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Review

Spent brewer's yeast (*Saccharomyces cerevisiae*) as a potential source of bioactive peptides: An overview

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ABSTRACT

Bioactive peptides become popular in several economic sectors over the years as they have demonstrated important biological benefits in digestive, immune, cardiovascular, and nervous human systems. Although many commercial peptides are chemically synthesized, they can also be obtained from natural protein sources such as spent brewer's yeast (*Saccharomyces cerevisiae*). The recovery of this fermentation by-product for production of functional ingredients is an important step in the increasingly demand to implement and promote a circular economy-based industry. Bioactive peptides can be found in protein-rich extracts produced from *S. cerevisiae*, and several studies have described their positive impact of human body. In this line, the present review highlights and discuss the reported biological properties of *S. cerevisiae* bioactive peptides in terms of antihypertensive, antioxidant and antimicrobial effects, although other bioactivities are also described. Concerning the growing interest in yeast protein-rich products by agri-food and cosmetic sectors, some of the products currently on the market are also pointed out and their potential source is discussed.

1. Introduction

Bio-functional peptides have been described as characteristic protein fragments that have a positive impact on body biological processes and may ultimately lead to health benefits [1]. In fact, pharmaceutical research is increasingly focusing on these molecules to the point of being candidates for clinical trials, due to their fewer and/or lower side-effects when compared with equivalent synthetic drugs [2]. Also, these small peptides have other advantages owing to their biospecificity to targets, wide action spectrum, low accumulation and toxicity, and high structural diversity [3].The high incidence and prevalence of metabolic diseases has valorised peptide therapeutics market, valued at $25 billion in 2018, and which is expected to increase at a compound annual growth rate (CAGR) of 7.9% from 2019 to 2027 [4].

Nutraceutical and functional food markets have also showed great interest in these molecules since the gastrointestinal digestion of food proteins contribute to the production of physiologically active peptides. They are involved in regulatory activities in humans, affecting particularly the digestive, immune, cardiovascular, and nervous systems when taken orally [1]. Bioactive peptides are made up of 3 to 20 amino acids, and their bioactivity is a result of their amino acid composition and sequence. In general, they are highly specific in their biological functions and choice of biological and metabolic targets [5].

Diverse food protein sources, such as meat, eggs, cereals, bone collagen, legumes, vegetables, seafood, fish, yeast, seaweed and fungi, have demonstrated the presence of wide range of bioactive peptides in their composition, being some of them particularly rich in these biomolecules. Different types and amounts of peptides are produced during digestion, according to the variety of dietary proteins consumed. Although the exogenous peptides, such as those food-derived, may have a lower affinity for cellular receptors than the endogenous equivalents, their physiologically significant effect has been highlighted over the years [6].

Since spent yeast it is one of the main brewing by-products, generating 2 to 4 kg of spent yeast waste per 100 L of beer [7], their recovery and reuse to obtain functional ingredients promotes environment

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protection and waste management in agri-food sector, thus contributing to a truer circular economy (Fig. 1). Despite the high nutritional value of yeast biomass, their bio-functional properties were described as very weak when compared with yeast extract [8]. Therefore, in the last decades, with the increasing interest in bioactive peptides for the nutraceutical and functional food markets, the low performance of raw yeast biomass meant that extraction of these bioactive peptides from yeast has become a topic of high relevance in research [9–11]. Yeast market is currently dominated by *S. cerevisiae*, despite the thousands of described yeast species. Its bioactive peptides can be obtained by physical, chemical or enzymatic extraction processes, where proteins are cleaved and freed from the cell envelope, followed by purification processes [12].

Antihypertensive, antioxidant and antimicrobial activities, among others, have been associated with *S. cerevisiae* protein extracts and specific bioactive peptides, mainly in cosmetic and agri-food sectors [9,10,14–16]. Regarding the application of protein-rich extracts in agri-food sector, a wide range of products are currently available, mainly due to their high nutritional value. Fermentation processes, animal feed, human dietary supplements and flavour enhancers are the main target for these products (Table 4). However, a new trend for yeast bioactive peptides application has emerged as well, since they have become popular on cosmetic formulations in order to treat aging skin (Fig. 1) [16–18].

One of the main challenges in food science and technology is the recovery of valuable and bio-functional ingredients from industry by-products. Yeast, as one of the most important by-products of brewing industry offers a wide spectrum of bioactive and biologically significant biopeptides. Therefore, the aim of the present review is to outline the potential bioactivities of peptides extracted from *S. cerevisiae* and to explore the economic sectors where they can and are currently applied, paving the way for the potential applications of spent yeast sourced extracts. Additionally, a summary of commercial products available is also discussed to overview the current yeast protein-rich extracts market and, again, the potential applications for spent yeast sourced-extracts.

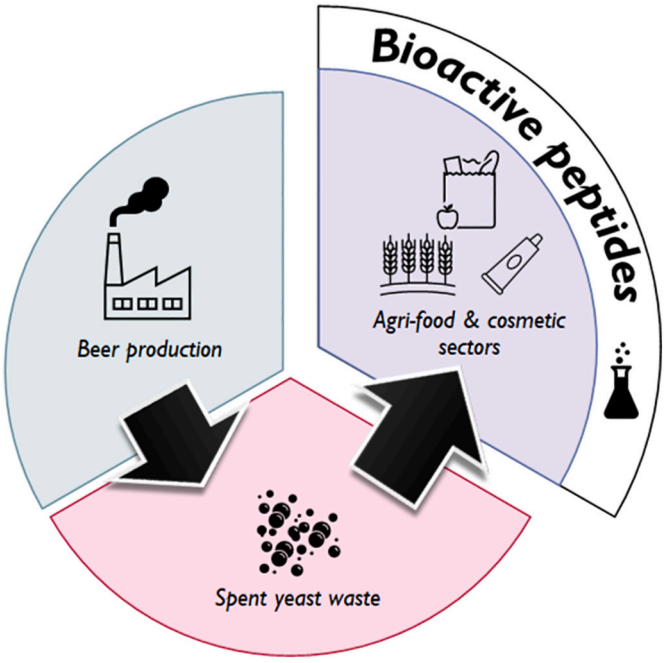

Fig. 1. Valorisation of spent yeast from beer production and its application on agri-food and cosmetic sectors.

2. Biological activities

Several different biological activities have been reported for protein and peptide extracted from spent brewer's yeast. Each activity has its own mechanism of action and while some peptide or protein extract have been described with one particular activity, one peptide can have more than one activity within its structure. On the following sections the most commonly found activities will be discussed.

2.1. Antihypertensive

Hypertension represents one of the major risks for cardiovascular diseases in the developed world [13]. It is well established that blood pressure is regulated by the angiotensin-converting enzyme (ACE) and by the kinin-kallicrein system [19]. In fact, the rennin-agiotensin system (RAS) is physiologically responsible for the control of blood pressure, where ACE plays the conversion of angiotensin I to the potent vasopressor angiotensin II, releasing aldosterone and increasing of sodium plasma concentration [20]. Bioactive peptides from spent brewer's yeast have been described as an alternative for several ACE inhibitory drugs, such as captopril and enalapril [5,21]. Some authors suggested a structure–activity relationship between the peptide and ACE inhibition, since their binding might be influenced by the hydrophobic amino acid nature of C-terminal tripeptide sequence of the substrate (peptides with 2–20 amino acids residues) that can interact with the subsites S1, S19, and S29 at the ACE active site [22]. However, several discovered peptides with ACE-inhibitory activities do not fit in with this model, exposing the weakness of this assumption, since it was only based on amino acids sequence [23]. For this reason, the relationship of antihypertensive activity with yeast peptide structure and sequence is yet to be confirmed [5].

In Table 1, several peptides extracted from *S. cerevisiae* are described as ACE activity inhibitors. In order to produce antihypertensive yeast hydrolysates with high protein, Huang et al. [24] optimized the hydrolysis conditions with a crude enzyme containing protease and β-glucanase activities produced by *Bacillus subtilis* fermentation. They obtained a peptide rich hydrolysate with 67.3% of peptides in a range of 460 to 2145 Da, being 91.2% of these smaller than 1500 Da. The extract exhibited a strong ACE-inhibitory activity with IC50 value of 26.13 μg/mL, decreasing the blood pressure *in vivo* after single oral administration (100 mg/kg in spontaneously hypertensive rats - SHR) and exhibiting a more persistent anti-hypertensive effect than captopril in long-term administration at 1200 mg/kg. Like some previous studies, the authors suggested a theoretical structure-function relation of peptides since they detected 428 of peptides with hydrophobic amino acid at N-end and 79 including Trp, Tyr and Pro at C-end. Kohama et al. [19] extracted three decapeptides from yeast glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with IC50 values between 0.4 and 18 μM, where the decapeptide PANLPWGSSNV contained the sequence homologous to vertebrate ACE inhibitors. The two other ACE-inhibitory decapeptides corresponded to different forms of yeast GAPDH with quite different sequences. Also Kim et al. [20] obtained an ACE inhibitory decapeptide (1178 Da; YDGGVFRVYT) from *S. cerevisiae* enzymatic hydrolysis which presented a stronger antihypertensive activity (IC50: 0.07 mg) than that of *Ganoderma frondosa* (IC50: 0.10 mg), but was slightly lower than that of the commercial antihypertensive drug captopril. However, the decapeptide was still considered a good candidate for antihypertensive

Table 1
Antihypertensive activity of bioactive peptides from *S. cerevisiae*.

Extraction and purification method	Extract characterization	Activity	Reference
Enzymatic hydrolysis of yeast powder (enzyme/substrate ratio of 7000 U/g, 55 °C, 5 h) followed by spray drying	Yeast hydrolysate (67.3% peptides)	ACE inhibitory activity: - IC50 value of 26.13 μg/mL. *In vivo*: -Decrease of blood pressure in SHR after single oral administration of yeast hydrolysate from dosage of 100 mg/kg. At 1200 mg/kg, the blood pressure reduction was similar in SHR and WKY. —In long-term administration,1200 mg/kg dosage of yeast hydrolysate seemed to exhibit a more persistent anti-hypertensive effect than captopril.	Huang et al. [24]
GAPDH EDTA-extraction followed by acid hydrolysis and SEC and RP-HPLC purification	Three GAPHD decapeptides (GHKIATFQER, GKKIATYQER, PANLPWGSSNV)	ACE inhibitory activity: -IC50 values of 0.4, 2 and 18 μM, respectively.	Kohama et al. [19]
Enzymatic hydrolysis (pepsin, trypsin, protease; 12 h) followed by 5 kDa ultrafiltration and SEC and RP-HPLC purification	Decapeptide (1178 Da; YDGGVFRVYT)	ACE inhibitory activity: - IC50 value (0.07 mg) was slightly lower than commercial antihypertensive drug (17.9 nM). *In vivo*: - Decreased of blood pressure in SHR (1 mg/kg dosage) with similar effects to positive control at the end of 2 h.	Kim et al. [20]
Alkaline enzymatic hydrolysis (alcalase; 12 h; 50 °C) followed by IEC, SEC and RP-HPLC purification	- Yeast peptide extract - Two peptide fractions: - Ala:Phe (1:1) - Gly:Phe (1:1– 1:2)	ACE inhibitory activity: - IC50 value of 3.0 and 3.4 μmol/L for Ala:Phe and Gly:Phe fractions, respectively. *In vivo*: - Decreased of blood pressure in SHR by administration of yeast extract (0.4% in diet) and two peptide fractions (8 mg/rat) in comparison with non-treated animals. - The effect of two peptide fractions estimated to be 62% of the activity of the captopril group (5 mg/rat).	Kanauchi et al. [25]
NM	Hexapeptide (TPTQQS)	ACE inhibition in a non-competitive manner by displacement of the zinc ion from the active site.	Ni et al. [23]
Sonication-trypsin hydrolysis followed by 10, 5 and 3 kDa ultrafiltration and SEC and RP-HPLC purification	- Yeast protein hydrolysates - Peptide fraction (< 3 kDa) - Decapeptide (1057.45 Da; YGKPVAVPAR)	ACE inhibitory activity: - Trypsin hydrolysate had the highest activity in antihypertensive assays (IC50 = 0.84 ± 0.01 mg/mL) in comparison with sonication-chymotrypsin hydrolysates and autolysates. - Peptide fraction (< 3 kDa) exhibited the strongest ACE inhibitory (IC50 = 0.32 mg/mL) activities in comparison with high MW peptide fractions. - Purified decapeptide increased the antihypertensive (IC50: 0.42 ± 0.02 mg/mL) activitiy	Mirzaei et al. [13]
10 kDa ultrafiltration followed by enzymatic hydrolysis (4% proteases) and 3 kDa nanofiltration	- Four peptide hydrolysed fractions: - Retentate >3 kDa - Retentate <3 kDa - Permeate >3 kDa - Permeate <3 kDa	ACE inhibitory activity: -Retentate fractions showed low IC50 (84.2 to 158 μg/mL) than retentates (198 to 259 μg/mL) -Fractions <3 kDa were the main responsible for ACE-inhibitory activity since they showed IC50 values of 84.2 and 198 μg/mL compared with peptides >3 kDa (158 and 259 μg/mL) (retentate and permeate, respectively). *In vivo:* - Retentate <3 kDa decreased systolic, diastolic and mean blood pressure in SHR (300 mg/kg) with similar effects to captopril (50 mg/kg) after 2 h administration, being this effect maintained throughout 24 h.	Amorim et al. [5]
Incubation of yeast powder with water (1:15) (pH 7.7, 60 °C, 30 min) followed by enzymatic hydrolysis (3.5% *w/v*, 6 h), ethanol precipitation (2 h, 4 °C) and 5 kDa filtration	-Yeast protein hydrolysates -Yeast peptide <5 kDa	ACE inhibitory activity: -Both samples showed high ACE activity with about 80% of enzyme inhibition -Ultrafiltration process increased the ACE inhibitory activity from IC50 of 55.39 mg/mL for protein hydrolysate and 29.32 mg/mL for peptide <5 kDa.	Hu et al. [26]

NM – Not mentioned; GAPDH - Glyceraldehyde-3-phosphate dehydrogenase; SHR - Spontaneously hypertensive rats; WKY - Wistar-Kyoto rats.

drugs and functional foods, since it did not induce the side effects usually associated with captopril, such as coughs and allergies. On the other hand, Kim et al. [20] when applying it, at a dosage of 1 mg decapeptide/kg, to spontaneously hypertensive rats (SHR), observed a decreased of blood pressure, similar to the captopril, at the end of 2 h, and without allergic reactions or coughing. Kanauchi et al. [25] observed a similar decrease in blood pressure in the same *in vivo* model by administration of yeast peptide hydrolysate (0.4% in diet) and two peptide fractions of Ala:Phe and Gly:Phe (8 mg/rat). In fact, the obtained decrease for the two fractions corresponding to 62% of captopril positive control effect (5 mg/rat) and an *in vitro* ACE-IC50 of 3.0 and 3.4 μmol/L was observed for Ala:Phe and Gly:Phe, respectively. In order to understand the ACE inhibition mechanism, Ni et al. [23] studied a docking simulation of hexapeptide ACE-inhibitor based on its sequence to find reference molecules for drug design that target ACE. The authors showed binding interactions between N-terminal Thr1, Thr3, and Gln4 residues of hexapeptide (TPTQQS) and the residues on the lid structure of ACE leading to enzyme inhibition in a non-competitive way by dislocation of the zinc ion from the active site (C-terminal Ser6) which is critical for ACE catalysis.

Table 2
Antioxidant activity of bioactive peptides from *S. cerevisiae*.

Extraction and purification method	Extract characterization	Activity	Reference
Autolysis (20 h; 47 °C)	Yeast autolysates (1000–2000 Da; 16.06 g free amino acids/100 g)	- Increased with the duration of the autolysis process, where the 20 h-autolysate showed the highest activity in ABTS (32.73 mMol TE/100 mL).	Podpora and Swiderski [9]
Enzymatic hydrolysis (papain; 24 h; 50–60 °C)	Yeast extracts (703–1740 Da; 60% protein)	- High activity in ABTS assay with concentrations ranging from 461.5 mmol TE/100 mg to 506.9 mmol TE/100 mg.	Podpora et al. [10]
Enzymatic alkaline hydrolysis (protease; 8 h; 60 °C) followed by 3 and 1 kDa ultrafiltration	- Eight peptide fractions from normal and Se-rich brewer's yeast (< 3 kDa and 1 kDa)	- Se-rich peptides fraction (MW < 1 kDa) with the highest activity in ABTS (85.4 ± 0.63%) compared with Se-rich peptides (MW < 3 kDa) or normal peptide fractions. - Inhibitory effect of Se-rich peptide fractions in lipid peroxidation was significantly higher than normal yeast peptide fractions, suggesting a synergistic effect between Se and yeast peptides. - Decrease of malonaldehyde level in liver and serum of mice after oral treatment with Se-rich yeast peptide fraction (peptide dose of 150 mg/kg body weight/day and Se dose: 100 μg/kg body weight/day during 30 days). - Se-rich yeast peptide fraction showed a protective effect UVB radiation-induced skin damage in mice and H_2O_2-induced cytotoxicity in human epidermal keratinocytes.	Guo et al. [28]
Sonication-trypsin hydrolysis followed by 10, 5 and 3 kDa ultrafiltration and SEC and RP-HPLC purification	- Yeast protein hydrolysates - Peptide fraction (< 3 kDa) - Decapeptide (1057.45 Da; YGKPVAVPAR)	- Trypsin hydrolysate had the highest activity in DPPH (179.2 ± 4.8 μM TE/mg protein) and ABTS (4653 ± 50 μM TE/mg protein) in comparison with sonication-chymotrypsin hydrolysates and autolysates. - Peptide fraction (< 3 kDa) exhibited the strongest DPPH (489.12 ± 0.001 μM TE/mg protein) and ABTS (7718.30 ± 57 μM TE/mg protein) activities in comparison with high MW peptide fractions. - Purified decapeptide increased the ABTS (26.25 μM TE/μg protein) activity.	Mirzaei et al. [13]
10 kDa ultrafiltration followed by enzymatic hydrolysis (4% proteases) and 3 kDa nanofiltration	-Four peptide hydrolysed fractions: - Retentate >3 kDa - Retentate <3 kDa - Permeate >3 kDa - Permeate <3 kDa	- Retentate fraction <3 kDa with the highest antioxidant activity (7.25 μM TE/ mg sample), followed by permeate <3 kDa (5.50 μM TE/ mg sample) in ORAC-FI assay.	Amorim et al. [5]
Incubation of yeast powder with water (1:15) (pH 7.7, 60 °C, 30 min) followed by enzymatic hydrolysis (3.5% *w/v*, 6 h), ethanol precipitation (2 h, 4 °C) and 5 kDa filtration	-Yeast protein hydrolysates -Yeast peptide <5 kDa	-The ultrafiltration process enhanced the superoxide scavenging activity from IC50 of 3.98 mg/mL for protein hydrolysate and 2.60 mg/mL for peptide <5 kDa. -Both extracts showed good DPPH radical scavenging activity but no differences were observed between extracts.	Hu et al. [26]
Enzymatic hydrolysis (48 h; enzymes:yeast of 1/100; 50 °C) followed by acid treatment and a combined ultrafiltration and activated carbon treatment	-Yeast hydrolysate with high content of CHP (64.9% protein)	- Strong scavenging activity in DPPH and ABTS assays (IC50 values of 1.9 and 0.9 mg/mL, respectively).	Jung et al. [30]
Enzymatic hydrolysis (up to 8h, enzyme:substrate ratio up to 0.12, and up to 55ºC)	-Yeast protein hydrolysates	- antioxidant capacity from 0.65 to 1.65 g TE- Antimicrobial activity versus *Aeromonas salmonicida*, *Bacillus cereus*, *B. subtilis* and *Salmonella enterica*	San Martin et al. [29]

TE – Trolox equivalent capacity; CHP – Cyclo-His-Pro.

2.2. Antioxidant

Antioxidant ingredients are capable to inhibit oxidation reactions. By doing so, they protect cells against oxidative stress, caused by reactive oxygen species (ROS) and nitrogen species, which are capable of damaging important cell components such as proteins, lipids and DNA, ultimately preventing the occurrence of several diseases. Many *S. cerevisiae* peptides have been reported by their antioxidant properties, as described in Table 2.

Podpora and Swiderski [9] and Podpora et al. [10] observed high antioxidant activity on protein *S. cerevisiae* extracts produced from autolysis (32.73 mMol TE/100 mL) and enzymatic hydrolysis (506.9 mmol TE/100 mg), with the increase of ABTS scavenging activity with the autolysis duration. However, the authors related these results with the presence of phenolic compounds [9].

Selenium-rich extracts from brewer's yeast are currently used for animal and human Selenium (Se) supplementation. Given the known oxidative stress properties of selenium, Guo et al. [28] studied the effect of combining bioactive peptides with Se. All tested yeast protein hydrolysates presented strong ABTS scavenging activity, with scavenging rates from 70.15% to 85.43%. However, the Se-rich peptide fractions with molecular weight (MW) < 1 kDa showed the highest antioxidant activity (85.4 ± 0.63%) compared with Se-rich peptides with MW < 3 kDa or higher yeast peptide fractions. Therefore, free radical scavenging activities seemed to be strongly influenced by MW. Furthermore, lipid peroxidation was also inhibited by all peptide fractions, with the Se-rich fraction presenting a significantly higher effect (maximum of 40% in liposome system and 70% in linoleic acid system). For this reason, the authors have suggested the use of Se and yeast peptides in a synergetic manner for lipid peroxidation prevention. Furthermore, Se-rich yeast peptide fraction showed excellent antioxidant activity *in vivo* (oral administration for 30 days: peptide dose of 150 mg/kg body weight/day and Se dose: 100 μg/kg body weight/day) by significantly decreased the level of malonaldehyde in liver and serum. The protective effect against skin damage caused by UVB radiation was also tested in mice (topical treatment for 15 days: peptide dose of 150 mg/kg body weight/day and Se dose of 100 μg/ kg body weight/day) with results showing an increase in glutathione peroxidase, catalase activities and glutathione content. In *in vitro* studies, using epidermal keratinocytes (HaCaT) cells it has been shown a protective effect against cytotoxicity caused by hydrogen peroxide, which the authors believe is caused by increased aquaporin-3 expression and attenuation of the phosphorylation of p38 MAPK. From the abovementioned results, it can be postulated that Se-rich yeast peptides may be a great and promising functional food and

cosmeceutical additive, given the reported antioxidative and skin oxidative damage protection properties.

Together with antioxidant properties, several studies have also been reported for *S. cerevisiae* peptides as antihypertensive and antidiabetic ingredients. In fact, the incidence of hypertension, aging and other diseases such as diabetes, cancer and neurodegenerative disturbances has been also related with antioxidant activity [5]. For that reason, the combination of ACE inhibition, antioxidant activity and/or antidiabetic activity in one single product could be very useful for the control of these chronic diseases. Mirzaei et al. [13] prepared different fractions of *S. cerevisiae* protein hydrolysates and purified a decapeptide (YGKP-VAVPAR; 1057.45 Da) that could potentially replace the current antioxidant and antihypertensive agents of chemical origin. The trypsin hydrolysate had the highest activity in DPPH scavenging (179.2 ± 4.8 μM TE/mg protein), ABTS radical scavenging (4653 ± 50 μM TE/mg protein) and antihypertensive assays (IC50 = 0.84 ± 0.01 mg/mL) in comparison with sonication-chymotrypsin hydrolysates and autolysates [13]. As described above, the authors hypothesized a structure–activity relationship due to the total content of hydrophobic amino acids in the trypsin hydrolysate since some previous studies described these features among potent antioxidant and ACE inhibitors protein hydrolysates with DPPH and ABTS radical-scavenging activities. However, there is no scientific evidence proving this relationship. The peptide fraction with MW under 3 kDa of trypsin hydrolysates exhibited the strongest DPPH (489.12 ± 0.001 μM TE/mg protein), ABTS (7718.30 ± 57 μM TE/mg protein) and ACE inhibitory (IC50 = 0.32 mg/mL) activities in comparison with high MW fractions. In fact, small peptides are more efficient antioxidants and ACE inhibitors than macro peptides or proteins because of their higher accessibility to the oxidant/antioxidant test system, and better binding to the ACE active site. From the purification of this fraction resulted the decapeptide that was identified to be highly potent ABTS radical-scavenging (26.25 μM TE/μg protein) and ACE-inhibitory peptide (IC50: 0.42 ± 0.02 mg/mL).

Amorim et al. [5] also produced four peptide fractions from spent brewer's yeast (retentate > and <3 kDa; permeate > and <3 kDa) by 10 kDa ultrafiltration followed by enzymatic hydrolysis and 3 kDa nanofiltration that showed strong antihypertensive and antioxidant activities. The authors observed an inhibition of ACE activity *in vitro* with retentates showing the lowest IC50 values fractions (84.2 to 158 μg/mL) compared with permeates (198 to 259 μg/mL). For this reason, the authors chose the retentate peptide fractions to study their effect in hypertension on spontaneously hypertensive rats (SHR) in a short-term oral exposure. The retentate <3 kDa decreased systolic, diastolic and mean blood pressure in SHR (300 mg/kg) with similar effects to captopril (50 mg/kg) after 2 h administration, being this effect maintained throughout 24 h. Likewise, this fraction showed the highest protective effect against peroxyl radicals (7.25 μM TE/mg sample), followed by permeate <3 kDa (5.50 μM TE/mg sample) following the same tendency of ACE inhibition. After MALDI-TOF/TOF analysis, tri and tetrapeptides were identified in this fraction (507 to 582 Da) with hydrophobic amino acid residues (SPQW, PWW and RYW) which can suggest again the binding affinity of peptide to ACE, but the structure-activity relationship was not confirmed yet. Besides, antioxidant activity can also be related with amino acids sequence of peptides (tyrosine, tryptophan, phenylalanine and the imidazole group of histidine) which are able to quench the free radicals through a direct electron transfer mechanism, while the proline's pyrrolidine ring can interact with secondary structure of peptides, increasing its flexibility. It can also quench oxygen singlets thanks to its low ionization potential.

Also Hu et al. [26] performed an 5 kDa filtration preceded by enzymatic hydrolysis and ethanol precipitation for production of hydrolysates from yeast powder, revealing that ultrafiltration process is an important step in order to enrich bioactivity of yeast peptides. Yeast peptides hydrolysate and peptide fraction <5 kDa allowed a strong ACE inhibition (about 80%) with IC50 values of 55.39 μg/mL and 29.32 μg/mL, respectively, confirming an increase of ACE-inhibitory activity using ultrafiltration process, being the fraction <5 kDa mainly constituted by 1 kDa oligopeptides (80.4%). The same increase of activity in 5 kDa fraction was observed in superoxide anion scavenging and α-glucosidase inhibitory activities (IC50 values of 2.60 mg/mL and 10.62 mg/mL, respectively). On the other hand, no differences were found in DPPH radical scavenging activity probably related with the lack of polypeptide specificity for this assay. The authors also performed a stability study of the potential extracts, observing a good heat and pH stability and high resistant effect against gastrointestinal proteases in peptides <5 kDa. On a more simple approach, San Martin *et al.* [29] produced yeast hydrolysates produced by enzymatic means with a protein extraction yield between 13.7 to 29.7%, which exhibit antioxidant activity, which range from 0.65 to 1.65 g of Trolox equivalent, by ABTS method. Due to this data, and to the fact that these hydrolysates also exhibit antimicrobial activity against *Aeromonas salmonicida*, *B. cereus*, *B. subtilis* and *Salmonella enterica*, lead the authors to conclude that this hydrolysates have great potential for functional food ingredients.

Concerning the therapy of metabolic disorders, Jung et al. [30] produced a yeast hydrolysed rich in Cyclo-His-Pro (CHP) since the supplementation of this dipeptide combined with zinc improved insulin sensitivity and glucose clearance. The extract prepared with Flavourzyme showed the highest level of CHP (674 μg/g, 64.9% protein) and strong scavenging activity in DPPH and ABTS assays (IC50 values of 1.9 and 0.9 mg/mL, respectively). Besides, the authors observed a significant decrease in glucose level of mice hyperglycemic models treated with yeast hydrolysate (100 mg/kg) compared with control at 30, 60, 90, and 120 min. Together, these results demonstrate the possibility to use a CHP-rich yeast extract as an antioxidative and/or antidiabetic ingredient in functional foods but further studies need to be performed to understand the role of CHP in antioxidant and antidiabetic mechanism.

2.3. Antimicrobial

In recent past we have witnessed an increase in the number of antibiotic resistant pathogens, with multiple antimicrobial classes becoming ineffective to combat infections in medical and agricultural fields [31]. In order to address this problem, antimicrobial peptides have been produced, isolated and purified from different sources such as microorganisms, invertebrates and other species [14]. Antimicrobial peptides are small oligopeptides, usually with a MW under 10 kDa, and encoded within the sequences of native protein precursors with a net positive charge and an amphipathic structure [14,32]. They are involved in the growth inhibition and killing of several microorganisms such as bacteria and fungi [32]. Their properties, such as amphipathicity, amino acid composition, cationic charge and size, are key factors in the attachment and infusion into pathogen membrane bilayers, allowing the formation of pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. However, it has been speculated that these mechanisms are not the sole reason for the antimicrobial properties of these peptides: the cytoplasmic membrane septum formation can be altered by translocated peptides, and other effects have been described such as the inhibition of cell-wall synthesis, nucleic-acid and protein synthesis or of enzymatic activity in general [33].

Many studies have reported the production of antimicrobial peptides from spent *S. cerevisiae* (Table 3). Enzymatic hydrolysis or cell physical disruption technique, followed by ultrafiltration and SEC or IEC purification were the methods mostly used for antimicrobial peptides production [15,36–38].

The malolactic fermentation is essential for certain types of wine, having a strong impact in their final performance, mainly in sensorial aspects such as decreasing acidity [39]. However, it has been described that yeast can produce antibacterial factors that can be responsible for the non-occurrence of malolactic fermentation [38]. In fact, Dick, Molan and Eschenbruch [38] showed that two cationic proteins isolated by

Table 3
Antimicrobial activity of bioactive peptides from *S. cerevisiae*.

Extraction and purification method	Extract characterization	Activity	Reference
French press followed by SEC, cation IEC purification and 500 Da ultrafiltration	Two cationic proteins - Small protein with high isoelectric point - Lysozyme-like	Antimicrobial activity: - Strong inhibitory action against lactic acid bacteria.	Dick, Molan and Eschenbruch [38]
10 kDa Ultrafiltration and dialysis (16 h, 4 °C)	*S. cerevisiae* compounds from proteinaceous nature (< 10 kDa)	Antimicrobial activity: - Strong inhibition growth and malic acid degradation of malolactic bacteria.	Comitini et al. [37
Enzymatic hydrolysis (trypsin, alkaline protease mixture; 72 h; 37 °C) followed by 10 kDa and 2 kDa ultrafiltration	2–10 kDa Peptide fraction (4.0, 4.5 and 6.0 kDa)	- Antimicrobial properties against *H. guilliermondii*. - Fungistatic effect against *K. marxianus*, *K. thermotolerans*, *T. delbrueckii* and *H. guilliermondii*. - Fungicidal effect against *K. marxianus*.	Albergaria et al. [34]
10 and 2 kDa Ultrafiltration followed by SEC and IEC purification	- 2–10 kDa Peptide fraction - Fragments of the *S. cerevisiae* GAPDH isoenzymes: GAPDH2/3 and GAPDH1; 1.638 and 1.622 kDa; VSWYDNEYGYSTR and ISWYDNEYGYSAR)	Antimicrobial activity against a wide variety of wine-related yeasts and bacteria related with GAPDH activity.	Branco et al. [15]
10 and 2 kDa Ultrafiltration followed by SEC purification	- 2–10 kDa Peptide fraction - "Saccharomycin"	- *S. cerevisiae* secretes several GAPDH-derived peptides with antimicrobial activity during alcoholic fermentation, namely a natural biocide "saccharomycin". - "Saccharomycin" exhibited a fungicidal effect against several wine-related non-*Saccharomyces* yeasts. - Antimicrobial activity of GAPDH-derived peptides was significantly higher than synthetic analogues. - The death of sensitive yeast cells (*H. guilliermondii* and *D. bruxellensis*) is related to the peptides capacity of cell penetration membrane.	Branco et al. [35] Caldeira et al. [36]
10 kDa and 2 kDa ultrafiltration followed by SEC purification	Peptide (9770 Da; thermostable at 50–90 °C for 30 min; tolerated a pH range of 5–7 at 4 °C and 25 °C for 24 h)	Antimicrobial activity: - Inhibition of 2 to 2.3 and 1.5 to 1.8 log units of gram-negative (*E. coli* and *K. aerogenes*) and gram-positive (*B. subtilis* and *S. aureus*) bacteria during 24 h of incubation, respectively.	Gddoa Al-sahlany et al. [14]

GAPDH - Glyceraldehyde-3-phosphate dehydrogenase.

cation exchange chromatography from *S. cerevisiae* strongly inhibited the lactic acid bacteria which are in charge of malolactic fermentation in wine. Comitini et al. [37] analysed the interactions between *Oenococcus oeni*, the dominant specie of lactic acid bacteria, and different yeasts in order to understand the mechanisms underlying the basis of the yeast–bacteria interactions in wine. They found that *S. cerevisiae* compounds of proteinaceous nature (MW < 10 kDa) inhibited the growth of different strains of *O. oeni* in a dose-dependent way, being related to yeast metabolic activity rather than to a competition for nutritional requirements. The proteinaceous factors of yeast can be explored on the inhibition of lactic bacteria, which offers new prospects for malolactic fermentation and its management. Beyond the inhibition of lactic acid bacteria, Albergaria et al. [34] observed antimicrobial activity of *S. cerevisiae* secreted peptides with a MW between 2 and 10 kDa against *Hanseniaspora guilliermondii*, a non-*Saccharomyces* yeast that contributes to increase the sensory complexity of wines.Conversely, a growth inhibitory effect *versus Kluyveromyces marxianus*, *K. thermotolerans*, *Torulaspora delbrueckii* and *H. guilliermondii* and a fungicidal effect *versus k. marxianus* was found in the same study, which suggests the use of bioactive peptides as a way to prevent wine spoilage caused by yeasts.

In their work, Branco et al. [15] Have shown the antimicrobial potential of a 2–10 kDa peptide fraction, testing it against a large range of wine related yeasts. Two peptides have resulted from the purification of this fraction with 1.638 and 1.622 kDa (VSWYDNEYGYSTR and ISWYDNEYGYSAR, respectively). These correspond to fragments from the *S. cerevisiae* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoenzymes, namely GAPDH2/3 and GAPDH1. The authors denominated the GAPDH-derived peptides fraction as "Saccharomycin", a natural biocide with fungicidal effect against several wine-related non-*Saccharomyces* yeasts during alcoholic fermentation [36,35]. It was also found that "Saccharomycin" antimicrobial activity was significantly higher when compared to synthetic analogues while being dependent on a conjugated action of GAPDH2/3 and GAPDH1 (ideally at ration 4:1) for maximum antimicrobial action [35]. Furthermore, in contrast to synthetics alternatives, "Saccharomycin" is active under acidic conditions, suggesting molecular adaptations, likely involving the formation of aggregates of a number of peptide units in order to maintaining their solubility and bioactivity. When studying the death mechanism induced by GAPDH-derived peptides, these molecules were chemically synthetized, and it was observed their internalization by cell membrane permeabilization. These peptides were able to enter the cytoplasm of sensitive yeast cells (*H. guilliermondii* and *Dekkera bruxellensis*) by crossing the cell membrane.

In addition to production of antimicrobial peptides by *S. cerevisiae* related to fermentation processes, Gddoa Al-sahlany et al. [14] isolated an antimicrobial peptide from *S. cerevisiae* culture medium suitable for use in sterilization and thermal processes in food production. After 24 h incubation, the peptide inhibits from 2 to 2.3 log units of gram-negative (*Escherichia coli* and *Klebsiella aerogenes*) and 1.5 to 1.8 log units of gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria, respectively. The authors found that the mode of action fundamentally depends on the electrostatic interaction between peptides and bacteria cells membrane, since peptides were adsorbed by bacterial cell membrane leading to its complete damage. In order to establish a relation between MW and inhibition of bacteria, the authors also analysed the fraction from 3 to 10 kDa ultrafiltration where they observed smaller peptides with antibacterial biological activity as well.

2.4. Other bioactivities

In addition to antihypertensive, antioxidant, antidiabetic and antimicrobial bioactivities, widely described for *S. cerevisiae* peptides, other bioactivities related to treatment and prevention of chronic diseases have also been studied (Fig. 2).

Hoz et al. [40] demonstrated the iron-binding capacity of peptide hydrolysates from sugarcane yeast (*S. cerevisiae*) (MW < 5 kDa) which

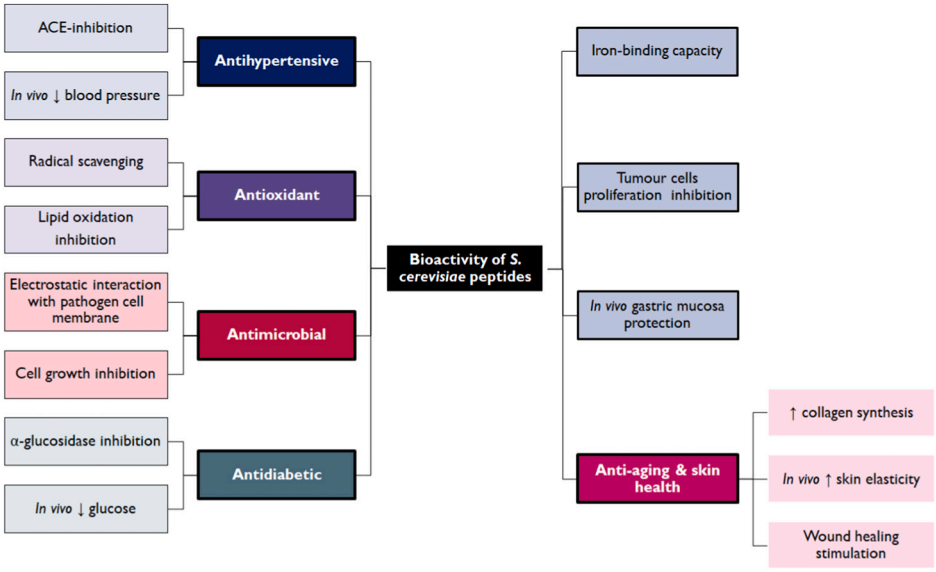

Fig. 2. Main bioactivities and possible mechanisms reported for peptides from *S. cerevisiae*.

enhanced iron bioavailability. The authors observed iron stability during *in vitro* digestion by evaluation of iron dialyzability (amount of soluble and stable iron until intestinal digestion) after the production of iron-peptide chelates, concluding that yeast peptide extracts were a promising iron-delivery component to produce supplements targeted for anti-anemic market.

On the other hand, Amorim et al. [41] observed the protection of gastric mucosa of rats against ulcerative lesions when treated with peptide hydrolysates from spent brewer's yeast. The peptide fraction under 3 kDa was able to reduce gastric injuries at the effective dose of 816 mg/kg, being pointed out a relation between a prostaglandin-mediated mechanism and the cytoprotective effect observed. However, this mechanism seems to be nonspecific. Furthermore, a cytotoxic effect against leukemia cells (K-562) was also revealed with the inhibition of more than 50% in concentration values (IC_{50}) of 2.5 μg/mL for this peptide fraction (25 μg/mL for fraction above 3 kDa). The authors suggested that the minerals also present in the extract may be the responsible for inhibition of tumor cells proliferation.

Recently, Jacob et al. [42] also hypothesized the potential use of yeast autolysates for stimulating immune cells and preventing diabetes due to the high amount of amino acid γ-aminobutyric acid (GABA) (10 mg protein/g yeast extract), since it acts as a strong secretagogue of insulin from the pancreas [43]. Fig. 2 presents an overview of the abovementioned potential bioactivities properties.

3. Applications

3.1. Agri-food sector

Beyond the use of yeast peptides as bioactive ingredients for specifically targeted applications, some studies exploited the benefits of yeast protein extracts introduction in the food market and winemaking industry [44,45]. Caballero-Córdoba and Sgarbieri [45] evaluated the nutritional and toxicological performance of yeast protein concentrates (extracted with sodium perchlorate) as single source of dietary protein. In fact, the authors observed an *in vivo* poor nutritional performance, since the food intake and growth rate were lower than that of the group fed by yeast biomass. Nevertheless, these results can be explained by a decrease in amino acid bioavailability such as lysine and methionine, and/or the presence of residual sodium perchlorate (from extraction process) in the diet, which led to some evidenced liver toxicity. On the other hand, a yeast protein extract, with 50% > 15 kDa, was proposed as an effective alternative as a fining agent in red wine clarification and stabilization by Gaspar et al. [44]. This alternative to traditional agents is health risk free, enhance phenolic compound extraction, promotes a significant brilliance and turbidity decrease, as well as colour improvement, when compared with reference traditional compounds such as albumin, bentonite or casein [44].

Yeast protein-rich extracts have also been used as human dietary supplements, flavour enhancers or additives in fermentation processes and animal feed. Table 4 lists some of the protein-rich products currently on the market derived from yeast (*S. cerevisiae* and *Cyberlindnera jadinii* (Torula)) obtained from different extraction processes (hydrolysis and autolysis) and/or spray-dried. Beyond the high protein content described on the product information, these extracts are also rich in amino acids and bioactive peptides, among others, which are responsible for some of the bioactive properties observed. In fact, with the exception of being used as protein sources, several of the remaining applications of these extracts (*e.g.* flavour enhancement) are dependent on the smaller molecules present (amino acids), and the choice of commercializing the whole protein extract is usually mainly economic. Since the source of those commercial products is not usually provided in the label, it is difficult to ascertain which ones come from spent yeast; however, such labelling could even be a positive differentiation parameter, due to the growing consumer awareness and valorisation on sustainability issues and circular economy.

3.2. Cosmeceuticals

Cosmeceuticals are bioactive compounds with medical benefits which are used in the cosmetic industry and are applied alongside cosmetical formulations, such as topical creams or lotions. They are not yet recognized, tested and regulated as drugs by U.S. Food and Drug Administration (FDA) [46], therefore the demonstration of their theoretical benefits is mostly conducted in *in vitro* studies with the active ingredients [47].

Peptides have become a popular active ingredient to incorporate in cosmeceutical products mainly in anti-aging skin formulations [17] since they incorporate a wide range of bioactivities, such as antioxidant and antimicrobial activities as described above, apart from inhibition of enzymes involved in aging process (elastase, collagenase, tyrosinase and hyaluronidase) [48]. Their action mechanism can involve the signalling and regulation of molecules in different skin physiological pathways; accordingly, they can be classified in signal, carrier and

Table 4
Protein rich-extracts from commercially available yeast.

Company	Product		Composition	Use and function
Ohly	Yeast peptone[a]		Yeast peptone rich in B-vitamins and naturally bound minerals.	Alternative to peptone sources.
	X-SEED®	Peptone[b]	Hydrolyzed yeast extract with 80% amino acids available in peptide form (MW < 2 kDa). Excellent source of B-vitamins and naturally bound mineral.	For some strains or in certain production processes, improving productivities and yields.
		Nitroboost[c]	Autolyzed and hydrolyzed yeast extract with balanced composition of free amino acids and easily available peptides.	Growth factors for demanding production processes with strains.
Biomin	LEVABON®[d]	Rumen E	Spray-dried and autolyzed *S. cerevisiae*, rich in bioactive ingredients and nutrients such as nucleotides, essential amino acids, peptides, cell wall carbohydrates and B-vitamins	- Feeding of dairy cows during the lactation and close-up period. Also suitable for calves and beef cattle - Improves milk and meat production - Improves feed efficiency and organic matter digestibility - Creates a better rumen environment
		Aquagrow E	Spray-dried and autolyzed *S. cerevisiae*, rich in bioactive ingredients and nutrients such as essential amino acids, peptides, cell wall carbohydrates, nucleotides and B-vitamins.	- Aquaculture feeds - Contributes to immunity development - Improves animals'stress resistance - Maintains intestinal integrity
Angel Yeast and Co., Ltd.	GroPro	Swine[e]	Provides young animal digestible proteins in the form of free amino acids/peptides and functional nucleic acids.	- Replaces SDPP in creep feed. - Promotes growth in weaned pigs.
		Poultry[f]	Provides young animal digestible proteins in the form of free amino acids/peptides and functional nucleic acids.	- Feed ingredient - Promotes growth and reduces mortality in broilers. - Improves laying rate and egg quality.
		Aqua[g]	Provides young animal digestible proteins in the form of free amino acids/peptides and functional nucleic acids.	- Feed ingredient - Promotes growth and improves palatability.
		Rumen[h]	High quality of nucleic acid, small peptide, amino acid, polysaccharides and other bioactives.	- Feed ingredient - Creates a better rumen environment - Improves feed digestibility. - Increases milk yield.
		Pet[i]	Rich in flavoring attractants such as peptides, amino acids and nucleic acids.	- Feed ingredient - Enhances palatability of pets' food. - Promotes growth and development of young pets by improving digestion. - Promotes the absorption of trace elements thus brighten the coats.
	Fubon[j]		Spent brewer's yeast extract with high efficient microprotein (about 40%) and B-vitamins.	- Improve animal feed quality and growth performance
Kohjin Life Sciences Co., Ltd.	HITHION™[k]	YH-15	Torula extract containing at least 15% of glutathione.	- Dietary supplement - Anti-hang over and liver health - Skin whitening and skin beauty, anti-aging
		YH-D18	Torula extract containing at least 18% of glutathione which is mainly oxidized.	- Food applications: beverages, jelly and snacks - Anti-hang over and liver health - Skin whitening and skin beauty, anti-aging.
	AJITOP™[l]		Yeast extract rich in natural glutamate and other amino acids, peptides, and nucleotides.	- Adds strong savory taste in front - Provides the authentic taste of food materials
Nippon Paper Industries Co., Ltd.	SK Yeast Extract[m]	HU(T)	Torula hydrolyzed extract with high contents of natural 5'-IMP, 5'-GMP and 64.6% of peptides (8–10% of free amino acids).	- Flavour enhancer and suppressor of nasty taste - Fermentation nutrient
		HUAP (T)	Torula hydrolyzed extract with high contents of natural 5'-IMP, 5'-GMP and 40.4% of protein (15–17% free amino acids).	- Flavour enhancer - Beef extract-replacer - Fermentation nutrient - Feed attractant for fish and animal
Alltech	NuPro®[n]		Yeast extract rich in nucleotides, glutamic acid, amino acids, peptides and inositol (45% of crude protein).	- Increases feed intake and animal performance (beef cattle, dairy cows, poultry, pigs, aqua, pet and equine) due to improvement of palatability - Contains components that improve cell repair and growth

SDPP – Spray dried porcine plasma, 5′-IMP - Inosine 5′-monophosphate, 5′-GMP - Guanosine 5′-monophosphate.

[a] https://www.ohly.com/en/x-seed/yeast-peptone/
[b] https://www.ohly.com/en/x-seed/yeast-peptone/x-seed-peptone/
[c] https://www.ohly.com/en/x-seed/yeast-peptone/x-seed-nitroboost/
[d] https://www2.biomin.net/nz/products/levabon/
[e] https://en.angelyeast.com//products/animal-nutrition/gropro-swine.html
[f] https://en.angelyeast.com//products/animal-nutrition/gropro-poultry.html
[g] https://en.angelyeast.com//products/animal-nutrition/gropro-aqua.html
[h] https://en.angelyeast.com//products/animal-nutrition/gropro-rumen.html
[i] https://en.angelyeast.com//products/animal-nutrition/gropro.html
[j] https://en.angelyeast.com/products/animal-nutrition/fubon-brewers-yeast.html
[k] https://www.kohjinls.com/en/business/healthfoodmaterial/hithionextract_yh/
[l] https://www.kohjinls.com/en/business/foodmaterial/ajitop/
[m] http://www.npchem.co.jp/english/product/yeast_extract/index.html
[n] https://www.alltech.com/nupro

Table 5
Cosmeceutical applications of bioactive peptides from *S. cerevisiae*.

Extraction and purification method	Extract characterization	Bioactivities	Reference
Hydrolysis with methanol (2 h, 60 °C) followed by SEC and IEC purification	Four peptide fractions	The fraction from 6000 to 17,000 Da caused angiogenesis in the chick embryo and the rabbit eye angiogenesis models. Stimulation of the wound healing in rats was also observed at dose levels of 200 and 20 μg/d.	Bentley et al. [50]
NM	Peptamide-6® (signal peptide; FVAPFP)	Increase of collagen synthesis and upregulation of growth factors, transmembrane, matrix and cell shock proteins.	Gorouhi and Maibach [17]
Microfluidizer followed by 3 kDa ultrafiltration and C18-HPLC purification	Hexapeptide-11 (FVAPFP)	*In vitro* increase of the Type 1A1 collagen expression in normal human dermal fibroblasts.	Gruber [18]
NM	Hexapeptide-11 (FVAPFP)	- *In vivo* improvement of skin elasticity in human. - Significant protection in fibroblasts against oxidative-stress. - Genes activation involved in the proteostasis network regulation.	Sklirou et al. [16]

NM – Not mentioned.

neurotransmitter inhibitor peptides [49]. Signal peptides can be responsible for triggering collagen synthesis and activating cell growth and migration, acting as growth factors, whereas carriers transport trace elements involved in angiogenesis, wound healing and other enzymatic processes (such as cooper and manganese). On the other hand, the peptides that slightly increase minimal muscular activity are named neurotransmitter inhibitors, since they specifically inhibit neurosecretion which can lead to wrinkles and fine lines smoothing [17,47,49]. Bioactive peptides skin barrier permeability remains a challenge, since only low MW peptides can permeate the skin. Besides, peptides formulation stability may also be an issue because of their susceptibility to pH and temperature and antioxidant peptides may lose their antioxidative capacity if left in contact with air.

Bioactive peptides isolated from *S. cerevisiae* have been described as having beneficial effects on wound healing and collagen synthesis (Table 5). Bentley et al. [50] extracted four different peptide fractions from yeast and screened their biological activity by using the chick embryo angiogenesis assay and the rabbit eye angiogenesis model. The most active fraction (6000–17,000 Da) caused angiogenesis in both assays and was chosen for wound healing assays in rats at dose levels of 200 and 20 μg/d, where an increase in wound healing stimulation was observed. Other studies focused on extraction of a specific hexapeptide (FVAPFP, Hexapeptide-11, Peptamide-6®) from *S. cerevisiae* [16–18] that was patented to be used in a personal care composition patch [17]. Gruber [18] observed an *in vitro* increase of the Type 1A1 collagen expression in normal human dermal fibroblasts by stimulation of the extracellular matrix protein production. For this reason, the peptide was suggested for use in topical application and for regulating skin condition. Gorouhi and Maibach [17] also reported an increase of collagen synthesis and upregulation of growth factors, transmembrane, matrix and cell shock proteins by Peptamide-6® from Arch Chemicals (Lonza Ltd.). It allowed an improvement of skin elasticity and deformation response at week 4 in 25 healthy subjects after application onto half-face (periorbital and cheek) of the product twice daily. In fact, Peptamide-6® is currently recognized as a natural firming hexapeptide, ideal for incorporation in face, body and eye creams or other anti-aging products since it reduces appearance of fine lines. Sklirou et al. [16] also observed an improvement of skin elasticity with *in vivo* skin deformation assays in human by hexapeptide-11 treatment, beyond a significant protection in fibroblasts against oxidative-stress. The authors found an activation of genes involved in proteostasis network regulation related with proteasome, autophagy, chaperones and antioxidant responses, suggesting the hexapeptide-11 as a promising anti-aging agent.

As occurs with the large majority of commercial yeast derived products, in cosmetic area the label information does not provide information on the source of yeast.

4. Conclusions

Over the years, due to increase interest owing to its health benefits in important physiological human processes, different sources of bioactive peptides have been ever more explored. *S. cerevisiae* has been described as an important and potentially less expensive source of bioactive peptides, since it can be obtained as a brewing by-product; indeed, its recovery and reuse are becoming an essential milestone for circular economy and environment protection.

In the last decades, several processes for extraction and purification of yeast protein and peptides have been studied, aiming to obtain the highest yield and molecules bioactivity at the lowest economic cost. However, this is still an ongoing process and there is room for much improvement.

Yeast bio-functional peptides are mainly characterized by antihypertensive, antioxidant and antimicrobial properties, although other studied bioactivities are being added to the portfolio. Several authors suggested yeast peptides as dietary supplement for treatment and prevention of chronic diseases, since they have a strong ACE inhibition activity, thus becoming an alternative for several ACE inhibitory drugs (such as captopril) in hypertension treatment, with the added bonus of having lower or no side effects. The protection of gastric mucosa is also pointed out as a health benefit, since yeast peptides were able to reduce gastric injuries *in vivo* by prostaglandin-mediated mechanism. Furthermore, the high iron-binding capacity of yeast peptides also makes them a promising iron-delivery and anti-anemic source to produce supplements with iron-peptide chelates. Recently, some authors hypothesized their use for preventing diabetes, because of the high amount of GABA detected in yeast extracts. Several yeast protein-rich extracts are already on the market, being used as human dietary supplements, flavour enhancers or additive in fermentation processes and animal feed, although their source is usually not provided. The labelling of the source as a sustainable one could be a promotion factor for the product, due to the increasing awareness and concern from general consumers to the environmental issues.

On the other hand, the strong antioxidant activity of yeast peptides with *in vitro* and *in vivo* models, together with beneficial effects on wound healing and collagen synthesis, makes them a very attractive ingredient to incorporation in cosmetic products for treatment of aging skin.

In conclusion, the exploration of spent brewer's yeast (*S. cerevisiae*) for new biomolecules, namely proteins and peptides, with great biological and economical value, is fuelled by several factors: spent yeast is a cheap and readily available by-product of the brewing industry, and its use would contribute for a more sustainable and circular economy; reported bioactivities of such proteins and peptides recovered from spent *S. cerevisiae* are promising agents against hypertension, skin aging, resistant microorganisms, and as functional foods; the financial potential of extraction of these molecules is high, due to the low cost of the raw material and potential simplicity of extraction methods used. Thus, the use of spent yeast as the preferential source of bioactive peptides will certainly imply more research in extraction and purification processes in order to lower their costs. However, the final economic and environmental benefits will certainly overmatch the investment. The market for yeast bioactive peptides already exists, now is time to pave the way for

those sustainably sourced.

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