**

243 Protein functions can be modulated in different ways, including their expression level, PTMs,
244 metabolites, and PPI. MS-based proteomics offers many different approaches for PPI
245 identification (Smits, 2016). Comprehensive and reproducible information about PPI is necessary
246 to build networks of interacting molecules (genes – their products – proteins – cofactors –
247 messenger molecules – metabolites) as a basis for quantitative/dynamic analyses (Bensimon,
248 2012). Dynamics of these networks that are modulated at different time scale by internal (*e.g.*
249 genomic alterations) and external factors (*e.g.* environmental, food) is believed to determine the
250 phenotype (Aebersold, 2016). Building of interaction networks, their analysis and comparison,
251 fusion, harvesting of information from networks and other sources in order to understand how
252 network capture and process information induce a specific response or phenotype, are complex
253 tasks for bioinformatics scientists (Gligorijević, 2016). Concerning food safety and quality,
254 network includes, as external factors, food processing and storage conditions, as well as the
255 effect of food on its consumer.

256 **2.4. Chemical proteomics**

257 Protein quantities do not necessarily represent their activities. A toxic protein whose toxicity is
258 based on its enzyme`s activity, or an enzyme whose activity is of a particular interest for food
259 processing or food value can be present in a denatured or inactivated form. Hence, measuring of
260 total amount will not provide information about quantity of active form. Quantitative information
261 about enzyme active form can be obtained by MS-based proteomics by means of activity-based

protein profiling (ABPP). Chemical probes are specially designed to contain a reactive group that targets a specific enzyme class by forming an irreversible covalent bond and a reporter group that enables their enrichment and/or detection (Wright, 2016). ABPP are developed for different enzyme classes (Cravatt, 2008), such as proteases (Fonović, 2008), kinases and phosphatases (Ruprecht, 2015), glycosydases, cytochrome P450. Proteins in low abundance that exhibit enzyme activity, can especially be assessed by ABPP (Cravatt, 2008).

Chemical probes can be designed based on small molecules derived from natural products or food and used for identification of their interaction partners in proteome (Wright, 2016). Proteomic approaches for the same purpose, but without chemical labelling of small molecule, are also described (Guo, 2017).

3. Sample preparation

Proper sampling, sample preparation and sample handling are seen as among the main problem areas of proteomics (Nilsson, 2010b). The division of labour between those who control sampling and sample preparation and those who work with the MS can result in serious data quality issues, due to the lack of accountability and management of the data generated (Nilsson, 2010b) and lack of standardization (Poste, 2011). Protocol should be thoroughly discussed between MS-based proteomics specialists and team members (biologists, chemists, food technologists, nutritionist, clinicians, etc.). Correct sampling requires knowledge of complex structure of the food matrix and the corresponding analytical protocol (Jongenburger, 2015). Sampling methods are not equally useful, and there is no universal method, thus making the choice during experimental design is a critical point (Skold, 2013). After sampling, a proteome can be rapidly modified by released (or activated) proteases, other protein- modifying enzymes (*e.g.* phosphatases) and metabolites (*e.g.* polyphenols, glutathione, organic acids) which are

naturally present in analysed food material. Sample preparation itself can be a significant bias of a foodomic method since the accuracy of the experimental data, and both their reproducibility and confidence essentially depend on the accuracy and quality of the clean-up technique. Consequently, sampling, sample handling and sample preparation has to be known and considered during interpretation of MS data (Skold, 2013). MS-based proteomic specialist is the one who must ensure high fidelity of the platform through routinely performing checks and balances (Bittremieux, 2017; Nilsson, 2010b). For this purpose, different standards should be in regular use to control purification efficiency (Gallien, 2014), protease digestion (Lebert, 2015), peptide retention time during LC (Beri, 2015; Escher, 2012), as well as the ionization efficacy. Automated pipelines for quality control of LC-MS/MS are in development (Bereman, 2015a). An overview of techniques in food analysis and sample preparation was recently published (Galloa, 2016).

MS-based proteomic techniques can detect about 2000 proteins in 0.1 ug of protein digest. However, detection of more than 9000 proteins requires more than 1 mg (>$5x10^6$ average human cells) of starting material (Mallick, 2010; Zubarev, 2013). Protein extraction is a first step in sample preparation, and it is a great source of variation, its design strongly influencing proteomic results (Dhabaria, 2015). An number of different procedures usually involve physical homogenisation (mechanical force, ultrasound (Kadama, 2015), increased pressure, heating/cooling, etc.), the use of buffers, detergents, chaotropic agents for protein extraction and solubilisation, application of reducing agents, as well as different substances for enzyme inhibition (Bodzon-Kulakowska, 2007). Some of these, alone or in combination with others, can introduce chemical or physical changes of amino acids such as carbamylation or the Maillard reaction (Kollipara, 2013). Sample preparation in foodomics is the topic of a comprehensive

overview about sample preparation in foodomics that also includes complex approach by use of different proteolytic enzymes and other methods for protein cleavage before further analysis by LC-MS/MS (Andjelković, 2017).

4. Acquisition of mass spectra in bottom-up proteomics

The term MS-based proteomics is used most often in the context of bottom-up approach (Fig.2). In bottom-up approach proteins are extracted and digested by a sequence-specific protease. Resulting highly complex mixture of peptides is supplied to mass spectrometer in the form, amount and time frame that will enable successful examination using a particular MS technique. Currently, mass spectra can be acquired in three different ways: data dependent, targeted and data independent acquisition (Fig.3).

4.1. Data dependent acquisition

The most common MS-based proteomic approach in food investigation is the shotgun bottom-up approach**,** also known as "discovery based" (Zhang, 2013), Fig.2. In this approach, an adequately extracted and prepared sample containing a protein mixture is digested to peptides with trypsin or with other site-specific proteases (Switzar, 2013). The obtained peptide mixture is subsequently separated into fractions by LC or other separation techniques, such as capillary electrophoresis. These fractions are either on-line electrosprayed (LC-ESI-MS/MS), or off-line spotted and after addition of proper matrix ionised by MALDI (LC-MALDI-MS/MS), and introduced into the corresponding mass spectrometer. In a further step, the generated ions are scanned (MS scan) and in so-called data dependent acquisition (DDA) mode, usually 3-20 most abundant ("top") ions are selected by predetermined rules (dynamic exclusion, detection window, charge state selection, base line subtraction etc.) in a time dependent manner (Fig.3). Selected ions are then fragmented (CID, HCD, ETD, EThcD) (Frese, 2011). The EThcD

fragmentation technique is implemented into the latest hybrid mass spectrometers and substantially improves the level of peptide backbone fragmentation (Frese, 2012). The generated fragments are subsequently analysed by an MS/MS scan. In the final step, the data from MS and MS/MS scans are matched with sequence databases, by means of different algorithms, in order to identify peptides and, subsequently, proteins (Audain, 2017; Ting, 2015). Interpretation of shotgun proteomic data is a complex task that can lead to ambiguities in determining the identities of sample proteins (Nesvizhskii, 2005). Information obtained at the level of peptides has to be analysed in detail in order to make correct conclusions about protein/s (isoforms, proteoforms, sequence redundancy) that contain particular peptide (Nesvizhskii, 2005).

4.1.1. Capacity of shotgun bottom-up approach

MS-based proteomics shows a brilliant development over last 10 years. Seven years ago a standard shotgun LC-MS/MS analysis of a single cell line lysate, over 3-h, elute more than 100,000 isotope features, likely representing peptides, and they could be detected with a HRMS scan. However, just 16% of these were targeted by an MS/MS scan and only 9% of them were identified by "top 10" DDA (Michalski, 2011). With a standardized analysis platform, the achieved degree of repeatability and reproducibility was about 70-80% (Tabb, 2010). A higher degree of reproducibility (>90%) with this technology could be achieved by repeating the analysis 7-10x until virtually every peptide has been observed, however, only when results of all subsequent runs have a very high overlap with already collected data (Mitchell, 2010). Application of a longer LC gradient or intensive sample fractionation with subsequent analysis of each fraction could also improve the reproducibility of DDA. However, this strategy requires more time and increases costs (Domon, 2010). Major limiting properties of mass spectrometers

for the detection of a larger number of peptides in a short time are: sequencing speed (duty cycle), sensitivity, and precursor ion isolation (Michalski, 2011).

Five years ago, an advanced commercial instrument under carefully optimized conditions could identify more than 37,000 peptides (belonging to ~5,000 proteins) in a 4-h single dimension LC-MS/MS run (Pirmoradian, 2013). This is about a half of the expressed proteome of an average human cell line. Multidimensional protein identification technology (MudPIT; combination of several separation techniques) (Fournier, 2007) could provide >10,000 proteins, but operational costs, sample preparation and consumption, and working time of LC-MS/MS of more than 24-h were still high (Pirmoradian, 2013). Latest Orbitrap Fusion MS system could analyse ~90% of yeast proteome (~4500 proteins) in 1.5-h of nanoLC work (Hebert, 2014).

Mass spectrometers handle a proteome dynamic range of 4-5 orders of magnitude (Domon, 2010). However, the proteome dynamic range stretches over at least 6 orders of magnitude, approaching 11 orders of magnitude in the best investigated case of blood plasma (Anderson, 2002). A wide dynamic range is one of the most challenging problems in MS-based proteomics and it is still not satisfactory solved. The complexity of analysed peptide mixtures is increased by the proteolytic background coming from ions of peptides that are results of an unspecific tryptic (proteolytic) cleavage. Relative abundance of nonspecific peptide ions is about one order of magnitude lower than the expected abundance of specific ones (Picotti, 2007). Proteolytic digestion increases the dynamic range of signal intensities of peptides for at least one order of magnitude (Zubarev, 2013). This is the intrinsic limitation of the shotgun technique that covers low abundant peptides and impairs their identification (Picotti, 2007). A portion of generated peptides can also be modified during sample preparation (Table 2). These modifications also occur in an undefined fraction of peptides. That means that the modifications are not

stoichiometric. Moreover, there are chemical modifications like racemization or isomerization (Table 2) of amino acid side chains that do not change the molecular mass but may influence chromatographic behaviour. All listed modifications are lowering the amount of a particular peptide (or a proteoform) and they also increase the complexity of an analysed mixture. Thus, identification of both low abundant proteins and highly hydrophobic proteins is a complex task that requires a specially designed sample preparation procedure, the choice of an optimal proteomic approach, an optimal LC-MS/MS system and corresponding data analysis (Josić, 2007, 2014; Vučković, 2013; Zubarev, 2013).

4.1.2. Future task

The exclusive use of trypsin in proteomics could be a reason why our view of the proteome still remains incomplete. For the sake of broadening this view, a parallel analysis with alternative proteases or other cleavage strategies shall be considered in the future. This will enable to access more information rich sequences important for the identification of protein isoforms and proteoforms (Giansanti, 2016; Trevisiol, 2016). Up to date, LysargiNase has been evaluated for its application in shotgun bottom-up proteomics (Tsiatsiani, 2017). It cleaves proteins at the N-terminal side of Arg and Lys. Comparing to the products of tryptic digestion, these peptides contain two protons, following ESI, positioned at the N-terminus. The consequence is a completely different fragmentation pattern that provides additional structural information (Tsiatsiani, 2017). Protection of Lys,\ by a chemical modification before trypsin digestion restricts cleavage to Arg (except when it is followed by Pro) and it could also be an alternative to the conventional trypsin digestion (Golghalyani, 2017). An even better might be achieved by use of protease GingisREX^{TM} that cleaves only the C-terminal side of Arg, regardless of Pro.

Stochastic nature of the precursor ion selection in DDA is biased toward the more abundant component in the sample. The consequence is that the changes caused by single nucleotide polymorphisms, mutations, splicing variants, some PTM`s and other protein modifications are mostly inaccessible. Additionally, the low sequencing speeds of mass spectrometers that are applied for analyses cause additional problems with reproducibility of DDA (Domon, 2010; Picotti, 2013). Application of dynamic exclusion in DDA maximizes the number of unique ions to be isolated for fragmentation and MS/MS scan. In the same time, application of dynamic exclusion strongly reduces the probability of isolation of a precursor ion at the apex of its chromatographic elution peak. The repercussion is a negative effect on the quality of the acquired spectra and consequently, on both qualitative and quantitative analysis. Efforts to overcome these problems have led to the development of targeted and directed approaches (Domon, 2010).

4.2. Targeted acquisition

Targeted proteomic approaches ("hypothesis driven proteomic approaches") were developed for accurate and reproducible quantification of any protein or a set of proteins in any biological sample (Picotti, 2013). First targeted approach was based on a MS acquisition technique called selected reaction monitoring (SRM) developed on QqQ mass spectrometers. The first step in a targeted approach is the selection of proteins that are objectives of a particular research (formulation of a hypothesis) and that will be targeted with the MS analysis. For each selected protein, at least one peptide with 2-4 characteristic transitions (pair of m/z values associated with the precursor and one of its fragment ions) is carefully selected for monitoring (Brusniak, 2011; Carr, 2014; Colangelo, 2013). When more than one transition is monitored, SRM is known as multiple reaction monitoring (MRM). If food proteins are analysed, selection of peptides for

421 SRM additionally has to take in consideration modification of proteins presented in Table 2. The
422 total number of targeted peptides that can be reliably quantified is limited by the time available
423 for the transition scan at a particular mass spectrometer, the amount of a particular peptide ion
424 and by the chromatographic elution profile of a peptide. Thus, the total number of proteins that
425 can be reliably quantified in one LC-MS/MS run on QqQ is around 100 (Picotti, 2013).
426 Sensitivity of SRM allows the identification of down to 100 or ~7500 copies per cell in non-
427 fractioned yeast or human proteomes respectively, in a 1-h LC run (Picotti, 2013; Ebhardt, 2012;
428 Picotti, 2009). The limit of detection of SRM can be further improved by sample fractionation or
429 enrichment. This enables the detection of low abundant proteins, isoforms and proteoforms (Liu,
430 2013). Better management of time available for mass spectra acquisition during an LC run can be
431 achieved using scheduled SRM. In this technique the detection window for a particular peptide
432 opens only around its elution time and as a consequence the number of quantified proteins can be
433 increased (Escher, 2012).

434 A **directed MS approach** consists of two distinct experiments. In the first run the sample is
435 analysed in MS mode and peptides are identified by bioinformatics tools. An integrative
436 approach providing any additional information from genome-wide mRNA analysis or
437 metabolome data would be extremely beneficial (Vehmas, 2014). In the second run, only
438 peptides of interest are included in the list of ions which will be selected for fragmentation and
439 MS/MS scan. Consequently, directed MS approach focuses ion selection on non-redundant and
440 information-rich precursor ions. Essentially, with better management of available mass
441 spectrometer time, the duty cycle was directed to the peptides of interest (Schmidt, 2008), and
442 not exclusively to the most intensive ions. Thus, partially removing bias toward more abundant
443 components increases the depth of analysis and the reliability of quantification (Domon, 2010;

444 Schmidt, 2008). Benefits of directed MS in proteomics can only be realized if the sample
445 complexity is high in relation to the duty cycles available, and if the samples are available in
446 amounts that allow multiple LC–MS/MS runs (Schmidt, 2008). This is the case in food research
447 where samples are of high complexity and their amounts are usually not restricted.

448 **4.3. Data independent acquisition**

449 Numerous technical advances, like the development of HRMS, improvements in ion collection,
450 transmission optics and selection, and increase of scan speed, enabled the development of data
451 independent acquisition (DIA) technique. DIA uses a defined window size to systematically, in
452 repeated cycles during a chromatographic run, sample precursor ions from an analysed mass
453 range. All sampled precursor ions are simultaneously fragmented and MS/MS spectra are
454 collected (Bilbao, 2015; Chapman, 2014). The window size in different DIA approaches ranges
455 from a wide window comprising the whole mass range to a very narrow one, down to 0.4 Th. In
456 this way DIA approaches generate very complex MS/MS spectra, especially when wide
457 precursor isolation windows were used. DIA does not provide direct link between the precursor
458 ion and its fragment ions. Hence, the analysis of data acquired with DIA require complex
459 processing strategies as well as software solutions and large informatics resources (Bilbao, 2015;
460 Egertson, 2015; Escher, 2012). Once acquired data with DIA can be later refined and re-mined.
461 Many different DIA approaches are developed and implemented on different mass
462 spectrometers, Table 3. Each of listed approaches has unique characteristics and choosing one
463 over the other involves trade-offs in sensitivity, selectivity and number of samples analysed in
464 certain time frame. Compared to DDA strategies in shotgun proteomics, DIA increased the
465 visibility of low abundant and isobaric peptides, and as a consequence increased the
466 identification of proteins containing these peptides. The dynamic range of DIA spans over 4-6

orders of magnitude (Aebersold, 2016; Gillet, 2012), up to 8 with CSI-PAcIFIC DIA (Table 3). Selective enrichment and purifications of peptides containing PTMs is a usual strategy for their analysis, (Fig. 2). However, this is not possible for all PTMs and DIA methods could be applied as an alternative.

5. Alternatives to MS-based bottom-up proteomic approach

Alternative and complementary approaches to MS-based bottom-up proteomic approaches are top-down and middle down MS-based approaches.

In top-down proteomics (Fig.2), intact proteins or large protein fragments (>15 kDa) are analysed by MS scan to obtain the molecular weight of a particular protein and its proteoforms. The MS scan is performed on an ultra-HRMS, in first line FT-ICR and Orbitrap. However, TOF mass analysers can also be used for certain top-down analyses. Upon MS scan the protein ions are fragmented and fragments are analysed in a consequent MS/MS scan. Fragmentation techniques such as ETD/ECD are of special importance, since they are able to preserve the information about PTM`s. Combination of ECD with CID (EtciD) or HCD (EthcD) can provide high protein sequence coverage that increases the confidence in proteoform identification (Brunner, 2015; Frese, 2012). Infrared multiphoton dissociation or ultraviolet photodissociation exhibited an additional potential to improve *de novo* protein sequencing (Shaw, 2016). Due to the current inefficiency of MS/MS techniques, limit of protein size that can be efficiently sequenced in a time-constraint experiment is around 50 kDa (Laskay, 2013). The MS scan of intact protein contains a large number of highly charged ions which originate from the same molecular species. In the presence of proteoforms and other proteins, isolation of single ion species is a hardly feasible task and the resulting MS/MS spectra are highly convoluted (Laskay, 2013). Hence, the top-down approach requires intensive fractionation to obtain less complex

protein mixtures (Tran, 2011; Zhang, 2014). The characterization of proteoforms in identified proteoform–spectrum matches still relies mainly on manual annotation (Kou, 2016), but recent technological advances of mass analysers towards ultra-high resolution, as well as new algorithms for data processing, are now making top-down the method of choice when studying complex proteoforms (Kou, 2016; Vyatkina, 2015).

Middle down proteomics analyse large peptides with a size of about 7-15 kDa, compared to less than 3 kDa in bottom-up approach, and 3-7 kDa in extended bottom-up proteomics. This strategy combines the advantages of bottom-up and top-down approaches and minimizes their shortcomings (Laskay, 2013). The advantage of longer peptides is their larger chance to contain higher charge, resulting in a more efficient ECD/ETD. The result of increased fragmentation efficiency is the higher sequence coverage. Additionally, larger peptides have a higher chance to contain individual mutations and PTM`s hence it is beneficial for the identification of isoforms and proteoforms. Moreover, the complexity of a peptide mixture is reduced when longer peptides are generated, rendering more time for mass analysers during an LC run, and resulting in a higher resolution for larger number of peptides. Consequently, the analysis of large proteins that still cannot be analysed by top-down approach, as well as the analysis of proteoforms that are difficult to separate, can benefit from the middle down approach (Zhang, 2014). Peptides of average mass >3.4 and >6.3 kDa can be generated by the Sap9 (Srzentić, 2014) and OmpT (Wu, 2012) proteases respectively.

6. Quantitative high-throughput proteomics

Mass spectrometry can provide relative quantitative information (a quantitative comparison of proteins between different samples expressed in a fold of change of a particular protein between

analysed samples) or an absolute (exact concentration or number of individual protein/s in a given sample). Different technologies and approaches were developed for this purpose, Table 4. The importance of MS-based quantitative proteomics is emphasized with recent studies demonstrating that the identity of cells and tissues seems to be determined primarily by the abundance at which they express their constituent proteins, and perhaps by the manner how the proteins are organized in the proteome, rather than by the presence or absence of certain proteins (Aebersold, 2016).

Quantitative changes of the food proteome may be influenced by different factors such as plant or animal strain, genetic engineering, growing conditions, quality of animal food, particular food processing, storage conditions, etc. Consequently, individually or collectively these factors can influence food quality and/or safety (Agrawal, 2013; D'Alessandro, 2012; Piras, 2016; Tedesco, 2014).

Biological variations of interest in proteomics are often very small. Consequently, the requirements for the precision of quantitative proteomic experiments are very high (Lyutvinskiy, 2013). Accurate mass measurement is of a major concern in the development of MS-based proteomics (Aebersold, 2016; Tabb, 2010). MS-based proteomics is an example of a multivariate process with the potential for highly correlated variables as performance declines (Bereman, 2015). Major sources of variability are extraction, instrumental variance, instrumental stability and protease digestion (Piehowski, 2013). Every source of variation can be detrimental to the extract of meaningful biological information. Different tools are available to monitor the system suitability and to improve proteomic workflows (Bereman, 2015; Walzer, 2014). The importance of this topic is promoted by HUPO within the proteomic standard initiative specialized quality control working group that has been founded in order to define a community data format and

associated controlled vocabulary terms, facilitate data exchange and archiving of MS derived quality control metrics (http://www.psidev.info/groups/quality-control). Quality control methods and standard operating procedures are necessary parts of proteomics, unfortunately still frequently neglected. The evaluation of performance can be achieved by sharing and exchanging results between reference laboratories, by use of common samples, different methodologies and experimental designs (Bereman, 2014; Tabb, 2016). If laboratories deploy different methodologies to analyse the differences between the same two complex samples, then they will assuredly see differences in the gene or protein lists produced by the two technologies (Tabb, 2016).

Quantitative comparison of proteins from different samples (relative quantification) is mostly performed by two basic technologies (Ong, 2005), Table 4.

1. Directly comparing ion abundance between samples while applying different strategies to minimize different mass spectrometer response and differences in sample preparation; this group of approaches are known as label-free quantification (LFQ) (Cox, 2014; Neilson, 2011);

2. Upon labelling (metabolic, chemical or enzymatic) of proteins (or peptides) in each of the few analysed samples using unique stable isotopes, samples are mixed and analysed together in the same run; this technology is known as stabile isotope dilution (Ciccimaro, 2010). Most known approaches based on the labelling technology are listed in Table 4.

6.1. Label-free strategies

In label free proteomics, quantification can be performed using different approaches at the MS scan level (area under the curve or signal intensity measurement) or at the MS/MS scan level (spectral counting) (Ahrné, 2013; Arike, 2014; Neilson, 2011). Comparative studies of LFQ

557 approaches demonstrated certain advantages the two former approaches (Ahrné, 2013; Arike,
558 2012; Dowle, 2016).

559 In a label-free quantitative proteomic experiment each sample is prepared and analysed
560 independently. Discrepancy in sample preparation procedure (sampling, sample handling,
561 extraction efficiency, protease digestion efficacy, clean-up efficacy, etc.) is a source of
562 variations. These variations can be reduced by procedure design, training of personnel and
563 application of robotics. Variability of instrument response (*e.g.* variation in the current of ESI
564 during an LC-MS/MS run, ion suppression during ionization, reproducibility of retention times,
565 fluctuation in instrumental sensitivity) can be reduced by use of internal standards (Lyutvinskiy,
566 2013; Piehowski, 2013). In order to standardize sample preparation, the extent of digestion and
567 performance of an LC-MS/MS system, a universal protein standard called DIGESTIF was
568 developed (Lebert, 2015). However, the use of internal standards introduces another level of
569 complexity and increases the costs of the analysis (Lyutvinskiy, 2013). Instrumental response
570 can be corrected by *in silico* post-processing. This significantly improves the accuracy and
571 precision of LFQ (Cox, 2014; Lyutvinskiy, 2013; Tu, 2017). An MS-based proteomic
572 experiment will highly benefit from every step undertaken towards the reduction or correction of
573 the coefficient of variation (CV). Reduction of CV improves efficacy (probability to detect
574 quantitative difference between proteomes) by reducing time and costs of experiments
575 (Lyutvinskiy, 2013). Detailed optimization of parameters for LFQ could provide relative
576 quantification of up to 2900 proteins in 4-h for samples analysed in triplicate (Pirmoradian,
577 2013).

578 **6.2. Label-based strategies**

579 Concerning both precision and accuracy, SILAC is the "golden standard" for relative
580 quantification in discovery proteomics (Lyutvinskiy, 2013; Zhang, 2013). This approach,
581 described in 2002 for *in vitro* non- isobaric metabolic labelling, was subsequently adjusted for
582 many different applications, including *in vivo* labelling of animals and plants, as well as tissue
583 analysis (Ong, 2007; Table 4). In SILAC method, labelled samples are concomitantly analysed
584 by LC-MS/MS and relative quantitative comparison is obtained from the MS scan. High
585 accuracy of this method is a consequence of several facts: mixing of differently labelled samples
586 early in the experimental process, which enables simultaneous sample preparation and LC-
587 MS/MS analysis; the fact that every protein is quantified several times through multiple MS
588 scans and usually (85%) through multiple peptides; and 100% efficiency of metabolic labelling.
589 High costs of an *in vivo* labelling were reduced with the development of spike-in SILAC, while
590 high complexity of tissue proteomes was addressed with the use of "super SILAC", for
591 references see Table 4.

592 When TMT/iTRAQ are used, relative quantitative information is obtained when peptides that are
593 chemically labelled with an isobaric tag upon fragmentation release low m/z reporter ions. These
594 reporter ions are compared in the subsequent MS/MS scan. Peptides labelled with an isobaric tag
595 have the same mass in an MS scan, thus they do not increase the complexity of the MS scan
596 spectrum as it is the case with peptide labelling with non-isobaric tags and SILAC. Comparing to
597 MS-based quantification, a higher dynamic range can be assessed with MS/MS based
598 quantification (Rauniyar, 2014). Currently, the advantage of TMT/iTRAQ over SILAC is
599 multiplexing that allows a simultaneous quantitative analysis of 10 samples (Weekes, 2014).
600 Application of triple-stage MS (MS3) was proposed to eliminate interference in iTRAQ, which
601 comes from near-isobaric ions that are co-isolated and co-fragmented with the selected peptide

(Ting, 2011). However, application of MS3 takes a penalty in sensitivity. When the MultiNoch MS3 method developed on the Orbitrap Velos mass spectrometer was applied, the sensitivity of MS3 could be increased 10x without a significant loss of selectivity (McAlister, 2014). Another method that eliminates accuracy and precision problems of TMT/iTRAQ exploits high accuracy and resolution of modern mass spectrometers using complement TMT fragment ion clusters as an alternative to reporter TMT fragment ions (Wühr, 2012).

Chemical dimethyl labelling introduces non-isobaric tag to peptides that allow quantification at the level of an MS scan. The main advantages of dimethyl labelling are inexpensive reagents, as well as the labelling procedure that can be easily automated, performed on-line, and applied in a high-throughput manner (Altelaar, 2013). Labelling with different isobaric tandem mass tags or with non-isobaric mass tags is performed at the level of peptides, after protease digestion of a sample. This includes more independent sample preparation steps (that can be significant sources of variability) before mixing differently labelled samples for further simultaneous sample preparation and LC-MS/MS analysis.

Additionally, special isobaric tags are developed for cysteine and PTMs, such as carbonyl and glycan modifications (Rauniyar, 2014).

Neutron encoding (NeuCode) is a new quantification approach which benefits from ultra-high resolution of FT-ICR and Orbitrap mass analysers that is capable of distinguishing a mDa mass difference in a neutron mass signature of different isotopes (Hebert, 2013). Neutron mass signatures can be encoded in metabolically, chemically or enzymatically introduced tags. Using neutron encoding, the multiplexing capacity of SILAC was increased, currently up to 9-plex in NeuCode SILAC. It combines the accuracy of SILAC with multiplexing capacity of isobaric tagging and does not suffer from the problem of precursor interference which reduces the

accuracy of isobaric tagging (Rose, 2013). The number of mechanisms for increasing multiplexing capacity (number of samples which can be simultaneously analysed) of isobaric regents was described (Braun, 2015; Frost, 2015). Results of the latest study evaluating the reproducibility of LFQ and iTRAQ showed an encouraging degree of conformity that suggests a degree of the maturity of proteomic methods (Tabb, 2016).

6.3. Absolute quantification with isotope labelled standards

Absolute quantitative information about individual proteins is a prerequisite for modelling studies of biochemical systems (Malmström, 2009), for understanding the complex interplay of the system (food or consumer) components or interplay between components of two systems (food and consumer), as well as for the quality and safety control of food. As in the case of relative quantification, absolute quantification strategies are based on the technology of stable isotope dilution (Brun, 2009; Villanueva, 2014). Essentially, signal intensity of a mass spectrometer is standardized with a known concentration of an isotope labelled reference. This reference can be produced by labelling a standard sample containing a known amount of peptides of interest tagged with isobaric (or non-isobaric) tags. Also, the reference can be an isotope labelled peptide of identical structure as the peptide of interest. The reference isotope labelled peptide can be supplied to the sample using different strategies: AQUA, QconCAT or PSAQ (reference in Table 4).

A synthetic isotope labelled peptide can be added into the sample before protease digestion or immediately before LC-MS/MS analysis. This strategy is known as AQUA (absolute quantification). If a subsequent immunoaffinity step is performed, in order to enrich the low abundance peptide of interest, the strategy is known as SISCAPA (Stable Isotope Standards and

647 Capture by Anti-Peptide Anti-bodies) (Anderson, 2004). The selection of peptides that will be
648 used as internal standards is important for the success of quantification, and different methods
649 are developed for this purpose (Brusniak, 2011; Eyers, 2011). Peptide standards for AQUA may
650 also contain PTMs if these are of interest for quantification. Quantification accuracy of AQUA
651 strategy may be compromised by incomplete protease digestion of proteins or if pre-fractionation
652 steps are used in sample preparation. Thus, efficiency of digestion has to be monitored, as well
653 as the yield (recovery) after each fractionation step (Gallien, 2014).

654 Quantification concata-mer (QconCAT) strategy uses polypeptide constructs (concatmers)
655 composed of many different isotope labelled peptides. These constructs are biologically
656 synthetized. Concatmers are added to the sample before protease digestion and protease releases
657 isotope labelled peptides. In the same time, these peptides serve as a control for digestion and
658 also as internal standards for quantification. QconCAT enables simultaneous quantification of
659 several proteins and is less cost-intensive than AQUA. However, this method also suffers from
660 the same problem caused by an insufficient protease digestion efficiency and possible low yield
661 during pre-fractionation.

662 In order to provide a reliable absolute quantification, a good internal standard should behave as
663 closely as possible as the analysed protein, following it throughout all sample preparation steps.
664 As a part of the strategy for overcoming problems with accuracy caused by protease digestion
665 efficiency and sample pre-fractionation, protein standard absolute quantification (PSAQ) strategy
666 uses isotope labelled intact proteins. Providing multiple peptide standards for target protein,
667 PSAQ provides also higher efficacy. Production costs of protein standards are limiting factors for
668 a wide application of this strategy. However, cell free systems for protein synthesis now offer a
669 way to reduce them (Madono, 2011). High-throughput system for synthesis of protein standards

for quantification of highly hydrophobic transmembrane proteins was also developed (Takemori, 2015). Nevertheless, problems with synthesis of protein standards containing particular PTMs are still present.

When introduced, S/MRM acquisition technique offered the highest sensitivity, a wide dynamic range, and the high selectivity, highest reproducibility and precision that are necessary for absolute quantification. Quantitative information in SRM is given by the intensity of the fragment ion of targeted transition. Nowadays, main problems with SRM are the number of proteins that can be simultaneously monitored and selectivity due to the resolution of QqQ mass spectrometers. The attempt to increase the number of proteins quantified by SRM requires the sacrifice of some selectivity or sensitivity. The addition of a third stage of mass filtering to MRM with multiple reaction monitoring cubed (MRM^3) method on a hybrid QqQ/LIT mass spectrometer (Fortin, 2009) increased the discrimination of interferences compared to regular S/MRM and limit of quantification. Increased selectivity with MRM^3 has as a consequence a lower number of proteins that can be simultaneously analysed, since a part of the available cycling time was sacrificed to a third stage of mass filtering (Gallien, 2013).

In order to increase the number of absolutely quantified proteins, relative and absolute quantification strategies were combined. In one combination, a small group of specially selected proteins was quantified using AQUA SRM. These anchor proteins are used as further calibration points for translating relative abundance measurements into absolute abundance measurements, for a large part of the proteome (Malmström, 2009).

Strategies for absolute quantification can be combined with SILAC for absolute quantification of individual proteins in complex mixtures. As a result, “absolute SILAC” (Hanke, 2008) and PrEST SILAC (Zeiler, 2012) were developed.

693 Absolute quantification may be performed by use of isobaric and non-isobaric mass tags, if
694 peptide standards are used as one, or more, channels in a multiplexed analysis.
695 High resolution of the hybrid quadruple-Orbitrap mass spectrometer enabled development of an
696 approach called parallel reaction monitoring (PRM) (Gallien, 2013). PRM uses a 2-24 Th wide
697 isolation windows on a quadruple for selecting ions for fragmentation and recording
698 fragmentation products in the Orbitrap mass analyser. High resolution of OT increases selectivity
699 by separating ions of interest from interferences leading to partially improved quantification
700 performance compared to SRM (Gallien, 2014a; Gallien, 2013). By use of internal standards and
701 the on-the-fly adjustment of acquisition parameters, it is possible to organize acquisition time in
702 PRM and quantify 600-1000 peptides in complex samples in ~1-h (Bourmaud, 2016; Gallien,
703 2015).

704 **6.4. Data independent acquisition in quantitative high-throughput proteomics**

705 The problem with precursor ion isolation window width that is required to ensure sufficient
706 sensitivity in mass spectrometers (Michalski, 2011) makes that MS/MS spectra, that are obtained
707 when samples of high complexity analysed, is actually a mixture ("chimera") spectra due to the
708 co-isolation of all ions (originating from co-eluted peptides in the previous LC or capillary
709 electrophoretic separation) falling within the mass isolation windows width (Luethy, 2008). This
710 problem with selectivity may cause difficulties in the following peptide identification since it
711 may increase the complexity of MS/MS spectra (Houel, 2010). Additionally, if fragments of co-
712 isolated ions are similar to the fragments of the selected peptide they will also impair
713 quantification accuracy, and this problem is more pronounced if mass spectrometers with low
714 resolution are used. The level of interference this creates depends on abundances of analysed

peptides. Taking in to account these facts, DIA makes an attempt to take advantage of these facts.

The DIA provide the possibility to overcome limitations of S/MRM and PRM in absolute quantification. The use of this acquisition technique substantially increases the number of proteins that can be simultaneously quantified; it simplifies the experimental design and provides a flexible post acquisition data analysis. Extraction of quantitative information from data acquired with DIA (DIA fragment ion maps) can be performed with a targeted or an untargeted approach (Egertson, 2015; Li, 2015; Röst, 2014; Ting, 2015; Tsou, 2015).

In targeted extraction (peptide-centric matching approach), spectral libraries are used to mine DIA fragment ion maps for constellations of signals that precisely correlate with the known coordinates of a targeted peptide, thus uniquely identifying the peptide in the map. Coordinates that spectral libraries contain are: retention time information and, reference MS/MS spectra with relative intensities of ions (Egertson, 2015; Gillet, 2012; Rosenberger, 2014). Retention time normalization has to be performed for each run according to reference peptides, in order to enable a comparison of the analysed sample and peptide library (Escher, 2012; Parker, 2015; Röst, 2014). This allows for acquired data to be analysed in the same way as by SRM by targeted data extraction of transitions of interest (Egertson, 2015; Gillet, 2012; Parker, 2015). Available software types for peptide-centric matching are Open SWATH, Skyline, Spectronaut, PeakView, and SwathProphet. DIA permits quantification of (at least) as many compounds as those typically identified by regular shotgun proteomics with the accuracy and reproducibility of SRM across many samples (Gillet, 2012). Generation of spectral libraries is a current limitation of the targeted data extraction approach. The problem with coverage and the quality of spectral libraries is particularly pronounced with new food samples and complex food samples containing new

738 proteins, their isoforms and proteoforms, especially those proteoforms generated upon food
739 processing. Therefore, targeted data processing of complex food samples is currently restricted
740 and has to be combined with other approaches, such as iterative data mining based on theoretical
741 knowledge to account for previously undetected proteins (Bilbao, 2015; Gillet, 2012). However,
742 once developed, spectral libraries for a particular food sample will be permanently available.

743 In untargeted data extraction (spectrum-centric matching approach), real time correlation,
744 based on the retention time between the MS signal (precursors) and the MS/MS signal
745 (fragments), is performed (Ting, 2015; Tsou, 2015). In that way, the established relationship
746 between the precursor and corresponding fragments enables searching and matching with
747 sequence databases in the same way as DDA spectra. Different software is available for
748 spectrum-centric matching (PLGS, DIA-Umpire, MSPLIT-DIA, Group-DIA).

749 The SWATH MS is a combination of DIA and targeted data analysis, developed on a QqTOF
750 mass spectrometer, which vastly extends the number of proteins that can be quantified in
751 complex sample (Gillet, 2012). The size of the sampling window for precursor ions in SWATH
752 MS is 25(+1) Th. Recently, attempts were made to adjust the sampling window size to the
753 density of precursors across the mass range in order to increase selectivity, depth of coverage and
754 data quality. Using SWATH MS, 2500 proteins of yeast were quantified in a 3-h LC-MS/MS run
755 with reproducibility, precision and accuracy comparable to S/MRM (Selevsek, 2015). The same
756 analysis with S/MRM would take 48-h. The SWATH MS demonstrates high sensitivity (detected
757 >300 proteins more than Western blot) (Selevsek, 2015).

758 **6.5. Selection of quantification strategy, quality control and validation**

759 Many different MS-based quantification approaches were developed. There is no 'one-size-fits-
760 all' proteomic strategy that can be used to address all biological questions (Mallick, 2010).

761 Consequently, an adequate choice of quantitative approach is important for the success of an
762 MS-based proteomic analysis. In order to make the correct choice it is necessary to be well
763 versed in the technology and know the limitations and the advantages of MS-based approaches
764 (Domon, 2010; Mallick, 2010). Moreover, the selection should consider factors such as the type
765 of sample (source and complexity), the number of samples, necessary accuracy and
766 reproducibility, available personnel and equipment, and finally, both the available time and total
767 costs.
768 Quality control is an integral part of high-throughput MS-based proteomic experiments.
769 Inadequate validation or absence of any validation was blamed for wrong conclusions of many
770 high-throughput proteomic studies (Mitchell, 2010), and in combination with problems in
771 reproducibility, that were caused by reckless and incorrect application of this technology, it also
772 was a source of scepticism towards proteomics (Editorial, 2008; Nilsson, 2010b). The main
773 sources of these irregularities are sample preparation, sample handling, data analysis and data
774 evaluation (Nilsson, 2010b). When these tasks are divided between different professions, without
775 consultation and coordination with specialist for MS-based proteomics who best know the
776 limitations and pitfalls of the technology, and who should also take care about all quality control
777 steps and provide practical instructions about data interpretation, they can become a serious
778 source of problems (Bell, 2009; Nilsson, 2010b). Consequently, successful proteomic analysis
779 should be performed in a systematic, accurate and reproducible manner.
780 Antibody-based techniques are standards for the validation of MS-based proteomic experiments
781 (radioimmunoassay, immunoblot, ELISA, immunofluorescence etc.). Moreover, validation can
782 be performed with other methods such as cryo-electron tomography or morphological
783 measurements at the single-cell level (Malmström, 2009). However, high sensitivity and

784 selectivity of SRM make this technique known as "the mass spectrometrist's ELISA"
785 (Aebersold, 2013; Picotti, 2013). The advantage that SRM offers over antibody-based techniques
786 is a fast and cost-effective assay development. The main problems with commercially available
787 antibodies are that they may not work effectively or that they are not available for a particular
788 protein of interest. The complex nature of protein and food matrix modifications may evoke
789 cross-reactivity or reduced affinity in antibody-based techniques. Both can lead to an over- or
790 under-estimation of a particular protein (Koeberl, 2014). Moreover, different protein isoforms, as
791 well as proteoforms cannot be easily distinguished by use of antibodies (Picotti, 2013). MS-
792 based quantification provides a possibility to establish metrological traceability which enables a
793 meaningful comparison of results among laboratories across the globe (Cryar, 2013; Smit, 2014).
794 All of the mentioned advantages of SRM lead to a proposal for the validation of antibody based
795 techniques with SRM (Aebersold, 2013; Nilsson, 2010a) and the SRM approach is now also in
796 use for the validation of orthogonal proteomic approaches (Selevsek, 2015).

797 **7. Recent technical developments important for MS-based proteomics**

798 The quality and reliability of qualitative and quantitative information obtained from a sample in
799 MS-based proteomics, are particularly influenced by the skill of the analyst, the sample
800 preparation procedure, selected MS technique and approach, the type of mass spectrometer used,
801 and data analysis (Mitchell, 2010). MS-based proteomics are still driven by advances in both
802 chromatographic and MS technology (Helm, 2014). Eight years ago, two main properties of
803 mass spectrometers, sequencing speed (cycling time – number of spectra per second (Hz)) and
804 ion current (efficiency of ionisation and ion transmission to detector) were seen as main limiting
805 parameters for the development of shotgun bottom-up proteomics (Michalski, 2011; Mitchell,

2010). Since then, different technical improvements tackling these two parameters were commercialized within new LC-MS/MS systems.

The mostly employed LC-MS/MS system in shotgun proteomics is nanoESI-LTQ-Orbitrap. During the last five years, the commercial hybrid OT MS has doubled its speed and increased its resolution. Currently, tribrid OT mass spectrometers achieve 20 Hz with ultra-high resolution of 500,000 (at 200 m/z) and accuracy better than 1ppm. However, the amount of ions that can be trapped in OT is still a limiting factor for achieving higher dynamic range (Aebersold, 2016). While commercial QqTOF systems can achieve scanning speed of 100 Hz, their resolution is for an order of value lower, with accuracy up to 1ppm. Mass spectra obtained on QqTOF under such high speed usually do not contain a sufficient number of fragment ions to enable productive peptide identification (Helm, 2014; Meier, 2016). However, in recent years, the number of improvements in technology (improved collision cell, orthogonal accelerator scheme, reflectron, and detector) made QqTOF resolution and accuracy compatible with shotgun bottom-up proteomics (Beck, 2015). Both high resolution and high speed are advantageous properties, but at the current technical level, their combination in a single mass spectrometer is still reversely proportional. High resolution and high accuracy are advantageous properties crucial for shotgun, while sequencing speed and the amount of usable ions are very important for targeted proteomics.

Development of ion mobility spectrometry (IMS) introduced an additional dimension of separation to the standard m/z scans. IMS separates ion based on their size and shape (size of collisional cross section). Ion separation by IMS is fast (~100Hz) (Helm, 2014). Incorporation of IMS in a QqTOF, after the collision cell, provided MS systems which have an improved duty cycle, therefore, improved sensitivity, up to 10-fold. Consequently, faster data acquisition

improved peptide identification and quantification (Distler, 2016; Helm, 2014). Recently, a trapped ion mobility spectrometry (TIMS) device was incorporated in a QqTOF before the first quadruple (Meier, 2016). Synchronization of the quadruple with TIMS enables the accumulation and "elution" of accumulated ions, while quantification capacity is preserved. This can result in a better signal-to-noise ratio and provides additional separation of precursor ions, which minimizes the problem with precursor ion isolation (*vide supra*). The application of method called Parallel accumulation - serial fragmentation on TIMS-QqTOF MS system increased MS/MS scan speed 12-20x without losing sensitivity, providing a 10-fold gain in shotgun proteomics (Meier, 2016).

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1460 **Figure captions**

1461 Fig. 1. Contribution of proteomics to foodomics.

1462 Fig. 2. Current MS-based proteomic workflow, from the first step – material sampling to the last
1463 step – data analysis. Different sample preparation techniques can be combined with different
1464 MS-based proteomic approaches, as it is depicted by arrows, in order to design a method for a
1465 particular analytical problem.

1466 Fig. 3. Techniques for acquisition of mass spectra in high-throughput bottom-up proteomics.

Table 1

A) Examples of application of MS-based proteomics in food research and assurance of food quality and safety

MS-based proteomic app.		Application in foodomics	Reference
High-throughput proteomics	Bottom-up	Identification of orange proteome	Lerma-García, M. J., D'Amato, A., Simó-Alfonso, E. F., Righetti, P. G., & Fasoli, E. (2016). Orange proteomic fingerprinting: From fruit to commercial juices. *Food Chemistry, 196*, 739-749.
		Identification and comparison of proteomes of milk whey from different animals	Yang, Y., Bu, D., Zhao, X., Sun, P., Wang, J., & Zhou, L. (2013). Proteomic Analysis of Cow, Yak, Bu□alo, Goat and Camel Milk Whey Proteins: Quantitative Di□erential Expression Patterns. *Journal of Proteome Research, 12*, 1660–1667.
	Top-down Middle-down	Characterization of allergenic 2S albumin and its proteoforms in Hazelnut cultivars	Korte, R., Happe, J., Brümmer, I., & Brockmeyer, J. (2017). Structural Characterization of the Allergenic 2S Albumin Cor a 14: Comparing Proteoform Patterns across Hazelnut Cultivars. *Journal of Proteome Research, 16*, 988-998.
Identification of primary structure of protein and proteoforms		Characterization of Mustard 2S albumin allergens	Hummel, M., Wigger, T., & Brockmeyer, J. (2015). Characterization of Mustard 2S Albumin Allergens by Bottom-up, Middle-down, and Top-down Proteomics: A Consensus Set of Isoforms of Sin a 1. *Journal of Proteome Research, 14*, 1547–1556.
		Identification of structural changes of milk Gal d 1 allergen upon lipid peroxidation	Nikolić, J., Nešić, A., Čavić, M., Đorđević, N., Anđelković, U., Atanasković-Marković, M., Drakulić, B., & Gavrović-Jankulović, M. (2017). Effect of malondialdehyde on the ovalbumin structure and its interactions with T84 epithelial cells. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1861*, 126-134.
PTM	Glysco-proteomics	Characterization of glycoproteome of wheat flour albumins and its potential effect on wheat beer quality	Dedvisitsakul, P., Jacobsen, S., Svensson, B., Bunkenborg, J., Finnie, C., & Hägglund, P. (2014). Glycopeptide Enrichment Using a Combination of ZIC-HILIC and Cotton Wool for Exploring the Glycoproteome of Wheat Flour Albumins. *Journal of Proteome Research, 13*, 2696-2703.
	Phospho-proteomics	Influence of phosphoproteome changes on meet quality	Huang, H., Larsen, M. R., Palmisano, G., Dai, J., & Lametsch, R. (2014). Quantitative phosphoproteomic analysis of porcine muscle within 24 h postmortem. *Journal of Proteomics, 106*, 125-139.
Conformational proteomics		Investigation of correlation between	Nyemb, K., Jardin, J., Causeur, D., Guérin-Dubiard, C., Dupont, D., Rutherfur, S. M., & Nau, F. (2014). Investigating the impact of

		aggregate morphology and digestibility of ovalbumin	ovalbumin aggregate morphology on in vitro ovalbumin digestion using label-free quantitative peptidomics and multivariate data analysis. *Food Research International, 63*, 192-202.
Protein – protein interactions		Identification of 14-3-3 proteins interaction partners in rice proteome	Zhang, Z., Zhang, Y., Zhao, H., Huang, F., Zhang, Z., Lin, W. (2017). The important functionality of 14-3-3 isoforms in rice roots revealed by affinity chromatography. *Journal of Proteomics, 158*, 20-30.
Chemical proteomics		Identification of curcumin interaction partners in human proteome	Abegg, D., Frei, R., Cerato, L., Prasad Hari, D., Wang, C., Waser, J., & Adibekian, A. (2015). Proteome-Wide Profiling of Targets of Cysteine reactive Small Molecules by Using Ethynyl Benziodoxolone Reagents. *Angewandte Chemie Int. Ed. 54*, 10852–10857.
B) Examples of different acquisition modes			
Data dependent acquisition	"Top 8"	Identification of allergens and glycation products in thermally processed peanut	Hebling, C. M., McFarland, M. A., Callahan, J. H., & Ross, M. M. (2013). Global Proteomic Screening of Protein Allergens and Advanced Glycation Endproducts in Thermally Processed Peanuts. *Journal of Agricultural and Food Chemistry, 61*, 5638-5648.
	"Top 20"	Identification of beer proteome	Grochalová, M., Konečná, H., Stejskal, K., Potěšil, D., Fridrichová, D., Srbová, E., Ornerová, K., & Zdráhal, Z. (2017). Deep coverage of the beer proteome. *Journal of Proteomics, 162*, 119-124.
Targeted acquisition	SRM / MRM	Detection of wheat contamination in foods	Colgrave, M. L., Goswami, H., Byrne, K., Blundell, M., Howitt, C. A., & Tanner, G. J. (2015). Proteomic Profiling of 16 Cereal Grains and the Application of Targeted Proteomics To Detect Wheat Contamination. *Journal of Proteome Research, 14*, 2659–2668.
		Identification of banned processed animal proteins in meat and bone meal	Marbaix, H., Budinger, D., Dieu, M., Fumière, O., Gillard, N., Delahaut, P., Mauro, S., & Raes, M. (2016). Identification of Proteins and Peptide Biomarkers for Detecting Banned Processed Animal Proteins (PAPs) in Meat and Bone Meal by Mass Spectrometry. *Journal of Agricultural and Food Chemistry, 64*, 2405-2414.
	PRM	Rapid detection of parasite (Anisakids) in fishery products	Carrera, M., Gallardo, J. M., Pascual, S., González, Á. F., & Medina, I. (2016). Protein biomarker discovery and fast monitoring for the identification and detection of Anisakids by parallel reaction monitoring (PRM) mass spectrometry. *Journal of Proteomics, 142*, 130-137.
Data independent acquisition	SWATH	Quantification of barley gluten in selectively bred barley lines	Colgrave, M. L., Byrne, K., Blundell, M., Heidelberger, S., Lane, C. S., Tanner, G. J., & Howitt, C. A. (2016). Comparing Multiple Reaction Monitoring and Sequential Window Acquisition of All Theoretical Mass Spectra for the Relative Quantification of Barley Gluten in Selectively Bred Barley Lines. *Analytical Chemistry, 88*, 9127–9135.
	WiSIM	Identification of tomato	Martin, L. B., Sherwood, R. W., Nicklay, J. J., Yang, Y., Muratore-Schroeder, T. L., Anderson, E. T., Thannhauser, T. W., Rose, J. K., &

		fruit proteins regulated by transcription factor CD2	Zhang, S. (2016). Application of wide selected-ion monitoring data-independent acquisition to identify tomato fruit proteins regulated by the CUTIN DEFICIENT2 transcription factor. *Proteomics, 16*, 2081-2094.

C) Examples of different quantification approaches and strategies

LFQ	MS signal intensity measurement	Characterization of muscle tissue from farmed and wild fish	Chiozzi, R. Z., Capriotti, A. L., Cavaliere, C., La Barbera, G., Montone, C. M., Piovesana, S., & Laganà, A. (2018). Label-Free Shotgun Proteomics Approach to Characterize Muscle Tissue from Farmed and Wild European Sea Bass (Dicentrarchus labrax). *Food Analytical Methods, 11*, 292-301.
		Quantification of proteins that influence meet quality	Gallego, M., Mora, L., Aristoy, M. C., & Toldrá, F. (2016). The use of label-free mass spectrometry for relative quantification of sarcoplasmic proteins during the processing of dry-cured ham. *Food Chemistry, 196*, 437-444.
	MS/MS spectral counting	Authentication of processed meet products	Montowska, M., & Fornal, E. (2017). Label-free quantification of meat proteins for evaluation of species composition of processed meat products. *Food Chemistry, 237*, 1092-1100.
Metabolic labeling based relative quant.	SILAC	Studying of mechanisms of resveratrol action in cell	Alayev, A., Doubleday, P. F., Berger, S. M., Ballif, B. A., & Holz, M. K. (2014). Phosphoproteomics Reveals Resveratrol-Dependent Inhibition of Akt/mTORC1/S6K1 Signaling. *Journal of Proteome Research, 13*, 5734-5742.
	In vivo SILAC (SILAM)	Studying interaction between host and microbiome important for pre- or prebiotic treatment	Oberbach, A., Haange, S. B., Schlichting, N., Heinrich, M., Lehmann, S., Till, H., Hugenholtz, F., Kullnick, Y., Smidt, H., Frank, K., Seifert, J., Jehmlich, N., & von Bergen, M. (2017). Metabolic in Vivo Labeling Highlights Differences of Metabolically Active Microbes from the Mucosal Gastrointestinal Microbiome between High-Fat and Normal Chow Diet. *Journal of Proteome Research, 16*, 1593-1604.
Chemical labeling based relative quant.	TMT / iTRAQ	Quantification of changes in proteome during fruiting process in *F.velutipes*	Liu, J. Y., Chang, M. C., Meng, J. L., Feng, C. P., Zhao, H., & Zhang, M. L. (2017). Comparative Proteome Reveals Metabolic Changes during the Fruiting Process in Flammulina velutipes. *Journal of Agricultural and Food Chemistry, 65*, 5091-5100.
	Dimethyl labeling	Characterization of muscle tissue from farmed and wild fish	Piovesana, S., Capriotti, A. L., Caruso, G., Cavaliere, C., La Barbera, G., Chiozzi, R. Z. & Lagana, A. (2016). Labeling and label free shotgun proteomics approaches to characterize muscle tissue from farmed and wild gilthead sea bream (Sparus aurata). *Journal of Chromatography A, 1428*, 193-201.
Enzym. labeling based	Proteolytic labeling with ^{18}O	Quantification of bovine and porcine gelatin	Sha, X. M., Tu, Z. C., Wang, H., Huang, T., Duan, D. L., He, N., Li, D. J., & Xiao, H. (2014). Gelatin Quantification by Oxygen-18 Labeling and Liquid Chromatography–High-Resolution Mass Spectrometry.

relative quant.			*Journal of Agricultural and Food Chemistry, 62*, 11840–11853.
Absolute quant.	AQUA	Quantification of Pru av 2 allergen in sweet cherry and other food	Ippoushi, K., Sasanuma, M., Oike, H., Kobori, M., & Maeda Yamamoto, M. (2016). Absolute quantification of Pru av 2 in sweet cherry fruit by liquid chromatography/tandem mass spectrometry with the use of a stable isotope-labelled peptide. *Food Chemistry, 204*, 129-134.
		Microfluidic – MS system for quantification of peanut allergens in complex food matrices	Sayers, R. L., Gethings, L. A., Lee, V., Balasundaram, A., Johnson, P. E., Marsh, J. A., Wallace, A., Brown, H., Rogers, A., Langridge, J. I., & Mills, E. N. C. (2018). Microfluidic separation coupled to mass spectrometry for quantification of peanut allergens in a complex food matrix. *Journal of Proteome Research, 17*, 647-655.
	QconCAT	Quantification of proteins in spore coat of food contaminant *B.cereus*	Stelder, S. K., Benito de Moya, C., Hoefsloot, H. C. J., de Koning, L. J., Brul, S., & de Koster, C. G. (2018). Stoichiometry, Absolute Abundance, and Localization of Proteins in the Bacillus cereus Spore Coat Insoluble Fraction Determined Using a QconCAT Approach. *Journal of Proteome Research, 17*, 903-917.
	PSAQ	Quantification of milk allergens in baked food samples	Newsome, G. A., & Scholl, P. F. (2013). Quantification of Allergenic Bovine Milk αS1-Casein in Baked Goods Using an Intact 15N-Labeled Protein Internal Standard. *Journal of Agricultural and Food Chemistry, 61*, 5659-5668.

Table 2.
Some examples of physical and chemical changes, introduced during food processing, food storage and sample preparation, of particular concern for MS based proteomic identification and quantification of food proteins or their proteoforms

- denaturation
- aggregation
- reduction of protein solubility in water due to structural changes of food matrix
- physical separation and removal of proteins
- unspecific and partial hydrolysis
- partial deglycosylation
- phosphorylation and dephosphorylation
- degradation of other PTMs
- activation or inactivation of enzymes
- reduction of disulfide bonds, or their formation
- formylation, methylation, acetylation (N-terminal amino acid, Lys)
- chemical reactions between proteins and different constituents of food matrix
  - modification of proteins (on: Cys, His, Lys, Met, Phe, Trp, Tyr) with reactive molecular species (reactive oxygen species, reactive nitrogen species, reactive carbonyl species, reactive sulphur species)
    - carbonylation (Arg, Lys, Pro, Thr)
    - oxidation (most frequently of Met, Cys, Phe, His, Pro, Trp, Tyr)
    - hydroxylation (Val, Phe, Trp, Leu)
    - nitration (Trp, Phe, His, Tyr)
    - nitrozylation (Tyr,)
  - modification of proteins (on: Cys, His, Lys, Arg, Gln, Asn) by lipoxidation products
  - glycation of proteins (usually on Lys) with reducing sugars (Maillard reaction)
  - formation of acrylamide from Asn and its subsequent interaction with proteins
- crosslinking (oligomerization and polymerization)
- isomerization and racemization (Asp->isoAsp, L-Pro->D-Pro and other amino acids)
- degradation of amino acids (most frequently deamidation of Asn->Asp and Gln-> Glu)
- carbamylation by urea (N-terminal amino acid)
- formation of dehydro and cross-linked amino acids such as dehydroalanine, methyldehydroalanine, beta-aminoalanine, lysinoalanine, ornithinoalanine, histidinoalanine, phenylethylaminoalanine, lanthionine, and methyl-lanthionine

Table. 3.
Different data independent acquisition (DIA) setups

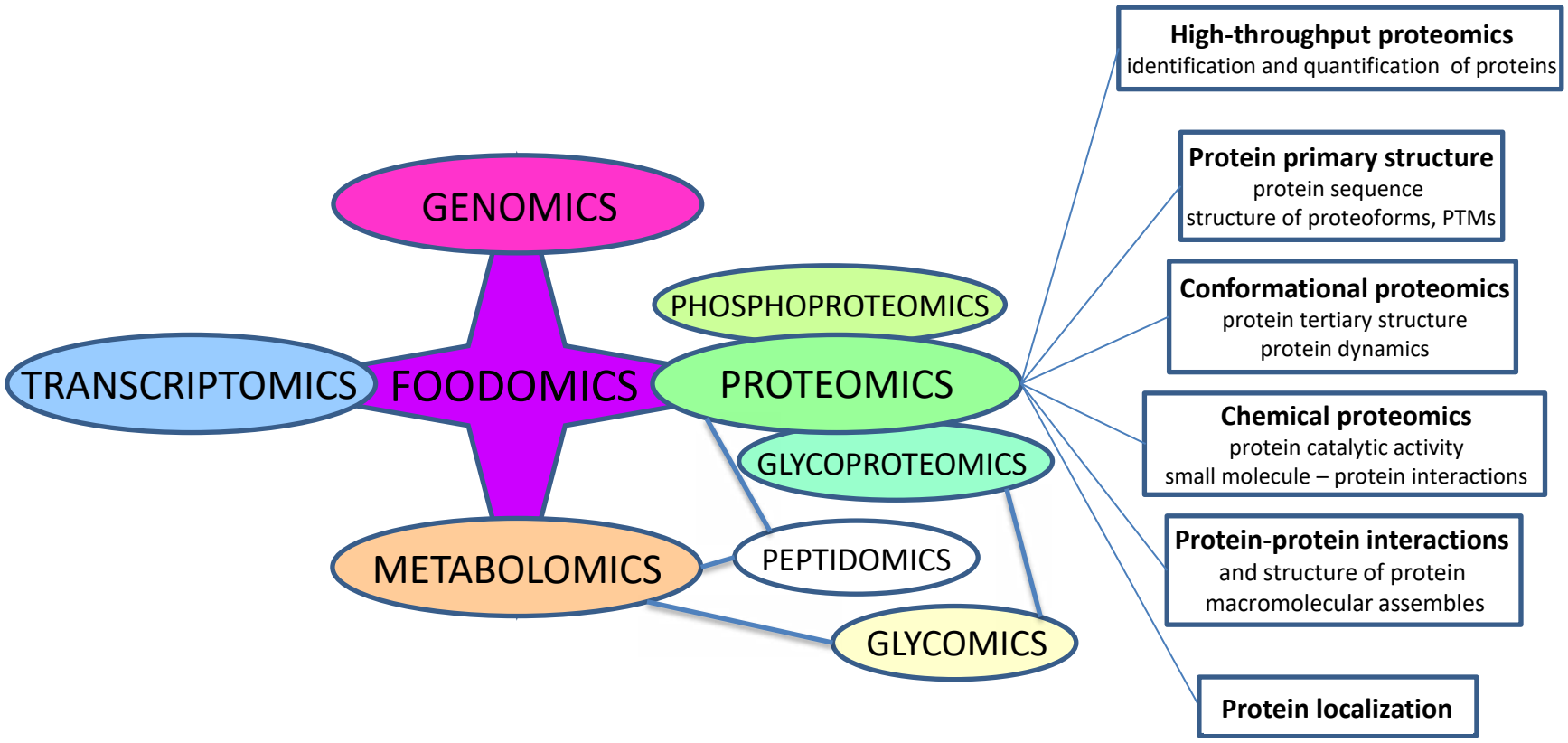
DIA setup	MS system	Reference
Multiplexed MS/MS	ESI-FT-ICR	Masselon, C., Anderson, G. A., Harkewicz, R., Bruce, J. E., Paša-Tolić, L., & Smith, R. D. (2000). Accurate Mass Multiplexed Tandem Mass Spectrometry for High-Throughput Polypeptide Identification from Mixtures. *Analytical Chemistry*, 72, 1918-1924.
Shotgun CID *(Shotgun collision-induced dissociation)*	µLC-µESI-TOF µLC-µESI - QIT µLC-µESI - QqTOF	Purvine, S., Eppel, J. T., Yi, E. C., & Goodlett, D. R. (2003). Shotgun collision-induced dissociation of peptides using a time of flight mass analyzer. *Proteomics*, 3, 847-850.
Original DIA	µLC-µESI - LTQ	Venable, J. D., Dong, M. Q., Wohlschlegel, J., Dillin, A., & Yates III, J. R. (2004). Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra. *Nature Methods*, 1, 39-45.
MS^E	µLC-µESI-High resolution QqTOF	Silva, J. C., Denny, R., Dorschel, C. A., Gorenstein, M., Kass, I. J., Li, G. Z., McKenna, T., Nold, M. J., Richardson, K., Young, P., & Geromanos, S. (2005). Quantitative proteomic analysis by accurate mass retention time pairs. *Analytical chemistry*, 77, 2187–2200.
p^2CID *(parallel collision-induced-dissociation)*	ESI-QqTOF	Ramos, A. A., Yang, H., Rosen, L. E., & Yao, X. D. (2006). Tandem parallel fragmentation of peptides for mass spectrometry. *Analytical Chemistry*, 78, 6391–6397.
AIF *(All ion fragmentation)*	nLC-nESI-Q-OT (Exactive)	Geiger, T., Cox, J., & Mann, M. (2010). Proteomics on an Orbitrap benchtop mass spectrometer using all-ion fragmentation. *Molecular & Cellular Proteomics*, 9, 2252–2261.
XDIA	ESI-LTQ-OT	Carvalho, P. C., Han, X., Xu, T., Cociorva, D., da Gloria Carvalho, M., Barbosa, V. C., & Yates, III, J. R. (2010). XDIA: improving on the label-free data-independent analysis. *Bioinformatics*, 26, 847–848.
PaCIFIC *(Precursor acquisition independent from ion count)*	nLC-nESI-LTQ nLC-nESI-LTQ-OT (XL)	Panchaud, A., Jung, S., Shaffer, S. A., Aitchison, J. D., & Goodlett, D. R. (2011). Faster, quantitative, and accurate precursor acquisition independent from ion count. *Analytical chemistry*, 83, 2250–2257.
MXS *(multiplexing strategy DIA)*	nLC-nESI-Q-OT (Exactive)	Egertson, J. E., Kuehn, A., Merrihew, G. E., Bateman, N. W., MacLean, B. X., Ting, Y. S., Canterbury, J. D., Marsh, D. M., Kellmann, M., Zabrouskov, V., Wu, C. C., & MacCoss, M. J. (2011). Multiplexed MS/MS for improved data-independent acquisition. *Nature Methods*, 10, 744-746.

FT-ARM *(Fourier transformation all ion monitoring)*	nLC-nESI-LTQ-OT nLC-nESI-LTQ-FTICR	Weisbrod, C. R., Eng, J. K., Hoopmann, M. R., Baker, T., & Bruce, J. E. (2012). Accurate peptide fragment mass analysis: Multiplexed peptide identification and quantification. *Journal of Proteome Research*, 11, 1621-1632.
SWATH-MS *(sequential windowed acquisition of all theoretical fragment ion mass spectra)*	nLC-ESI-High resolution QqTOF	Gillet, L. C., Navarro, P., Tate, S., Rost, H., Selevsek, N., Reiter, L., Ron Bonner, R., & Aebersold, R. (2012). Targeted Data Extraction of the MS/MS Spectra Generated by Data-independent Acquisition: A New Concept for Consistent and Accurate Proteome Analysis. *Molecular & Cellular Proteomics*, doi: 10.1074/mcp.O111.016717.
HDMS^E *(High definition MS^E)*	nLC-nESI-Q-TWIMS-TOF	Shliaha, P. V., Bond, N. J., Gatto, L., & Lilley, K. S. (2013). Effects of Traveling Wave Ion Mobility Separation on Data Independent Acquisition in Proteomics Studies. *Journal of Proteome Research*, 12, 2323-2339.
UDMS^E *(Ultra-high definition MS^E)*	nLC-nESI-Q-TWIMS-TOF	Distler, U., Kuharev, J., Navarro, P., Levin, Y., Schild, H., & Tenzer, S. (2014). Drift time-specific collision energies enable deep-coverage data-independent acquisition proteomics. *Nature Methods*, 11, 167-170.
WiSIMDIA *(wide selected-ion monitoring DIA)*	nLC-nESI-Q-LIT-OT (Fusion, Fusion Lumos)	Kiyonami, R., Patel, B., Senko, M., Zabrouskov, V., Egertson, J., Ting, S., MacCoss, M., Rogers, J., & Hühmer, A. F. R. (2014). Large Scale Targeted Protein Quantification Using WiSIM-DIA on an Orbitrap Fusion Tribrid Mass Spectrometer. Thermo Scientific Application Note 600.
CSI PaCIFIC *(captive spray ionization PaCIFIC)*	nLC-nESI-CSI-LTQ-OT	Chapman, J. D., Edgar, J. S., Goodlett, D. R., & Ah Goo, Y. (2016). Use of captive spray ionization to increase throughput of the data-independent acquisition technique PAcIFIC. *Rapid Communications in Mass Spectrometry*, 30, 1101-1107.

Table 4. MS-based proteomic approaches and strategies for quantification of individual proteins and proteome.

Relative quantification	LFQ (Label free quantification)		MS scan level	Area under the curve	Ahrné, E., Molzahn, L., Glatter, T., & Schmidt, A. (2013). Critical assessment of proteome-wide label-free absolute abundance estimation strategies. *Proteomics, 13*, 2567-2578. Neilson, K. A., Ali, N. A., Muralidharan, S., Mirzaei, M., Mariani, M., Assadourian, G., Lee, A., van Sluyter, S. C., & Haynes, P. A. (2011). Less label, more free: approaches in label-free quantitative mass spectrometry. *Proteomics, 11*, 535-553. Dowle, A., A., Wilson, J., & Thomas, J. R. (2016). Comparing the Diagnostic Classification Accuracy of iTRAQ, Peak-Area, Spectral-Counting, and emPAI Methods for Relative Quantification in Expression Proteomics. *Journal of Proteome Research, 15*, 3550-3562. Arike, L., & Peil, L. (2014). Spectral Counting Label-Free Proteomics. *Methods in Molecular Biology, 1156*, 213-222.
				Signal intensity measurement	
			MS/MS scan level	Spectral counting	
	Label-based quant.	Metabolic	SILAC (Stable isotope labeling with amino acids in cell culture)		Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular & Cellular Proteomics, 1*, 376-386. Mann, M. (2014). Fifteen Years of Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC). *Methods in Molecular Biology, 1188*, 1-7.
			In vivo SILAC (SILAM)		Krüger, M., Moser, M., Ussar, S., Thievessen, I., Luber, C. A., Forner, F., Schmidt, S., Zanivan, S., Fässler, R., & Mann, M. (2008). SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell, 134*, 353-364. Zanivan, S., Meves, A., Behrendt, K., Schoof, E. M., Neilson, L. J., Cox, J., Tang, H. R., Kalna, G., van Ree, J. H., van Deursen, J. M., Trempus, C. S., Machesky, L. M., Linding, R., Wickström, S. A., Fässler, R., & Mann, M. (2013). In Vivo SILAC-Based Proteomics Reveals Phosphoproteome Changes during Mouse Skin Carcinogenesis. *Cell Reports, 3*, 552-566.
			Plant SILAC		Lewandowska, D., ten Have, S., Hodge, K., Tillemans, V., Lamond, A. I., & Brown, J. W. S. (2013). Plant SILAC: Stable-Isotope Labelling with Amino Acids of Arabidopsis Seedlings for Quantitative Proteomics. *PLOS One, doi: 10.1371/journal.pone.0072207* Matthes, A., Köhl, K., & Schulze, W. X. (2014). SILAC and Alternatives in Studying Cellular Proteomes of Plants. *Methods in Molecular Biology, 1188*, 65-83.
			Super SILAC		Geiger, T., Cox, J., Ostasiewicz, P., Wisniewski, J. R., & Mann, M. (2010). Super-SILACmix for quantitative proteomics of human tumor tissue. *Nature Methods, 7*, 383-385.

					Shenoy, A., & Geiger, T. (2015). Super-SILAC: current trends and future perspectives. *Expert Review of Proteomics, 12*, 13-19.
			Spike-in SILAC		Geiger, T., Wisniewski, J. R., Cox, J., Zanivan, S., Kruger, M., Ishihama, Y., & Mann, M. (2011). Use of stable isotope labeling by amino acids in cell culture as a spike-in standard in quantitative proteomics. *Nature Protocols, 6*, 147-157.
		Chemical	Isobaric	TMT / iTRAQ	Christoforou, A., & Lilley, K. S. (2011). Taming the isobaric tagging elephant in the room in quantitative proteomics. *Nature Methods, 8*, 911-913.
				Other	Rauniyar, N., & YatesIII, J. R. (2014). Isobaric Labeling-Based Relative Quantification in Shotgun Proteomics. *Journal of Proteome Research, 13*, 5293-5309.
			Non-isobaric	Dimethyl labeling	Boersema, P. J., Raijmakers, R., Lemeer, S., Mohammed, S., & Heck, A. J. R. (2009). Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics. *Nature Protocols, 4*, 484-494. Lau, H. T., Suh, H. W., Golkowski, M., & Ong, S. E. (2014). Comparing SILAC- and Stable Isotope Dimethyl-Labeling Approaches for Quantitative Proteomics. *Journal of Proteome Research, 13*, 4164-4174.
				mTRAQ	DeSouza, L. V., Taylor, A. M., Li, W., Minkoff, M. S., Romaschin, A. D., Colgan, T. J., & Siu, K. W. (2008). Multiple Reaction Monitoring of mTRAQ-Labeled Peptides Enables Absolute Quantification of Endogenous Levels of a Potential Cancer Marker in Cancerous and Normal Endometrial Tissues. *Journal of Proteome Research, 7*, 3525-3534. Oppermann, F. S., Klammer, M., Bobe, C., Cox, J., Schaab, C., Tebbe, A., & Daub, H. (2013). Comparison of SILAC and mTRAQ Quantification for Phosphoproteomics on a Quadrupole Orbitrap Mass Spectrometer. *Journal of Proteome Research, 12*, 4089-4100.
		Enzymatic	Proteolytic labeling with ^{18}O		Hajkova, D., Sekhar Rao, K. C., & Miyagi, M. (2011). Recent Technological Developments in Proteolytic ^{18}O Labeling. *Current Proteomics, 8*, 39-46.
Absolute quantification	Spike-in of isotope-labeled standards		AQUA (Absolute quantification)		Gerber, S. A., Rush, J., Stemman, O., Kirschner, M. W., & Gygi, S. P. (2003). Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proceedings of the National Academy of Sciences, 100*, 6940-6945.
			QconCAT (Quantification concata-mer)		Beynon, R. J., Doherty, M. K., Pratt, J. M., & Gaskell, S. J. (2005). Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nature Methods, 2*, 587-589.
			PSAQ (Protein standard for absolute quantification)		Brun, V., Dupuis, A., Adrait, A., Marcellin, M., Thomas, D., Court, M., Vandenesch, F., & Garin, J. (2007). Isotope-labeled protein standards: toward absolute quantitative proteomics. *Molecular & Cellular Proteomics, 6*, 2139-2149.


GENOMICS
TRANSCRIPTOMICS
FOODOMICS
PHOSPHOPROTEOMICS
PROTEOMICS
GLYCOPROTEOMICS
METABOLOMICS
PEPTIDOMICS
GLYCOMICS
High-throughput proteomics
identification and quantification of proteins
Protein primary structure
protein sequence
structure of proteoforms, PTMs
Conformational proteomics
protein tertiary structure
protein dynamics
Chemical proteomics
protein catalytic activity
small molecule – protein interactions
Protein-protein interactions
and structure of protein
macromolecular assembles
Protein localization

MATERIAL FOR ANALYSIS (Cells, tissue, fluid)

Sampling and sample handling:
- Biopsy
- Laser-capture microdissection
- Cell sorting (FACS)
- Gradient centrifugation
- Free-flow electrophoresis

SAMPLE

Homogenization, protein extraction and/or solubilisation

PROTEIN EXTRACT

Protein fractionation

Gel-based proteomics *

- Native PAGE (polyacrylamide gel electrophoresis)
- SDS-PAGE (Sodium dodecylsuplhate)
- Isoelectric focusing (IEF)
- ALS-PAGE (Acid labile surfactant)
- 2D-PAGE

Electroelution

Individual gel spots or lines containing proteins

Liquid chormatography (LC)
- Size exclusion (SEC)
- Strong cation exchange (SCX)
- Anion exchange (AXC)
- Hydrophobic interaction
- Hydrophilic interaction (HILIC)
- Reversed phase (RP)
- Immobilized metal affinity (IMAC)

Capillary electrophoresis (CE)

Capillary isoelectricfocusing (CIEF)

Proteins separated to fractions

Protein depletion

- Immunoprecipitation
- Affinity depletion
- Combinatorial peptide ligand libraries (CPLL)

Protein enrichment

- Affinity chromatogrphy (AC)
- Affinity capture
- Chemical capture

Low-abundant proteins

Glycoproteins

Selected proteins

BOTTOM-UP PROTEOMICS

Proteolysis:
Trypsin or alternative specific proteases or chemical

Deglycosylated Proteins

PEPTIDES

PHOSPHOPROTEOMICS

Phosphopeptide enrichment:
TiO_2 / ZrO_2
IMAC, Indium tin-oxide,
Hydroxyapatite chromatogr.

PHOSPHOPEPTIDES

Enrichment:
Affinity capture
COFRADIC

SELECTED PEPTIDES

GLYCOPROTEOMICS

Enrichment:
Affinity capture
Chemical capture

GLYCOPEPTIDES

GLYCOMICS

Release of glycans:
Enzymatic
Chemical

GLYCANS

Modification :
Chemical
Enzymatic

Modif. GLYCANS

TOP-DOWN PROTEOMICS

Peptide fractionation:
1D or 2D LC (RP, SCX, AXC, HILIC, IMAC), CE

Glycan fractionation:
LC (PGC, HILIC), CE

Mass spectrometry

Ionization (ESI, nanoESI, Native ESI, MALDI)
MS scan (Q, QIT, LIT, TOF, OT, FT-ICR)
Fragmentation of ions (CID, HCD, ETD, ECD, EThcD, IRMPD, UVPD, PSD, ISD)
MS/MS scan (QqQ, QIT, LIT, QqTOF, TOF-TOF, Q-OT, LIT-OT, Q-FTICR, LIT-FTICR) or MS^n scan (QIT, LIT, LIT-OT, LIT-FTICR, FTICR)

Ultra-high resolution mass analysers
(FTICR, OT)

Data analysis

Peptide identification (spectrum centric, peptide centric, manual *de novo* sequencing)
Protein and proteoform identification
Quantification

Data analysis

Glycan identification
Quantification

Data analysis

Proteoform identification
Quantification

P E P T I D E S
(SAMPLE DIGESTED WITH PROTEASE)

ELECTROSPRAY IONIZATION

MASS ANALYSERS

QIT, LIT, QqTOF, Q-OT, LIT-OT, Q-LIT-OT, Q-FTICR, LIT-FTICR | QqQ

DATA DEPENDENT ACQUISITION (DDA)

Top “n”
(n ≤ 20)

DATA INDEPENDENT ACQUISITION (DIA)

Different DIA setups are listed in Table 2

TARGETED DATA ACQUISITION

SRM / MRM, PRM

RAW DATA

RAW DATA

RAW DATA

UNTARGETED DATA EXTRACTION
SPECTRUM CENTIRC MATCHING APPROACH

TARGETED DATA EXTRACTION
PEPTIDE CENTRIC MATCHING APPROACH

IDENTIFIED and QUANTIFIED PEPTIDES

IDENTIFIED and QUANTIFIED PROTEINS

IDENTIFIED and QUANTIFIED PEPTIDES

IDENTIFIED and QUANTIFIED PROTEINS

Highlights:

Mass spectrometry based proteomics, as one of the four main sources of data in foodomics, are presented.

MS-based proteomic approaches applicable in food research, quality and safety control are described.

Improvements in sample preparation and in the technology of mass spectrometers are presented.

Critical points for application of MS-based proteomics in food analysis are described.

Continuously growing capabilities of MS-based proteomics and future directions are discussed.