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## REVIEW ARTICLE

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## Future production of yeast biomass for sustainable proteins: a critical review

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Yeast biomass has untapped potential as a sustainable source of nutritional protein. To realise this, current production capacity and the demand for yeast-based products in food applications must be increased. This review explores the possibility of increasing yeast supply using low-cost and sustainable substrates such as lignocellulosic sugars, starch hydrolysates and waste lactose, and by utilising the waste yeast biomass that will be generated from future recombinant protein production. Candidate yeast strains and processes for producing biomass from these substrates are reviewed in relation to production efficiency and product functionality. The opportunity to lower production costs and control yeast cell properties using continuous cultivation is highlighted. Current knowledge of how yeast diversity, metabolism and physiology are influenced by growth conditions is brought together to understand how yeast biomass can be produced with desirable functional properties. In particular, this review provides insights into how the variety and adaptability of yeast can make it possible to adjust attributes such as protein and cell wall composition through strain selection and controlled production. Major gaps are identified as targets for future research, in particular understanding the functional properties of non-*Saccharomyces* yeast biomass that could be produced from lignocellulosic sugars, lactose and precision fermentation. Specific, controlled studies of yeast biomass functionality in relation to species and growth are now needed to help expand the scale of production and associated environmental benefits of nutritional yeast.

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## Sustainability spotlight

This article aims to enable expanded production of yeast biomass as a sustainable source of nutritional protein. The review focussed on addressing key limits to current yeast supply by outlining paths to utilisation of low-cost and sustainable feedstocks and efficient production processes. Achieving this at large scale would contribute to three UN SDGs: 2: zero hunger: increasing the world's supply of nutritional protein in a sustainable and economic manner will help alleviate hunger. 12: responsible consumption and production: ensuring more sustainable production of nutritional proteins while better utilising wastes. 15: life on land: freeing up land currently used to inefficiently produce protein will help reduce pressures leading to deforestation.

## 1. Introduction

## 1.1 Yeast as a sustainable source of protein

The growing global demand for protein is straining key resources such as land, water and phosphorous, and is significantly contributing to greenhouse gas (GHG) emissions.<sup>1</sup> While plant-based proteins can displace more resource- and GHG-intensive animal-derived proteins, microbial protein production from organisms such as yeast, can be even more resource-efficient.<sup>2,3</sup> Microbial biomass is produced in fermenters, which enables complete utilisation of nitrogen and phosphorus while eliminating run-off pollution.<sup>4</sup> Further, microbes have significant inherent advantages over plants and animals as a source of protein, including very short generation times, the ability to manipulate metabolism and composition, and the possibility of

continuous production independent of climatic factors. Although heterotrophically grown microbes such as yeast require an organic carbon source, this can involve utilisation of low-value by-products or wastes<sup>5</sup> such as molasses and lactose, sugars obtained from high-yielding crops such as sugarcane, or sugars derived from second-generation feedstocks such as agricultural wastes or lignocellulosic crops such as switchgrass.<sup>6</sup> Thus, utilising microbial protein production methods can provide major reductions in land, water and GHG footprints compared to animal protein production.<sup>2,4,6</sup> The potential of microbial protein production for food (often referred to a single cell protein, SCP) has been recognised for almost a century, with the term 'single cell protein' (SCP) first coined at Massachusetts Institute of Technology in 1966.<sup>7–10</sup> However, production and utilisation of microbial proteins must be dramatically increased to realise the potential environmental benefits. Further, large-scale production of yeast biomass should preferably utilise second generation feedstocks,<sup>11</sup> leveraging the experience and achievements from industrial scale bioethanol production.<sup>12</sup>

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Yeast is a particularly promising source of microbial protein. Yeasts such as *Saccharomyces* have been domesticated for millennia, studied in detail, and are robust and fast-growing in a variety of industrial applications.<sup>13</sup> Traditionally, the catalytic power of yeast has been used to leaven bread<sup>14</sup> and produce ethanol and flavours in beer and wine.<sup>15</sup> More recently, the ability of recombinant yeast to express foreign proteins is being developed to produce specific food-related products such as proteins and vitamins, using so-called 'precision fermentation'.<sup>16</sup> As will be discussed below, a variety of yeast species can be used for food ingredient production, including *S. cerevisiae* (Baker's yeast and ale yeast), *Saccharomyces pastorianus* (lager yeast),<sup>17,18</sup> *Kluyveromyces* and *Candida* (lactose-fermenting yeasts), *Komagataella phaffii* (methylotrophic yeast)<sup>19</sup> and *Yarrowia* (oleaginous yeast). The focus of this review is on future sustainable production of yeast biomass as a source of nutritional proteins for food applications<sup>20</sup> such as extruded food products,<sup>21,22</sup> processed meats<sup>23</sup> and mayonnaise.<sup>24</sup> This includes both purpose-grown yeast and yeast biomass as a byproduct from precision fermentation.

## 1.2 Yeast proteins as food ingredients

Yeast are a rich source of proteins, which typically represent 40–60% of the cell biomass.<sup>20</sup> As a food ingredient, yeast proteins comprise the entire proteome of the organism including a multitude of enzymes and structural proteins. This is contrast to most plant proteins used as food ingredients, which typically comprise a small number of highly abundant storage proteins (e.g. glycinin and  $\beta$ -conglycinin in soybeans,<sup>25</sup> globulin, glutelin, albumin and prolamin in chickpeas<sup>26</sup> and glutenins and gliadins in wheat<sup>27</sup>). The limited structural diversity with different plant protein ingredients makes them relatively straightforward to understand in terms of food functionality and nutritional characteristics such as amino acid profile. In comparison, the diversity of proteins within yeast cells coupled with variability of protein profiles as a function of both species and growth conditions makes it much more challenging to characterise and predict their behaviour as food ingredients. It also provides an opportunity to tailor the properties of the produced yeast proteins through species selection and process design.

Currently, yeast serves as the raw material for so-called yeast extracts,<sup>28</sup> which are derived from autolysed yeast cells and used as ingredients for a range of food applications including savoury spreads, soups, snacks, sauces and prepared meals.<sup>29</sup> As food ingredients, the hydrolysed yeast proteins, peptides and amino acids have desirable taste attributes such as umami and kokumi, participate in Maillard reactions and contribute to the nutritional value of the products.<sup>30</sup> Proteins from yeast such as *S. cerevisiae* and *Pichia* sp. are generally considered to not pose concerns in terms of allergenicity or toxicity.<sup>31</sup> Further, the proteins in *S. cerevisiae* contain high amounts of essential amino acids.<sup>32,33</sup> The content and profile of essential amino acids in *S. cerevisiae*, *Yarrowia lipolytica* and *Candida utilis* compared favourably against wheat, egg, cow milk and FAO requirements.<sup>20</sup> While the high content of nucleic acids in yeast would limit safe dietary intake, there are established methods

involving heat, enzyme or alkali treatments to breakdown or extract the RNA.<sup>34</sup> Increasing global consumption of yeast biomass to accrue meaningful environmental benefits, will rely on both reducing the cost of production to commodity levels and expanding the range of applications for yeast products. Developing yeast products with desirable attributes will be key, and will require careful control of yeast biology during large-scale production and bioprocessing to optimise the properties of yeast cells for specific food applications and products.

The functionality of yeast biomass as a food ingredient for various formulations such as baked goods, snacks and processed meat has been recently reviewed,<sup>35</sup> and includes properties such as solubility, flavour enhancement, nutritional supplementation, foaming and emulsification. While these reviews provide a good overview of the relevant functional properties of relevance and the recent literature, it is notable that the information was not discussed in relation to different yeast species or growth conditions.<sup>35</sup> Overall, research into food functionality of yeast is still in its infancy, with studies by food scientists starting to emerge, but mostly on generic 'yeast proteins'. For example, a recent study investigating the foaming and emulsifying properties of yeast protein did not mention the species of yeast, the growth conditions, nor any characterisation information of the proteins.<sup>36</sup> Further, the digestibility of one yeast protein concentrate powder has recently been studied, however the species and growth conditions of the yeast were not detailed.<sup>37</sup> Further, there appears to be a lack of comparative analyses of the functional properties between or within different yeasts or of different individual or classes of yeast proteins from within the proteome. Although not specifically targeted to nutrition, one early study compared the amino acid profiles of eight yeast species including *Saccharomyces cerevisiae*, *Candida utilis*, *Kluyveromyces fragilis*, finding some broad similarities but also variability.<sup>38</sup> As discussed below, the protein and amino acid profiles of different yeasts grown in different ways on different media will be highly variable and to some extent able to be manipulated. Producing yeast biomass with desirable properties will require understanding how yeast proteomes are influenced by controllable production parameters, in the context of scalable and economic feedstocks and processes. There have been major recent advances in our understanding of yeast metabolism<sup>39–41</sup> that can now be applied to controlling the yeast proteome as a raw material for food applications grown on substrates sourced from sustainable feedstocks.

## 2. Future sources of edible yeast biomass

Current production of potentially edible protein from baker's and brewer's yeasts is a little under 1 M tonne per year (assuming 1.7 Mt per year of yeast containing ~50% protein<sup>42</sup>). This is equivalent to ~0.3% of current global protein demand (260–290 Mt per year).<sup>1</sup> Approximately 2/3 of this yeast is baker's yeast, and 1/3 is brewer's spent yeast (BSY) that is produced as a by-product from alcoholic beverage production.<sup>28,43</sup> However,





only a small proportion of this yeast biomass is converted into food ingredients such as yeast extracts. Most baker's yeast is used in baking applications, while the majority of BSY is used as an animal feed.<sup>42</sup> Yeast biomass is also generated in wine (*i.e.* lees)<sup>44</sup> and spirit making (*i.e.* spent yeast from distilleries), but these processes produce relatively low amounts of biomass. Further, yeast biomass is a byproduct of industrial bioethanol production, however these processes are not operated under food-grade conditions, limiting the potential of the yeast biomass as a source of human nutritional protein.

Future expansion of yeast food products will require major new sources of yeast. While it is possible to increase the use of available BSY for yeast-based food ingredients, as a by-product, brewer's yeast production is ultimately limited by the demand for beer. There is much greater scope to expand the production of baker's yeast as a food ingredient. This would require construction of new dedicated fermentation facilities to the increase production capacity of baker's yeast biomass for food applications. Production of baker's yeast has traditionally used molasses as the carbon source, however the sustainability and price of molasses is becoming a limiting factor.<sup>45,46</sup> Baker's yeast can also be produced using hydrolysed starch from crops such as cassava<sup>47</sup> or rice,<sup>48</sup> which can greatly expand the amount of available substrate.

To further expand future yeast protein production, it is increasingly being acknowledged that it is preferable to use second generation feedstock materials that don't compete with food production and that production of protein should be considered over fuels.<sup>11</sup> In particular, yeasts that are able to utilise pentose sugars (natively or *via* genetic modification) could be produced from lignocellulosic sugars, and spent yeast resulting from production of recombinant food proteins could be utilised. There is also potential to produce other edible yeasts such as *Kluyveromyces* using the lactose in dairy waste streams such as whey, or oil-bearing yeasts such as *Yarrowia lipolytica*. These opportunities represent a range of new yeast production processes that will differ from each other in many respects, including the species/strain of yeast, growth medium composition, reactor mode and operational conditions (Table 1). In this section, the key distinguishing features of the different yeast production options will be critically reviewed. This provides the practical context for the subsequent analysis of the effects of controllable yeast production parameters on biomass properties.

## 2.1 Expanded production of baker's yeast

As an active ingredient for commercial and domestic baking applications, the major aim of conventional processes is to produce high yields of active Baker's yeast from low-cost medium ingredients.<sup>51,52</sup> This has led to a tightly defined and well-established production process. Future expanded production of baker's yeast biomass for nutritional proteins can be expected to follow a similar process, aimed at maximising biomass yield. To achieve a high biomass yield baker's yeast is produced using aerobic fed-batch fermentation. The yeast must be grown under aerobic conditions to enable more efficient

aerobic respiration to occur in place of fermentation. When grown aerobically, the biomass yield can reach up to  $0.5 \text{ g g}^{-1}$  compared to around  $0.1 \text{ g g}^{-1}$  under anaerobic conditions under which much of the substrate carbon is converted to ethanol instead of new cell matter.<sup>53</sup> To be aerobic, active aeration is required to maintain the oxygen level above a critical dissolved oxygen concentration, the value of which varies in response to adaptive yeast metabolism.<sup>54</sup>

Aerobic growth is a necessary but not sufficient requirement for avoiding ethanol production by baker's yeast. To grow rapidly when high concentrations of glucose are present, some yeast including *S. cerevisiae* invoke a form of overflow metabolism, referred to as the Crabtree effect, to generate energy *via* aerobic fermentation rather than the more efficient respiration.<sup>55,56</sup> This occurs when the concentration of glucose in the medium exceeds a critical value, which has been reported to be as low as 40 and  $50 \text{ mg L}^{-1}$ .<sup>53</sup> This means that batch cultivation of *Saccharomyces*, even under aerobic conditions, will result in the formation of ethanol as high sugar concentrations are present in the initial growth media. Therefore, to avoid the Crabtree effect and resulting ethanol production, fed-batch cultivation is used in which low concentrations of glucose are maintained by controlled feeding of substrate during the cultivation to provide the necessary flow of carbon.<sup>57–59</sup> Industrial fed-batch production of baker's yeast is performed in large bubble column bioreactors ( $>100 \text{ m}^3$ )<sup>53</sup> operated at temperatures between 28–30 °C.<sup>60</sup> The reactors are typically seeded with about  $20 \text{ g L}^{-1}$  of yeast, which increases to a final concentration of 55–60  $\text{g L}^{-1}$ .<sup>53</sup> The growth medium is typically cane or beet molasses, which contains sucrose that is rapidly hydrolysed to glucose and fructose by the yeast's invertase, with ammonia fed as a nitrogen source.<sup>53</sup> Very large-scale production of yeast biomass will require cheap and sustainable sources of fermentable sugars that extend beyond the limited supply of molasses. Starch-based feedstocks that can be hydrolysed to glucose would appear compatible with current molasses-based production processes involving *S. cerevisiae*. In contrast to production of biofuels from these crops that presents a food *versus* fuel issue, production of yeast biomass represents an upgrading of food supply. While new facilities dedicated to the production of baker's yeast biomass for food applications from starch-based feedstock could use a similar fed-batch cultivation process, there is also the scope to increase efficiency using aerobic continuous cultivation operated to maximise productivity while avoiding the Crabtree effect, as discussed in Section 3.2.

## 2.2 Yeast production from lignocellulosic biomass

While producing yeast protein from edible sugars can be considered an upgrade in food supply, it would be preferably to increase protein supply using non-edible materials *i.e.* second generation feedstocks.<sup>11</sup> In particular, it is possible to grow yeast on highly abundant lignocellulosic materials, which have been intensely studied as a feedstock for bioethanol production.<sup>61</sup> Importantly, the high abundance of lignocellulosic biomass means there is essentially no limit to the amount of biomass



Table 1 Comparative summary of future options for expanding the production of yeast biomass as a food ingredient

		Starch hydrolysates	Lignocellulosic sugars	Precision fermentation yeast	Lactose-fermenting
Yeast	Genus/Species	<i>S. cerevisiae</i> or others	Various, including <i>Saccharomyces</i> (recombinant), <i>Komagataella</i> , and <i>Yarrowia</i>	<i>S. cerevisiae</i> or <i>Komagataella phaffii</i> (formerly <i>Pichia pastoris</i> )	<i>Kluyveromyces</i> or <i>Candida</i>
GMO Reactor mode		No Fed-batch or continuous	Maybe Batch (non-Crabtree yeast), fed-batch (Crabtree yeast) or continuous (Crabtree or non-Crabtree yeast)	Yes Fed-batch	No Batch (non-Crabtree yeast), fed-batch (Crabtree yeast) or continuous (Crabtree or non-Crabtree yeast)
Harvest growth state		Decelerating growth (batch/fed-batch); controlled $\mu = D$ for continuous	Decelerating growth (batch/fed-batch); controlled $\mu = D$ for continuous	Decelerating growth	Decelerating growth (batch/fed-batch); controlled $\mu = D$ for continuous
Feedstock	Source	Corn, wheat, tubers, rice	Grasses, forestry, food crop residues	Sugarcane/beet (molasses), various (methanol and other)	Dairy
	Sustainability	Up-grading of food supply (edible sugar to protein)	Increased food supply (non-edible sugar to protein)	Increased food supply (waste by-product to protein)	Increased food supply (waste by-product to protein)
Growth medium	C-source	Molasses (sucrose); or starch hydrolysates (glucose, maltose)	Lignocellulosic sugars including glucose, xylose, mannose, galactose and arabinose	Various, including glucose, molasses and methanol	Lactose from whey
	N-source Phenolics and inhibitors	Urea or ammonia/um Molasses phenolics (e.g. caffeic acid, ferulic acid, gallic acid) <sup>49</sup>	Urea or ammonia/um Various including furfural and HMF	Various Minimal	OrgN in whey Not significant
Extracellular Environment	Temperature (°C)	~30	Various	Various	~30
	Dissolved O <sub>2</sub> pH	Aerobic ~4.5	Aerobic 4–6	Aerobic 4–6	Aerobic 4–5 (ref. 50)
Future scope	Manipulable conditions to tailor yeast biomass	Yes	Yes	No	Yes
	Potential scale of production	Very large	Very large	Large	Moderate

that could be produced from this resource. Further, lignocellulosic material can be sourced as wastes from current crop production, or produced at high-efficiency on marginal lands.<sup>6</sup> While lignocellulosic must undergo pretreatment using methods such as acid and enzyme hydrolysis to release the fermentable sugars, these processes are well established and have been proven at scale in a range of countries using various feedstocks.<sup>12</sup> In addition, biorefinery pathways have been established to produce multiple products in addition to the fermentable sugars, for example from the lignin component, which can improve process economics.<sup>12,62</sup> In contrast to bio-ethanol production, growing yeast biomass as a protein source requires aerobic conditions to maximise biomass yield. Further, for yeast subject to the Crabtree effect, fed-batch or continuous cultivation will be required to avoid high sugar concentrations in the growth medium. An additional consideration for yeast biomass production from lignocellulose is the need to supplement the carbohydrate-rich hydrolysates with a balanced supply of an economical nitrogen source.

A specific challenge for yeast production from lignocellulosic materials, is that the hydrolysates contain a mix of sugars including glucose, xylose, mannose, arabinose and galactose.<sup>63</sup> While many yeasts including *Candida utilis* can grow aerobically on xylose<sup>64</sup> and others such as *Scheffersomyces stipitis* (formerly *Pichia stipitis*) can even grow anaerobically,<sup>63</sup> *S. cerevisiae* is unable to utilise xylose and arabinose necessitating genetic modification for growth on these sugars.<sup>65,66</sup> The resulting yeast biomass would therefore be considered a genetically modified food, which could limit its application in some jurisdictions. Theoretically any yeast that can grow on xylose<sup>67</sup> could be produced, although due consideration would be needed for the use of any yeast strains that have not yet approved for human consumption.

Another challenge is that lignocellulosic material must be hydrolysed to release the fermentable sugars. In addition to increasing the cost, thermochemical pretreatment used to facilitate enzymatic of hydrolysis of lignocellulose produces toxic byproducts such as furfural and hydroxymethyl furfural (HMF).<sup>68</sup> As furfural is toxic to yeast, there is active research into



metabolic engineering of furfural-tolerant yeast.<sup>69</sup> The potential presence of furfural and HMF in the edible yeast biomass should also be considered. As products of the Maillard reaction, furfural and HMF are inevitably present in many foods, with considerable research being undertaken into concerns for the potential toxicity of these chemicals.<sup>70</sup> Although warranting verification, it should be possible to ensure sufficiently low concentrations of these compounds remain in the yeast following washing of the harvested biomass.

Despite these challenges, there are many recent examples of yeast being successfully grown on different biological wastes. For example, *Candida utilis* was produced as SCP from ammonia-pretreated and enzymatically hydrolysed spent brewers grain<sup>71</sup> and from orange peel residues.<sup>72</sup> *Candida maltose* was grown on volatile fatty acids produced from acidogenic bacterial fermentation of coffee grinds processed by alkaline pretreatment and subjected to enzyme hydrolysis.<sup>73</sup> Five different species of yeast (*Candida utilis*, *Candida tropicalis*, *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, and *Geotrichum candidum*) were grown separately and in combination on the sugars contained starch processing wastewater.<sup>74</sup> It has also been demonstrated that nitrogen-rich biogas slurry from anaerobic digestors could be used to grow *Debaryomyces hansenii*, a yeast able to tolerate the high alkali/saline conditions.<sup>75</sup> Beyond the yeast strains mentioned so far, oil-bearing yeast such as *Yarrowia*<sup>76</sup> could be grown as a source of both protein and nutritional oils. Importantly, *Yarrowia* is commonly found in food such as cheese, yoghurt, kefir, and soy sauce<sup>66</sup> and has been concluded to be a safe organism.<sup>77</sup> *Yarrowia lipolytica* biomass has been produced by direct conversion of food waste and by indirect conversion of volatile fatty acids produced during initial anaerobic bacterial fermentation.<sup>78</sup> *Yarrowia lipolytica* and another oleaginous yeast *Rhodospiridium toruloides* have been cultivated on cocoa fatty acid distillates.<sup>79</sup>

While the use of waste feedstocks can increase the extent and sustainability of yeast protein production, it must also be economic. A recent technoeconomic analysis of the production of single-cell protein organisms including *Candida utilis* from steam explosion pretreated and enzymatically hydrolysed wheat straw has been conducted.<sup>80</sup> Encouragingly, it was concluded from the analysis that yeast protein production from lignocellulosic biomass had potential to be both technically and economically feasible.<sup>80</sup> The promise of producing yeast protein from lignocellulose is further supported by the increasing number of commercial plants producing ethanol,<sup>12</sup> a much lower value product than protein, from lignocellulosic feedstocks. Overall, there appears to be vast potential for future production of yeast biomass from a variety of waste feedstocks. Nonetheless, there is much work to be done performing more technoeconomic assessments and life-cycle analyses of yeast biomass production from different feedstocks to provide confidence to establish an industry. This should occur in parallel with the establishment of yeast protein and biomass as bulk food ingredient, which will help to define the market value of the products, a key component in meaningful technoeconomic assessments.

### 2.3 Yeast as a by-product of recombinant food proteins ('precision fermentation')

As mentioned above, there is growing interest in expanding the production of specific food ingredients such as milk proteins and leghemoglobin using genetically modified microorganisms including yeast such as *S. cerevisiae* and *Komagataella phaffii* (formerly *Pichia pastoris*) using so-called 'precision fermentation'.<sup>3,16,81,82</sup> In this case the residual yeast biomass is a by-product that is produced under food-grade conditions. As such, expansion of this industry could result in a significant new source of yeast biomass for food production. If production of animal-free milk proteins expressed by recombinant yeast is scaled to the extent that it replaces even a small fraction of global milk production, it could result in large amounts of residual yeast biomass. For example, if recombinant yeast were used to produce the equivalent of 1% of global milk protein supply, and assuming a 40 : 1 ratio of biomass-to-recombinant protein,<sup>83</sup> >5 Mt per year of yeast biomass would be produced. While recombinant proteins expressed by yeast have been approved for food applications in some jurisdictions such as the USA, it does not appear that the yeast itself has been approved for human consumption and the implications of the recombinant DNA remaining in the yeast cells would need to be considered. In this case, as the yeast growth systems will be optimised with respect to recombinant protein production there is minimal scope to tailor the cellular proteome and physiology of the residual yeast. Both the yeast strain and growth conditions will be selected based on optimisation of the recombinant protein expression rather than biomass productivity or properties. For example, while protein expression platforms have often been developed using strains that grow most rapidly at 30 °C or above, it may be preferable for protein expression and stability and energy efficiency to operate at a lower temperature.<sup>84,85</sup>

### 2.4 Lactose fermenting yeast

Lactose is a disaccharide sugar present in whey, an abundant waste in cheese manufacturing<sup>86,87</sup> that is a potential low-cost feedstock for yeast biomass production. However, *Saccharomyces* is not able to metabolise lactose unless it has been pre-hydrolysed into glucose and galactose.<sup>88</sup> While this can be done using the enzyme lactase ( $\beta$ -galactosidase),<sup>89</sup> to avoid the cost and complication of lactose hydrolysis, it is instead possible to grow yeasts that have the ability to consume lactose directly. Such lactose-utilising yeast include such as certain species of *Kluyveromyces*<sup>90</sup> and *Candida*<sup>91</sup> that can produce lactase.<sup>92,93</sup>

Much research has been devoted to investigating the use of lactose-fermenting yeast to produce ethanol from whey.<sup>50,67,88,94,95</sup> In this case, the yeast is grown anaerobically to maximise ethanol yield, with the yeast biomass a by-product. Yeast biomass is a more valuable product than ethanol, and there have been a few recent studies of the production of yeast extracts and ingredients from lactose fermenting yeast, in particular *Kluyveromyces marxianus*. This includes production of yeast biomass as single cell protein,<sup>96</sup> for yeast extracts,<sup>97</sup> and



for the production of RNA-rich extracts.<sup>98</sup> Importantly for aerobic biomass production, *K. marxianus* does not exhibit the Crabtree effect, whereas *K. lactis* produces ethanol under aerobic conditions, but to a lesser extent than *S. cerevisiae*.<sup>99</sup> Importantly for food ingredient production, *K. marxianus* is considered a GRAS organism (generally recognised as safe), owing to its long history of use in products such as cheese and kafil.<sup>100</sup>

*Candida* is another lactose-fermenting yeast that has been investigated for the production of ethanol from acid whey.<sup>95</sup> *Candida* species are also encountered in various food applications<sup>101</sup> and beer production,<sup>102</sup> with a recently published review of literature pertaining to the potential application of *Candida* yeasts in food applications available.<sup>103</sup> However, as some *Candida* species, in particular *Candida albicans*,<sup>104</sup> are known to be pathogenic, it has been recommended that suitable testing must be done before using a given strain of *Candida* for applications.<sup>101</sup> Arguably the most important species for food applications is *Candida utilis*, which is the name of asexual/anamorphic form of the yeast *Cyberlindnera (Pichia) jadinii*.<sup>105,106</sup> *Cyberlindnera jadinii/Candida utilis* has long been used for animal feed applications, has GRAS status, and is considered to have much promise for food applications.<sup>106</sup> Importantly for aerobic yeast biomass production, species of *Candida* including *Cyberlindnera jadinii/Candida utilis*,<sup>107</sup> *Candida tropicalis*<sup>108</sup> and *Candida albicans*<sup>109</sup> are Crabtree negative, meaning that high biomass yields can be obtained in batch cultures.

Whey is the primary source of lactose, and it can be used directly as the growth medium for yeast production. The composition of whey varies depending on the cheesemaking process, but all forms contain significant amounts of lactose 4.5–6%.<sup>94</sup> The concentration of lactose in combination with the growth yield define the yeast biomass concentration that can be obtained. While the lactose concentration can theoretically be increased (e.g. by membrane filtration or evaporation), the additional process step adds complication and cost. The use of concentrated whey can also place osmotic stress on the yeast.<sup>110</sup> Whey also contains nitrogen sources including proteinaceous and non-protein nitrogen compounds such as urea.<sup>87</sup> The pH of sweet whey is higher (pH 6–7) than that of acid whey (pH < 5).<sup>87</sup>

### 3. Controlled production of future yeast biomass supplies

For any future dedicated yeast production facility, there is the opportunity to design the process to tailor the properties of the biomass or to lower the cost of production. Controllable parameters that will influence the properties and cost of the produced biomass include yeast strain selection, reactor design and operation, growth medium composition, the timing of yeast harvesting and the mode of cultivation (Fig. 1). The remaining sections of this review will explore the interactions between yeast cultivation, metabolism and physiology in the context of yeast as a food ingredient. The review focusses on the yeast properties of most importance to food ingredient functionality; the proteome, which determines the amino acid

profile and content,<sup>32</sup> and the cell wall composition (including  $\beta$ -glucan content) which influences cell strength.<sup>111</sup>

#### 3.1 Leveraging yeast diversity

As a model organism *S. cerevisiae* has been very widely studied in comparison to other yeast species. Even within this species there is diversity, with measurable metabolic differences between different strains of *S. cerevisiae*.<sup>112,113</sup> As discussed above, accessing alternative feedstocks requires looking beyond *S. cerevisiae*, and there is an enormous diversity of yeast that could be exploited including 10–16 species of *Saccharomyces* and >150 species of *Candida*.<sup>105</sup> Metabolic traits such as the Crabtree effect<sup>56</sup> and the ability to utilise various sugars differ amongst yeast,<sup>63</sup> which directly influences yeast production. In addition, yeast strains and species vary in other production-related parameters such as the maximum specific growth rate<sup>114</sup> and tolerance to inhibitors<sup>115</sup> including ethanol.<sup>116</sup> As such, selection of a yeast species must consider the metabolism and growth performance in relation to the available substrate and overall medium composition.

In addition to affecting the yeast production process, differences in metabolic traits will affect the biochemical composition and physiology of the cells, which will in turn influence the ingredient properties (Fig. 1). Most of the scientific literature pertaining to the key traits of relevance to food ingredient functionality, have been performed on *S. cerevisiae*. Currently, there is significantly less knowledge of the functional properties of non-*Saccharomyces* biomass including *Yarrowia*, *Kluyveromyces* and *Candida* as food ingredients. Only some of the many yeast species have been studied in relation to functional food properties, such as probiotic capability and bioactive metabolites.<sup>117</sup> However, comparative studies of different yeasts in relation to food ingredient production are lacking and represent a worthwhile topic for future research. One recent study compared yeast extract production from *S. cerevisiae*, *S. boulardii* and *K. marxianus* grown aerobically on glucose in fed-batch culture.<sup>97</sup> Levels of volatile metabolites, proteins and amino acids varied between the species, but all yeast extracts contained high levels of flavour-enhancing amino acids including glutamic acid.<sup>97</sup> The limited knowledge of non-*Saccharomyces* yeasts is an impediment to the production of yeast biomass from lactose and lignocellulosic sugars.

The cell wall is a key component of yeast to consider in relation to food ingredient production, typically comprising around 15–30% of the dry weight of yeast cells.<sup>105,118</sup> The cell walls of yeasts make the cells physically robust and help control interactions with the extracellular environment.<sup>119</sup> They are generally considered to be complex interconnected network comprised of  $\beta$  1,3-glucan fibres, branched  $\beta$  1,6-glucan, chitin and glycoproteins.<sup>118</sup> While yeast cell walls have the same overall biochemical components, the relative abundance differs considerably.<sup>118,120</sup>  $\beta$ -Glucan is a major biochemical component of yeast cell walls (typically 50–70 wt% for *S. cerevisiae*)<sup>121,122</sup> that has received ongoing interest in regard to potential health benefits.<sup>123–125</sup> Importantly, the cell walls of *Candida*<sup>126</sup> and *Yarrowia*,<sup>126</sup> *Cyberlindnera/Pichia jadinii*<sup>127</sup> and *Kluyveromyces*<sup>128</sup>





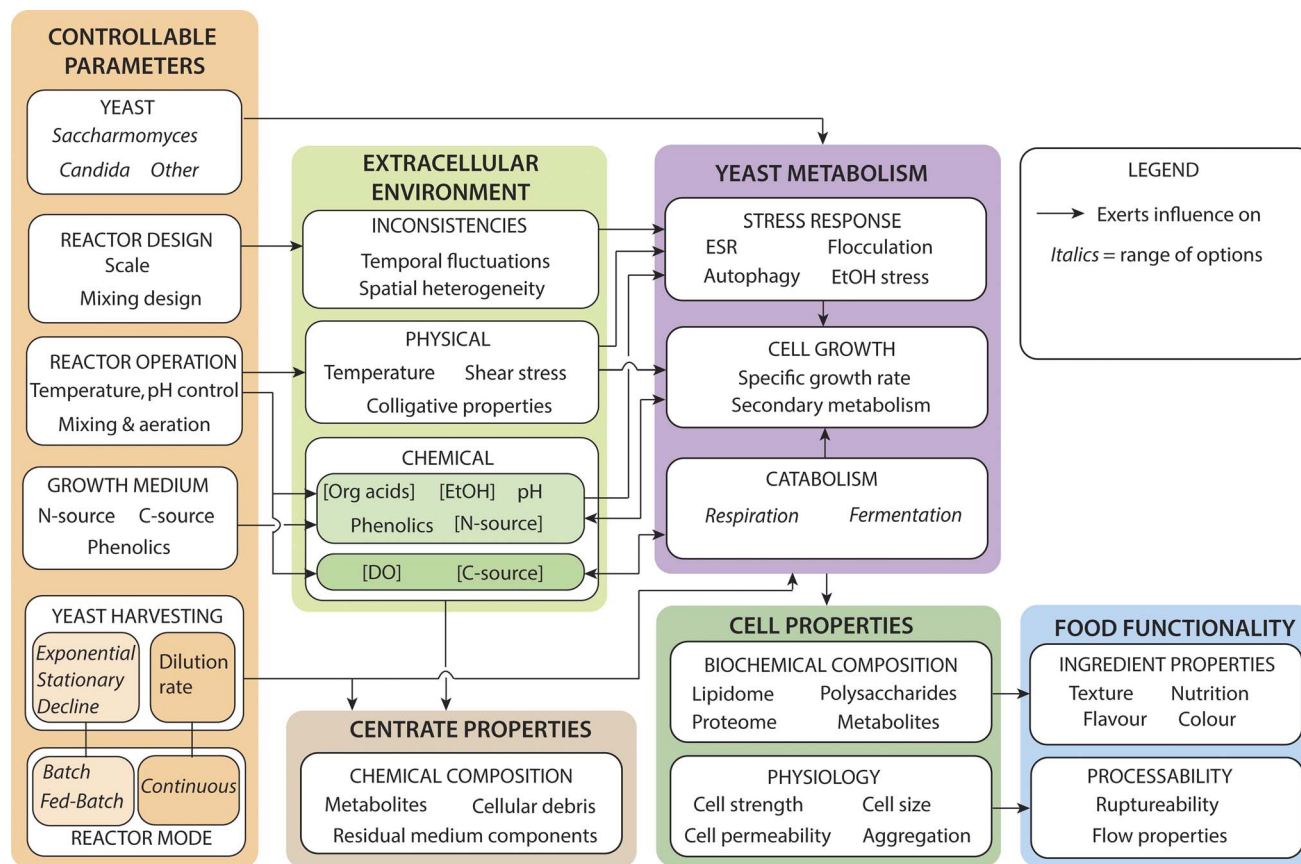


Fig. 1 Diagram illustrating key connections between the controllable parameters for yeast production, the extracellular environment, yeast metabolism, the properties of the harvested cells and centrate, and the functionality of the harvested yeast for food ingredient production.

all include  $\beta$ -glucan, although the exact composition varies. Yeast cell walls also contain proteins as a key structural unit that contributes significantly to the overall protein content of the biomass. A recent *in silico* proteomic study comparing cell wall proteins expressible amongst 92 different yeasts found that proteins required for cell wall biosynthesis and remodelling were more highly conserved between the species than those involved in flocculation and aggregation.<sup>119</sup>

### 3.2 Maximising yeast production efficiency

Expanded yeast production will require the use of less costly and more abundant substrates than molasses. In addition, highly efficient and cost-effective processes that maximise biomass yield and productivity will be needed to commoditise yeast proteins as a food ingredient. Yeast biomass can be produced using three different fermenter operational modes: batch, fed-batch and continuous. These reactor modes have inherent differences that are consequential to many aspects of yeast ingredient production (Fig. 2). Most importantly, continuous fermentation offers much higher volumetric productivities ( $\text{g L}^{-1} \text{d}^{-1}$ ) compared to batch and fed-batch operations due to the avoidance of process interruptions and the sustained high biomass concentrations in the bioreactors.<sup>8</sup> As such continuous cultivation has the potential to greatly reduce the cost of future yeast production compared to conventional fed-

batch production of baker's yeast. However, prolonged cultivation in continuous cultures can increase the risk of contamination by foreign organisms or genetic variants in the production strain arising from repeated cell division cycles.

Continuous baker's yeast production has been attempted industrially in the past,<sup>129</sup> however it has not been favoured industrially,<sup>130</sup> presumably due to operational challenges such as increased risk of contamination and genetic variants. As baker's yeast must be tightly controlled to ensure consistent activity in baking applications,<sup>131</sup> genetic variation and contamination (even by non-pathogenic microbes) presents a major issue. However, these issues may represent less of a problem for yeast biomass produced solely as a protein supply. In which case, the possibility of open, continuous cultivation not involving tight aseptic controls could also be considered to further reduce the costs.<sup>132</sup> This could be applicable if strains of yeast can be used that are tolerant to extreme conditions such as low pH or the presence of inhibitory compounds.

Batch and fed-batch operations, which are relevant to beer fermentation and conventional baker's yeast production respectively, are dynamic, involving inconstant extracellular environments (e.g. cell, substrate and metabolite concentrations) and corresponding changes in yeast metabolism and cell physiology (e.g. altered rate cell division, or environmental





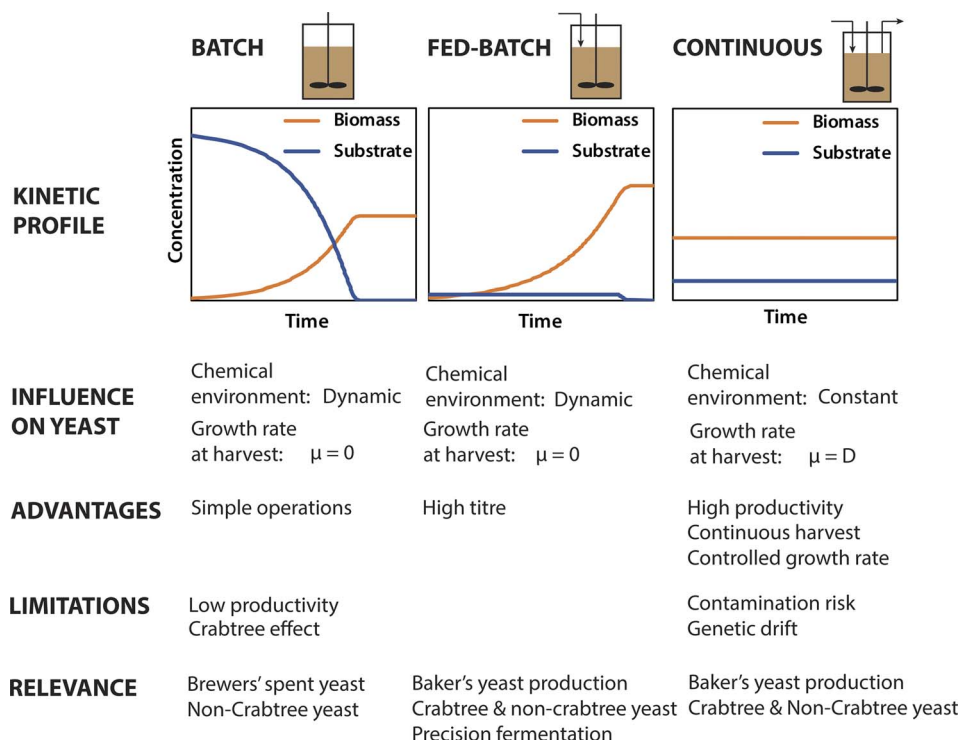


Fig. 2 Comparison of batch, fed-batch and continuous yeast production modes.

stress responses). Cells are harvested periodically at the end of batch and fed-batch operations at which point the cells have ceased replication.<sup>133</sup> In current practice, baker's yeast is typically grown in fed-batch culture for 12–15 hours, with the cells harvested just 1–2 hours after cessation of substrate feeding,<sup>53</sup> representing an early stationary phase. In contrast, continuously harvested biomass is still growing at the specific growth rate ( $\mu$ ) corresponding to the chosen dilution rate ( $D$ ). Further, continuous cultures are typically operated at steady state, in which the extracellular environment and yeast metabolism are constant and controlled by operational parameters such as the dilution rate, feed substrate concentration, pH, and temperature. As such, the properties of the harvested cells can be tuned to some extent by controlling operational parameters, in particular the dilution rate (as  $\mu = D$ ). However, as maximal biomass productivity is achieved at high dilution rates close to  $\mu_{\max}$ <sup>134</sup> it may not be practical to operate at low dilution rates even if biomass with more favourable functional properties could be produced.

### 3.3 Effect of production parameters on yeast cell properties

The physiological and metabolic state of the yeast cells upon harvesting define the raw material properties for ingredient manufacture. The effect of production parameters on the properties of the harvested yeast is therefore important to understand. During exponential growth yeast cells divide *via* budding, using a highly regulated cell cycle.<sup>135</sup> Cells have characteristic proteomes and cell physiology tuned to their growth in different environmental conditions. In *S. cerevisiae*,

the rate of cell division (as controlled by chemostat dilution rate) has recently been shown to directly control the allocation of proteins devoted to biosynthesis.<sup>136</sup> This is consistent with another study that showed a greater percentage of the proteome of rapidly growing *S. cerevisiae* is allocated to translation than in slower growing yeast, and that an excess of ribosomes (beyond the active translational capacity) is maintained in order to rapidly respond to more favourable growth conditions.<sup>137</sup> Thus, both the overall amount and the relative abundance of different proteins in the *S. cerevisiae* proteome is directly influenced by growth rate. Proteomic remodelling in relation to growth rate should occur in other relevant yeasts, and this could be a topic for future research.

As explained above, the growth rate of yeast harvested from batch and fed-batch fermenters will be zero, whereas it can be controlled in continuous fermentation (Fig. 2). This has important implications for yeast biomass as a food ingredient, in which protein is the major desirable nutrient. For example, the protein content and amino acid profile of yeast biomass is important to its nutritional value. Importantly, the amino acid content of *S. cerevisiae* changes as a function of growth conditions, with the total protein and free amino acid content decreasing during the stationary phase.<sup>138</sup> More recently, it was shown that differences in the proteomes of yeast grown under different conditions (aerobic *versus* anaerobic) significantly influenced the amino acid profile of yeast hydrolysates, and therefore their nutritional and taste attributes.<sup>32</sup> With further research it may be possible to deliberately influence the amino acid profile of yeast biomass by selecting growth conditions and media for specific strains that result in proteomes with



characteristic amino acid profiles, for example rich in specific amino acids.

Yeast cells multiply *via* budding, which is a complex, highly regulated process requiring controlled growth and division of the cell wall.<sup>139</sup> The cell wall composition and strength are important in relation to yeast as a food ingredient. In one study of both lager and ale yeasts there was no significant difference in the mechanical strength of cells harvested at the start, middle or end of a 100 hours batch fermentation.<sup>140</sup> However, in a study on baker's yeast,<sup>133</sup> cells grown in chemostats at low dilution rates ( $D = 0.06\text{--}0.12\text{ h}^{-1}$ ) were found to be stronger than those grown at high dilution rates ( $D = 0.22\text{--}0.28\text{ h}^{-1}$ ). Likewise, cells harvested in the late stationary of batch cultures were stronger than those harvested earlier in the batch cycle. It was proposed that the increase in strength at low  $D$  could be due to lower proportion of freshly budded cells and/or stronger walls in slower growing yeast.<sup>133</sup> Significant differences in the mechanical strength of other microalgae cells has been observed as a function of a prolonged stationary phase (resulting from nitrogen source depletion), which could be attributed to thickened cell walls,<sup>111</sup> which is also known to occur in the early stationary phase for *S. cerevisiae*.<sup>121</sup> The physical strength of cells is an important property for ingredient production, which is dependent on the cell wall composition and thickness.<sup>111</sup> The mechanical strengths of yeast cells such as *S. cerevisiae*,<sup>141</sup> *Pichia pastoris*,<sup>142</sup> *Yarrowia lipolytica*<sup>143</sup> are all high, requiring multiple passes through a high-pressure homogeniser at over 1000 bar to achieving a high degree of cell rupture. Data describing the comparative strength of yeast in other genera such as *Kluyveromyces* and *Candida* appear to be lacking. Cell strength has been found to be highly dependent on the strain/species of yeast, with twice as much force required to rupture a strain of lager yeast compared to a strain of ale yeast.<sup>140</sup> This has major implications for the processability of yeast biomass, as cell rupture is an energy intensive process<sup>144</sup> which is dependent on cell strength.<sup>111</sup> Therefore, if mechanical cell rupture is to be used to release intracellular components such as whole proteins, it is important to consider cell strength as a parameter in the selection of a candidate yeast strain. Further, as dynamic entities, yeast respond to environmental stimuli *via* adaptive cellular physiology,<sup>145</sup> resulting in divergent physical properties of the cells.

In the stationary phase present in fed-batch cultivation, cells respond to challenges such as starvation or high ethanol concentrations. This is relevant to yeast biomass that is stored following production and prior to processing. These conditions represent environmental stresses that can trigger an environment stress response (ESR) that results in adaptive reprogramming of cell metabolism and physiology including the cell wall.<sup>146</sup> The ESR in *S. cerevisiae* has been studied extensively and involves a complex array of changes, including the expression of various stress response proteins.<sup>147,148</sup> In *S. cerevisiae* the expression of ~900 genes can be altered as part of the ESR.<sup>149</sup> Despite the apparent magnitude of changes in the proteome that result from the expression of various stress proteins, this does not appear to have yet been considered in relation to the functional properties of yeast as food, for example the amino

acid profile of the yeast. This is an important aspect to consider, as environmental stresses could be imposed incidentally or deliberately during production to alter the properties of the yeast for food applications.

### 3.4 Effect of reactor conditions, growth medium and accumulation of metabolites

The extracellular environment in which the yeast grow is determined by the composition of the growth medium and the operation bioreactor or fermenter. As the yeast respond metabolically and physiologically to their environment, yeast biomass properties can be influenced in deliberate ways based on an understanding of yeast responses.

Temperature is an important and controllable parameter that has a major effect on yeast growth rate, metabolite production, physiology and proteome.<sup>150</sup> Each strain of yeast has a preferred temperature range,<sup>150</sup> below which the growth rate is suboptimal and above which temperature stress occurs.<sup>151</sup> For dedicated yeast biomass production, productivity is favoured by operating at optimal growth temperatures. However, for beer and wine production the metabolite profiles are crucial for flavour profiles, and high cell yields are not required. As such, the operational temperature is dictated by flavour considerations and is often suboptimal with respect to growth.<sup>17</sup> For recombinant protein production ('precision fermentation'), the aim is to maximise protein expression, which depends on the concentration of cells in combination with the expression rate per cell. In this case, the growth of yeast is intermediate to the main objectives, and the optimal temperature may<sup>152</sup> or may not<sup>153</sup> coincide with that which confers maximal growth. Temperature is a controllable parameter in industrial yeast production, although in very large reactors there can be limits to the extent and consistency of cooling that can be provided. As aerobic metabolism is much more exothermic than anaerobic fermentation, the cooling capacity must be much higher. As heat generation is proportional to cell concentration, the cooling load will increase during batch and fed-batch operation, while remaining constant in continuous mode.

Beer fermentation is predominantly anaerobic, as the aim to produce alcohol and flavours rather than biomass. Oxygen is only made available at the start of beer brewing to boost cell growth and shorten the lag phase. For the other yeast production processes including baker's yeast, lactose fermenting yeast and recombinant protein production, aerobic growth is desired to maximise biomass and protein yield. It has recently been shown that the presence or absence of oxygen also influences the protein composition of the yeast with important implications for the flavour and nutritional properties of the biomass.<sup>32</sup> Maintaining dissolved oxygen levels is critical to maximising productivity, and for obligate aerobes such as *Komagataella phaffii*<sup>19</sup> it is essential. In *S. cerevisiae* there is not a straightforward threshold dissolved oxygen threshold, as it is a combination of dissolved oxygen and glucose concentrations that dictate whether the cells respire aerobically or undergo fermentation.<sup>54</sup> Further, high concentrations of dissolved



oxygen can result in oxidative stress due to the presence of radical oxygen species.<sup>154</sup> The dissolved oxygen concentration in the bioreactor is dependent on the mixing and aeration system (supply) and the oxygen demand, which will increase as a function of cell concentration and specific oxygen demand. Providing oxygen can be a challenge in high-density, aerobic processes, particular at the very large scales of relevance to yeast food production, and can ultimately limit the scale of the reactor.<sup>51</sup> Large fermenters also subject the cells to greater environmental fluctuations due to the challenges involved in mixing large volumes, which can influence the physiology of the cells.<sup>155</sup>

The growth medium is the repository of the required substrate (carbon source) and nitrogen source, and potentially contains other constituents such as phenolics that may influence yeast properties. As shown in Table 1, there is much variation in the characteristics of growth medium used for current and future yeast biomass production. A recent review has highlighted the significant variations in the total protein content (30–70%) of different yeast species grown on different waste substrates, confirming the important influence of growth media on yeast biomass properties.<sup>20</sup> Although much effort has been spent on developing ideal growth media for baker's yeast production,<sup>156</sup> to a large extent the formulation of media is dictated by external factors such as the cost and availability of the raw materials. The carbon source is the major component of the growth media and a significant contributor to the cost of yeast production. While molasses is less expensive than refined sugar, the use of waste sugars such as lactose or lignocellulosic hydrolysates could help reduce the cost. More needs to be understood about the influence different substrates have on the properties of the resultant yeast, to assist media formulations aimed at balancing product quality and the cost of production.

In addition to the components needed for yeast growth, the media may contain phenolics (e.g. from molasses,<sup>49</sup> malt<sup>157</sup> or hops<sup>158</sup>). Phenolics can adsorb onto the cells<sup>159</sup> or carry over into the final product in the extracellular growth medium. Yeast metabolites also accumulate in the growth medium that can influence the organoleptic properties of the biomass. While most of extracellular metabolites are removed upon harvesting of the yeast for nutritional applications, their presence can affect the physiology of the yeast cells. In particular, ethanol can invoke stress responses in yeast,<sup>160</sup> including altering gene expression and remodelling their lipidome to adapt membranes to high ethanol concentrations.<sup>161</sup> Yeasts also have cell wall-associated responses to a range of environmental stresses relating to growth medium components, such as osmotic and chemical stresses.<sup>122</sup> For example, *S. cerevisiae* has been shown to adapt its cell wall (increased stiffness and  $\beta$ -glucan content) in response to acetic acid stress.<sup>162</sup> Accumulation of yeast-derived organic acids put a downward pressure on pH, however this can be actively controlled to stay within favourable limits.

### 3.5 Effect of yeast biomass storage

In addition to the stationary phase occurring the fermenter, it is likely in some instance that yeast cells will be subjected to prolonged periods of storage under starved conditions prior to ingredient processing. This could include transportation from a brewery or yeast production facility and/or storage at a processing plant. Although the yeast can be stored at cold temperatures to slow down metabolic activity and cell death, extended periods of nutrient starvation can eventually trigger autophagy and ultimately cell death. Autophagy is a cellular self-digestion process that occurs in all eukaryotic cells that can help survival by recycling cell resources to essential functions during starvation.<sup>163–165</sup> Autophagy can ultimately lead to cell death, which can also occur *via* programmed necrotic or apoptosis processes.<sup>166,167</sup> Cell death results in loss of cellular function, homeostasis and integrity.<sup>168</sup> Cell death is linked with autolysis in which hydrolytic enzymes are released from vacuoles to degrade cell constituents, and which is a process that can be intentionally triggered as part of yeast extract production.<sup>30,32</sup> Although autophagy and cell death have been extensively studied, there appear to be few dedicated studies investigating these processes in relation to yeast storage and the resulting effect of cell and protein degradation on food ingredient production.

There are clearly very large differences in the metabolism and physiology of yeast growing under different cultivation modes and conditions. Such differences would directly affect any processing performed immediately following harvest. Further, it is also likely that any differences in the harvested yeast would continue to have an influence during subsequent processing or biomass storage (which would represent an extended stationary phase). In this regard, it is important to understand to what extent the metabolic and physiological state of the cells during growth has a permanent effect on the composition or physiology of the harvested cells. However, the dynamics of these changes and the implications for food ingredients have not yet been considered in literature. Most investigations into the dynamics of yeast adaptations have so far involved transcriptomic studies,<sup>121,169</sup> which provide insights into how quickly the cells' genetic expression system changes. However, the effects of gene expression take time to materialise in the proteome and cell physiology, which are the characteristics of practical interest for food ingredients. One previous study has been performed on the dynamical response of yeast to anaerobiosis, revealing the changes in the transcriptome occurred much more rapidly (within a single cell cycle) than the resultant remodelling of the cell wall (occurring over at least 4 cell generations).<sup>170</sup> The persistence of various cell properties over multiple generations, such as cell wall and protein composition, has important implications for harvested yeast biomass, in which the cells are unlikely to continue cell division during cold storage or processing stages such as autolysis. As such, undertaking further targeted research into the dynamics and persistence of post-production changes in yeast will be important.





## 4. Research gaps and future direction

There is a wealth of scientific knowledge of yeast that can be applied to production of food ingredients. There is also a rapidly increasing amount of research specifically relating to the production and use of yeast in food. Nonetheless, as a complex and emerging area, there are some important research gaps that should be targeted for future studies.

### 4.1 Non-*Saccharomyces cerevisiae* yeasts

While *S. cerevisiae* has been very widely studied, much less is known about other species making them more difficult to predict and control. In particular, *Kluyveromyces*, *Candida*, *Yarrowia* and *Komagataella phaffii* have much future relevance for utilisation of lactose and lignocellulosic sugars, and biomass generated from lipid and recombinant protein production and therefore warrant comprehensive investigation.

### 4.2 Yeast proteome

The extent to which yeast protein and amino acid profiles can be influenced by strain selection or genetic modification, media composition, growth conditions and storage is yet to be fully understood. Further, while the amino acid profile is understood to affect nutrition and flavour of yeast ingredients, the influence of differences in yeast proteomes on other functional properties is less clear and requires controlled studies to be undertaken. The rise of proteomics and genomics presents a new opportunity to understand the extent of protein divergence between different yeast species. It will be interesting to see whether strain selection/modification or controlled growth will be the most effective lever for tailoring yeast biomass with specific protein or amino acid profiles.

### 4.3 Yeast cell wall

The composition and strength of the cell wall will affect downstream processing and the nutritional and textural properties of yeast ingredients. These cellular properties have been shown to vary between species and as a function of environmental conditions, and further investigation is needed to fully understand how they can be beneficially influenced during production.

### 4.4 Continuous culture

Continuous culture has potential advantages over conventional fed-batch operation for producing food-grade yeast supplies, including higher productivity and the ability to tune and control growth parameters. To take advantage of this, further research is needed to understand the rates of genetic drift in relation to potential impacts on biomass quality, and how specific growth rates relate to desirable attributes amongst different yeasts.

### 4.5 Downstream processing

Following production of yeast, the biomass must be harvested and stored, and may be subjected to further downstream processing to convert the biomass into useful products. For

example, cell rupture may be required to recover and purify intracellular proteins and extract glucan from cell walls. There is much still to be learned in relation to downstream processing of yeast, in particular for novel strains.

### 4.6 Yeast as a food

A significant amount of research in the discipline of food science is now needed to characterise the functional properties and digestibility of yeast biomass in the context of different food systems and formulations. It is still not yet properly understood how complex mixtures of yeast proteins and cell wall materials interact with different food matrices. There will be variations in the properties of biomass generated from different feedstock-species-process combinations that are yet to be understood. These variations will represent both a challenge and an opportunity for food scientists making yeast-based protein ingredients. Comparative studies should carefully report the species and growth conditions of the biomass used in experiments to enable links between production and functionality to be determined. Research to inform regulation of not-yet-approved yeast species and GMO yeast is also required, particularly in relation to potential allergenicity and toxicity.

### 4.7 Technoeconomic assessments (TEA) and life cycle analyses (LCA)

While internal analysis of these technologies can be performed internally by commercial entities, there would be value in publicly available, rigorous TEAs and LCAs that can provide confidence for investment in scaling up of such technologies. Such work can build upon relevant elements of TEAs and LCAs that are already available for lignocellulosic biorefineries<sup>171–173</sup> or yeast-derived oils.<sup>174</sup>

## 5. Conclusions

Overall, realising the potential of yeast to reduce the environmental impact of food production will require vast increases in the scale of both production and consumption. Major challenges to achieving this include the bringing cost of production down, ensuring adequate supplies of raw materials in particular the carbon source, increasing global fermenter capacity, and understanding how to more extensively utilise yeast protein in human foods. The first challenge will require utilising new sustainable feedstocks, yeast strains and production processes such as continuous cultivation that can drastically reduce the cost of production. The latter challenge will require innovation to incorporate large quantities of yeast proteins into palatable consumer products, and overcoming any regulatory barriers associated recombinant yeast biomass. Leveraging our extensive knowledge of yeast will help realise the opportunity to significantly expand the nutritional yeast industry.

## Author contributions

G. Martin wrote and finalised the manuscript. S. Chan contributed to the initial drafting.



## Conflicts of interest

The authors have no competing interests to declare.

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