Send Orders for Reprints to reprints@benthamscience.net

Protein & Peptide Letters, 2022, *29*, 379-383

PERSPECTIVE

Recent Advances in Utilizing Omics Approach to Identify the Bioactive Peptides and Ripening Metabolism in Plant-based Food

Kah Yaw Ee[1,2,*], Ming Quan Lam[2,3] and Chun Shiong Chong[4]

[1]*Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia;* [2]*Centre for Agriculture and Food Research, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia;* [3]*Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia;* [4]*Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*

ARTICLE HISTORY

Received: January 20, 2022
Revised: February 07, 2022
Accepted: February 13, 2022

DOI:
10.2174/0929866529666220328125151

Abstract: Bioactive peptides with potential health benefits and metabolic functionality have been identified from plant-based food. The aim of this perspective is to report the recent progress in the research of plant-derived bioactive peptides using the combination of omics technologies and bioinformatics tools. Studies examining bioactive peptides with identified amino acid sequences and well-characterized biological functionalities are highlighted. Various software, webtools and workflows for analyzing and interpreting the biological data acquired from different omics approaches are discussed. The emerging evidence from the integration of proteomics and metabolomics data with advanced laboratory analytical methods supports more potential applications in the envisioned development of nutraceutical and therapeutic products. Notwithstanding, much works are mandatory to resolve those lied-ahead challenges before realizing the proposed applications of plant peptides.

Keywords: Omics, bioinformatics, bioactive peptides, fruit ripening, metabolism, plant-based food.

1. INTRODUCTION

Plant-based food has been actively investigated as source of protein and bioactive peptides. The conventional hydrolysis of proteins can be carried out using chemical, enzymatic, or fermentation methods to produce functional peptides, containing 2-20 amino acid residues, which are known as bioactive peptides. The degree of hydrolysis affects the overall size, structural configuration, and amino acid composition of the peptides. These bioactive peptides possess different health promoting benefits, such as immunomodulatory, antihypertensive, antioxidant, antiproliferative, hypocholesterolemic, metal chelating and anti-inflammatory activities. Several factors, such as different proteases, duration of hydrolysis, enzyme-to-substrate (E/S) ratio, and types of protein substrates, have an impact on the degree of hydrolysis and the emergence of these biological activities [1]. To identify bioactive peptides, many approaches have been utilized previously, such as conventional extraction, fractionation and purification. However, these approaches have many shortcomings, such as limited quantities of bioactive peptides being purified and the precise identity of these bioactive peptides is not known.

*Address correspondence to this author at the Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia;
Tel.: +60 5 468 8888 (Ext. 4503); E-mail: eeky@utar.edu.my

To investigate bioactive peptides systematically, the integration of omics technologies and bioinformatics tools has been paid considerable attention. In recent years, the unveiling of omics based information provides new insights into functional potential, mechanism of action, metabolism, and safety of specific biological functioning proteins and peptides [2]. This information assists the future development process of nutraceutical products and avoids the occurrence of toxicity. Therefore, these combination approaches enhance the quality and functionality of bioactive peptides.

Recent advances in high-throughput omics technologies bring new opportunities and challenges for the precise integration of bioinformatics data with cutting-edge analytical methods. The omics approaches are powerful tools in determining biological data at the molecular level. They are generally categorized into genomics, transcriptomics, proteomics, and metabolomics based on analysis of total DNA, expressed RNA, transcripts, proteins, and metabolites, respectively [2, 3]. Due to their extensive applications in different fields, a surge in multi-omics disciplines, such as foodomics, nutrigenomics, and lipidomics, is gaining large momentum [4]. The use of omics technology in the discovery of bioactive peptides has accelerated the advancement of skills and knowledge into a broader perspective. For instance, reversed-phase liquid chromatography mass spectrometry (RPLC-MS) acts as a golden standard in proteomics and metabolomics. For proteomics, the use of hydrophilic interaction chromatography (HILIC) or electrostatic repulsion

1875-5305/22 $65.00+.00 © 2022 Bentham Science Publishers

Protein & Peptide Letters

Table 1. Identification of bioactive peptides using various omics approaches.

Source	Peptide(s)	Bioactivity	Omics Approach	Refs.
Distilled spent grain	PR	ACE inhibition	Proteomics	[8]
Zein	PP, PPP, LPP, HL, PPPVHL, FY, GI; PP, LP, PV, HL, VH, PF, PL, PPPP, MP; PPPV, VHLP, PPPVHL, LPPPV; HLPPPV, LPPPVH; HL, PW, LLPF, MM; IL, LL, VL, LI; YP, VW	ACE inhibition; DPP-IV inhibition; Anti-amnestic activity; Antioxidant activity; Glucose uptake stimulation; α-glucosidase inhibition	Proteomics	[9]
Salacca zalacca fruits	SZ1, SZ2, SZ3, SZ4	α-glucosidase inhibition; Ferric reducing antioxidant power	Metabolomics	[11]

hydrophilic interaction chromatography coupled with mass spectrometry (ERLIC-MS) to characterize complex proteomes has been explored at peptide and protein levels, revealing the unique separation and orthogonality of HILIC compared with RPLC. HILIC resolves peptides or proteins with the same amino acid composition but having different post-translational modifications (PTMs), such as acetylation, glycosylation, phosphorylation, deamidation and methylation. For example, the application of UHPLC-HILIC-MS/MS method to quantify 40 endogenous metabolites of amino acids and their derivatives in cell lysates within 10 min [5]. Metabolomics approaches are used to discover the organism's metabolism before and after the intervention of functional compounds, identify effective metabolites/biomarkers, and predict the interventional effects and mechanism. For metabolomics, the use of HILIC-MS shows efficacy in the analysis, identification, and quantitation of more polar bioactive compounds. However, it will not be efficient in resolving the complexity of the proteome without the integration of bioinformatics.

To cope with the numerous data generated from omics technologies, bioinformatics plays a crucial role in this term. Various software, webtools and workflows were developed to filter, process, analyze, and interpret the biological data acquired from different omics approaches [2]. For instance, Mascot is a popular and robust software search engine that is widely utilized in identifying proteins from peptide sequence databases based on MS data [6]. Similarly, a more recent software, ACD/Spec Manager v.12.00 can serve for a similar functions to process MS data either manually or automate routine pipelines [5]. Apart from that, bioinformatics tools such as AutoDock, Vina, GOLD, and MOE-Dock also have been developed to reveal the interactions between two biomolecules, especially between inhibitors and proteins, which are termed molecular docking [7]. Furthermore, to investigate the physiological changes and metabolism in plant-based food, comparative studies using different omics approaches are mandatory. This has involved higher computing power and more sophisticated bioinformatics tools compared to a single set of omics data alone. Thus, it is important to address the role of bioinformatics while discussing omics approach in food science.

Due to the advances in technology, many recent research articles addressed the use of omics approaches combined with sophisticated bioinformatics tools, especially proteomics and metabolomics, individually or in combination in identifying bioactive peptides (Table **1**). Recently, a new bioassay-guided proteomics, *de novo* Biolynx peptide sequencer based on ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS), and electro-spray ionization with triple-quadrupole mass spectrometry (ESI-QQQ-MS) were applied in identifying 22 peptides with angiotensin converting enzyme (ACE) inhibitory or anti-ACE activities from distilled spent grain. PR (272 Da) was determined as the most abundant (92.14 $\mu g\ g^{-1}$ dry weight) anti-ACE (IC_{50} 50 mmol/L) peptide using molecular docking simulation [8]. *In silico* proteolytic digestion together with nano-LC-MS/MS analysis on prolamine proteins revealed 420 peptides originating from 71 proteins, of which 116 were predicted to be bioactive peptides. Some of them possessed more than one functionality, including anti-ACE (PP, PPP, LPP, HL, PPPVHL, FY, GI), anti-DPP IV (PP, LP, PV, HL, VH, PF, PL, PPPP, MP), anti-amnestic (PPPV, VHLP, PPPVHL, LPPPV, HLPPPV, LPPPVH), antioxidant (HL, PW, LLPF, MM), anti-α-glucosidase (YP, VW), and glucose uptake stimulation (IL, LL, VL, LI) activities [9]. On the other hand, applying conventional procedures on *Zizyphus jujuba* fruit protein fractions [10] revealed only two peptides, IER (IC_{50} 0.060 mg/mL) and IGK (0.072 mg/mL), with potent ACE inhibition. This further indicates that the utilization of omics technology can facilitate the exploration and study of bioactive peptides in terms of quantity and functionality within a short period of time. In a study of metabolite profiling of *S. zalacca* fruit flesh using LC-QTOF-MS/MS, 4 bioactive peptides, SZ1, SZ2, SZ3 and SZ4, were identified [11]. Their molecular interactions were predicted using *in silico* docking simulation method to elucidate the possible mechanisms of α-glucosidase inhibition and ferric reducing antioxidant power. The hydrophobic and hydrogen bonding interactive sites for both functions, such as THR274, SER298, ILE272, GLN239, LYS156, ASP242, ASN415, HIE280, ARG315 and GLY309, were disclosed.

Furthermore, omics approaches have been currently applied to investigate physiological changes in fruits at different growing stages (Table **2**). These studies aimed to

Table 2. Peptides, proteins and metabolites detected during the ripening metabolism using various omics approaches.

Source	Protein/Peptide/Metabolite	Metabolism	Omics Approach	Refs.
Exogeneous methyl jasmonate (MeJA) treated tea leaves	337 (12h), 246 (24h), 413 (48h) DEPs; 266 nonvolatile and 100 volatile metabolites	Aroma biosynthesis and catabolism	Proteomics and metabolomics	[12]
Peach fruit	1663 DEPs	Cell wall and sugar metabolism; Aroma and color changes; Glycogen and isocitrate metabolism; Protein localization	Transcriptomics and proteomics	[13]
Lycium barbarum, *Lycium ruthenicum* fruits	DEPs	Chromoplast-biogenesis; Plastoglobules localization; Anthocyanin synthesis and accumulation	Genomics and transcriptomics	[14]
Tomato fruit	145 DEPs	Cell wall metabolism; Vesicle-mediated transport; Hormone biosynthesis; Secondary metabolism; Lipid metabolism; Protein synthesis and degradation; Carbohydrate metabolic processes; Signaling and response to stress	Transcriptomics and proteomics	[15]
Tomato fruit	70 cys-containing peptides 13 redox-sensitive proteins	Cell wall degradation; Ethylene biosynthesis	Proteomics	[16]
Mangosteen	277 DEPs	Ethylene biosynthesis; Carbohydrate metabolism; Cell wall modification; Secondary metabolite biosynthesis	Proteomics	[17]
Prunella vulgaris L.	1910 DEPs	Stress-responsive; Chlorophyll degradation; Inhibition of chlorophyll biosynthesis; Increased abundance of transketolase; Tricarboxylic acid cycle; Phenylpropanoid biosynthesis and metabolism	Proteomics	[18]

Abbreviation: DEP, differentially expressed proteins.

monitor the postharvest ripening, preservation of fresh produces and nutritional values. For example, iTRAQ-based proteomics analysis was performed [12] to identify proteins that expressed differently and were involved in the physiological changes of tea leaves during the priming process with exogenous methyl jasmonate (MeJA). It was followed by MS-based metabolomics analysis to identify different metabolites that assisted the understanding of tea aroma biosynthesis and catabolism. In terms of tea leaf quality improvement, these integrated proteomic and metabolomic techniques can be applied to further investigate bioactive peptides in different processing steps. With the aid of LC/MS-MS, 1663 differentially expressed proteins (DEPs) were identified from 2740 proteins (131435 spectra) of peach fruit [13]. Transcriptomics approaches elucidated the 26% gene codes for proteins that were highly expressed in ripe fruit. Quantitative results showed 15% of proteins involved in glycogen and isocitrate metabolism, protein localization in mature fruit, and cell wall modification in ripe fruit. These data could be potentially used in developing methods to monitor the shelf life of fresh produces during storage and transportation. Similarly, a study [14] reported that the increase of DEP is related to anthocyanin synthesis and accumulation in the ripening of *Lycium ruthenicum* fruit. High number of photosynthesis-related proteins [IIL1 (HG16024.t1, UGT73B3 (HG12463.t1), ZDS (HG26423.t1), DXR (HG24457.t1), MECPS (HG26684.t1), CYCB2 (HG22121.t1), ZISO (HG08646.t1)] in *Lycium barbarum* significantly discriminated both of them in terms of nutritional quality and antioxidant compound production.

Another study [15] integrated proteomics and transcriptomics data from the membrane proteome of tomato fruit pericarp during ripening. Several software and databases have served different purposes, for example, homology-based comparisons with TAIR10 protein database, prediction of an N-terminal signal peptide for translocation into the endoplasmic reticulum by SignalP, expanding annotation data from Gene Ontology and

Reactome pathways, obtaining information on annotated genes from TOMATOMICS database, and gaining information on pathways and biochemical reactions from SolCyc database. Abundances of DEPs (145) were identified from 1315 proteins. They are involved in the process of fruit ripening, such as cell wall metabolism, vesicle-mediated transport, hormone biosynthesis, secondary metabolism, lipid metabolism, protein synthesis and degradation, carbohydrate metabolic processes, signaling and response to stress. A study [16] reported 70 cysteine-containing peptides that were released from 51 proteins of tomato fruit as redox-sensitive sites of targeted enzymes in moderating the ripening process. The researchers applied iodoacetyl tandem mass tag (iodoTMT)-based redox proteomic techniques to elucidate molecular interactions between reactive oxygen species (ROS) and peptides in different metabolic pathways. The significant change of 13 redox-sensitive proteins could be used as biomarkers in cell wall degradation and ethylene biosynthesis. Moreover, a total of 3397 proteins were identified from mangosteen fruit using Sequential Windowed Acquisition of Theoretical-Mass Spectra (SWATH-MS) analysis [17]. Furthermore, 277 DEPs were determined during the ripening process and associated with ethylene biosynthesis by 1-aminocyclopropane-1-carboxylate oxidase (ACO), carbohydrate metabolism by pyruvate kinase (PK), cell wall modification by polygalacturonase (PG) and secondary metabolite biosynthesis by phenylalanine ammonia-lyase (PAL). Although 5 benzophenone synthase (BPS) proteins were identified, they were not expressed differently. Further study is suggested to analyze the xanthone biosynthesis pathways. Similar proteomic techniques were also applied for studying the growing stages of traditional medicinal plants to understand the molecular mechanisms during maturation and to monitor the harvesting process [18]. A total of 1910 DEPs from 7655 proteins were identified with associations with energy synthesis and metabolism, post-translational modifications, and molecular chaperone functions. Specifically, the study discussed stress-responsive proteins in fruit ripening and seed development, degradation, and inhibition of chlorophyll, increased transketolase, tricarboxylic acid (TCA) cycle, phenylpropanoid biosynthesis and other metabolisms during ripening.

CONCLUSION

The combination of omics approach and bioinformatics brings new perspectives into bioactive peptides and fruit ripening metabolism research. However, challenges still lie ahead in developing a strong foundation for the predicted applications of these plant-based peptides in nutraceuticals. Producing reliable data is one of the major concerns, which depends on appropriate experimental designs, up-to-date databases, and bioinformtics software. Moreover, in-depth *in vivo* and clinical studies are still lacking to realize the absorption, distribution, metabolism, and excretion (ADME) profile of all bioactive peptides [19]. Specifically, the integration of various omics technologies should be considered as next generation research to construct a comprehensive understanding on the biological processes of nutraceuticals intervention on metabolic disorders [20]. Projections on the use of safer, more efficient, and sustainable analytical methods to produce high sensitivity, accuracy, and precision data can assist in establishing regulations on applying bioactive proteins or peptides in food products. In this way, it can assure the safety and well-being of the consumers.

AUTHORS' CONTRIBUTIONS

Kah Yaw Ee: Conceptualization, Formal analysis, Writing – original draft, review & editing; Ming Quan Lam: Conceptualization, Validation, Writing – review & editing; Chun Shiong Chong: Conceptualization, Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Chai, T-T.; Ee, K.Y.; Kumar, D.T.; Manan, F.A.; Wong, F-C. Plant bioactive peptides: Current status and prospects towards use on human health. *Protein Pept. Lett.,* **2021**, *28*(6), 623-642. http://dx.doi.org/10.2174/0929866527999201211195936 PMID: 33319654

[2] Prakash, B.; Singh, P.P.; Kumar, A.; Gupta, V. Prospects of omics technologies and bioinformatics approaches in food science. In: *Functional and Preservative Properties of Phytochemicals*; Elsevier, **2020**, pp. 317-340. http://dx.doi.org/10.1016/B978-0-12-818593-3.00010-5

[3] Barros-Velázquez, J. *Foodomics: Omic Strategies and Applications in Food Science*; Royal Society of Chemistry: London, UK, **2021**. http://dx.doi.org/10.1039/9781839163005

[4] Balkir, P.; Kemahlioglu, K.; Yucel, U. Foodomics: A new approach in food quality and safety. *Trends Food Sci. Technol.,* **2021**, *108*, 49-57. http://dx.doi.org/10.1016/j.tifs.2020.11.028

[5] Zhu, B.; Li, L.; Wei, H.; Zhou, W.; Zhou, W.; Li, F.; Lin, P.; Sheng, J.; Wang, Q.; Yan, C.; Cheng, Y. A simultaneously quantitative profiling method for 40 endogenous amino acids and derivatives in cell lines using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry. *Talanta,* **2020**, *207*, 120256. http://dx.doi.org/10.1016/j.talanta.2019.120256 PMID: 31594590

[6] Koenig, T.; Menze, B.H.; Kirchner, M.; Monigatti, F.; Parker, K.C.; Patterson, T.; Steen, J.J.; Hamprecht, F.A.; Steen, H. Robust prediction of the MASCOT score for an improved quality assessment in mass spectrometric proteomics. *J. Proteome Res.,* **2008**, *7*(9), 3708-3717. http://dx.doi.org/10.1021/pr700859x PMID: 18707158

[7] Pagadala, N.S.; Syed, K.; Tuszynski, J. Software for molecular docking: A review. *Biophys. Rev.,* **2017**, *9*(2), 91-102. http://dx.doi.org/10.1007/s12551-016-0247-1 PMID: 28510083

[8] Wei, D.; Fan, W-L.; Xu, Y. Identification of water-soluble peptides in distilled spent grain and its angiotensin converting enzyme (ACE) inhibitory activity based on UPLC-Q-TOF-MS and proteomics analysis. *Food Chem.,* **2021**, *353*, 129521. http://dx.doi.org/10.1016/j.foodchem.2021.129521 PMID: 33735773

[9] Li, M.; Zheng, H.; Lin, M.; Zhu, W.; Zhang, J. Characterization of the protein and peptide of excipient zein by the multi-enzyme digestion coupled with nano-LC-MS/MS. *Food Chem.,* **2020**, *321*, 126712. http://dx.doi.org/10.1016/j.foodchem.2020.126712 PMID: 32247179

[10] Memarpoor-Yazdi, M.; Zare-Zardini, H.; Mogharrab, N.; Navapour, L. Purification, characterization and mechanistic evaluation of angiotensin converting enzyme inhibitory peptides derived from *Zizyphus jujuba* fruit. *Sci. Rep.,* **2020**, *10*(1), 3976. http://dx.doi.org/10.1038/s41598-020-60972-w PMID: 32132600

[11] Saleh, M.S.; Siddiqui, M.J.; Alshwyeh, H.A.; Al-Mekhlafi, N.A.; Mediani, A.; Ibrahim, Z.; Ismail, N.H.; Kamisah, Y. Metabolomics-based profiling with chemometric approach to identify bioactive compounds in *Salacca zalacca* fruits extracts and *in silico* molecular docking. *Arab. J. Chem.,* **2021**, *14*(4), 103038. http://dx.doi.org/10.1016/j.arabjc.2021.103038

[12] Shi, J.; Wang, J.; Lv, H.; Peng, Q.; Schreiner, M.; Baldermann, S.; Lin, Z. Integrated proteomic and metabolomic analyses reveal the importance of aroma precursor accumulation and storage in methyl jasmonate-primed tea leaves. *Hortic. Res.,* **2021**, *8*(1), 95. http://dx.doi.org/10.1038/s41438-021-00528-9 PMID: 33931596

[13] Nilo-Poyanco, R.; Moraga, C.; Benedetto, G.; Orellana, A.; Almeida, A.M. Shotgun proteomics of peach fruit reveals major metabolic pathways associated to ripening. *BMC Genom.,* **2021**, *22*, 17.

[14] Zeng, S.; Huang, S.; Yang, T.; Ai, P.; Li, L.; Wang, Y. Comparative proteomic and ultrastructural analysis shed light on fruit pigmentation distinct in two *Lycium* species. *Ind. Crops Prod.,* **2020**, *147*, 112267. http://dx.doi.org/10.1016/j.indcrop.2020.112267

[15] Pontiggia, D.; Spinelli, F.; Fabbri, C.; Licursi, V.; Negri, R.; De Lorenzo, G.; Mattei, B. Changes in the microsomal proteome of tomato fruit during ripening. *Sci. Rep.,* **2019**, *9*(1), 14350. http://dx.doi.org/10.1038/s41598-019-50575-5 PMID: 31586085

[16] Wang, P.; Li, X.; Wang, Y.; Wang, W.; Tian, S.; Qin, G. Redox proteomic analysis reveals the involvement of oxidative post-translational modification in tomato fruit ripening. *Postharvest Biol. Technol.,* **2021**, *178*, 111556. http://dx.doi.org/10.1016/j.postharvbio.2021.111556

[17] Jamil, I.N.; Sanusi, S.; Mackeen, M.M.; Noor, N.M.; Aizat, W.M. SWATH-MS proteomics and postharvest analyses of mangosteen ripening revealed intricate regulation of carbohydrate metabolism and secondary metabolite biosynthesis. *Postharvest Biol. Technol.,* **2021**, *176*, 111493. http://dx.doi.org/10.1016/j.postharvbio.2021.111493

[18] Zhang, Z.; Xia, B.; Li, Y.; Lin, Y.; Xie, J.; Wu, P.; Lin, L.; Liao, D. Comparative proteomic analysis of *Prunella vulgaris* L. spica ripening. *J. Proteomics,* **2021**, *232*, 104028. http://dx.doi.org/10.1016/j.jprot.2020.104028 PMID: 33129985

[19] Sosalagere, C.; Adesegun Kehinde, B.; Sharma, P. Isolation and functionalities of bioactive peptides from fruits and vegetables: A reviews. *Food Chem.,* **2022**, *366*, 130494. http://dx.doi.org/10.1016/j.foodchem.2021.130494 PMID: 34293544

[20] Fu, J.; Zhang, L-L.; Li, W.; Zhang, Y.; Zhang, Y.; Liu, F.; Zou, L. Application of metabolomics for revealing the interventional effects of functional foods on metabolic diseases. *Food Chem.,* **2022**, *367*, 130697. http://dx.doi.org/10.1016/j.foodchem.2021.130697 PMID: 34365248