Bioactive peptides from yeast: A comparative review on production methods, bioactivity, structure-function relationship, and stability

Mahta Mirzaei[a,b*], Amin Shavandi[a*], Saeed Mirdamadi[c*], Nazila Soleymanzadeh[c], Paria Motahari[d], Niloofar Mirdamadi[e], Muriel Moser[f], Gilles Subra[g], Houman Alimoradi[h], Stanislas Goriely [i]

a) BioMatter unit, École polytechnique de Bruxelles, Université Libre de Bruxelles (ULB), Avenue F.D. Roosevelt, 50 - CP 165/61, 1050 Brussels, Belgium.

b) Department of Food Science and Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

c) Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

d) Biopharmaceutical Research Center, Aryogen Pharmed, Inc., Alborz university of Medical Science, Karaj, Iran.

e) School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

f) Laboratory of Immunobiology, Faculty of Sciences, Université Libre de Bruxelles, Gosselies, Belgium.

g) IBMM, Univ. Montpellier, CNRS, ENSCM Montpellier, France.

h) School of Biomedical Sciences, University of Otago, Dunedin, New Zealand.

i) ULB Center for Research in Immunology (U-CRI), Laboratory of Immunobiology, Université Libre de Bruxelles, Gosselies, Belgium.

Contents

Abstract

Background: Yeast cells are a rich source of protein and have long been investigated for the production of yeast extract as a source of bioactive peptides with different documented bioactivity, e.g. antioxidant, ACE-inhibitory, antidiabetic, as well as prevention of chronic diseases and providing immune responces. Furthermore, yeast cells are known to contribute to generation of bioactive peptides due to their proteolytic activity during fermentation processes, and also release of antimicrobial peptides during growth.

Scope and approach*:* Although reports on preparation and characteristic of yeast extract increased tremendously, research on the functional properties of yeast extract attributed to the content of bioactive peptides and production methods lack a systematic review. Also, the contribution of yeast cells to the production of bioactive peptides during the growth and fermentation process has not been summarized previously. This review summarizes previous studies on yeast-derived peptides, the production methods, the bioactivity, the mechanism of action, as well as the structure-function relationship, and stability of identified peptides. This article would be helpful to promote the application of yeast-derived peptides in research and commercialization.

Key finding: Yeast cells and yeast extract have great potentials for producing bioactive peptides with multiple functionalities. Current scientific evidence regarding the potential health benefit of yeast highlights the need for additional investigation on the bioactivity of peptides as influenced by production and purification methods. Also, predicting and designing new peptide sequences with specific functionality with the aid of bioinformatics tools, animal and human studies will effectively transfer these findings into practical and market applications.

***Keywords*:** Bioactive peptides, Yeast cells, Yeast extract, Enzymatic hydrolysis, Fermentation.

1. Introduction

Bioactive peptides are protein fragments that exhibit different functionalities once released from the original proteins and could have significant pharmacological effects. Based on amino acid composition, size, sequences, and physicochemical properties, bioactive peptides could present as antihypertensive, antioxidant, antimicrobial, opioid, mineral binding, antithrombotic, or immunomodulatory agents (Ahmed & Hammami, 2019; Sánchez & Vázquez, 2017). So far, 4283 bioactive peptides have been registered on the BIOPEP database (Minkiewicz, Iwaniak, & Darewicz, 2019). They can be released from the precursor protein either by the enzyme's proteolytic activity, fermentation or after gastrointestinal digestion. A classical workflow has been followed to exploit bioactive peptides from different protein sources, including preparation of protein hydrolysate or fermented product, assessing the biological activities, purification, and characterization of the peptides (Wu, Wang, Qi, & Guo, 2019). Researchers have looked at producing bioactive peptides from various protein sources, including animal, plant, and microbial proteins. Bioactive peptides can be obtained from yeast cells which are composed of 30-60% protein of its dry mass, (M. Amorim, Marques, et al., 2019; de la Hoz, et al., 2014). Yeast cell extract as a food additive is a Generally Regarded as Safe (GRAS) ingredient by the U.S Food and Drug administration and can be produced from different yeasts, mainly *Saccharomyces cerevisiae* (Mirzaei, Mirdamadi, Ehsani, Aminlari, & Ebrahim, 2015), occasionally *Kluyveromyces marxianus* (Mirzaei, Mirdamadi, Ehsani, & Aminlari, 2018) and *Candida utilities* (E.Y. Jung, et al., 2011) by hydrolysis, autolysis, or combination of both (M. Amorim, Pinheiro, & Pintado, 2019). The high hydrophobic and basic amino acids content make yeast cell extract an important source of bioactive peptides, especially angiotensin-converting-enzyme inhibitor (ACE-I) and antioxidant peptides (M. Amorim, Marques, et al., 2019). Furthermore, antimicrobial peptides released by different strains of yeasts are identified as important to biocontrol undesired and spoilage microorganisms and have been suggested as an alternative to the chemical preservatives commonly applied in various food and beverage industries (P. Branco, et al., 2017; Rizk, et al., 2016).

This review focuses on the current state-of-the-art of yeast-originated peptides. We initially categorized yeast-originated peptides into three different groups and summarized different scientific research carried out in this field, including 1) Bioactive peptides produced through autolysis or hydrolysis of yeast cells which are responsible for functional properties of yeast

extract (Fig. 1A-I). 2) Bioactive peptides produced by yeast cells during the fermentation process and are responsible for the functional properties of yeast fermented products (Fig.1A-II). 3) Antimicrobial peptides released by yeast cells during the growth phase (Fig.1-III). In explaining these sections, the parameters influence the bioactivity of the final products including type of the protein and enzyme, enzyme to substrate ratio (E/S), time, temperature, and pH in hydrolysis and autolysis processes and type of yeast strain and protein source, temperature, time, percent of inoculum, and rotation speed in fermentation process are considered.

Then we reviewed the reports on the bioactivity of yeast-derived peptides, focusing on production and corresponding mechanisms. This review also considers the reports on the structure-function relationship of bioactive peptides, stability, and toxicity. Bioinformatic methods are introduced as a new strategy for designing new peptide sequences and investigating the activity mechanisms of identified yeast-derived bioactive peptides.

2. Yeast extract as a source of bioactive peptides

Yeast extract is the soluble fraction of yeast cells widely used in the food industry, cosmetic materials, animal feed, and microbial culture media (Takalloo, Nikkhah, Nemati, Jalilian, & Sajedi, 2020). Yeast cell is protected by a thick cell wall rupturing, which is a major challenge in extracting the intracellular contents and producing yeast extract (Liu, Ding, Sun, Boussetta, & Vorobiev, 2016). Depending on the cell disruption method such as mechanical (e.g. ball milling, and ultrasonication) or nonmechanical (e.g. enzymatic or chemical), it can affect peptides and protein recovery values, the downstream unit operations, the economy of the process, and bioactivity of yeast extract (Takalloo, et al., 2020). (Fig.1 B)

Mechanical methods including sonication, bead lysis, and high-pressure treatment or homogenization are usually used in yeast extract industrial scale production. High energy requirements, difficulty in downstream processing, low selectivity and the risk of protein degradation are among the disadvantages of mechanical methods. In the chemical approach, chemical substances such as acids, alkaline, and detergents are used for this purpose. The toxicity of chemical agents, the risk of protein denaturation, and the high salt content of final products are reasons that limited the application of this method (Liu, et al., 2016; Takalloo, et al., 2020).

In an enzymatic method, the yeast cell wall is disrupted without destroying the cell integrity. Commercially available lytic enzymes such as lysozyme and gluconase, are used to lyse the

glucan structure and mannoprotein complex of the cell wall, resulting in the release of the soluble compounds. In contrast, in an autolysis method usually used to produce yeast extract, the cell wall is disrupted using endogenous cellular enzymes (de la Hoz, et al., 2014; Liu, et al., 2016).

Autolysis is commonly carried out by incubating the yeast cells at 37-60 ºC for 8-72 h (Takalloo, et al., 2020; Tangüler & Erten, 2009). The bioactivity of yeast extract depends on the type of biomolecules produced via the proteolytic activity of yeast cells, e.g. phenolic compounds, β-glucans, vitamin, enzymes from yeast cells, products of Maillard reaction, amino acids and peptides (Tangüler & Erten, 2009). The yeast cells contain various proteolytic enzymes, active in a wide range of pH (Jacob, Striegel, Rychlik, Hutzler, & Methner, 2019). The proteolytic activity of endogenous enzymes is influenced by autolysis conditions (time and pH) and affects directly the yield of solid and protein recovery, free amino acid concentration and bioactivity of yeast extract (Annunziato & Costantino, 2020; Tangüler & Erten, 2009; E.F. Vieira & Ferreira, 2017).

Tangüler and Erten (2009) reported autolysis of baker's yeast at 50 ºC for 24 h as the optimal situation for producing yeast autolysate with 3.7% of α-amino nitrogen, 52.5% protein and 1.98% total solid content. Podpora, Swiderski, sadowska, pitotrowska, and Rakowska (2015) reported an increase from 11.2% to 77.5 % of the free amino acids after 46 h of autolysis at 47 ºC. The authors reported a high antioxidant activity (22.18 - 32.73 mmol TEAC/100 mL) for yeast autolysate, related to polyphenolic compounds (228.3 to 336.1 mg GAE/100 mL) derived from hops and malts, peptides, Maillard products and glutathione. The content of glutathione with known antioxidant ability to scavenger electrophilic and oxidant species has increased in Baker's yeast from less than 1% to about 5% post-fermentation.

E. F. Vieira, Melo, and Ferreira (2017) optimized the autolysis process of *Saccharomyces pasturianus* using response surface methodology (RSM) to produce autolysates with the highest ACE-I, antioxidant activities, and total phenolic contents (TPC). The ACE-I activity (IC_{50}= 375 µg/mL) peaked after 6 h of autolysis at 25 °C and then decreased at higher temperatures (38-40 °C), probably due to the degradation of ACE-I bioactive peptides. Although the TPC and ferric reducing antioxidant capacity (FRAP) activities were higher at 38-40 °C due to the release of bound phenolic compounds. Therefore 36°C for 6 h was reported as the optimum autolysis condition, in which IC_{50}, FRAP, and TPC values were measured as 379 µg/mL, 374 mM TE/mL, and 385 mM Gallic acid equivalent (GAE)/mL, respectively.

Although extensive autolysis may increase the release of bioactive compounds from the yeast cells, but it can breakdown the previously generated bioactive peptides and reduce the yeast extract bioactivity. The selection of autolysis condition is a crucial step for producing yeast extract with the highest value of bioactive compounds. Different research results show that mild autolysis is a better choice for producing yeast extract with desirable bioactivity.

Enzymatic hydrolysis with a variety of proteolytic enzymes, also mechanical cell disruption, following by enzymatic hydrolysis, are other ways for yeast extract production. A combination of autolysis and enzymatic hydrolysis is also used for this purpose (Takalloo, et al., 2020). Our group, Mirzaei et al. (2015), reported that autolysis of *S. cerevisiae* and *Kluyveromyces marxianus* at 52°C, pH 5, 96 h in the presence of ethyl acetate (1.5%) resulted in a higher degree of hydrolysis (DH) in comparison with the sonicated assisted enzymatic hydrolysis with trypsin (1300 BAEE unit/mg solid) and chymotrypsin (55 unit/mg solid) at E/S: 1/10 pH 7.8, 37 °C for 5 h. Despite the higher value of DH, the yeast extract obtained after autolysis of *S.cerevisiae* exhibited lower ACE-I and DPPH• and ABTS•+ radical scavenging activity compared to yeast extracts obtained after sonication and enzymatic hydrolysis with trypsin and chymotrypsin (Mirzaei, et al., 2015). It is worth noting that yeast autolysate which produced in the presence of a chemical solvent such as ethyl acetate has various drawback, including a possible carcinogenic risk, a high salt content and poor sensory features (Jaeger, Aredt, Zannini, & Sahin, 2020).

M. Amorim, Pinheiro, et al. (2019) also examined the combination of autolysis (70 °C for 5 h) of SBY with enzymatic hydrolysis using an extract from *Cynara cardunculus* (a flower from the wild thistle) at E/S: 4% (v/v) for 4.5 h to produce yeast extracts with ACE-I activity. According to the molecular weight (MW) analysis by fast protein liquid chromatography (FPLC), MW of the peptides from the autolysates were in the range of 5-10 kDa and < 3 kDa for the hydrolysates. However, due to the degradation of bioactive peptides, excessive hydrolysis did not result in the lowest IC_{50}.

Takalloo, et al. (2020) compared the efficiency of three cell lysis methods of autolysis (pH 5.5, 55 °C, 48 h), plasmolysis (autolysis in the presence of 1.5% ethyl acetate) and alcalase hydrolysis (0.2 %, pH 7, 55 °C) for the release of soluble solids, protein, and intracellular molecules. The alcalase hydrolysis was the most effective technique in accelerating yeast cell lysis and releasing valuable compounds with free radical scavenging and bacterial binding capacity. Enrichment with trace elements such as selenium and tellurium was also the subject of some research to improve yeast extracts' bioactivity. Guo, Guo, and Liu (2020) reported the higher inhibition activity of selenium-rich yeast peptide fractions against lipid peroxidation

either in a liposome system or in the linoleic acid system, compared to the peptide fraction obtained from the normal yeast peptide fractions. When applied topically on the dorsal skin of mice, selenium-enriched peptide fractions effectively alleviated ultraviolet B (UVB) radiation-induced skin damage. They showed a protective effect against H_2O_2-induced oxidative stress in human keratinocyte cell lines.

In another research, the tellurium-enriched peptides were produced by growing *S. cerevisiae* (ATCC 7752) in the yeast mold (YM) medium containing sodium tellurite at the non-toxic concentration (50 ppm). The protein was extracted by sonication followed by hydrolysis at different acidic (HCl, at 80 °C), enzymatic (proteinase K and pronase E, 37 °C, 3 h), and autolysis (50 °C, 48 h) conditions. The obtained tellurium-enriched peptides exhibited glutathione peroxidase (GPX)-like activity (Morya, Dong, & Kim, 2014).

It is difficult to critically evaluate the reported results concerning the optimum condition for the production of yeast extracts with improved functional properties, mainly due to the various experimental conditions that have been used for the preparation and different methods that have been used to measure the bioactivity. In addition, in most studies, *in vitro* cell assays and *in vivo* evaluations are needed to confirm the reported bioactivities further.

Summing up, intensive hydrolysis does not necessarily lead to higher bioactivity. An uncontrolled autolysis process causes degradation of the bioactive peptides that have been produced. Hence, specific enzymatic hydrolysis under controlled conditions regarding type of enzyme, E/S ratio, reaction time, pH, and temperature would be a more reliable choice for producing yeast extracts with improved functional properties.

3. Production of bioactive peptides through fermentation with yeasts

Bioactive peptides, which are protein fragments with 2-20 amino acid residues, are well known to be produced during food fermentation. Yeast cells contribute to the production of bioactive peptides during fermentation due to their proteolytic activity, improving the functional properties of fermented foods such as Kefir, Kumis, Viili, and Laban (Chaves-López, et al., 2012; Rai, Kumari, Sanjukta, & Sahoo, 2016; D. Zhang, et al., 2017). While there is ample literature on Lactic Acid Bacteria (LAB) such as *L. rhamnosus, L. fermentum, L.helveticus* and Lb. *paracasei* for producing bioactive peptides (Moslehishad, Mirdamadi, Ehsani, Ezzatpanah, & Moosavi-Movahedi, 2013; Soleymanzadeh, Mirdamadi, & Kianirad, 2016), the role of yeast and co-culture of yeast and LAB in producing bioactive peptides only reported in a few

studies(Chaves-López, et al., 2012; Hamme, Sannier, Piot, Didelot, & Bordenave-Juchereau, 2009). An early report by Nakamura, et al. (1995) indicated the contribution of *S.cerevisiae in combination with L.helveticus* (CP790) for producing two well-known ACE-I peptides, VPP (IC_{50}: 9 µM) and IPP (IC_{50}: 5 µM) after fermentation of skim milk with Calpis sour milk starter at 37 ºC for 24 h. Rai, et al. (2016) reported the role of proteolytic yeasts isolated from Chhurpi (a traditional cheese) in improving total antioxidant activity (44.08-59.63 µg ascorbic acid equivalent (AAE)/mg), and reducing power (increased form 11.05 µg AAE/mg protein to 81.06-107.4 µg AAE/mg) of fermented milk. The antioxidant and ACE-I activity of the products fermented with *Candida lipolytica* MIUG D67 (10^6 CFU/100 mL) was improved compared to the control sample fermented with kefir grains (2.5%). In another study, multiple cultures of *Candida lipolytica* MIUG D67, MIUG D99 (10^6 CFU/100 mL), and kefir grains (2.5%) improved the antioxidant and ACE-I activity of bovine colostrum after 24 and 48 h of fermentation (Gaspar-Pintiliescu, et al. (2020). The DPPH• and ABTS•+ radical scavenging activity of the product fermented with *C. lipolytica* and kefir grains was measured as 2.69 mM TE/g and 0.99 µM Trolox/mg, respectively, in comparison with the values of 0.14 mM TE/g and 0.59 µM Trolox/mg measured for the control samples. All reported results reflect the positive role of yeast in increasing the metabolites content with biological activities in the fermented products.

Yeast species/strains express carboxypeptidases and aminopeptidases according to their amino acid auxotrophy resulting in a diverse range of biologically active peptides (Rai, et al., 2016; D. Zhang, et al., 2017). A total of 93 yeast strains isolated from Kumis in different geographical regions of Colombia showed intra-species variability for their proteolytic activities leading to the release of peptides with various ACE-I activities (8.69-89.19%) during skim milk fermentation at 28 ºC for 52 h. They concluded that the peptide profile produced by each yeast strain is unique to that strain based on its proteolytic activity. Among the tested strains, *K. marxianus* had the highest levels of proteolytic activity (Chaves-López, et al., 2012). So, the type of yeast strain plays the most important role in producing bioactive peptides during fermentation. Although most documented reports show the contribution of namely *S. cerevisiae, K. marxianus and Candida lipolytica* in the production of bioactive peptides, the possible role of other yeast species/strains should not be overlooked. In addition, the co-culture of yeast and LAB may improve the fermentation process by allowing higher production of bioactive peptides. For example, kappa-casein and alpha-S1-casein were more degraded in a mixed fermentation system with *Lactobacillus: Streptococcus* and the yeast *Kluyveromyces* (ratio of 15:15:1), compared to the control sample fermented with the *Lactobacillus*.

Moreover, the content of lower MW peptides (<1 kDa) increased significantly at the end of fermentation. This degradation of the peptides may indicate a better digestibility and absorption of milk peptides resulting from a mixed fermentation with yeast (D. Zhang, et al., 2017).

As depicted in Fig. 1A, type of protein substrate, time, reaction temperature, inoculum level, and mixing speed are major factors affecting the bioactivity of the peptides produced during fermentation. The study of Li, Sadiq, Liu, Chen, and He (2015) showed that the most significant factors with the yeast *K. marxianus* Z17 for producing ACE-I peptides during fermentation were temperature, inoculum level, and rotation speed. Using response surface methodology (RSM), authors reported that the highest ACE-I activity (81.23%) was obtained at 32 ºC, pH=6.5, inoculum 6%, and a rotation speed of 189 rpm.

Hamme, et al. (2009) reported the co-culture of *K. marxianus* with *Lactobacillus rhamnosus* fully hydrolyzed α-lactoalbumin and to a less extent β-lactoglubulin in an aerobic fermentation. The ACE-I activity reached 23% after 24 h and 61% (IC_{50}:72 µg/mL) after 168 h of fermentation. Chaves-López, et al. (2012) reported the increase of the content of free amino groups and the improvement from zero to values between 60-80% of ACE-I activity of skim milk fermented by *K. marxianus, Torulospora delbruckii, Clavispora iusitaniae, Pichia kudriavzevii, Pichia kudriavzevil, Galactomyces geotrichum* by increasing fermentation time to 52 h at 28 ºC. However, after 52 h of the reaction process, a considerable activity drop was observed which could be due to hydrolysis and probable utilization of peptides for cell growth.

Yeast extracted enzymes have also been used by some researchers for producing bioactive peptides from different protein sources. For example, protease extract from SBY (0.725 U/mL) was used for the hydrolysis of spent brewers' grain (E. Vieira, Teixeira, & Ferreira, 2016) and sardine sarcoplasmic protein (E.F. Vieira & Ferreira, 2017) to produce antioxidant and ACE-I peptides. Pokora, et al. (2017) also reported the ability of serine protease enzyme from yeast *lipolytica* to produce antioxidant peptides from egg white protein, indicating the capacity of yeast enzymes to be used for *in vitro* protein digestion and release of bioactive peptides.

Yeast cells can hydrolyze proteins, which improves the functional properties of fermented products. They are also well known for manufacturing aroma precursors, flavoring substances such as alcohol and glycerol. These characteristics highlight yeast`s importance as a starter culture in the manufacture of functional fermented foods. However, most of the research has concentrated on dairy products, with only a few studies resulted in discovering new bioactive peptide sequences. More research is needed to figure out the ideal fermentation conditions for a variety of fermented foods. Fermented products and bioactive peptides derived from the yeast fermentation process are summarized in Table 1.

Table 1. A summary of bioactive peptides produced by yeasts or derived from mixed cultures with lactic acid bacteria (LAB) during fermentation.

Fermented product	Peptide sequence	Starter (S)	Fermentation condition	characteristics	Methods of purification	Ref.
Fermented milk	IPP ,VPP	Calpis sour milk starter containing *L.helveticus* (CP790) and *S.cerevisiae*	37 ºC for 24 h	ACE-I activity IPP (IC50:5 µM) VPP (IC50:9 µM)	Ultrafiltration of sour milk supernatant (Cut off :10 kDa), Four steps RP-HPLC	*(Nakamura, et al., 1995)*
Soft chhurpi	Not identified	*125* isolated yeasts from Chhurpi *Kluyveromyces marxianus* YMP45 and *S. cerevisiae* YAM14 resulted in the higher antioxidant activity	Inoculum 5%, 30 °C for 48 h	-DPPH• radical scavenging IC_{50}:0.38-0.75 mg protein -Superoxide radical scavenging IC_{50}:0.29-0.32 mg pro -Reducing power 81.6-107.4 µg AAE/mg -Total antioxidant activity 44.08-59.63 µg AAE/mg	-	(Rai, et al., 2016)
Colombian kumis	Not identified	*Galactomyces geotrichum Pichia kudriavzevii Clavispora lusitaniae Candida tropicali*	Inoculum 5%, 28 °C for 52 h	ACE-I activity (Ranging from 8.69-88.19%)	-	(Chaves-López, et al., 2012)
Colostrum	Not identified	Co-cultivation of *Candida lipolytica* (MIUG D67)(10^6 CFU/mL) and Kefir grain (2.5%)	30°C for 48 h	Improving DPPH• (2.69 mM TE/g), ABTS•+ (0.99 µM Trolox/mg) radical scavenging, and ACE-I activity in comparison with samples fermented with kefir grain	Ultrafiltration	(Cotârleț, et al., 2019)
Milk	Not identified	*K. marxianus (Km)* in combination with *L. bulgaricus (Lb) and St.thermophilus (St) Lb/St/Km:15/15/1*	35°C until pH 4.6	The number of peptides with MW 0-1 KDa increased in milk fermented by *K. marxianus* compared with milk fermented only by *lactobacillus.*	-	(D. Zhang, et al., 2017)

Milk	VLSRYP (κ-casein f3136) and LRFF (αs1-casein f21-24)	*K. marxianus Z17*	Optimum condition obtained by RSM analysis (32 ºC, pH=6.5, Inoculum 6%, rotation speed of 189 rpm)	ACE-iI activity (IC_{50}) VLSRYP(36.7 μM) LRFF(116.9 μM). Competitive Inhibitory effect	Sephadex g-15 gel filtration, reversed phase-high performance liquid chromatography and MALDI TOF/TOF MS/MS.	(Li, et al., 2015)
Acid goat whey	Not identified	Microflora extracted from *Bamalou des pyrenees* cheese containing *K. marxianus* and *Lb. rhamnosus*	Aerobic fermentation At 37 °C, 168 h	ACE-I activity IC_{50}:72 µg/mL	-	(Hamme, et al., 2009)
Bovine colostrum	Not identified	Subsequent fermentation with *Candida lipolytica (MIUG D67 and MIUG D67)(*10^4 CFU/mL) and kefir grain *(2.5%)*	30 °C for 48 h and 72 h 150-18 rpm	Bovine colostrum fermentation yielded products with improved ACE-I and antioxidant activity in compared with the non fermented sample	Amicon Ultra centrifugal filters (Cutoff 10 kDa)	(Gaspar-Pintiliescu, et al., 2020)

4. Peptides released by yeast

Antimicrobial peptides (AMPs), a type of oligopeptides with variable sequence lengths from 10 to 100 amino acids, are known to be released by yeast cells as they grow. AMPs are found in most living kingdoms (bacteria, yeasts, fungi, insects, vertebrates, and plants) and can be active against bacteria, molds, yeasts, and some viruses (Annunziato & Costantino, 2020).

Yeast antimicrobial activity was first linked to the production of Mycocins, extracellular protein or glycoprotein metabolites with inhibitory growth activity against yeasts closely related to producer strains. The ability of yeasts to produce extracellular peptides that inhibit Gram-positive and Gram-negative bacteria are widely reported in the literature and summarized in Table 2.

352

353 Table 2- Antimicrobial peptides released by yeast strains

Peptides or peptide fractions	**Producer M.O**	**Activity**	**Target M.O**	**Ref.**
Peptide fraction with MW ≤14 kDa	*S. cerevisiae* R107	Lytic activity	*E. coli and St. aureus* and malolactic bacteria	(Dick, Molan, & Eschenbruch, 2015)
VSWYDNEYGYSTR and ISWYDNEYGYSAR MW of 1.638 and 1.622 kDa	*S. cerevisiae* CCMI 885	Fungicidal activity	*H. guilliermondii, L. thermotolerans, K. marxianus,* *T. delbrueckii,* *D. bruxellensis*	(P. Branco, et al., 2013; P. Branco, et al., 2017)
Peptide fraction with MW ≤ 10 kDa	*C. intermedia* LAMAP1790	Antifungal activity	*B. bruxellensis*	(Peña & Ganga, 2019)
Peptide fraction with MW< 9770 Da	*S. cerevisiae* ATCC 36858	Bacteriostatic	*St. aureus* ATCC 25923, *Klebsiella aerogenes* ATCC 13048, *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 23857	(Thyab Gddoa Al-sahlany, et al., 2020)
Peptide fraction with MW about 5.9 kDa	*S. cerevisiae RUBY*	Bacteriolytic	*O. oeni Viniflora oenos*	(Osborne & Edwards, 2007)
Peptide fraction with MW between 5 and 10 kDa	*S. cerevisiae* strain D	Bacteriostatic	*O. oeni* strain X	(Nehme, Mathieu, & Taillandier, 2010)
DDDDDNDDDDEEGNxKTKSAAT and CNATQACPEDKPCCSQYGEcGTG	*Saccharomyces cerevisiae* Uvaferm BDX	Bacteriostatic	*Oenoccus oeni*	(Rizk, et al., 2018)
Not identified	Different yeast strains isolated from dairy products including: *Candida intermedia Candida kefir, Candida tropicalis*	Bacteriostatic	*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*	(Younis, Awad, Dawod, & Yousef, 2017)
Not identified	*Candida tropicalis* LMA-693,	Bacteriostatic	*Listeria ivanovii* *HPB28*	(Hatoum, Labrie, & Fliss, 2013)

	Debaromyces hansenii LMA-916, *Pichia fermentans* LMA-256 and *Pichia anomala* LMA-827

According to Nehme (2010), *S.cerevisiae* Uvaferm BDX produces a 5-10 kDa peptide fraction that inhibits the growth and malate consumption of *Oenococus oeni* during malolactic fermentation. Rizk, et al. (2016) later discovered that the peptide fraction released by *S. cerevisiae* Uvaferm BDX is gradually released during alcoholic fermentation, peaking at the later stages of the stationary phase and inhibiting the malate consumption by directly inhibiting the malolactic reaction. Malolactic fermentation is the primary source of energy during wine making, and its inhibition results in growth inhibition of *O.oeni.*

P. Branco, et al. (2013) discovered antimicrobial peptides produced by *S. cerevisiae* CCMI 885. These AMPs were identified as two fragments of the C-terminal amino acid sequence of *S. cerevisiae* glyceraldehyde 3-phosphate dehydrogenase (GAPDH) isoenzymes and were active against several non-*Saccharomyces* yeasts and one strain of *O.oeni*. Two main anionic peptides have been identified: AMP2/3 and AMP1, with the amino acid sequences of VSWYDNEYGYSTR and ISWYDNEYGYSAR, MW of 1.638 and 1.622 kDa, and a theoretical pI of 4.37. Later, they discovered that several other *S.cerevisiae* strains secret natural biocide fractions during alcoholic fermentation, albeit at varying levels, which correlates with the antagonistic effect exerted against non-*Saccharomyces* yeasts (P. Branco, et al., 2017).

A recent study showed that *S. cerevisiae* Uvaferm released two anionic antimicrobial peptides with pI of 3.5 and 4. Amino acid alignment revealed that peptide sequences matched the two yeast protein sequences of Wtm2p and Utr2, DDDDDNDDDDEEGNxKTKSAAT and CNATQACPEDKPCCSQYGEcGTG, respectively(Rizk, et al., 2018).

Thyab Gddoa Al-sahlany, et al. (2020) also identified a thermostable antimicrobial peptide with Mw of 9.77 kDa produced by *S. cerevisiae* ATCC 36858. This peptide was active within a pH range of 4-7 *against Gram-positive and Gram-negative bacteria, including Bacillus subtilis, E. coli, Klebsiella aerogenes, and Staphylococcus aureus*.

Different mechanisms have been proposed for the antimicrobial activity of peptides released by yeast cells. Cationic peptides kill microbes by interacting with anionic components of target cell membranes; however, anionic peptides use a variety of mechanisms such as translocation across the membrane and cell membrane permeabilization via pore formation, resulting in loss

of vital components and cell death (P. Branco, et al., 2013; Lis, et al., 2013). P. Branco, Viana, Albergaria, and Arneborg (2015) reported that small peptide fractions (<10 kDa) corresponding to the C-terminal amino acid sequence of the GAPDH protein produced by *S. cerevisiae* CCMI 885 disrupts the integrity of the target cell plasma membrane (*H. guilliermondii*) and possibly the vacuole membrane. As a result, sensitive cells could not maintain their pH homeostasis, resulting in decreased pH value from 6.5 (healthy cells) to 5.4 (damaged cells) after 20 min of exposure to the antimicrobial peptide.

The antimicrobial activity of K1 killer toxin (19 kDa α/β heterodimer protein) produced by different strains of *S. cerevisiae* is attributed to the disruption of the plasma membrane integrity and uncontrolled influx of protons and efflux of potassium ions in sensitive target cells; this is most likely caused by α- domain dependent formation of membrane channels and the subsequent selective leakage of monovalent cations from the cytoplasm (Gier, Lermen, Schmitt, & Breinig, 2019).

Rizk, et al. (2018) reported the antibacterial activity of cationic GAPDH-derived peptides (5–10 kDa) produced by *S. cerevisiae* Uvaferm BDX against *O. oeni* is due to inhibition of malolactic reaction without affecting membrane integrity. It has been reported that peptide enters the *O. oeni* Vitilactic F cytoplasm via an unknown mechanism and binds to NAD^+ binding site of the malolactic enzyme and inhibits its activity, maybe by chelating Mn^{++}, its cofactor.

As previously stated, yeasts with the ability to release antimicrobial peptides can control the growth of spoilage M.O during the fermentation process, reducing the need for chemical antiseptics. These yeasts, however, may also be active against beneficial M.O (P. Branco, et al., 2013). As a result, to avoid interfering with the fermentation process by inhibiting beneficial M.O, the use of yeasts or yeast-derived peptides as bio-preservatives must be done selectively.

5. Bioactivity of yeast-derived peptides

Yeast-derived peptides obtained from different enzymatic hydrolysis, autolysis or those produced during yeast fermentation exhibited different biological activities are summarized in Fig. 2.

5.1. Antioxidant activity of yeast-derived peptides

High concentrations of reactive oxygen species (ROS) and reactive nitrogen species (NOS) are mediators of different diseases. Antioxidant compounds can protect cells against oxidative stress and damage macromolecules such as protein, lipid, and DNA (Sarmadi & Ismail, 2010; Wen, Zhang, Zhang, Duan, & Ma, 2020).

Natural antioxidant compounds with free radicals scavenging, metal ion chelating, inhibition of lipid oxidation, reducing power and cytoprotective effects against oxidative stress have been widely studied in recent years as an alternative to synthetic antioxidants (Hęś, Dziedzic, Górecka, Jędrusek-Golińska, & Gujska, 2019; Unuofin & Lebelo, 2020). Protein hydrolysates and bioactive peptides derived from various protein sources are also well known for antioxidant activity (I. D. Nwachukwu & R. E. Aluko, 2019) (Table 3).

Various recent studies reported the antioxidant activity of yeast extract, related to different bioactive compounds including phenolic compounds, β-glucans, glutathione (GSH) as the most important thiol in cells, and antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) (Đorđević, et al., 2018; Rakowska, Sadowska, Dybkowska, & Swiderski, 2017; E. Vieira, Carvalho, et al., 2016). Thirteen different phenolic compounds, including gallic acid, caffeic acid, vanillic acid and catechin, were found in yeast extract obtained from the mechanical cell disruption method. The mean value of antioxidant activity evaluated by FRAP, DPPH· scavenging activity and Ferricyanic reducing power were 261, 59.7, and 127 mg TE/100 gr dry weight (E. Vieira, Carvalho, et al., 2016).

Enzymatic hydrolysis of yeast cells by proteases and autolysis disrupts the cell wall and improves yeast extract's bioactivity by producing peptides and free amino acids. The specific cleavage activity of the proteolytic enzyme for a substrate is typically based on the sequence of amino acids directly surrounding the cleaved bond. The specific activity of enzymes and the hydrolysis conditions influence the size, composition, and sequence of peptide chains, and thus the biological activity of yeast extract (R. J. S. de Castro & H. H. Sato, 2015; Sarmadi & Ismail, 2010).

Several proteolytic enzymes such as flavourzyme, trypsin, and chymotrypsin have successfully produced antioxidant peptides from yeast cells (E.Y. Jung, et al., 2011; Mirzaei, et al., 2018; Mirzaei, et al., 2015). For example, E.Y. Jung, et al. (2011) compared flavourzyme to neutrase (endo protease from B.amyloliquifaciens), alcalase, protamex, and flavourzyme, and ficin in

the production of yeast extract with antioxidant activity. They reported hydrolysis of yeast cell suspension with flavourzyme at 50 ºC, E/S:1/100 yielded extract with the highest DPPH• (IC_{50}:19.1 mg/mL) and ABTS•+˙ (IC_{50}:9 mg/mL) scavenging activity, as well as the highest concentration of histidyl-proline diketopierazine [cyclo(His-Pro)] (674 µg/mL). CHP is widely used in the treatment of metabolic disorders.

Our study compared the efficacy of trypsin and chymotrypsin (E/S:1/10, 5 h) in producing *S.cerevisiae* yeast extract with antioxidant activity after protein extraction using sonication (600 W, 20 KH, 10 min). The trypsin hydrolysate exhibited higher ABTS•+˙ scavenging activity than chymotrypsin hydrolysate at a similar DH value (18%) obtained after 5h hydrolysis at an E/S ratio of 1/10, while the DPPH• scavenging activity was the same for both (Mirzaei, et al., 2015). In a similar study on extracted soluble protein from *K. marxianus*, chymotrypsin hydrolysis resulted in an extract with a higher DH value (21.59%). Still, no significant difference in DPPH• and ABTS•+ scavenging activity was observed between the activities of extracts obtained from chymotrypsin and trypsin (Mirzaei, et al., 2018).

The antioxidant activity of yeast autolysate is also directly influenced by the specific activity of intracellular proteases and intensity of autolysis. In comparison between autolysis (96 h, 52°C) and enzymatic hydrolysis under control conditions with trypsin and chymotrypsin (E/S: 1/10, 37°C, 5 h), our research group detected lower DPPH• (52.23 µM TE/mg protein) and ABTS•+ (2211.59 µM TE/mg protein) scavenging activity for *S. cerevisiae* autolysate sample, possibly due to degradation of produced antioxidant peptides through intensive autolysis process (Mirzaei, et al., 2015). M. Amorim, Marques, et al. (2019) reported the high oxygen radical absorbance capacity (ORAC) of peptide fraction with MW<3 kDa obtained after autolysis and enzymatic hydrolysis of SBY with an extract from *Cynara cardunculus*.

Despite several reports on the antioxidant activity of yeast extract, only the best of a few of them led to the identification of peptide sequences with antioxidant activity. YRGKPVAVPAR(YR-10) and VLSTSFPPK (VL-9) with ABTS•+ scavenging activity (26.25 and 5.568 µm Trolox equivalent (TE)/µg protein) were purified from *S. cerevisiae* and *K.marxianus*, respectively (Mirzaei, et al., 2018; Mirzaei, et al., 2015).

Besides the *in vitro* assay for antioxidant activity, the cellular antioxidant activity of yeast-derived peptides was also the subject of some studies. Protective effects against oxidative stress, modulation effects of peptides on the antioxidant gene expression, and antioxidant enzymes activity are the main subjects investigated in this context.

The yeast extract of *S. cerevisiae* obtained by sonication-trypsin hydrolysis (E/S: 1/10, 37°C, 5 h) exhibited cytoprotective activity against H_2O_2-mediated lipid and protein oxidation in Caco-2 cells. We also highlighted the beneficial role of ultrafiltration (UF) peptide fractions with MW<3 kDa and 3-5 kDa in inhibiting H_2O_2 -mediated lipids and proteins oxidation in Caco-2 cells (Mirzaei, Mirdamadi, & Safavi, 2019). Furthermore, the antioxidant synthetic peptides VLSTSFCPK (VCK-9), VLSTSFYPK (VYK-9) and STSFPPK (SK-7) were created using yeast-derived peptides as a template, demonstrated a positive role in lowering protein and lipid oxidation. They increased the viability of cells exposed to H_2O_2-induced oxidative stress at a final concentration of 1 mM by activating antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) and most likely by activation of the Keap1/nuclear factor erythroid 2- related factor 2 (Keap1-Nrf2) signalling pathway and overexpression of antioxidant enzyme genes (Mirdamadi, et al., 2021). Sklirou, et al. (2015) also investigated the antiaging properties of a synthetic hexa-peptide (FVAPFP) derived from *S. cerevisiae*. This peptide activated antioxidant-related genes in a dose and time-dependent manner. It primarily induced *NRF2* gene expression. Hexapeptide-11 also provided significant protection against stress-induced premature senescence (SIPS) caused by H_2O_2.

5.2.The antihypertensive activity of yeast-derived peptides

In response to reduce blood pressure, kidney granular cells, which contain Prorenin, convert it to renin and release it into the blood. Renin is a common component of renin-angiotensin aldosterone (RASS) and Kinin-kallikrein (KKS) systems. It acts on angiotensinogen and cleaves it into angiotensin I, which is the precursor of angiotensin II. This conversion is catalysed by the angiotensin-converting enzyme (ACE). Angiotensin II is a versatile effector molecule that has a vasoactive role in all blood vessels. ACE also breaks down bradykinin, a vasodilator substance. Therefore, in the case of hypertension as a chronic disease, synthetic drugs such as captopril and enalapril reduce blood pressure through ACE inhibition, which decreases the level of angiotensin II or increases bradykinin levels (Patel, Rauf, Khan, & Abu-Izneid, 2017).

The side effects of these synthetic drugs, such as cough, taste disturbances, and skin rashes, have encouraged the researchers to look for natural alternatives of ACE-I, such as ACE-I peptides (Chai, Voo, & Chen, 2020; R. S. de Castro & H. H. Sato, 2015; Halim, Yusof, & Sarbon, 2016; Wu, et al., 2019). Yeast proteins have also been an attractive source of ACE-I peptides (Mirzaei, et al., 2015; Ni, Li, Guo, et al., 2012). To produce ACE-I peptides from

yeast cells, enzymatic hydrolysis and autolysis have been routinely used, either alone or with physical cell disruption methods. Protein extraction enzymes such as lywallzyme (a complex cell wall lyticase) and other proteolytic enzymes such as pepsin, trypsin, protease, alcalase and crude enzymes obtained from bacteria have also been used for enzymatic hydrolysis of yeast and producing yeast extract with ACE-I activity (Huang, Wang, Hou, & Hu, 2020; Kanauchi, Igarashi, Ogata, Mitsuyama, & Andoh, 2005; Mirzaei, et al., 2018; Mirzaei, et al., 2015; Ni, Li, Guo, et al., 2012; E.F. Vieira & Ferreira, 2017).

The type of ACE-I peptides produced in yeast extract is determined by the protease used. Because various enzymes have distinct cleavage sites, they can produce different peptides from the same substrate. Hydrolysis duration, E/S ratio, and DH are all critical factors in the production of ACE-I peptides.

Sonication-enzymatic hydrolysis and autolysis (52°C, 96 h, pH 5, in the presence of ethyl acetate) were compared in a study by our research group to produce yeast extracts with ACE-I activity from *S. cerevisiae*. Sonication (600 W, 20 kHz, 10 min) followed by trypsin hydrolysis under controlled conditions (37°C, 5 h, pH 7.8) resulted in higher ACE-I activity (IC_{50}: 0.84 mg/mL) compared to autolysis (IC_{50}: 2.18 mg/mL) that relies on yeast intracellular enzymes activity and caused more intensive hydrolysis (DH: 48.75%) compared to enzymatic hydrolysis (DH:18.51%). Furthermore, trypsin was more effective than chymotrypsin (IC_{50}:1.71 mg/mL)(Mirzaei, et al., 2015). Although, in another study on *K.marxianus*, the effect of trypsin and chymotrypsin on ACE-I peptides production was reported to be the same (IC_{50}:0.51 and 0.49 mg/mL) (Mirzaei, et al., 2018).

In other researches, autolysate (70 ºC for 5 h) and subsequent hydrolysis (E/S: 4% for 4.5 h) of SBY by a protease from *Cynara cardunculus* produced yeast extract with the ACE-I activity (IC_{50}: 146 µg/mL). The IC_{50} of peptide fraction with MW< 3 kDa was 84.2 µg/mL (M. Amorim, Pinheiro, et al., 2019). Autolysis of *Saccaromyces pasturianus* at 36°C for 6 h and enzymatic hydrolysis of dry yeast cell with crude enzyme from *Bacillus subtilis* produced yeast extract with IC_{50} of 379 µg/mL and 266.13 µg/mL, respectively (Huang, et al., 2020; E. F. Vieira, et al., 2017)

So far, the IC_{50} for ACE-I activity in yeast extracts obtained under different conditions of hydrolysis ranged from 0.146-2.18 mg/mL. Following ultrafiltration, the inhibitory activity increased primarily for the fraction with MW< 5 kDa. Some studies went a step further and purified peptide sequences with ACE-I activity. The IC_{50} values measured for yeast-derived peptides are in the range of 12-465 µmol/L, as shown in Table 3. Peptide TPTQQS (IC_{50}: 73.25

µg/mL) was obtained by hydrolyzing *S. cerevisiae* yeast cells with lywallzyme and protease under optimal conditions (material-liquid ratio: 10%; E/S: 1%; 40 ºC; 2 h) and purified using ultrafiltration, FPLC, and ion-exchange chromatography (Ni, Li, Guo, et al., 2012). Our research group also produced and purified three new ACE-I peptides, YGKPVAVPAR (YR-10) , VLSTSFPPK (VL-9), and LPQSVHLDL (LL-9) with IC_{50} values of 139.6 µmol/l (0.42 mg/mL), 15.20 µmol/L, 22.88 µmol/L, respectively by sonication and trypsin enzymatic hydrolysis of *S. cerevisiae* and *K. marxianus*, ultrafiltration and RP-HPLC (Mirzaei, et al., 2018; Mirzaei, et al., 2015). Three ACE-I peptides (PWW, SQPW, and RYW) with IC_{50} values of 465.1, 12.0, and 77.2 µmol/L, respectively, were also produced by autolysis (70 ºC for 5 h) and enzymatic hydrolysis (E/S: 4% for 4.5 h) of yeast cell with a protease from *Cynara cardunculus* (M. Amorim, Pinheiro, et al., 2019).

More *in vivo* studies on antihypertensive effect of yeast-derived peptides were carried out in spontaneous hypertensive rats (SHRs) model. An ACE-I peptide (YDGGVFRVYT) with an IC_{50} of 0.07 mg/mL obtained after hydrolysis of the cell-free extract with pepsin was able to reduce average blood pressure in SHRs by 30 mmHg at the dosage of 1 mg /kg body weight (Kim, Lee, Jeong, Chung, & Lee, 2004). Kanauchi, et al. (2005) discovered that the peptide fraction of brewer's yeast obtained after alcalase hydrolysis (2.4 L, pH=7.5-8.5, 50 ºC, 12 h), and fractionation with Amberlite XAD-2 resin can significantly reduce the systolic blood pressure (SBP) in SHRs. Systolic, diastolic and mean blood pressure of SHRs were significantly reduced after oral administration of protein retentate hydrolysed with MW<3 kDa containing tri and tetra peptides with hydrophobic amino acids at 300 mg/kg body weight (M. Amorim, Marques, et al., 2019). Furthermore, rats treated with 1200 mg/kg of yeast hydrolysate obtained by hydrolysis of yeast powder by a crude enzyme from *B. subtilis* (7000 U/g, 55 ºC, 5 h) experienced a decrease in SBP from 185.2 mm Hg to 156.8 mm Hg after 4 h. On the 15th day after administration, SBP reduction was at its peak (20.5 mm Hg) (Huang, et al., 2020).

Given the importance of NO synthesis in endothelial cells, which relaxes the vessels, Huang and co-workers examined blood NO levels in SHRs plasma and found that long-term administration of medium and high doses of yeast hydrolysate obtained by hydrolysis of yeast powder by crude *B. subtilis* enzyme with protease and β-glucanase activity increased NO value by 140% (Huang, et al., 2020).

Various findings indicate that yeast protein can be a source of ACE-I peptides released under controlled proteolysis conditions. Thefore, yeast extract can be used as a functional food

ingredient. It is also possible to identify novel peptide sequences from yeast cells that could be used as drug or nutraceutical compounds.

5.3. Other identified activities

In addition to the features discussed in sections 5.1 and 5.2, some reports point to other properties of yeast-derived peptides, including reduction and prevention of chronic diseases, antidiabetic activity, and providing immune protection (M. M. Amorim, et al., 2016; Chakrabarti, Guha, & Majumder, 2018; E.Y. Jung, et al., 2011). For example, peptide fraction (MW<3 kDa) obtained after autolysis and enzymatic hydrolysis of Brewer's yeast *(Saccharomyces* sp.) reduced gastric injuries in ethanol-induced ulcer animal model with effective dose (DE_{50}) of 816 mg/kg bodyweight. The identified mechanism was the stimulation of prostaglandins and mucus production. Interestingly, the same extract inhibited the growth of the K-562 leukemic cell line by more than 50% at the concentration of 2.5 μg/mL (M. M. Amorim, et al., 2016). Research performed by Williams, Dias, Jayasinghe, Roessner, and Bennett (2016) showed that glycopeptide-rich yeast extracts obtained after sequential enzymatic hydrolysis of brewer's and baker's yeast (*Saccharomyces cerevisiae)* by glutaminase, corolase PN-L, alcalase 2.4 L, flavourzyme 1000 L and trypsin each at 5% w/w, suppressed production of IFN-γ in human whole blood assay, suggesting that yeast extract display anti-inflammatory properties.

de la Hoz, et al. (2014) compared the iron binding properties of hydrolysates of sugar cane yeast extract (*Saccharomyces cerevisiae)* by alcalase (from *B.lichenformis*, 2.4 AU/g), protex 51FP (*Aspergillus oryzae*, 400000 HU/g) and viscozyme L (from *Aspergillus aculeatus*, 100 FBG/g). The peptide fraction (MW< 5 kDa) obtained after enzymatic hydrolysis with viscozyme (pH:4.4, E/S: 2%, 51 °C) presented the highest iron-binding capacity (22.8 %). E.Y. Jung, et al. (2011) studied SBY hydrolysate with high content of Cyclo-His-Pro (CHP) obtained after enzymatic by neutras, alcalase, protamex, flavourzyme, and ficin (50 ºC, E/S:1/100) for antidiabetic activity. The hydrolysate significantly decreased the blood glucose levels in the oral glucose tolerance test on male ICR mice, up to 120 min after injection. E. Y. Jung, et al. (2014) also reported the effect of yeast hydrolysate obtained after enzymatic hydrolysis of *Saccharomyces cerevisiae* with bromelain at 30 ºC for 4 h on abdominal fat accumulation.

6. Structure-activity relationship

Although the structure-activity relationship (SAR) of antioxidant, ACE-I, and antimicrobial peptides is not fully understood, various parameters such as peptides size, hydrophobicity, electric charge, amino acids type and positioning in peptide sequence have been proposed to be important (Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017; Ifeanyi D. Nwachukwu & Rotimi E. Aluko, 2019; Ovchinnikova, 2019). The SAR of peptides identified from yeast protein is discussed herein.

6.1. Structure-activity relationship of antioxidant peptides

Several studies have reported the radical scavenging activity of yeast extract, and yeast-derived peptides via hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (E.Y. Jung, et al., 2011; Mirzaei, et al., 2018; Mirzaei, et al., 2015). Other mechanisms considered include ferric reducing capacity and inhibition of linoleic acid oxidation (Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019; E.F. Vieira & Ferreira, 2017). Furthermore, some studies have looked into the cytoprotective activity of yeast peptides against ROS by increasing antioxidant enzymes or overexpression of antioxidant genes (Mirdamadi, et al., 2021). Although, the exact mechanism underlying the antioxidant activity of yeast-derived peptides and the structure-function relationship of antioxidant peptides are not completely understood.

Peptide size has a significant impact on the antioxidant activity of peptides. Several studies have found that the <3 kDa fraction of yeast protein hydrolysate has higher antioxidant activity, and they typically contain less than ten amino acid residues (Table 3) (M. Amorim, Marques, et al., 2019; Guo, et al., 2020; Hu, Wang, Guo, Li, & Hou, 2014; Mirzaei, et al., 2018; Mirzaei, et al., 2015). It could be due to smaller peptides being more accessible to oxidant molecules, easier transport through intestinal epithelial cells, and increased bioavailability (Mirzaei, et al., 2015; Wen, et al., 2020).

Furthermore, the antioxidant activity of the peptide sequence is influenced by each amino acid residue (Sarmadi & Ismail, 2010). Tyr, Met, Lys, Cys and His are common amino acid residues in the structure of antioxidant peptides. Aside from the role of amino acids in the peptide sequence, it is thought that amino acid residues in N and C terminus play a more significant role in determining the antioxidant activity. With a higher occurrence of hydrophobic amino acids, the last three amino acids in C-terminal play an important role. The hydrophobic nature of peptides increases their solubility in lipids and allows them to interact with hydrophobic

radicals and lipids (Sarmadi & Ismail, 2010). Gly, Ala, Val, Leu, Ser, and Pro were discovered in the N-terminal and sequence of several antioxidant peptides in the hydrophobic amino acids. Gly in the sequence of YGKPVAVPAR (YR-10) and YGKHVAVHAR (YHR-10) and N-terminal of GKPVAVPA (GA-8) and GKHVAVHA (GHA-8) act as hydrogen donor and contribute to radical scavenging activity of peptides. Val and Leu in N-terminal of VLSTSFPPK (VL-9), VLSTSFPPF(VF-9), VLSTSFPPW(VW-9), VLSTSFPPY(VY-9) contribute to radical scavenging activity of peptides. Removing both of them from the sequence of VL-9 led to lower DPPH· and ABTS•+ radical scavenging activity of obtained peptide (STSFPPK(SK-7))(Mirdamadi, et al., 2021; Mirzaei, Mirdamadi, Safavi, Zare, et al., 2019).

Pyrrolidine ring of Pro was also identified as an important factor in quenching singlet oxygen due to its low ionization potential. In addition, it can interact with the peptide`s secondary structure, increasing flexibility. Because of its low ionization potential, it can also quench singlet oxygen. M. Amorim, Marques, et al. (2019) reported that Pro in the sequence of PWW, SQPW collaborate in antioxidant activity through the quenching of free radicals.

Aromatic amino acids (Tyr, His, Trp and Phe) can donate electrons to radicals and thus play an important role in the radical scavenging activity of peptides (M. Amorim, Marques, et al., 2019). They also promote metal ions chelating. They are, however, stable due to their resonance structure (Wen, et al., 2020).

The significant role of His in the antioxidant activity of peptides was also reported by several researchers (Chen, Muramoto, Yamauchi, Fujimoto, & Nokihara, 1998; Mirzaei, Mirdamadi, Safavi, Zare, et al., 2019). His contributes to the chelating of metal ions, scavenging hydroxyl radicals, and inhibiting lipid oxidation due to its imidazole group (Chen, et al., 1998).

Trp with Indolyl group and Phe also act as hydrogen donors, improving radical scavenging activity of peptides (Wen, et al., 2020). The presence of Trp and Phe in the sequence of VLSTSFPPW (VW-9) and VLSTSFPPF (VF-9) improved the radical scavenging activity of peptides. However, the side chains of Lys are also known for electron-accepting from free radicals, reducing Fe^{3+} to Fe^{2+} and chelating Fe^{2+} and Cu^{2+} ions (Wen, et al., 2020).

The thiol group of cysteine is also well known to contribute to the radical scavenging activity of peptides via SET mechanism. Previous research in our laboratory found that VLSTSFCPK (VCK-9) obtained by replacing one Pro with Cys in VLSTSFPPK (VL-9) sequence had higher radical scavenging activity as well as cellular antioxidant capacity. The ability of VCK-9 to inhibit lipid and protein oxidation in cells exposed to H_2O_2-induced oxidative stress was attributed to radical scavenging activity of peptide and also activation of keap1-NrF2/ARE

pathway and increasing the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Mirdamadi, et al., 2021).

Table 3- A summary of bioactive peptides obtained from yeast extract

Peptides sequences or peptide fractions	**Source**	**Bioactivity**	**Mechanism of activity**	**MW (kDa)**	**Method of production and purification**	**Ref.**
YHKPVAVPAR (YR-10)	*S. cerevisiae* (PTCC5269)	-ABTS•+ radical scavenging 26.25 µM TE/mg protein -ACE-I IC_{50}: 0.42 mg/mL - Decreasing the level of Malondialdehyde (MDA) and protein carbonyls and increasing the viability of Caco-2 cells exposed to oxidative stress	-Radical scavenging activity -Increasing the level of antioxidant enzyme (Cat) in cells exposed to oxidative stress	1.057	Protein extraction with sonication (600 W, 20 KHz, 10 min) and enzymatic hydrolysis by Trypsin (pH 7.8, 37 ºC, E/S:1/10, 5 h) Ultrafiltration (3, 5, 10 kDa) and RP-HPLC	(Mirzaei, et al., 2015) (Mirzaei, Mirdamadi, Safavi, Zare, et al., 2019)
VLSTSFPPK (VL-9)	*K. marxianus* (PTCC5195)	-ACE-I IC_{50}: 15.20 mmol/L -ABTS•+ scavenging 5568 µM TE/mg protein	Not reported	1.118	Protein extraction with sonication (600 W, 20 KHz, 10 min)and enzymatic hydrolysis by Trypsin (pH 7.8, 37 ºC, E/S:1/10, 5 h). Ultrafiltration (3, 5, 10 kDa) and RP-HPLC	(Mirzaei, et al., 2018)
LPQSVHLDL (LL-9)	*K.marxianus* (PTCC5195)	ACE-I activity IC_{50}: 22.88 mmol/L	Not reported	1.180	Protein extraction with sonication and enzymatic hydrolysis by Trypsin. Ultrafiltration (3, 5, 10 kDa) and RP-HPLC	(Mirzaei, et al., 2018)

TPTQQS	*S. cerevisiae*	ACE-I IC_{50}:73.25 µg/mL (0.11 mmol/L)	Non-competitive - Adjacent to subdomain I of the testis ACE (tACE) -A linker between the α helix of the enzyme and zinc ion in the active site of the enzyme	0.656	Enzymatic hydrolysis by yeast extracted enzymes including Lywallzyme and Protease (1%); 40 ºC; 2 h); ultrafiltration (10, 30 and 100 kDa), FPLC with Sephadex peptide column and anion exchange column.	(Ni, Li, Guo, et al., 2012) (Ni, Li, Liu, & Hu, 2012)
YDGGVFRVYT	*S. cerevisiae*	-ACE-I IC_{50} :0.07 mg/mL -Decreasing the average blood pressure in SHR by 30 mmHg after oral administration at the dose of 1 mg/kg body weight.	Competitive	1.178	Enzymatic hydrolysis of cell-free extract from *S. cerevisiae* culture by pepsin, trypsin and protease N (Enzyme ratio: 1% V/V), 12 h, optimum pH and temperature for each enzyme Ultrafiltration and FPLC	(Kim, et al., 2004)
PWW	*SBY*	ACE-I IC_{50}: 465.1 µM -Decreasing SBP, DBP and MBP in SHR after oral administration at the dose of 300 mg/kg body weight.	Not reported	<3	Autolysis (70 ºC, 5 h) and enzymatic hydrolysis (E/S:4%, 4.5 h) by protease from *Cynara cardunculus*. Ultrafiltration, Purification by ZipTip C18 pipette tips	(M. Amorim, Marques, et al., 2019)

SQPW	*SBY*	ACE-I IC_{50}: 12 µM -Decreasing SBP, DBP and MBP in SHR after oral administration at the dose of 300 mg/kg body weight.	Not reported	<3	Autolysis (70 ºC, 5 h) and enzymatic hydrolysis (E/S:4%, 4.5 h) by Protease from *Cynara cardunculus*. Ultrafiltration, Purification by ZipTipC18 pipette tips	(M. Amorim, Marques, et al., 2019)
RYW	*SBY*	ACE-I IC_{50}: 77.2 µM -Decreasing SBP, DBP and MBP in SHR after oral administration at the dose of 300 mg/kg body weight.	Not reported	< 3	Autolysis (70 ºC, 5 h) and enzymatic hydrolysis (E/S:4%, 4.5 h) by Protease from *Cynara cardunculus*. Ultrafiltration, Purification by ZipTipC18 pipette tips	(M. Amorim, Marques, et al., 2019)
Fraction < 3 kDa	*S. cerevisiae*	ACE-I IC_{50}: 84.2 µg/mL	Not reported	< 3	Autolysis (70 °C, 5 h) and subsequent hydrolysis (using extract of *Cynara cardunculus*, E/S: 4%, 4.5 h) *Ultrafiltration*	(M. Amorim, Pinheiro, et al., 2019)
Fraction < 5 kDa	*Yeast (S. cerevisiae) powder (Guangzhou, China)*	-ACE-I IC_{50} : 29.3 µg/mL -Super oxide onion scavenging IC_{50}: 2.60 mg/mL -α-glucosidase inhibitory: (IC_{50}:10.62 mg/mL)	Not reported	< 5	Enzymatic hydrolysis by yeast protein extraction enzyme (Lywallzyme and a Protease) (3.5% w/w) Ultrafiltration	(Hu, et al., 2014)

Whole hydrolysate	*S. pasturianus*	-ACE-I IC_{50} :379 µg/mL -FRAP activity: 374 µM TE/mL	Not reported	Whole fraction	Autolysis 36 ºC, 6 h	(E. F. Vieira, et al., 2017)
Not specified	*Brewer's yeast (Saccharomyces sp.)*	Protective effect on gastric mucosa	Cytoprotective effect by stimulation of prostaglandins and mucus production and other unclarified mechanisms	> 3 and < 3	Autolysis (70 °C, 5 h) and subsequent hydrolysis (using extract of *C. cardunculus*, E/S: 4%, 4.5 h)	(M. M. Amorim, et al., 2016)
Yeast hydrolysate	Dry yeast cell	ACE-I IC_{50}: 266.13 µg/mL Reducing SBP in SHRs by 20.5 mm Hg, 15 days after administration of 1200 mg/kg of the yeast hydrolysate	Improvement of the NO production.	Whole fraction	Hydrolysis of intracellular proteins from dry yeast using crude enzyme with protease and β-glucanase activities, generated from *Bacillus subtilis* Hu 528.	(Huang, et al., 2020)
Yeast hydrolysate	Sugar cane yeast extract (*S. cerevisiae*)	Iron binding properties	Not reported	< 5	Enzymatic hydrolysis of yeast extract by alcalase, Viscozyme L, and Protex 51FP.	(de la Hoz, et al., 2014)
Yeast hydrolysate with high content of Diketopiperazine (DKP)	SBY	-Glucose tolerance activity - DPPH• scavenging IC_{50}: 1.9 mg/mL -ABTS•+ scavenging IC_{50}: 0.9 mg/mL	Not reported	-	Enzymatic hydrolysis of yeast suspension with neutrase (endo protease from B.*amyloliquifaciens*), alcalase (endo protease from *B. licheniformis*), protamex (*Bacillus* protease complex), flavourzyme (Endo and exo protease from	(E.Y. Jung, et al., 2011)

					Asp.oryzae), and ficin (Endo protease from Fig latex). 50 ºC, E/S:1/100, optimum pH of each enzyme	
Selenium-rich yeast peptide fractions	Normal and selenium-rich (1900 µg Se/gr) *brewer's yeast* protein extract	In vitro, cellular and in vivo antioxidant activity	Not reported	<1	Enzymatic hydrolysis with yeast extract enzymes (8 h, pH 10, 60 ºC, E/S: 8000 U/g) and alkaline protease (6 h, pH 11, 60 ºC, 6000 U/g)	(Guo, et al., 2020)
Peptide fraction	*S. cerevisiae*	Antiulcer and antiproliferative properties against tumour cells	Not reported	< 3	Autolysis of the SBY (70 ºC, 5 h) and hydrolysis of ultrafiltrated fraction (10 kDa) with proteases from an aqueous extract of *C. cardunculus (E/S:4%, 4.5 h).*	(M. M. Amorim, et al., 2016)
YGKHVAVHAR (YHR-10)	Synthetic peptides designed according to the sequence of YR-10 as the template		- DPPH• scavenging (IC_{50}: 200 µM) and ferric reducing activity (820 µm$FeSO_4$/ mmol) -Reducing the level of MDA and protein carbonyl in cells under oxidative stress	1.137.31	Designing using YR-10 as template based on replacing Pro with HisSynthesis by solid phase method	
GKPVAVPA (GA-8)			- DPPH• scavenging (IC_{50}: 500 µm) and ferric reducing activity (80 µm $FeSO_4$/ml)	0.739	Designing after in silico simulation of gastrointestinal digestion of YR-10.	

			-Reducing the level of MDA and protein carbonyl in cells under oxidative stress		Synthesis by solid phase method.	
PAR-3			- DPPH• scavenging activity (IC_{50}: 2500 µm), Ferric reducing activity (220 µm $FeSO_4$/mmol), inhibition of linoleic acid oxidation -Increasing the viability of Caco-2 cells under oxidative stress	0.342	Designing based on three amino acids in the C-terminal of YR-10. Synthesis by solid phase method.	
YGKPVAVPAR (YR-10)	*S. cerevisiae*	ACE-I IC_{50}:139.554 µmol/L	Non-competitive (according to the result of molecular docking study and kinetic of enzyme activity	1.057	Synthetic peptide Originally obtained from *S.cerevisiae* protein hydrolysate	(Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019)
YGKHVAVHAR (YHR-10)	Synthetic peptides designed according to the sequence of YR-10 as a template	ACE-I IC_{50}: 61.91 µmol/L	Non-competitive (according to the result of molecular docking study and kinetic of enzyme activity	1.137	Designing based on replacing Pro with His using YR-10 as a template. Synthesis by solid phase method.	
GKPVAVPA (GA-8)		ACE-I IC_{50}: 463.230 µmol/L	Non-competitive (According to the result of molecular docking study)	0.739	Designing after in silico simulation of gastrointestinal digestion of YR-10. Synthesis by solid phase method.	
GKHVAVHA		ACE-I IC_{50}:135.135 µmol/L	Non-competitive	0.817	Designing based on replacing Pro	

(GHA-8)			(according to the result of molecular docking study and kinetic of enzyme activity) Changing the conformation of Zn in the active site of the enzyme		with His in the sequence of GA-8. Synthesis by solid phase method.	
PAR (PAR-3)		ACE-I IC_{50}: 514.024 µmol/L	Non-competitive (according to molecular docking analysis)	0.342	Designing based on three amino acids in the C-terminal of YR-10. Synthesis by solid phase method	
VLSTSFPPK (VL-9)	*K. marxianus*	ACE-I activity IC_{50}: 80.015 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	0.974	Synthetic peptides Originally obtained from *K.marxianus* protein hydrolysate	
VLSTSFPPY (VY-9)	Synthetic peptides designed according to the structure of VL-9 as the template	ACE-I activity IC_{50} : 75.08 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	1	Designing based on substitution of Lys with Tyr in the sequence of VL-9. Synthesis by solid phase method	(Mirzaei, Mirdamadi, & Safavi, 2020)
VLSTSFPPW (VW-9)		ACE-I activity IC_{50} : 98.08 µmol/L	Non-competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	1.032	Designing based on substitution of Lys with Trp in the sequence of VL-9. Synthesis by solid phase method.	
VLSTSFPPF (VF-9)		ACE-I activity IC_{50} : 139.56 µmol/L	Un-competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	0.99	Designing based on substitution of Lys with Phe in the sequence of VL-9. Synthesis by solid phase method.	

VLSTSFCPK (VCK-9)		ACE-I activity IC_{50}: 66.39 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	0.980	Designing based on substitution of Pro with Cys in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFYPK (VYK-9)		ACE-I activity IC_{50}: 78.32 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	0.89	Designing based on substitution of Pro with Tyr in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFHPK (VHK-9)		ACE-I activity IC_{50}: 80.27 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	1.14	Designing based on substitution of Pro with His in the sequence of VL-9. Synthesis by solid phase method.
STSFPPK (SK-7)		ACE-I activity IC_{50}: 57.65 µmol/L	Competitive inhibitor (according to kinetic of enzyme activity and molecular docking analysis)	0.76	Designing based on in silico simulation of gastrointestinal digestion of VL-9. Synthesis by solid phase method.
VLSTSFPPY (VY-9)	Synthetic peptides designed according to the structure of VL-9 as a template	ABTS•+ scavenging (82% /25 µmol/L) and ferric reducing (80 µmol $FeSO_4$ /L) activity		1.009	Designing based on substitution of Lys with Tyr in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFPPW (VW-9)		ABTS•+ radical scavenging (65% /25 µmol/L) and ferric reducing (125 µmol $FeSO_4$ /L) activity		1.03	Designing based on substitution of Lys with Trp in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFPPF (VF-9)		Ferric reducing (40 µmol $FeSO_4$ /L) activity		0.99	Designing based on substitution of Lys with Phe in

					the sequence of VL-9. Synthesis by solid phase method.
VLSTSFCPK (VCK-9)		DPPH• (80% /500 μmol/L of peptide) and ABTS•+ (85% /25 μmol/L of peptide) radical scavenging and ferric reducing activity, and ferric reducing (140 μmol $FeSO_4$/L) activity) Cytoprotective effect against oxidative stress in $Caco_2$ cell line	Reducing lipid and protein oxidation in Caco-2 cells Activation of Keap1-Nrf2/ARE signalling pathway Increasing the activity of Cat, SOD and GPx in cells	0.98	Designing based on substitution of Pro with Cys in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFYPK (VYK-9)		ABTS•+ radical scavenging (80 % /25 μmol/L of peptide) and ferric reducing (50 μmol $FeSO_4$/L) activity Cytoprotective effect against oxidative stress in $Caco_2$ cell line	Reducing lipid and protein oxidation in cells Activation of Keap1-Nrf2/ARE signalling pathway Increasing the activity of Cat and GPx in cells	0.89	Designing based on substitution of Pro with Tyr in the sequence of VL-9. Synthesis by solid phase method.
VLSTSFHPK (VHK-9)		Ferric reducing (40 μmol $FeSO_4$/L) activity		1.014	Designing based on substitution of Pro with His in the sequence of VL-9. Synthesis by solid phase method.
STSFPPK(SK-7)		Ferric reducing (45 μmol $FeSO_4$/L) activity Cytoprotective effect against oxidative stress in Caco2 cell line	Reducing lipid and protein oxidation in cells Activation of Keap1-Nrf2/ARE signalling pathway	0.768	Designing based on in silico simulation of gastrointestinal digestion in the sequence of VL-9. Synthesis by solid phase method.

			Increasing the activity of Cat and SOD in cells			
FVAPFP	*S. cerevisiae*	Antiaging	Promoting dose and time-dependent activation of proteasome, autophagy, chaperones and antioxidant responses related genes	Non reported	Originally isolated from yeast extract. Later was synthesized by solid phase method.	(Sklirou, et al., 2015)
Two peptide fractions A (Ala:Phe/1:1), B(Gly:Phe/1:1, 1:2)	*BY-G brewer's yeast*	ACE-I activity Fraction A IC_{50}: 3.0 µmol/L Fraction B IC_{50}: 3.4 µmol/L Antihypertensive activity in the SHR model	Non reported	Fraction A (0.237) Fraction B (0.223)	Hydrolysis of BY-G with Alcalase (2.4 L, pH:7.5-8.5, 50 ºC, 12 h). Amberlite XAD-2 resin was used for peptide absorption.	(Kanauchi, et al., 2005)
Peptide fraction	*K. marxianus* PTCC 5195	Bacteriostatic and bactericidal effect against *St. aureus* MIC: 13.3 mg/mL, MBC: 21.3 mg/mL and *Lis.monocytogenes* MIC:13.3 mg/mL	Not reported	5-10	Autolysis (2.5% yeast cells in distilled water, 52 ºC, pH 5, 120 RPM, 96 h) and ultrafiltration	(Mirzaei, Mirdamadi, Ehsani, & Aminlari, 2016)
glycopeptide-rich yeast extracts.		Anti-inflammatory activity (suppressing the production of IFN-γ in human whole blood assay)	Not reported		Sequence enzymatic hydrolysis of brewer's and baker's yeast (*S. cerevisiae)* by glutaminase, Corolase PN-L, Alcalase 2.4 L, Flavourzyme 1000 L and Trypsin each at 5% w/w.	(Williams, et al., 2016)

Yeast protein hydrolysate and peptide fractions	*S. cerevisiae IFO 2346*	Anti-obesity and anti-abdominal fat accumulation effect.	Not reported	<10	Enzymatic hydrolysis with 1000 units of Bromelain at 30 ºC for 4 h.	(E. Y. Jung, et al., 2014)

Notes: SBP: Systolic blood pressure ; DBP : Diastolic blood pressure; MBP: Mean blood pressure ; SHR: Spontaneously Hypertensive Rats.

6.2.Structure-activity relationship of ACE-I peptides

ACE is divided into two isoforms: somatic ACE (sACE) and testicular ACE (tACE). In almost all studies, sACE as a single polypeptide chain serves as the ACE model. It comprises a C-terminal cytosolic domain, a heavily glycosylated extracellular domain, and a hydrophobic transmembrane domain. The extracellular domain comprises two homologous N (612 residues) and C (600 residues) domains. Each one contains an active catalytic site in the middle, distinguished by a zinc-binding motif-HEXXH, where X can be any amino acid. *In vivo*, the C domain of ACE is known as the main active site of angiotensin I cleavage. ACE`s C-terminal hydrophobic sequence enables the enzyme to attach to the surface of the cell membrane. sACE has three main active site pockets, S1, S1', and S2. The S1 pocket includes three residues, Ala354, Glu384, and Tyr523, while S2 pockets include, Gln281, His353, Tyr520, His513, and Lys511 residues, and the S1' only contains Glu162 residue. ACE is a metalloproteinase with a zinc ion interacting with His383, His387, and Glu411. These regions are responsible for ACE`s bioactivity and the region for binding inhibitors (Xue, Yin, Howell, & Zhang, 2021) (Fig. 3A).

Most ACE-I interact with C and N domains of sACE and tACE through hydrogen and hydrophobic interactions or bind directly to the catalytic Zn^{2+}. Multiple hydrogen bonds lead to the formation of a stable enzyme-peptide complex and inhibition of the enzyme.

Most of the studies introduced peptide fraction MW < 3 kDa responsible for ACE-I activity of yeast protein hydrolysate and autolysate (M. Amorim, Marques, et al., 2019; M. Amorim, Pinheiro, et al., 2019; Hu, et al., 2014). Aromatic (Trp, Phe, Tyr, Pro) and branched aliphatic hydrophobic amino acids (Gly, Val, Leu, Ile) ILe) in the sequences of ACE-I peptides, especially at C-terminal, are known as important residues that contribute to the interaction of peptides with enzyme (M. Amorim, Marques, et al., 2019). In addition, the presence of positively charged amino acids (Lys, His, Arg) in peptide sequence contributes to ACE-I activity, particularly through interaction with Zn^{+2} (M. Amorim, Marques, et al., 2019).

In a recent study performed to analyse different ACE-I peptides after enzymatic hydrolysis of yeast powder with *Bacillus subtilis* (Hu 528)-derived enzymes, it was found that all 778 identified peptides contain 5-21 amino acids with MW between 460-2145 Da (91.18% of them were smaller than 1500 Da). Also, 428 peptides contained hydrophobic amino acid at N-terminus, and 79 peptides contained Trp, Tyr and Pro at the C-terminal (Huang, et al., 2020). After autolysis, ultrafiltration and further hydrolysis of SBY with proteases from *Cynara cardunculus*, SPQW, PWW and RYW were purified. The presence of hydrophobic amino acids in these peptide sequences, particularly at the C-terminal, aids in the interaction with the enzyme (M. Amorim, Marques, et al., 2019).

VLSTSFPPK (VL-9) and LPQSVHLDK(LL-9) identified from *K. marxianus*, with IC_{50} values of 15.20 and 22.88 µM, respectively, contained more than 40% hydrophobic amino acids (Mirzaei, et al., 2018). By considering the structure of these two peptides, it was found that the presence of two Pro in C-terminal and Val and Leu in N-terminal of VL-9, also the presence of Asp and Glu in the sequence of LL-9 and Lys in the C-terminal of both peptides play important roles in ACE-I activity. Following a molecular docking study, VL-9 exhibited interactions with the S2 and S1'pockets of ACE, owing to Val1, Leu2, Thr4, Ser5 and Lys 9. LL-9, Also interacted with S1 and S2 pockets of ACE via H-bonds with Glu3, His6, Asp8, and Lys9. None of them interacted directly with Zn^{2+} in the enzyme's active site but did cause some changes in the bond length of Zn^{2+} with surrounding atoms and disrupted its tetrahedral coordination (Mirzaei, et al., 2018).

Our research group investigated the role of some amino acids in the sequence of ACE-I peptides by replacing or omitting special amino acid residues from the original peptides, VL-9 and YR-10 and comparing their activity after chemical synthesis two different studies. Table 4 summarizes the results, indicating that Lys and Tyr play a more significant role in the C-terminus than Trp and Phe. Cys plays a more important role in the antepenultimate position peptides than Pro, Tyr, and His. The removal of Val and Leu from the N-terminus of VL-9, resulted in a significant increase in ACE-I activity. Furthermore, results obtained by substituting His for Pro in two different sequences (GA-8 and YR-10) demonstrated the importance of His in the sequence of ACE-I peptides. Furthermore, we discovered that a peptide containing only three C-terminus amino acid residues of the original peptide (PAR-3) had lower activity; confirming the importance of entire peptide sequence in ACE interaction (Mirzaei, Mirdamadi, & Safavi, 2020; Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019).

Table 4-Comparing ACE-I activity of peptides originated from VLSTSFPPK(VL-9) and YGKPVAVPAR (YR-10) (Mirzaei, Mirdamadi, & Safavi, 2020; Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019).

Peptide sname		Peptide sequence									ACE-I IC_{50}(µmol/l)	Mechanism of inhibition
VL-9		V	L	S	T	S	F	P	P	K	80.015	Competetive
VY-9		V	L	S	T	S	F	p	P	Y	75.08	Competetive
VW-9		V	L	S	T	S	F	p	P	W	98.08	Non-Competetive
VF-9		V	L	S	T	S	F	p	P	F	139.56	Un-Competetive
VCK-9		V	L	S	T	S	F	C	P	K	66.39	Competetive
VYK-9		V	L	S	T	S	F	Y	P	K	78.32	Competetive
VHK-9		V	L	S	T	S	F	H	P	K	80.27	Competetive
SK-7				S	T	S	F	p	P	K	57.65	Competetive
YR-10	Y	G	K	P	V	A	V	P	A	R	139.554	Non-competitive
GA-8		G	K	P	V	A	V	P	A		463.23	Non-competitive
YHR-10	Y	G	K	H	V	A	V	H	A	R	61.91	Non-competitive
GHA-8		G	K	H	V	A	V	H	A		135.13	Non-competitive
PAR-3								P	A	R	514.024	Non-competitive

In a similar research, Ni, Li, Guo, et al. (2012) reported the activity of TPTQQS was reduced by 40% when amino acids from N-terminal were deleted, and Gln4 was replaced with Leu4. Ser6 was the only amino acid that interacted with the Zn^{2+} ions in the enzyme`s active site.

Some researchers have characterized the inhibitory pattern of yeast-derived peptides. Considering the kinetic activity of the enzyme in the presence and absence of different concentrations of peptides and performing a Lineweaver-Burk plot brings the opportunity to find the inhibition mechanism of peptides. Also, molecular docking studies have been applied by some researchers to find the kind of interactions between peptides and ACE and evaluate the inhibition mechanism (Mirzaei, Mirdamadi, & Safavi, 2020; Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019).

As shown in Tables 3 and 4, several yeast-derived peptides, including YDGGVFRVYT, VL-9, VY-9, VCK-9, VYK-9, VHK-9, and SK-9 were known to be competitive inhibitors. Competitive inhibitors form hydrogen and hydrophobic interactions with ACE`s S1, S2 and S1' pockets, as well as directly with the catalytic $Zn^{+2}$ in the active site, inhibiting the enzyme`s interaction with its natural substrate. In this case, the k_m (substrate concentration when the reaction reaches half of its maximum value) of the enzyme reaction increased, but the V_{max} (the highest reaction rate) remained constant (Kim, et al., 2004; Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019). The stable interactions of VL-9, VY-9, VCK-9, VYK-9, VHK-9, and SK-9

peptides with amino acid residues in the enzyme's active site via hydrogen and hydrophobic bonds were also revealed by molecular docking studies (Mirzaei, Mirdamadi, & Safavi, 2020).

TPTQQS, VW-9, YR-10, YHR-10, GA-8, GHA-8, and PAR-3, on the other hand, inhibit the ACE via a non-competitive mechanism (Mirzaei, Mirdamadi, & Safavi, 2020; Mirzaei, Mirdamadi, Safavi, & Hadizadeh, 2019; Ni, Li, Guo, et al., 2012). Non-competitive inhibitors can bind with ACE at a site other than the active site, regardless of whether the substrate binds to enzymes. V_{max} decreases in the presence of non-competitive inhibitors, but k_m does not change. According to Ni, Li, Guo, et al. (2012), Thr1, Thr3, and Gln4 are important residues in orienting TPTQQS peptide onto the lid structure of ACE, keeping peptide out of the active site of the enzyme and hence responsible for non-competitive inhibition. The mechanism of inhibition became competitive after the deletion of Thr1, Pro2, Thr3, and Gln4 from the sequence of TPTQQS peptide. VF-9 has been identified as an uncompetitive inhibitor. This means that it only binds to ACE when the substrate is also bound to ACE. When uncompetitive inhibitors are present, V_{max} and K_m decrease (Xue, et al., 2021).

6.3. Structure-activity relationship of antimicrobial peptides

The antimicrobial activity of AMPs is affected by several factors, including size, charge, degree of helicity, hydrophobicity and sequence. Several researchers (Ahmed & Hammami, 2019; R. Zhang, et al., 2016) have previously reviewed the structural characteristics of AMPs. This section will briefly discuss some important parameters affecting the structure-function relationship of bioactive peptides.

AMPs are classified into four major classes based on their secondary structure: ß- sheet, α-helix, loop and extended peptides, with α-helix and ß -sheet being the most common forms. α-helical peptides are typically shorter than 40 amino acids long, free of Cys, and contain approximately 50% hydrophobic residues. In an aqueous solution, they have a linear structure that transforms into an amphipathic helical structure upon contacting with bacterial membrane. ß -sheet AMPs are usually cyclic molecules. They typically contain Cys residues and are stabilized by disulfide bonds (Ahmed & Hammami, 2019).

The vast majority of AMPs are cationic to target negatively charged components of the cell wall or plasma membrane (P. Branco, et al., 2015). Many anionic AMPs, on the other hand,

are known to be involved in the antimicrobial mechanism of yeast. Divalent metal cations such as Fe^{++}, Mn^{++} and Mg^{++} increase anionic AMPs activity by facilitating their binding to anionic membrane components (Rizk, et al., 2018). Having both hydrophilic and hydrophobic domains is another key element for interacting AMPs with target cytoplasmic membrane. Most AMPs contain 50% hydrophobic residues. The degree of hydrophobicity determines their antimicrobial activity and controls the penetration of peptides into lipid layer of cell membrane (Ahmed & Hammami, 2019; P. A. B. Branco, 2018).

7. Peptides' stability

Displaying a wide range of activities, bioactive peptides attracted the attention of consumers and food manufacturers as natural alternatives to synthetic drugs. An important challenge for using bioactive peptides in the formulation of functional food is to preserve their biological properties under different thermal and non-thermal conditions and during the digestion process (Agyei, et al., 2018). The peptide sequence and its secondary structure are important for its binding property on its target (i.e. enzymes, receptors, metal ions, antibodies), which dictates its further biological activity (Agyei, et al., 2018).

In some reported studies, the yeast protein hydrolysates, peptide fractions, and purified peptides have been investigated to stabilize their bioactivity after exposure to various thermal and non-thermal treatments. Peptides are inherently sensitive to heat treatments. Exposure to high temperatures may change the secondary structure of peptides. Peptides with a high proportion of β-sheet structure are supposed to be more sensitive to heat treatment (Asaduzzaman & Chun, 2015; Udenigwe & Fogliano, 2017). The concentration of salt may also influence the structure of peptides. The change in conformation of side chains in amino acid residue due to high salt concentration may lead to less bioactivity of peptides (Zhu, Zhang, Kang, Zhou, & Xu, 2014). In addition, exposure to acidic or alkaline conditions is another common reason for peptide instability. pH treatment may influence the aggregation properties of peptides. It may change the ionization of peptides and lead to structural denaturation, low solubility, cleavage of amide bonds, or damage to amino acids. Racemization may also occur under alkaline conditions, resulting in a loss of activity (Jang, Liceaga, & Yoon, 2016; B. Wang, Xie, & Li, 2019; X. Wang, et al., 2017). In a study performed by Thyab Gddoa Al-sahlany, et al. (2020), the peptide of 770 g/mol obtained from *S. cerevisiae* was investigated for the thermal and pH stability of antimicrobial activity against the antimicrobial activity *E.coli* and *S. aureus*. It was known to

be thermostable at 50-90 °C for 30 min. 93% and 95% stability activity was obtained after heating at 100 °C for 30 min. Although, heating at 120 °C for 30 min decreased the activity by 68% and 77%. The isolated peptide was stable at pH 5-7 at 4 °C and 25 °C during the first 24 h.

Peptides' stability during gastrointestinal digestion is essential for the applications of peptides as health-promoting agents. In vitro gastrointestinal incubation provides a good opportunity for researchers to assess the protein hydrolysates or peptides' stability.

In a limited number of research, peptide stability has been investigated with an insight into peptide sequences and the role of each amino acid. Our research group (Mirzaei, Mirdamadi, Safavi, & Soleymanzadeh, 2020) studied and compared the stability of antioxidant synthetic peptides including VLSTSFPPK (VL-9), VLSTSFCPK (VCK-9), VLSTSFHPK (VHK-9) and VLSTSFYPK (VYK-9) against heat, pH and NaCl treatments. We found that VL-9 is stable under heat (50, 70, 90 °C for 6 min) and salt (2, 4, 6, 8 gr/100 mL) treatment. However, peptides with His and Cys in place of Pro (VHK-9 and VCK-9) in the antepenultimate position had lower heat and NaCl stability, which could be due to peptide secondary structure differences, higher susceptibility of Cys to heat induced aggregation through disulfide bonds and production of dimers or trimers, side chains modification and cleavage of thiol bonds and formation of disulfide bonds upon exposure to heat, and also high susceptibility of His side chains to oxidation. Acidic and alkaline conditions (pH 2, 5, 9, and 11) were unfavourable for all synthetic peptides. The second group of peptides, including VLSTSFPPK (VL-9), VLSTSFPPY (VY-9), VLSTSFPPW (VW-9), VLSTSFPPF (VF-9) were compared for stability against a digestion process simulated using gastric fluid containing pepsin and intestinal fluid containing trypsin and chymotrypsin, according to the method of United States Pharmacopeia. When compared to peptides with no charges and with hydrophobic nature, VL-9 with a positive charge and hydrophilic property demonstrated the highest stability and remained stable during the first hour of gastric digestion. Finally, we discovered that positively charged and hydrophilic peptides have improved gastrointestinal digesting stability.

Another study looked at the stability of yeast protein extract and peptide fractions under the gastrointestinal tract conditions. The SBY yeast extract produced by autolysis (70 °C, 5 h) and subsequent hydrolysis (using the extract of *C. cardunculus*, E/S: 4%, 4.5 h) as well as peptide fractions (<3 and >3 kDa) were incubated under three digestion conditions: mouth (amylase 100 U/mL, for one minute at 37 °C), gastric (pepsin>250 U/mL, pH=2, for 60 min at 37 °C), and intestinal (pH=6, pancreatin 2 gr/L, bile salt: 0.25 mg/mL for 120 min at 37 °C). Results showed an increasing level of ACE-I activity of yeast autolysate after final digestion, indicating

further hydrolysis of protein or peptides and production of new peptide sequences with ACE-I activity (M. Amorim, Pinheiro, et al., 2019).

Some studies evaluate the digestion stability of peptide sequences through *in silico* simulation of digestion enzyme activities. However, in this type of study, the general conditions of gastrointestinal digestion, including the effect of acids, bile salts, etc., have been ignored. Among these, the stability of an ACE-I peptide, VLSTSFPPK (VL-9), against the digestion process was investigated by simulating the activity of digestive enzymes using *in silico* approach (www. expasy.org/tools/). The two peptide sequences before and after *in silico* simulated digestion were synthesized chemically and compared for ACE-I activity. The resulting digested peptide, STSFPPK (SK-7) exhibited a 27% higher ACE-I activity. The increased activity could be explained by the smaller size of the obtained peptides, which improves their interaction with the enzyme (Mirzaei, Mirdamadi, & Safavi, 2020). In another similar study, the digestion stability of antioxidant peptide, YGKPVAVPAR (YR-10) was investigated after *in silico* stimulation of enzymatic digestion activity. The activity of the digested peptide, GKPVAVPA (GA-8) was compared with the original one. The removal of Tyr and Arg, respectively at the N and C terminus of YR-10, decreased the DPPH•, ABTS•+radical scavenging and ferric reducing capacity of the resulting peptide. Although, GA-8 exhibited higher inhibitory activity of linoleic acid oxidation that could be explained by a higher hydrophobicity and higher values of the aliphatic index (the relative volume occupied by aliphatic side chains) compared to YR-10 (Mirzaei, Mirdamadi, Safavi, Zare, et al., 2019).

8. Bioinformatics tools to predict structure, physicochemical properties and bioactivity of peptides

The conventional methods used to identify bioactive peptides include protein extraction, hydrolysis, purification, identification, and synthesizing peptide sequences for confirming bioactivity. This strategy is time-consuming and expensive. To overcome these limitations, a range of integrated bioinformatics approaches have been used to predict the potential of different protein sources for generating bioactive peptides. Data-based bioinformatics tools are potential cost-effective approaches to screen and predict protein potency as a precursor of bioactive peptides. In this approach, the enzymatic hydrolysis of protein is simulated based on protease cleavage specificities. The databanks information for the sequences and physicochemical properties of various identified bioactive peptides are used to predict the bioactivity of produced peptides (Han, Maycock, Murray, & Boesch, 2019). Chemical

synthesis of anticipated sequences and experimental study of expected bioactivity have also been used by some researchers to verify the predicted activity of peptides (Pearman, Ronander, Smith, & Morris, 2020; Tran, et al., 2021).

Also, with knowledge of microbial protease activity and the aid of in silico modelling of fermentation, it would be possible to predict the optimum fermentation condition for improving the production of bioactive peptides (Chai, et al., 2020) (Fig. 3B-III).

The main databases of peptides and the list of available tools for predicting the physicochemical properties of peptides are introduced in Table 5.

Table 5. Main tools and database for in silico analysis of bioactive peptides.

Name	**Website**	**Description**
Peptide property calculator	https:// pepcalc.com/	Makes calculations and estimations on physiochemical properties of peptides
Pepdraw tool	http://www. tulane.edu/~biochem/ WW/PepDraw/	A tool to draw peptide primary structure and calculate theoretical properties
Protparam tool	http://web.expasy.org/protparam/	Computation of various physical and chemical parameters for a given protein
Peptide cutter	http://web.expassy.org/Peptide-cutter/	Predicts potential cleavage sites cleaved by proteases or chemicals in a given protein sequence
Peptideranker	http://distilldeep.ucd.ie/PeptideRanker/	Ranks peptide(s) by the predicted probability that the peptide will be bioactive.
ToxinPred	http://crdd.osdd.net/raghava/toxinpred/	Predict and design toxic/non-toxic peptides.
BIOPEP	http://www.uwm.edu.pl/biochemia	Bioactive peptide database
Uniprot	www.Uniprot.org	
SwePep	www.Swepep.org	

Also, computer-aided molecular modelling methods have gained lots of attention for finding the structure-function relationship of bioactive peptides. This finding can provide a new opportunity to design new peptide sequences with specific functionality. Quantitative structure-function relationship (QSAR) provides a mathematical model for predicting the

activity and conformation of peptides (Fig. 3B-II). For this purpose, many peptide sequences are collected and used for building a model, then structural parameters descriptors will be selected, and finally, a QSAR model will be established (Han, et al., 2019).

Molecular docking studies also provide the opportunity to study the interaction of peptides with different target molecules such as enzymes and receptors. This kind of study would help researchers investigate the possible interactions of peptides and receptors and identify the contribution role of different amino acids in the interaction between ligands and receptors. This information helps scientists explain the potential mechanisms of peptide activity and design new peptide structures with improved bioactivity. Molecular docking studies between peptide-protein is usually done in the following steps by providing the crystal structure of protein receptors from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do//), drawing peptide structure using programs such as Hyperchem and obtaining the PDB file , Performing docking of ligand on the active site of the receptor using different programs such as Autodock, HADDOCK, MOE, etc., and considering the interactions bonds between ligand and receptor using different viewer programs such as Lig plot, Studio Discovery, etc. (Fig. 3B-I).

However, to the best of our knowledge, no study was performed to screen peptides released from yeast protein, although molecular docking studies assess the mechanism of yeast-derived antioxidant and ACE-I peptides. For example, we investigated the role of each residue in ACE-I peptide sequence VLSTSFPPK (VL-9) obtained from *K. marxianus* for ACE binding and possible interaction with Zn^{++} in the active site (Mirzaei, et al., 2018). In another research, we used a molecular docking study to investigate the possible interaction of antioxidant peptides with Keap1 involved in activating the Keap1-Nrf2 signalling pathway (Mirdamadi, et al., 2021). It's worth mentioning that the accuracy of data obtained from *in silico* studies needs to be confirmed using experimental analysis.

9. Conclusion and future perspective

Production of yeast extract with high content of bioactive peptides and improved bioactivity has been the subject of some studies reviewed in this paper. Although autolysis has been the most cost-effective and widely used reported method for manufacturing yeast extract, a downside of this technology is the lack of control over the autolysis process. Alternatively, yeast extract with numerous health benefits has recently been produced by enzymatic hydrolysis with improved functional properties. The most frequent enzymes utilized to produce

antioxidant and ACE-I peptides from yeast protein have been trypsin, pepsin, lywallzyme, alcalse, and viscozyme. More researches on the roles of several other proteolytic enzymes seems to be required. It is also important to note that any changes in hydrolysis parameters, such as protein and enzyme type, E/S ratio, and time, can produce a completely different product. Thus, standardization of the hydrolysis, separation and purification steps is required to achieve a product with the expected properties.

Different researchers have reported on the positive roles of yeast cells alone or in combination with L.A.B in improving the functional properties of fermented foods, particularly dairy products, due to yeast's proteolytic activity and production of bioactive peptides, indicating the need for more research on producing various fermented foods with improved functional properties using fermentation under controlled conditions. Several studies have also verified yeast`s ability to release antimicrobial peptides. Although, for the most part, their sequence are unknown.

Some challenges remain in the preparation of yeast-derived peptides as a food additive. First, most studies are limited to laboratory production and are not up-scaled to the industry yet. Second, the traditional production methods for producing protein hydrolysate and peptides are time-consuming and more efficient peptides production and purification methods must be imagined. Chemical synthesis can be considered as an alternative method for the production of bioactive peptides with specific sequences. Despite its high cost, chemical synthesis allows for producing large quantities of peptides with consistent quality.

On the other hand, computer-based modelling techniques such as data basis-aided bioinformatics tools, QSAR, and molecular docking studies would be widely used to predict peptides in the protein hydrolysate and find the structure-function relationship peptides. Thirdly, only a few studies have looked at the stability of yeast-derived peptides under various treatments used in food industries and gastrointestinal digestion, so the way is open for more research in this area. Fourth, the sensory properties of yeast-derived peptides must be taken into account in the formulation of various food products. These issues must be addressed before yeast-derived peptides can be used for food applications.

Credit authorship contribution statement

Mahta Mirzaei: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization,. **Amin Shavandi, Saeed Mirdamadi:** Writing – review & editing, Visualization,. **Nazila Soleymanzadeh, Paria Motahari, Niloofar**

Mirdamadi: Conceptualization, Methodology, Validation, Writing – original draft, Writing, **Muriel Moser, Gilles Subra, Houman Alimoradi, and Stanislas Goriely**: review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

The first Author (Mahta Mirzaei) acknowledges the fellowship provided by the Europe Program in IF@ULB - MARIE SKŁODOWSKA-CURIE Cofound Action (European Horizon 2020). Saeed Mirdamadi gratefully acknowledges the supports of the Iranian Research Organization for Science and Technology (IROST) (Grant Number: 1012195004), Iran National Science Foundation (INSF) (Grant Number: 95824648), National Institute for Medical Research Development (Grant Number: 962563), and Iranian Research Organization for Science and Technology (Grant Number: 1012198002).

References:

Agyei, D., Acquah, C., Tan, K., Hii, H., Rajendran, S. R. C. K., Udenigwe, C. C., & Danquah, M. K. (2018). Prospects in the use of aptamers for characterizing the structure and stability of bioactive proteins and peptides in food. *Analytical and Bioanalytical Chemistry, 410*, 297-306.

Ahmed, T. A. E., & Hammami, R. (2019). Recent insights into structure-function relationships of antimicrobial peptides. *Journal of Food Biochemistry, 43*, e12546.

Amorim, M., Marques, C., Pereira, J. O., Guardão, L., Martins, M. J., Osório, H., Moura, D., Calhau, C., Pinheiro, H., & Pintado, M. (2019). Antihypertensive effect of spent brewer yeast peptide. *Process Biochemistry, 76*, 213-218.

Amorim, M., Pinheiro, H., & Pintado, M. (2019). Valorization of spent brewer's yeast: Optimization of hydrolysis process towards the generation of stable ACE-inhibitory peptides. *LWT-Food Science and technology, 111*, 77-84.

Amorim, M. M., Pereira, J. O., Monteiro, K. M., Ruiz, A. L., Carvalho, J. E., Pinheiro, H., & Pintado, M. (2016). Antiulcer and antiproliferative properties of spent brewer's yeast peptide extracts for incorporation into foods. *Food Function, 7*, 2331-2337.

Annunziato, G., & Costantino, G. (2020). Antimicrobial peptides (AMPs): a patent review (2015-2020). *Expert Opinion on Therapeutic Patents, 30*, 931-947.

Asaduzzaman, A. K. M., & Chun, B.-S. (2015). Recovery of functional materials with thermally stable antioxidative properties in squid muscle hydrolyzates by subcritical water. *Journal of Food Science and Technology, 52*, 793-802.

Branco, P., Francisco, D., Chambon, C., Hébraud, M., Arneborg, N., Almeida, M. G., Caldeira, J., & Albergaria, H. (2013). Identification of novel GAPDH-derived antimicrobial

peptides secreted by Saccharomyces cerevisiae and involved in wine microbial interactions. *Applied Microbiology and biotechnology, 98*, 843-853.
Branco, P., Francisco, D., Monteiro, M., Almeida, M., Caldeira, J., Arneborg, N., Prista, C., & Albergaria, H. (2017). Antimicrobial properties and death-inducing mechanisms of saccharomycin, a biocide secreted by Saccharomyces cerevisiae. *Applied Microbiology and biotechnology, 101*, 159-171.
Branco, P., Viana, T., Albergaria, H., & Arneborg, N. (2015). Antimicrobial peptides (AMPs) produced by Saccharomyces cerevisiae induce alterations in the intracellular pH, membrane permeability and culturability of Hanseniaspora guilliermondii cells. *International Journal of Food Microbiology, 205*, 112-118.
Branco, P. A. B. (2018). Purification and biochemical/molecular characterisation of antimicrobial peptides produced by Saccharomyces cerevisiae and evaluation of their mode of action. *Lisboa:ISA*, 246 P.
Chai, K. F., Voo, A. Y. H., & Chen, W. N. (2020). Bioactive peptides from food fermentation: A comprehensive review of their sources, bioactivities, applications, and future development. *Comprehensive Reviews in Food Science and Food Safety, 19*, 3825-3885.
Chakrabarti, S., Guha, S., & Majumder, K. (2018). Food-Derived Bioactive Peptides in Human Health: Challenges and Opportunities. *Nutrients, 10*, 1738.
Chaves-López, C., Tofalo, R., Serio, A., Paparella, A., Sacchetti, G., & Suzzi, G. (2012). Yeasts from Colombian Kumis as source of peptides with Angiotensin I converting enzyme (ACE) inhibitory activity in milk. *International Journal of Food Microbiology, 159*, 39-46.
Chen, H. M., Muramoto, K., Yamauchi, F., Fujimoto, K., & Nokihara, K. (1998). Antioxidative Properties of Histidine-Containing Peptides Designed from Peptide Fragments Found in the Digests of a Soybean Protein. *J Agric Food Chem, 46*, 49-53.
Cotârleț, M., Vasile, A. M., Cantaragiu, A. M., Gaspar-Pintiliescu, A., Crăciunescu, O., Oancea, A., Moraru, A., Moraru, L., & Bahrim, G. E. (2019). Colostrum-derived bioactive peptides obtained by fermentation with kefir grains enriched with selected yeasts. *Fascicle VI-Food Technology, 43*, 54-68.
Daskaya-Dikmen, C., Yucetepe, A., Karbancioglu-Guler, F., Daskaya, H., & Ozcelik, B. (2017). Angiotensin-I-Converting Enzyme (ACE)-Inhibitory Peptides from Plants. *Nutrients, 9*, 316.
de Castro, R. J. S., & Sato, H. H. (2015). Biologically active peptides: Processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. *Food Research International, 74*, 185-198.
de Castro, R. S., & Sato, H. H. (2015). Biologically active peptides: Processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. *Food Research International, 74*, 185-198.
de la Hoz, L., Ponezi, A. N., Milani, R. F., Nunes da Silva, V. S., Sonia de Souza, A., & Bertoldo-Pacheco, M. T. (2014). Iron-binding properties of sugar cane yeast peptides. *Food Chemistry, 142*, 166-169.
Dick, K., Molan, P., & Eschenbruch, R. (2015). The isolation from Saccharomyces cerevisiae of two antibacterial cationic proteins that inhibit malolactic bacteria. *Journal of Grapevine Research, 31*, 105-116.
Đorđević, N. O., Todorović, N., Novaković, I. T., Pezo, L. L., Pejin, B., Maraš, V., Tešević, V. V., & Pajović, S. B. (2018). Antioxidant Activity of Selected Polyphenolics in Yeast Cells: The Case Study of Montenegrin Merlot Wine. *Molecules, 23*, 1971.
Gaspar-Pintiliescu, A., Oancea, A., Cotarlet, M., Vasile, A. M., Bahrim, G. E., Shaposhnikov, S., Craciunescu, O., & Oprita, E. I. (2020). Angiotensin-converting enzyme inhibition,

antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. *International Journal of dairy Technoligy, 73*, 108-116.
Gier, S., Lermen, M., Schmitt, M. J., & Breinig, F. (2019). Substitution of cysteines in the yeast viral killer toxin K1 precursor reveals novel insights in heterodimer formation and immunity. *Scientific Reports, 9*, 13127.
Guo, H., Guo, S., & Liu, H. (2020). Antioxidant activity and inhibition of ultraviolet radiation-induced skin damage of Selenium-rich peptide fraction from selenium-rich yeast protein hydrolysate. *Bioorganic Chemistry, 105*, 104431.
Halim, N. R. A., Yusof, H. M., & Sarbon, N. M. (2016). Functional and bioactive properties of fish protein hydolysates and peptides: A comprehensive review. *Trends in Food Science & Technology, 51*, 24-33.
Hamme, V., Sannier, F., Piot, J. M., Didelot, S., & Bordenave-Juchereau, S. (2009). Crude goat whey fermentation by Kluyveromyces marxianus and Lactobacillus rhamnosus: contribution to proteolysis and ACE inhibitory activity. *Journal Of Dairy Research, 76*, 152.
Han, R., Maycock, J., Murray, B. S., & Boesch, C. (2019). Identification of angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory peptides derived from oilseed proteins using two integrated bioinformatic approaches. *Food Research International, 115*, 283-291.
Hatoum, R., Labrie, S., & Fliss, I. (2013). Identification and Partial Characterization of Antilisterial Compounds Produced by Dairy Yeasts. *Probiotics Antimicrob Proteins, 5*, 8-17.
Hęś, M., Dziedzic, K., Górecka, D., Jędrusek-Golińska, A., & Gujska, E. (2019). Aloe vera (L.) Webb.: Natural Sources of Antioxidants - A Review. *Plant foods for human nutrition (Dordrecht, Netherlands), 74*, 255-265.
Hu, S., Wang, X., Guo, S., Li, L., & Hou, Y. (2014). Preparation and Properties of Bioactive Peptides from Yeast Protein. In *Focusing on Modern Food industry*.
Huang, Y., Wang, J., Hou, Y., & Hu, S. Q. (2020). Production of yeast hydrolysates by Bacillus subtilis derived enzymes and antihypertensive activity in spontaneously hypertensive rats. *Food Biotechnology, 34*, 262-281.
Jacob, F. F., Striegel, L., Rychlik, M., Hutzler, M., & Methner, F.-J. (2019). Spent Yeast from Brewing Processes: A Biodiverse Starting Material for Yeast Extract Production. *Fermentation, 5*, 51.
Jaeger, A., Aredt, E. K., Zannini, E., & Sahin, A. W. (2020). Brewer`s spent yeast(BSY), and underutilized brewing by-product. *Fermentation, 6*.
Jang, H. L., Liceaga, A. M., & Yoon, K. Y. (2016). Purification, characterisation and stability of an antioxidant peptide derived from sandfish (Arctoscopus japonicus) protein hydrolysates. *Journal of Functional Foods, 20*, 433-442.
Jung, E. Y., Cho, M. K., Hong, Y. H., Kim, J. H., Park, Y., Chang, U. J., & Suh, H. J. (2014). Yeast hydrolysate can reduce body weight and abdominal fat accumulation in obese adults. *Nutrition, 30*, 25-32.
Jung, E. Y., Lee, H. S., Choi, J. W., Ra, K. S., Kim, M. R., & Suh, H. J. (2011). Glucose Tolerance and Antioxidant Activity of Spent Brewer's Yeast Hydrolysate with a High Content of Cyclo-His-Pro (CHP). *Journal of Food Science, 76*, C272-C278.
Kanauchi, O., Igarashi, K., Ogata, R., Mitsuyama, K., & Andoh, A. (2005). A yeast extract high in bioactive peptides has a blood-pressure lowering effect in hypertensive model. *Current Medicinal Chemistry, 12*, 3085-3090.
Kim, J. H., Lee, D. H., Jeong, S. C., Chung, K., & Lee, J. S. (2004). Characterization of Antihypertensive Angiotensin I-Converting Enzyme Inhibitor from Saccharomyces cerevisiae. *Journal of Microbiology and Biotechnology, 14*, 1318-1323.

Li, Y., Sadiq, F. A., Liu, T., Chen, J., & He, G. (2015). Purification and identification of novel peptides with inhibitory effect against angiotensin I-converting enzyme and optimization of process conditions in milk fermented with the yeast Kluyveromyces marxianus. *Journal of Functional Foods, 16*, 278-288.

Lis, M., Bhatt, S., Schoenly, N. E., Lee, A. Y., Nislow, C., & Bobek, L. A. (2013). Chemical genomic screening of a Saccharomyces cerevisiae genomewide mutant collection reveals genes required for defense against four antimicrobial peptides derived from proteins found in human saliva. *Antimicrobial Agents and Chemotherapy, 57*, 840-847.

Liu, D., Ding, L., Sun, J., Boussetta, N., & Vorobiev, E. (2016). Yeast cell disruption strategies for recovery of intracellular bio-active compounds — A review. *Innovative Food Science & Emerging Technologies, 36*, 181-192.

Minkiewicz, P., Iwaniak, A., & Darewicz, M. (2019). BIOPEP-UWM Database of Bioactive Peptides: Current Opportunities. *International Journal of Molecular science, 20*, 5978.

Mirdamadi, S., Mirzaei, M., Soleymanzadeh, N., Safavi, M., Bakhtiari, N., & Zandi, M. (2021). Antioxidant and cytoprotective effects of synthetic peptides identified from Kluyveromyces marxianus protein hydrolysate: Insight into the molecular mechanism. *LWT-Food Science and technology, 148*, 111792.

Mirzaei, M., Mirdamadi, S., Ehsani, M., & Aminlari, M. (2016). Antioxidant, ACE-inhibitory and antimicrobial activities of Kluyveromyces marxianus protein hydrolysates and their peptide fractions. *Functional Foods in Health and Disease, 6*, 425-439.

Mirzaei, M., Mirdamadi, S., Ehsani, M. R., & Aminlari, M. (2018). Production of antioxidant and ACE-inhibitory peptides from Kluyveromyces marxianus protein hydrolysates: Purification and molecular docking. *Journal of Food and Drug Analysis, 26*, 696-705.

Mirzaei, M., Mirdamadi, S., Ehsani, M. R., Aminlari, M., & Ebrahim, H. (2015). Purification and identification of antioxidant and ACE-inhibitory peptide from Saccharomyces cerevisiae protein hydrolysate. *Journal of Functional Foods, 19*, 259-268.

Mirzaei, M., Mirdamadi, S., & Safavi, M. (2019). Antioxidant activity and protective effects of Saccharomyces cerevisiae peptide fractions against H2O2-induced oxidative stress in Caco-2 cells. *Journal of Food Measurement and Characterization, 13*, 2654-2662.

Mirzaei, M., Mirdamadi, S., & Safavi, M. (2020). Structural analysis of ACE-inhibitory peptide (VL-9) derived from Kluyveromyces marxianus protein hydrolysate. *Journal of Molecular Structure, 1213*, 128199.

Mirzaei, M., Mirdamadi, S., Safavi, M., & Hadizadeh, M. (2019). In vitro and in silico studies of novel synthetic ACE-inhibitory peptides derived from Saccharomyces cerevisiae protein hydrolysate. *Bioorganic Chemistry, 87*, 647-654.

Mirzaei, M., Mirdamadi, S., Safavi, M., & Soleymanzadeh, N. (2020). The stability of antioxidant and ACE-inhibitory peptides as influenced by peptide sequences. *LWT-Food Science and technology, 130*, 109710.

Mirzaei, M., Mirdamadi, S., Safavi, M., Zare, D., Hadizadeh, M., & Asadi, M. M. (2019). Synthesis, in vitro and cellular antioxidant activity evaluation of novel peptides derived from Saccharomyces cerevisiae protein hydrolysate: structure-function relationship : Antioxidant activity and synthetic peptides. *Amino Acids, 51*, 1167-1175.

Morya, V. K., Dong, S. J., & Kim, E.-k. (2014). Production and Characterization Te-Peptide by Induced Autolysis of Saccharomyces Cerevisiae. *Applied Biochemistry and Biotechnology, 172*, 3390-3401.

Moslehishad, M., Mirdamadi, S., Ehsani, M. R., Ezzatpanah, H., & Moosavi-Movahedi, A. A. (2013). The proteolytic activity of selected lactic acid bacteria in fermenting cow's and camel's milk and the resultant sensory characteristics of the products. *International Journal of Dairy Technology, 66*, 279-285.

Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S., & Takano, T. (1995). Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *Journal of Dairy Science, 78*, 777-783.

Nehme, N., Mathieu, F., & Taillandier, P. (2010). Impact of the co-culture of Saccharomyces cerevisiae–Oenococcus oeni on malolactic fermentation and partial characterization of a yeast-derived inhibitory peptidic fraction. *Food Microbiology, 27*, 150-157.

Ni, H., Li, L., Guo, S. S., Li, H. H., Jiang, R., & Hu, S. Q. (2012). Isolation and Identification of an Angiotensin-I Converting Enzyme Inhibitory Peptide from Yeast (Saccharomyces cerevisiae). *Current Analytical Chemistry, 8*, 180-185.

Ni, H., Li, L., Liu, G., & Hu, S. Q. (2012). Inhibition Mechanism and Model of an Angiotensin I-Converting Enzyme (ACE)-Inhibitory Hexapeptide from Yeast (Saccharomyces cerevisiae). *PLOS ONE, 7*, e37077.

Nwachukwu, I. D., & Aluko, R. E. (2019). Structural and functional properties of food protein-derived antioxidant peptides. *Journal of Food Biochemistry, 43*, e12761.

Nwachukwu, I. D., & Aluko, R. E. (2019). Structural and functional properties of food protein-derived antioxidant peptides. *J Food Biochem, 43*, e12761.

Osborne, J. P., & Edwards, C. G. (2007). Inhibition of malolactic fermentation by a peptide produced by Saccharomyces cerevisiae during alcoholic fermentation. *International Journal of Food Microbiology, 118*, 27-34.

Ovchinnikova, T. V. (2019). Structure, Function, and Therapeutic Potential of Marine Bioactive Peptides. *Marine Drugs, 17*, 505.

Patel, S., Rauf, A., Khan, H., & Abu-Izneid, T. (2017). Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. *Biomedicine & Pharmacotherapy, 94*, 317-325.

Pearman, N. A., Ronander, E., Smith, A. M., & Morris, G. A. (2020). The identification and characterisation of novel bioactive peptides derived from porcine liver. *Curr Res Food Sci, 3*, 314-321.

Peña, R., & Ganga, M. A. (2019). Novel antimicrobial peptides produced by Candida intermedia LAMAP1790 active against the wine-spoilage yeast Brettanomyces bruxellensis. *Antonie Van Leeuwenhoek, 112*, 297-304.

Podpora, B., Swiderski, F., sadowska, A., pitotrowska, A., & Rakowska, R. (2015). Spent Brewer's Yeast Autolysates as a New and Valuable Component of Functional Food and Dietary Supplements. *Journal of Food Processing & Technology, 6*.

Pokora, M., Zambrowicz, A., Zabłocka, A., Dąbrowska, A., Szołtysik, M., Babij, K., Eckert, E., Trziszka, T., & Chrzanowska, J. (2017). The use of serine protease from Yarrowia lipolytica yeast in the production of biopeptides from denatured egg white proteins. *Acta Biochimica Polonica, 64*, 245-253.

Rai, A. K., Kumari, R., Sanjukta, S., & Sahoo, D. (2016). Production of bioactive protein hydrolysate using the yeasts isolated from soft chhurpi. *Bioresource Technology, 219*, 239-245.

Rakowska, R., Sadowska, A., Dybkowska, E., & Swiderski, F. (2017). Spent yeast as natural source of functional food additives. *Roczniki Państwowego Zakładu Higieny, 68*.

Rizk, Z., El Rayess, Y., Ghanem, C., Mathieu, F., Taillandier, P., & Nehme, N. (2016). Impact of inhibitory peptides released by Saccharomyces cerevisiae BDX on the malolactic fermentation performed by Oenococcus oeni Vitilactic F. *International Journal of Food Microbiology, 233*, 90-96.

Rizk, Z., Rayess, Y. E., Ghanem, C., Mathieu, F., Taillandier, P., & Nehme, N. (2018). Identification of multiple-derived peptides produced by Saccharomyces cerevisiae involved in malolactic fermentation inhibition. *FEMS Yeast Research, 18*, foy080.

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food Quality and Safety, 1*, 29-46.
Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: a review. *Peptides, 31*, 1949-1956.
Sklirou, A. D., Ralli, M., Dominguez, M., Papassideri, I., Skaltsounis, A. L., & Trougakos, I. P. (2015). Hexapeptide-11 is a novel modulator of the proteostasis network in human diploid fibroblasts. *Redox Biology, 5*, 205-215.
Soleymanzadeh, N., Mirdamadi, S., & Kianirad, M. (2016). Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy Science & Technology, 96*, 443-457.
Takalloo, Z., Nikkhah, M., Nemati, R., Jalilian, N., & Sajedi, R. H. (2020). Autolysis, plasmolysis and enzymatic hydrolysis of baker's yeast (Saccharomyces cerevisiae): a comparative study. *World Journal of Microbiology and Biotechnology, 36*, 68.
Tangüler, H., & Erten, H. (2009). The effect of different temperatures on autolysis of baker's yeast for the production of yeast extract. *Turkish Journal of Agriculture and Foresty, 33*, 149-154.
Thyab Gddoa Al-sahlany, S., Altemimi, A. B., Al-Manhel, A. J. A., Niamah, A. K., Lakhssassi, N., & Ibrahim, S. A. (2020). Purification of Bioactive Peptide with Antimicrobial Properties Produced by Saccharomyces cerevisiae. *Foods, 9*, 324.
Tran, T. T. N., Tran, D. P., Nguyen, V. C., Tran, T. D. T., Bui, T. T. T., & Bowie, J. H. (2021). Antioxidant activities of major tryptophyllin L peptides: A joint investigation of Gaussian-based 3D-QSAR and radical scavenging experiments. *Journal of Peptide Science, 27*, e3295.
Udenigwe, C. C., & Fogliano, V. (2017). Food matrix interaction and bioavailability of bioactive peptides: Two faces of the same coin? *Journal of Functional Foods, 35*, 9-12.
Unuofin, J. O., & Lebelo, S. L. (2020). Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. *Oxid Med Cell Longev, 2020*, 1356893.
Vieira, E., Carvalho, J., Pinto, E., Cunha, S., Almeida, A., & Ferreira, I. (2016). Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract. *Journal of Food Composition and Analysis, 52*.
Vieira, E., Teixeira, J., & Ferreira, I. M. P. L. V. O. (2016). Valorization of brewers' spent grain and spent yeast through protein hydrolysates with antioxidant properties. *European Food Research and Technology, 242*, 1975-1984.
Vieira, E. F., & Ferreira, I. M. P. L. V. O. (2017). Antioxidant and antihypertensive hydrolysates obtained from by-products of cannery sardine and brewing industries. *International Journal of Food Properties, 20*, 662-673.
Vieira, E. F., Melo, A., & Ferreira, I. M. P. L. V. O. (2017). Autolysis of intracellular content of Brewer's spent yeast to maximize ACE-inhibitory and antioxidant activities. *LWT - Food Science and Technology, 82*, 255-259.
Wang, B., Xie, N., & Li, B. (2019). Influence of peptide characteristics on their stability, intestinal transport, and in vitro bioavailability: A review. *Journal of Food Biochemistry, 43*, e12571.
Wang, X., Yu, H., Xing, R., Chen, X., Liu, S., & Li, P. (2017). Optimization of the Extraction and Stability of Antioxidative Peptides from Mackerel (Pneumatophorus japonicus) Protein. *BioMed Research International, 2017*, 6837285.
Wen, C., Zhang, J., Zhang, H., Duan, Y., & Ma, H. (2020). Plant protein-derived antioxidant peptides: Isolation, identification, mechanism of action and application in food systems: A review. *Trends in Food Science & Technology, 105*, 308-322.

Williams, R., Dias, D. A., Jayasinghe, N., Roessner, U., & Bennett, L. E. (2016). Beta-glucan-depleted, glycopeptide-rich extracts from Brewer's and Baker's yeast (Saccharomyces cerevisiae) lower interferon-gamma production by stimulated human blood cells in vitro. *Food Chemistry*, *197*, 761-768.

Wu, S., Wang, X., Qi, W., & Guo, Q. (2019). Bioactive protein/peptides of flaxseed: A review. *Trends in Food Science & Technology*, *92*, 184-193.

Xue, L., Yin, R., Howell, K., & Zhang, P. (2021). Activity and bioavailability of food protein-derived angiotensin-I-converting enzyme–inhibitory peptides. *Comprehensive Reviews in Food Science and Food Safety*, *20*, 1150-1187.

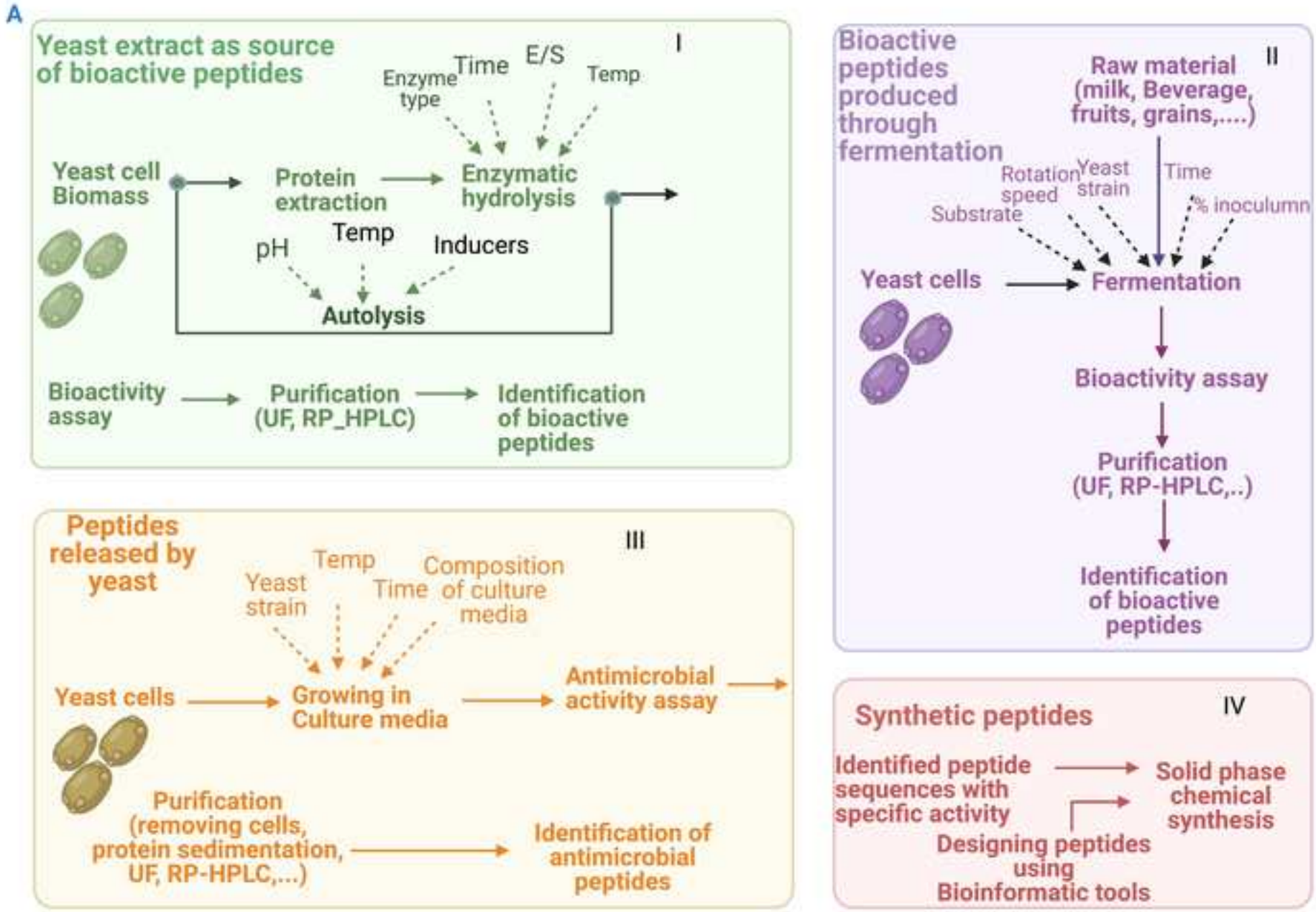
Younis, G., Awad, A., Dawod, R. E., & Yousef, N. E. (2017). Antimicrobial activity of yeasts against some pathogenic bacteria. *Vet World*, *10*, 979-983.

Zhang, D., Liu, J., Jiang, T., Li, L., Fang, G., Liu, Y., & Chen, L. (2017). Influence of Kluyveromyces marxianus on proteins, peptides, and amino acids in Lactobacillus-fermented milk. *food Science and Technology*, *26*, 739-748.

Zhang, R., Eckert, T., Lutteke, T., Hanstein, S., Scheidig, A., Bonvin, A. M., Nifantiev, N. E., Kozar, T., Schauer, R., Enani, M. A., & Siebert, H. C. (2016). Structure-Function Relationships of Antimicrobial Peptides and Proteins with Respect to Contact Molecules on Pathogen Surfaces. *Current Topics in Med icinal Chemistry*, *16*, 89-98.

Zhu, C., Zhang, W., Kang, Z., Zhou, G., & Xu, X. (2014). Stability of an antioxidant peptide extracted from Jinhua ham. *Meat Science*, *96*, 783-789.

Figure 1A



Figure

Click here to access/download;Figure;Fig 1.B.png

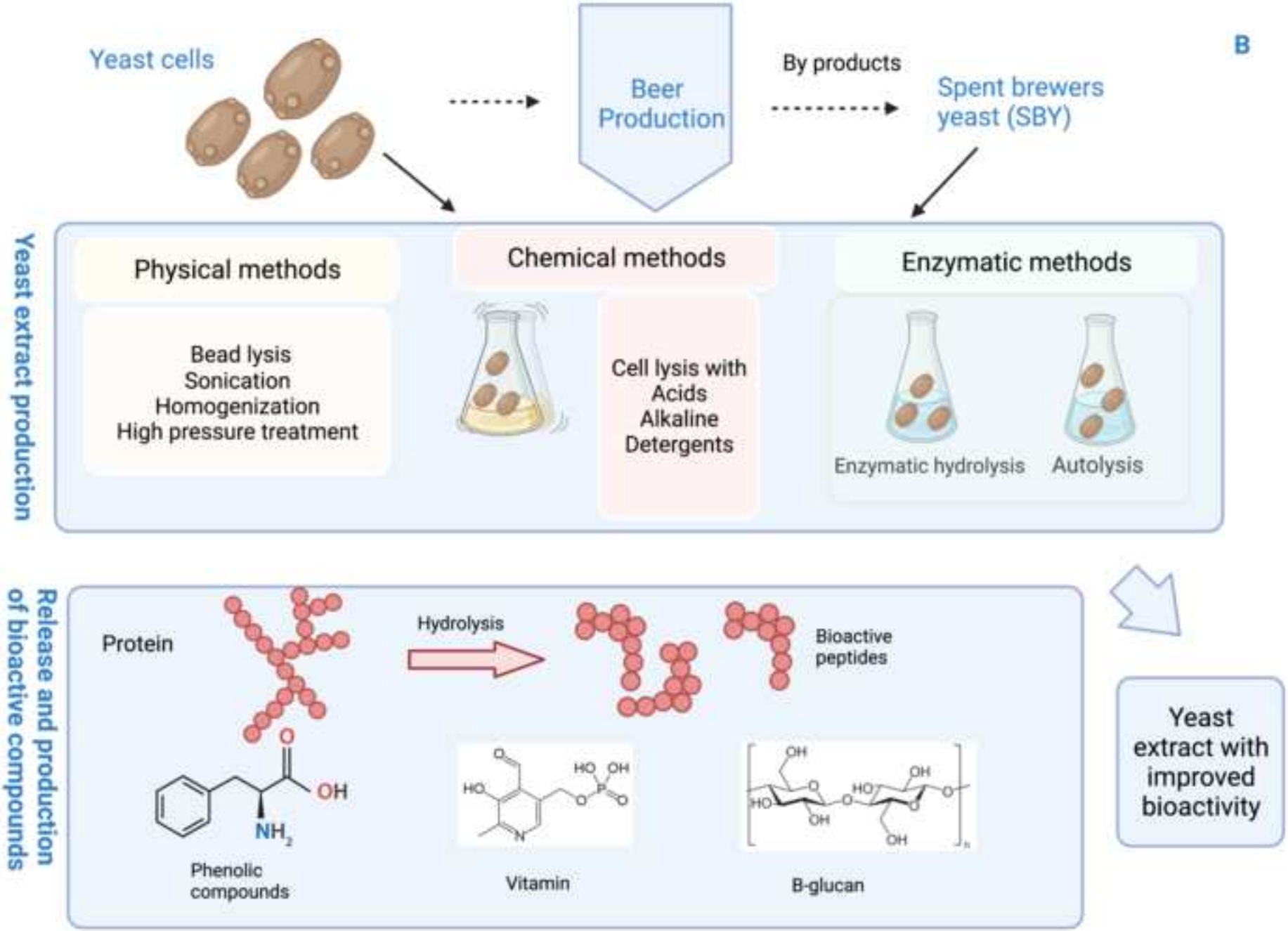


Figure 2

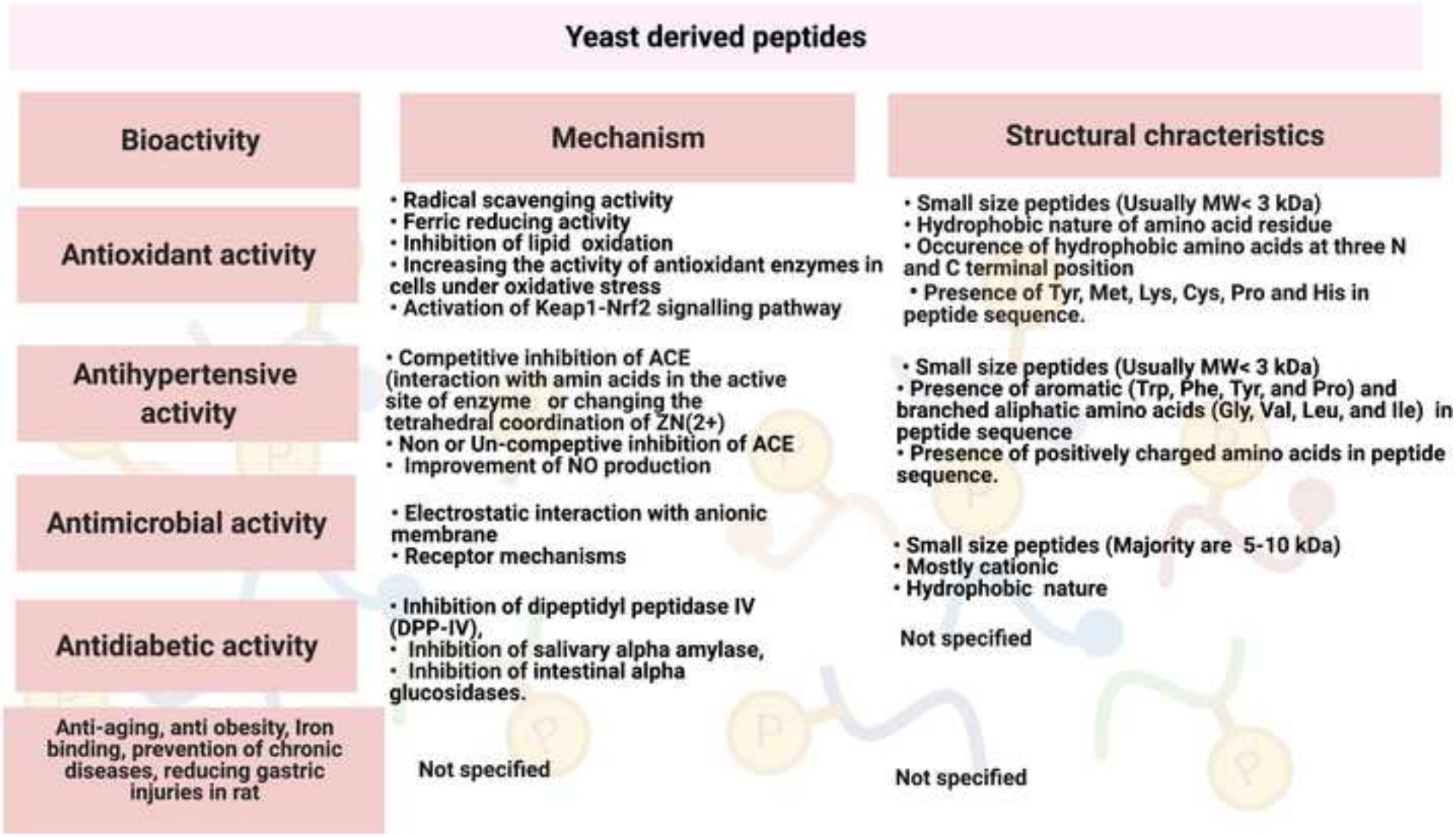


Figure 3A

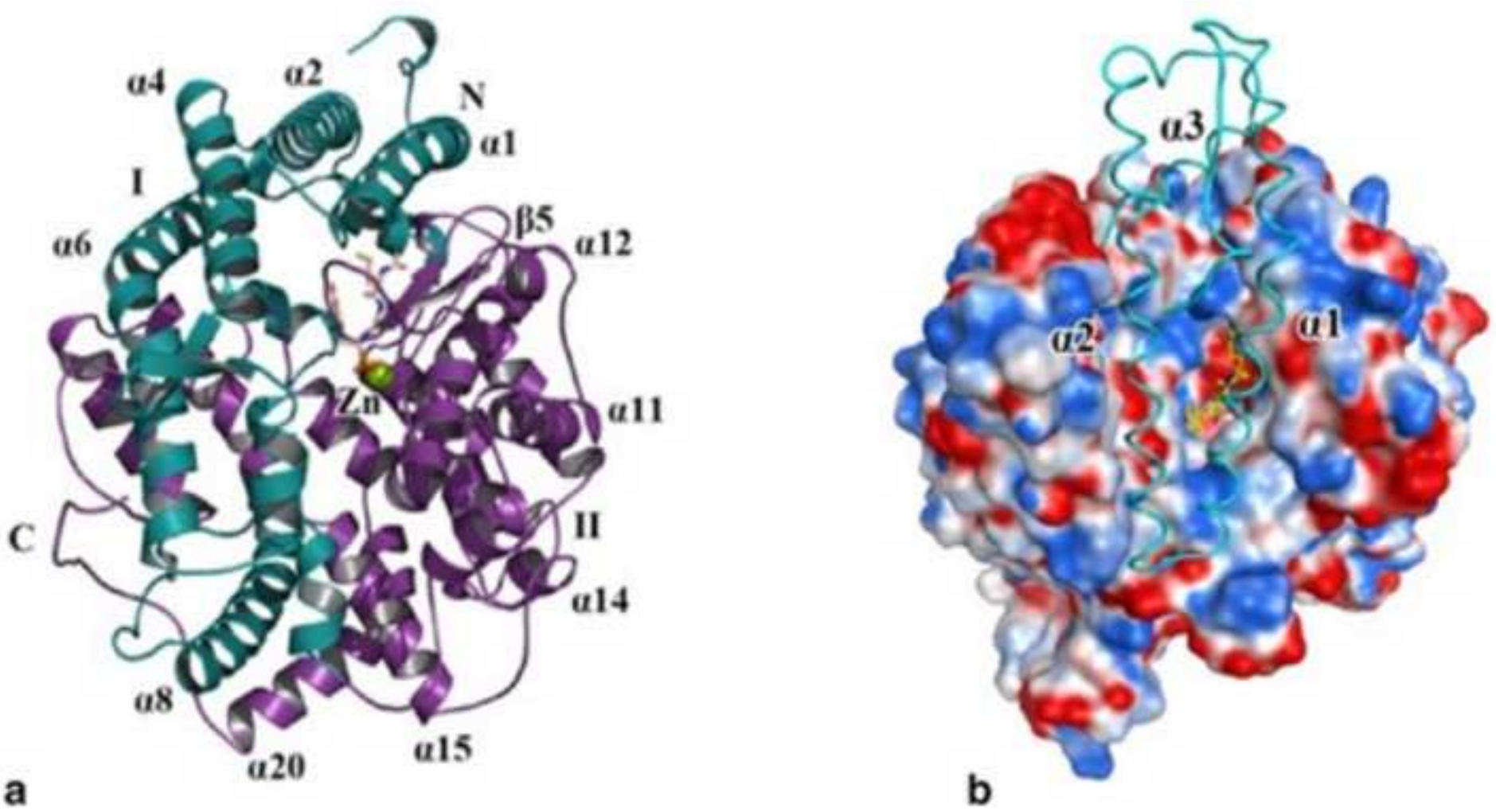


Figure 3B

Click here to access/download;Figure;Fig. 3B.jpeg