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The complexity of yeast extracts and its consequences on the utility in brewing: A review

From an international perspective, one particular approach to increase the profitability of the modern industrial brewing process is to ferment high-gravity worts with high proportions of unmalted grain. However, the nutrients provided for yeast in these methods are often insufficient to achieve a satisfactory fermentation result and product quality. Yeast extracts, referred to as "yeast foods", can optimize these fermentation processes because they provide a multitude of essential nutrients for the fermenting yeast such as free alpha-amino nitrogen, minerals and vitamins, or generate an added benefit for a product as a result of their antioxidative properties. In industrial manufacturing processes of yeast extracts, which take advantage of autolytic, hydrolytic, plasmolytic, thermal and mechanical methods, this nutrient composition can be controlled through the choice of process parameters and the used yeast quality. As a consequence, the complex yeast extract composition made from bioactive components varies greatly in commercially available products and can thereby influence fermentation in different ways. In beer production, the high number of physiologically effective yeast extract components stimulates the yeast's fermentation performance in various ways, depending on the oxygen supply and wort composition. Besides enhanced fermentation rates, individual nutrients of the yeast extract also influence the yeast secondary metabolism and thereby the overall flavor of the beer. Based on the specified complex interrelationships, this review intends to first provide an overview of the different bioactive substances of a yeast extract and explain the technical manufacturing background. It then discusses the physiologically effective components of the yeast extract when used in the brewing process. Various results of individual papers will also be examined for production-related nutrient variations.

Descriptors: yeast extract, brewer's yeast, autolysis, mechanical disruption, high gravity, fermentation performance

1 Introduction

Yeast beside lactic acid and acetic acid bacteria can be seen as oldest "pets" of human being. Fermentation does help to stabilize food since ten thousands of years [69]. It was only with the findings of *Pasteur* that the fermentative properties of yeasts gradually started to be exploited until *Hansen* finally introduced a "pure fermentation" in a brewery in 1883 [4]. That laid the foundation for the targeted use of different yeast strains in food industry. Today, yeasts are used as optimized starter cultures in beer, wine and bread production [4]. Furthermore, those yeasts are also used as nutritional supplements (nutritional yeast) due to their nutritionally valuable substance groups or used to selectively enrich bioactive substances in food and beverages [33]. Yeast's complex physical structure has caused the industry to develop a variety of methods to process and extract its ingredients [64]. One widespread and commercially used product is referred to as yeast extract. This can be defined as the soluble content of a yeast cell that remains following the destruction and removal of the cell wall [113] and which can also undergo enzymatic degradation processes [85]. Primarily, it is known as a flavor enhancer in various processed foods [82, 110]. A further area of application is the use of yeast extract in microbiological culture media in order to increase a multitude of different available nutrients [53]. In this context yeast extract is also called "yeast food" [53]. The technological processes during manufacture and the selective addition of further nutrients means that the composition of the yeast extracts/yeast foods is no longer restricted exclusively to the original yeast cell content [38].

A common malt wort in the beer-brewing process has a large number of nutrients to supply the yeast during propagation and fermentation when manufacturing beer [4, 75, 115]. As part of measures to increase process efficiency in breweries (high-gravity methods, use of high levels of unmalted grains in beer wort production, increase in cell count during propagation, reduction of process times during fermentation), good yeast performance requires an appropriate

https://doi.org/10.23763/BrSc19-04jacob

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Table 1 Analysis of the production-dependent proteinogenic composition of yeast extracts

Yeast species	Medium	Method	Results and comment	Reference
Saccharomyces cerevisiae	Beer wort	Autolysis (47 °C, 48 h)	Proportion of free amino acids 77.5 %; 74.25 % of peptides with a molecular mass of 1000–2000 Da	[83]
Saccharomyces cerevisiae *Kluyveromyces marxianus* *Scheffersomyces stipitis* *Pichia angusta*	Sugar beet pulp hydrolysate, sucrose, molasses	Autolysis (50 °C, 48 h)	Proportion of free amino acids 10.14–44.97 %; use of saponine for cell lysis	[10]
Saccharomyces cerevisiae	Beer wort	Autolysis (50 °C, 24 h) Cell mill Sonotrode	Proportion of free amino acids 11–30 %; hydrolytic decomposition of protein during the process using cell mill and sonotrode	[55]
Saccharomyces cerevisiae *Pichia anomala* *Pichia jadinii* *Pichia pini* *Pichia pastoris* *Yarrowia lipolytica*	Sugar cane molasses	Autolysis (50 °C, 7 d)	1.53–9.18 mg amino acids per mL cell lysate (15 % cell suspension); autolysis capacity depends on strain	[85]
Saccharomyces pastorianus	Beer wort	Cell mill	Protein content 64.1 %; alpha-amino nitrogen 3.79 %	[113]
Saccharomyces pastorianus	Beer wort	Cell mill	Protein proportion (69.8–76.5 %) is increased by higher number of repitching, while proteolytic activity is reduced	[111]
Saccharomyces species	Beer wort	Thermolysis (95 °C, 5 min) Autolysis (50 °C, 12 h)	Proportion of free amino acids 67.05 %; use of exo- and endoproteases increases protein proportion	[21]
Candida utilis	Glucose, ammonium sulfate, potassium, magnesium	Thermolysis (95 °C, 5 min) Autolysis (50 °C, 6 h; 50 °C, 6 h)	Proportion of free amino acids 9.26 %; protein proportion 32.7 %	[42]
Brewer's yeast	Beer wort	Autolysis (50–60 °C)	Proportion of free amino acids 28.60–35.28 %; Protein proportion 62.5–63.8 %	[84]
Brewer's yeast	Beer wort	Autolysis (50 °C, 20 h)	Total nitrogen content 8.2 %; alpha-amino nitrogen 4.5 %	[90]
Saccharomyces pastorianus	Beer wort	Autolysis (50 °C, 48 h, toluene; 60 °C, 48 h, toluene)	alpha-amino nitrogen 8.18 mg/g (50 °C), 6.15 mg/g (60 °C)	[44]
Baker's yeast	unknown	Autolysis (48 °C, 24 h, pH 4.0–8.5, chitosan, ethyl acetate)	Influence of pH and ethyl actetate on alpha-amino nitrogen content (2.2–5.2 %) and total nitrogen content (5–10.6 %)	[22]
Saccharomyces pastorianus	Beer wort	Cell lysis with exogenic enzymes	Analysis of temperature, pH and time influence on release and the breakdown of cell protein	[59]
Saccharomyces cerevisiae	Glucose, ammonium sulfate	French Press Autolysis (37 °C, 24 h)	Measurement of protein decomposition in fractions of different molecular sizes	[96]
Baker's yeast	unknown	Autolysis (48 °C, 72 h) Hydrolysis (lyticase, papain)	Influence of enzymes on protein content in the yeast extract	[71]
Brewer's spent yeast	Beer wort	Autolysis (70 °C, 4 h) Hydrolysis with exogenic enzymes	Ultra and nano-filtration of the liquid yeast extract and determination of proteinogenic composition of the fractions	[2]
Saccharomyces cerevisiae	Sugar cane molasses	High pressure treatment Autolysis (50 °C, 24 h) Hydrolysis (papain)	Increase in protein content using high pressure pre-treatment and exogenic enzyme (papain)	[109]
Candida lipolytica	2 % hexadecane medium	Autolysis (25 °C, 8 h) Hydrolysis (HCl, NaOH) High pressure treatment	Maximum protein content through high pressure treatment of alkali-treated yeast suspension	[105]

nutrient level in the fermentation medium [38, 53]. A variety of research papers have been published to clarify the influence of individual substance groups on fermentation performance and beer quality. These led to the development of various strategies to optimize the yeast's nutrient supply for relevant challenges in modern beer preparation [38, 115]. In this context, yeast extracts have also been used that represent a cost-effective and universal product for the brewing industry based on their complex nutrient composition, but which can also pose a challenge to brewing technologists. Researchers have repeatedly emphasized the highly fluctuating physical composition of yeast extracts and the effects on a fermentation process, which are therefore difficult to calculate [38, 53, 91, 95, 96, 121]. This calls for brewing technologists to acquire specific knowledge of the composition of a yeast extract [53]. In

order to select the right product, they need to first understand the complexity of the product but also the reasons and circumstances that cause the material composition to vary.

In this review, we therefore summarize the research work on the complex physical structure of yeast extracts and then the relevant, underlying technological reasons. Based on this overview, the second part of the review will re-examine the research work on using yeast extracts in the brewing industry and the results. Our objective in doing so is to provide an overview of the effective components of yeast extract. In addition, inconsistent results of works with a production-related variation in physical yeast extract composition will also be put into context.

2 Yeast extract composition

2.1 Nutrient fractions

Yeast extract with the purpose of a yeast food should provide the fermenting yeast cultures with various nutrients to guarantee optimal cell propagation and fermentation performance. Moreover, the various bioactive substances of yeast extract offer different additional benefits for the finished fermentation product. The high number of different metabolites in the yeast cell metabolism generates a correspondingly complex composition of yeast extract, which is also influenced by the manufacturing process. In the following, we will present the substances described in literature as relevant in yeast extract, which serve as a source of nutrients for the yeast cell, and which may also have an impact on the fermentation product, beer.

The quantitatively largest proportion of yeast extract nutrients is formed by proteinogenic amino acids which are either free or bound via peptide bonds as a complex protein. Details on concentrations of proteins and amino acids should be viewed in consideration of the analytics [55]. Usually for protein determination the nitrogen content measured by Kjeldahl method is multiplied by a factor based on the amino acid spectrum, the number of amino groups and the molecular weight of the respective amino acids. Using this method, non-proteinogenic nitrogen sources such as ribonucleic acid, deoxyribonucleic acids or ammonium of the yeast extract are also recorded and, in some circumstances, may distort the actual value [55]. Differentiation should also be made between free and bound amino acids, and not just to specify the total protein content [55]. Depending on the manufacturing method and starting yeast (see 2.2 and 2.3), the literature gives various details on the quantity of the proteinogenic material in yeast extract. According to *Sommer*, 73–75 % of an autolytically obtained yeast extract proteinogenic material, which consists of 35–40 % free amino acids, also consists of di-, tri- and tetra-peptides with a molecular mass below 600 Da (Dalton) (10–15 %) and oligopeptides in the range of 2000–3000 Da (40–45 %) [94]. Larger peptides only comprise 2–5 % [94]. The ratio of free to protein-bound amino acid in yeast extract is not usually constant [55, 94], whereby 85 % of the methionine, leucine, alanine and phenylalanine content is free and not protein-bound [94]. In contrast, just 14–37 % of asparagine acid, glycine and arginine is free and not protein-bound [94]. *Podpora* et al. showed, for a yeast extract from spent yeast in beer production, how the size distribution of peptides changed during autolysis and established an average amino acid number in the peptides of 20–30 [83]. After 48 hours, the level of free amino acids in their yeast extract was 77.5 % [83], compared with a level of 31 % for *Jacob* et al. as a result of the autolysis conditions [55]. *Berlowska* et al. determined values of between 10.14 and 44.97 % for different yeast strains [10]. Using a cell mill or ultrasonic sonotrode also produces protein sizes of between 3000–80,000 Da in the yeast extract and a proportion of free amino acids of between 11 and 15 % [55]. An overview of the values determined in the literature of proteinogenic material in yeast extracts is displayed in table 1.

A proportionally small share of yeast extract, but one that has high nutrient potential for yeast, is comprised of different macro-minerals and trace elements. In a yeast extract produced via cell mill from spent brewer's yeast, *Vieira* et al. established values for sodium, potassium, calcium and magnesium of 1228 mg, 9148 mg, 27.1 mg and 273 mg per 100 g yeast extract [113]. Trace elements include chromium, iron, manganese, cobalt, molybdenum, zinc, copper and selenium. The content of these elements was below 0.6 mg per 100 g with the exception of iron (1.76 mg) and zinc (11.9 mg) [113]. *Ingledew* et al. also analyzed the sulfur and phosphorus content at 744 mg and 1454 mg per 100 g respectively [53]. Just 38.9 mg/100 g was determined for magnesium compared with 0.7 mg/100 g for copper, which was double the figures Vieira et al. found [53, 113]. Following a selective membrane filtration of a yeast autolysate, *Amorim* et al. determined various minerals in the hydrolyzed permeate and retentate, wherein the fraction of the retentate at a molecular size of less than 3 kDa, displayed higher concentrations of phosphorus (1.03 %), magnesium (0.68 %), calcium (0.40 %), sodium (11.81 %) and potassium (7.95 %) than other fractions [2].

Vitamins can serve both as nutrients for yeast and also give the finished product an increased nutritional value. As yeast is generally considered to be a good source of vitamins, vitamins can also be detected in yeast extract [113]. Detailed studies have already been published on the vitamin content in intact yeast cells and their nutrient potential [49, 54]. The transfer of the vitamins into the yeast extract appears to depend on the manufacturing process based on their sensitivity to environmental influences [108, 113]. There are only a few references in literature on vitamin concentrations in yeast extracts [113]. In a mechanically disrupted yeast extract, B vitamins were evidenced as follows: vitamin B3 77.2 mg, B6 55.1 mg and B9 3.01 mg per 100 g [113]. Vitamin B2 and B12 were not detected [113]. *Sato* et al. referenced paradoxical results in their paper, which presumably were attributed to the varying vitamin contents (no absolute values specified) in the yeast extracts used from different manufacturers, even individual product batches [91]. According to *Leclerc* et al., vitamin components in yeast extract stimulate bacteria growth that form acetic acid in their semi-synthetic media, however, no vitamin concentrations of the applied yeast extract were measured [62].

Various carbohydrates and lipids of yeast extract can be absorbed as nutrients from yeast. The precise composition of all carbohydrates and fats in yeast extract used as yeast food has little been reported. Vieira et al. calculated a total carbohydrate content of 12.9 % in a mechanically produced yeast extract [113]. *Verduyn* et al. reported carbohydrate contents of between 8.5 and 20.6 % in the

yeast extract depending on different manufacturing methods [109]. In an autolytically obtained yeast extract from baker's yeast, *Münch* determined values of 3130 mg for glucose, 73 mg for fructose, and 267 mg for saccharose per kg of the used yeast dry weight [72]. An autolysate made from brewer's yeast showed measured values of 3800 mg glucose, 990 mg fructose and less than 10 mg sucrose [72]. More complex carbohydrates such as glycogen and trehalose, which each contributed 40 % and 25 % of the cell dry weight [11, 81], are not reported in the context of yeast extract production; though the optimized extraction of trehalose from yeast cells was reported [119]. In a commercial yeast extract, 1412 mg glucose, 133 mg fructose and less than 10 mg sucrose could be detected accordingly [72]. The fat content of yeast extract is generally described as being low (1 %) [4, 5]. Following mechanical disruption, a total fat content of 1.32 % was stated [113]. Only by using special lipid-storing yeast strains it is possible to achieve lipid levels of up to 65.23 % of dry cell weight [93]. To supply yeasts with unsaturated fatty acids and sterols it is common to use "hulls" in wine fermentation [73]. These are the empty cell hulls that are separated from the yeast extract at the end of its extraction [73].

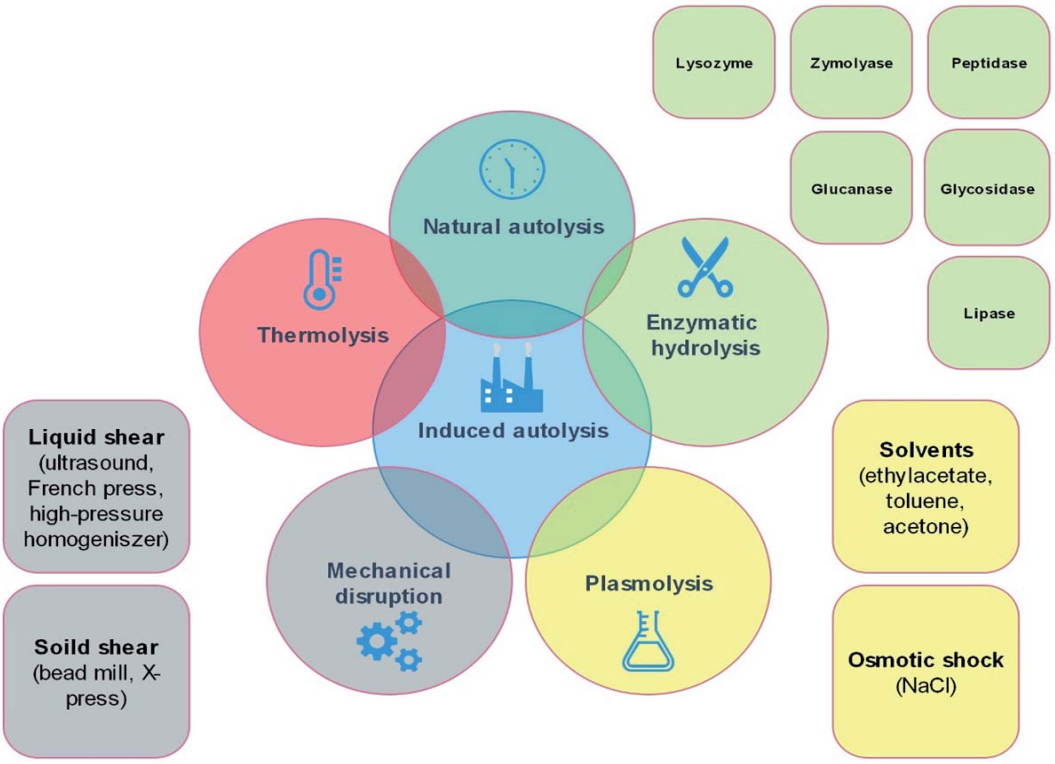

Fig. 1 **Technological interrelationships of individual cell disruption methods**

An added benefit for fermentation and the fermentation product is generated by adding yeast extract based on its antioxidative and reductive potential, which is ascribed to free amino acids, peptides (glutathione), polyphenols, minerals, ubiquinone, hydroquinone, and vitamins [13, 112]. For a mechanically disrupted yeast extract from spent brewer's yeast, values of between 59.7 mg TE (Trolox equivalent) and 261 mg TE per 100 g yeast extract were given, depending on the method of determination [113]. For autolytically produced yeast extract, 461.5 and 506.9 mmol TE per 100 mg yeast extract were measured, which correspond to 115.50 × 10^3 g and 126.87 × 10^3 g TE per 100 g yeast extract [84]. From a yeast extract, *Bastin* et al. and *Bourdaudhui* et al. isolated and concentrated fractions that displayed a high antioxidative potential [8, 13]. In addition, yeast extract can also show ACE (angiotensin-converting enzyme)-inhibiting activity [112]. Using various flavorings and flavor-enhancing substances in the yeast extract can influence the sensory properties of the fermentation product. Specifically, 5´-nucleotides are used, which develop from the enzymatic decomposition of RNA during autolytic and hydrolytic processes [110]. Münch, *Zhang* et al. and *Lin* et al. conducted studies into flavorings in yeast extracts and identified a wide range of substances accountable for these flavorings [63, 72, 122].

2.2 Influence of yeast extract manufacturing methods

Before extracting cell ingredients, the yeast starting material is separated from the medium in which it was propagated and then debittered as required [15]. This is done to remove hop components (resins and tannins) that are absorbed on the yeast cell wall or in the cell [17, 30, 74, 100]. An alkaline wash at pH 10 removes substances bound to the cell wall [74]. The debittering process can also be performed on disrupted yeast extract by adding synthetic adsorption materials [52] or by using microfiltration [92]. This way almost all the components with a bitter effect are removed [52]. However, these processes can start to have an impact on the composition of the yeast extract. *In* et al. reported of a modified amino acid composition [52]. As substances including polyphenols are removed by the debittering process, this can presumably also affect the antioxidative potential, which depends on these compounds [112, 113].

There are a variety of methods that can be used to release the content of yeast cells. In general, these can be differentiated into mechanical (cell mill, high-pressure treatment, ultrasonic, etc.) and non-mechanical methods (autolysis, hydrolysis, thermolysis, osmotic shock, solvents, enzymes, etc.) [37]. A precise differentiation of the individual methods and their technical details was listed by *Middelberg*, *Geciova* et al. and *Liu* et al. in their reviews [37, 64, 70], however, in practice, individual methods are often combined to produce yeast extracts [109]. A schematic overview of the technological overlaps of individual cell disruption methods can be found in figure 1. For the selective release and extraction of individual bioactive substances from yeast cells, methods are chosen according to selectivity of the target substances, recovery rates in the downstream process, and profitability [64]. When manu-

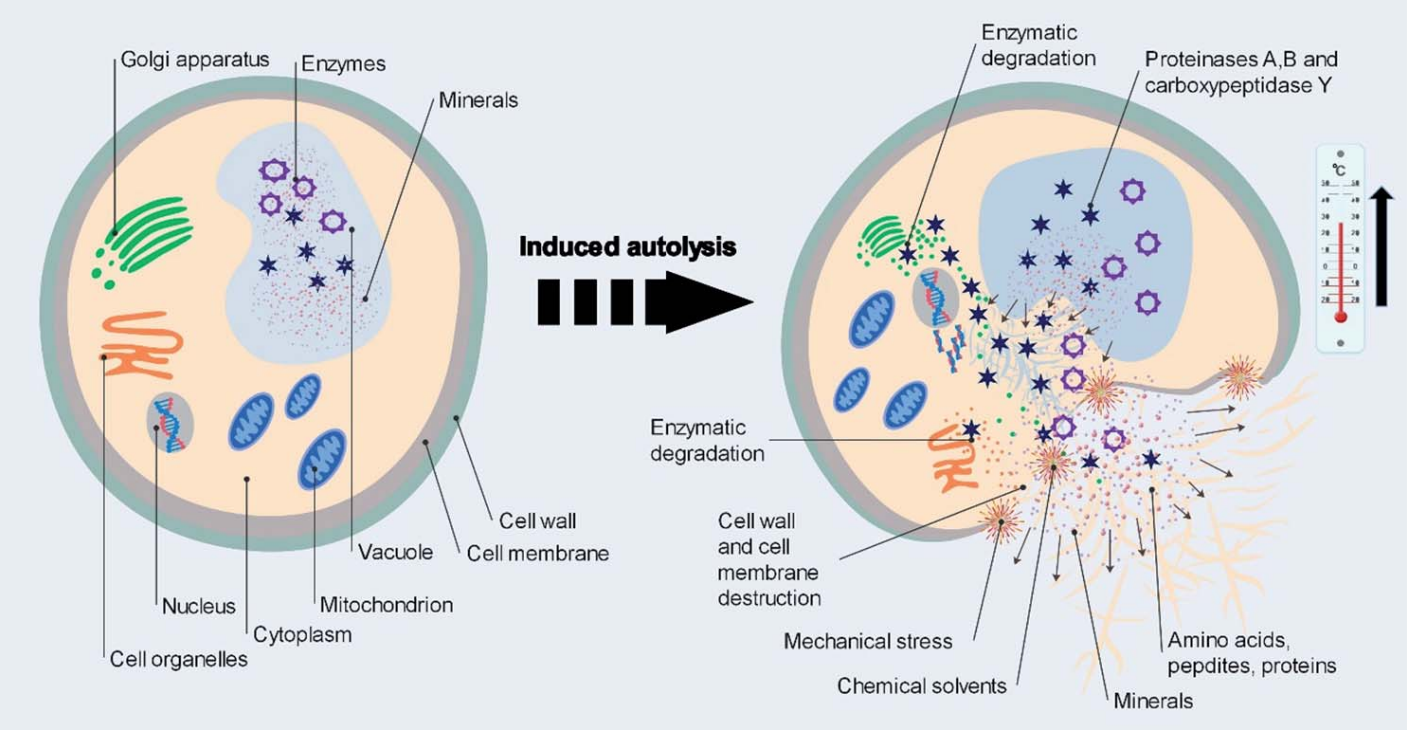

Fig. 2 Schematic representation of morphological changes during induced autolysis of yeast

facturing yeast extracts that are used as yeast foods, cost-intensive selective methods are avoided. The primary consideration is to produce large quantities profitably, ideally to transfer all nutritionally relevant ingredients from the yeast cell to the yeast extract, and to make these available in a metabolically effective form. Industry utilizes the principle of yeast cell autolysis [16, 94]. This normally begins naturally at the end of the stationary propagation phase of a population and can continue for a long period. At the start, endogenic cell structures are broken down, which results in the release of vacuolar proteases into cytoplasm [1, 5]. Once the initial inhibition of the cytoplasmatic proteases is complete, the activation phase starts [1, 5]. This is followed by decomposition of the intracellular polymers and their accumulation in the cell [1, 5]. If the hydrolytic products reach a particular molecular mass, they pass through the cell wall pores into the surroundings [1, 5]. For industrial purposes, autolysis needs to be accelerated and controlled, so the process is generally induced via physical methods, chemical and biological additives (Fig. 2).

The aim of industrial autolysis is firstly to release the cell content and secondly, the decomposition (hydrolysis) of proteins into peptides and amino acids using endogenic enzymes (vacuolar protease A, B and carboxypeptidase Y) [9]. A controlled temperature shock has proven to be particularly effective for the entire process and can be combined with osmotic stress (NaCl) [77]. By controlling the cell concentration, time, temperature and pH of medium, the process can then be influenced. A temperature of 40–55 °C and a duration of between 24 and 48 hours have shown to be optimal parameters for cell lysis and protein brake down [9, 16, 77, 101]. The greatest protein release and protein hydrolysis occurs at pH 5.5 [9, 22] and 7.0 [22]. A PEF (pulsed electric field) treatment can reduce the processing time [29] or a high-pressure treatment can be connected upstream for direct cell disruption [109]. A two-stage autolysis process with a pre-concentrated and then diluted yeast solution can also increase the yield [12]. The ratio of free amino acids to oligopeptides in the autolytically produced yeast extract is relatively constant according to Sommer [94]. This can only be influenced, and the protein release increased [25] by adding exogenic proteases and peptidases [94]. *Chae* et al. conducted studies on the optimum use of exogenic enzymes for protein extraction and the aroma composition [21]. Vieira et al. showed that a high RNA content can be extracted from spent brewer's yeast in the yeast extract at 60 °C and 24 h with potassium hydroxide [110], though at 37 °C and 6 h the yeast extract has high antioxidative and ACE-inhibiting properties [112]. According to *Alexandre*, 5´-adenosine monophosphate and 5´-guanosine monophosphate, which have a flavor-enhancing effect, are formed at greater quantities during autolysis at 50 °C (pH 7.0) or 40 °C (pH 4.0) [1]. If the yeast cells contain further fermentable sugar, this can be broken down in a pre-incubation at 38 °C in 4 hours and will not be part of the finished yeast extract [83].

The use of exogenic enzymes such as zymolyase, lysozyme, glycosidase, glucanase, peptidase and lipase enables a swifter, direct breakdown of the cell wall and a controlled and gentle release of cell constituents, without the endogenic hydrolytic enzymes being decomposed [59, 64, 71]. Rather than "autolysis", researchers also refer to "hydrolysis" [71]. *Hunter* et al. developed kinetics for the lysis of yeast cells, based on a two-layer model of the cell wall structure [51]. *Gaudreau* et al. were able to increase the release of zinc from the yeast cell into the yeast extract using glucanases [36]. The term "acid hydrolysis" is used to describe a non-enzymatic cell disruption process with acid treatment and thermal impact [72]. Plasmolysis, in which cell extract is released using high salt or solvent concentrations, can also be used to produce yeast extract. [94]. However, autolysis and plasmolysis are often used together in practice [16]. *Breddam* et al. described, how autolysis (for the purpose of extracting enzymes) can be accelerated with solvents

and tested a wide range of chemicals such as methanol, acetone, ethyl acetate and toluene [14]. Saponine and chitosan were also studied in terms of their effect during autolysis, the latter despite having an effect on membrane stability [120], had no significant impact [22]. Thermolysis, in which the yeast cell is destroyed by high temperatures, is also used [94]. According to this principle, the spent yeast in breweries can be disrupted when boiling the wort and the yeast extract released. Although in doing so, some of the cell wall components are also dissolved in the final wort [48].

The use of cell mills for cell disruption is one of the most frequently used mechanical processes in industry and plays a secondary role in producing yeast extract as a yeast food as the energy costs are higher compared with autolysis [64]. Using cell mills in the conventional way gently releases different proteins [46], enzymes [35] or cell wall components [39] and selectively separates them in the downstream process. β-Glucan is also extracted this way and has health benefits for humans [113]. To make the extraction process of this polysaccharide more profitable using a cell mill, the remaining cell extract can be used as a yeast extract [111, 113]. Vieira et al. characterized this and optimized it via subsequent autolysis [110-113]. The mechanical disruption method using a cell mill is characterized by high disruption efficiency in a single treatment process, good temperature controllability, it is simple to scale-up and is not very labor intensive [64]. Through compression and shear forces between the beads, energy is transferred to the yeast cells, destroying them [64]. The high friction energy produced must be discharged by cooling, depending on the substances being extracted [113]. Many different factors such as the shaking intensity [106], bead quota [89], bead size [26] and cell concentration [47] can also ultimately influence the release rate. A detailed overview of these factors is provided in a review by Middelberg [70]. Despite the thermally gentle cell mill method, chemical reactions occur in the yeast disruption during production. According to *Heim* et al., these reactions include long-chained molecules being truncated and intracellular components reacting with each other [47]. *Gohari* et al. reported in detail that thiol groups were oxidized during the process and form disulfide bonds [39]. Jacob et al. showed that, despite a constant process temperature at 7 °C, peptide bonds are cleaved and there are more free amino acids in the yeast extract than were originally present in the free amino acid pool of the yeast cell [55]. It can also result in protein denaturation and aggregation [80]. Vieira et al. noted that the vitamin content in the yeast extract was presumably influenced by the mechanical disruption process [113].

Following the release and solution of the nutrient-rich fractions, the insoluble components need to be separated out. The remaining liquid yeast extract is concentrated using a thin-film evaporator, sterilized for a short period or then spray dried for preservation and enzyme deactivation [94]. The procedure must be gentle to protect thermally sensitive substances such as vitamins [94]. If the yeast extract is heated more strongly, this may cause free amino acids to be lost in reactions with reducing sugars (Maillard reactions) and the formation of various flavorings [72]. For freeze-dried yeast cell extract and that stored at –25 °C, which is mechanically produced using a cell mill, the proteolytic activity could be kept constant for six months [111]. Resuspended and frozen at –25 °C, the activity reduced by half [111]. ACE-inhibiting properties, reduction and antioxidative potential remained unaffected in the specified storage methods for six months [111].

2.3 Influence of the yeast starting material

Yeast extract composition depends not only on the manufacturing method but also on the yeast starting material, which should contain large quantities of specific nutrients as well as good autolytic/hydrolytic activity. To produce large quantities cost effectively, *Saccharomyces cerevisiae* strains are mainly used in Europe, which are cultivated on molasses-based media [94]. In the United States of America and the United Kingdom, these are spent yeasts from the brewing process (*S. cerevisiae* and *S. pastorianus* strains), as well as strains of *Kluyveromyces fragilis* (fermented on whey) or *Candida utilis* (propagated on waste products that contain carbohydrate) [78, 94]. In addition, *K. marxianus* [88], *Scheffersomyces stipitis*, *Pichia* (*anomala, angusta, jadinii, pastoris, pini*) [10, 85], *Yarrowia lipolytica* [85] and maritime yeast isolates were tested [67]. If individual nutrients need to be present in selected quantities in the yeast extract, specific strains can be used [94].

The intact yeast cells of the yeast starting material already provide a pool of free amino acids, however, according to analyses by Berlowska et al. this comprises less than 1 % of the yeast extract [10]. However, the content can vary greatly as studies by *Hans* et al. showed [43]. Dramatic changes were observed during the diauxic phase and when entering the stationary phase [43]. Midway through the exponential growth is when the maximum content of free amino acids was achieved, which made up 10 % of the cell dry weight [43]. The greater the endogenic hydrolytic activity of the yeast cell during autolysis, the more amino acids can be released from proteins into the yeast extract [94]. It was revealed that the proteolytic activity for autolysis was highest after the second use of brewer's yeast [111]. Culture conditions (temperature and pH) of *S. cerevisiae* (grown on cane molasses) and *K. marxianus* (grown on whey permeate) had an impact on autolysis extent (cellular components solubilization, amino and nucleic nitrogen release) by affecting the lytic enzymes activities [3, 118]. The reuse (4 runs) of the yeast in the brewing process also resulted in an increased protein content in the yeast extract according to Vieira et al. [111]. A high level of proteinogenic material produces, among other things, high-protein strains of *S. cerevisiae* as a starting material for yeast extract production [55]. The use of yeasts which hold an intrinsic catalytic mechanism to decompose glutamic acid into gamma-aminobutyric acid during autolysis, can also change the range of free amino acids [67]. "Servomyces" is the trade name of an active and dried yeast (taxonomic name: *S. cerevisiae*) of Lallemand Inc., which has retained high concentrations of zinc in its metabolism [34]. By adding this zinc-enriched yeast to the wort kettle, the yeast is thermally disrupted, and the zinc-rich yeast extract is dissolved in the wort [34]. The composition of yeast extract can also be defined by the content of various carbohydrates. Under certain environmental conditions such as increased temperatures and a deficiency of individual nutrients, trehalose and glycogen are stored in the yeast cell [11].

According to *Barrette* et al. and *Champagne* et al., a minor to major contamination of the starting yeast with bacteria did not significantly influence the yeast extract yield, the degree of protein

Table 2 Investigations to improve the fermentation performance of yeasts using yeast extract as yeast food

Yeast food dosage	Fermentation medium	Active yeast extract components	Fermentation performance	Reference
0.3 %	Corn adjunct (percentage unknown) wort (28 °P)	Increase in free alpha-amino nitrogen (FAN) (quantity unknown)	Extract decomposition with yeast extract additive (up to 10 °P) within 5 days; without yeast extract additive within 9 days (up to 10 °P)	[53]
1.0 %	Corn adjunct (22.5 %) wort (27 g dissolved solids/100 mL)	Increase in FAN by 400 mg/L	Extract decomposition with yeast extract additive (up to 10 g/100 mL) within 8 days; without yeast extract additive within 10 days (up to 11 °P)	[20]
0.75 %	Corn adjunct (percentage unknown) wort (24 g dissolved solids/100 mL)	Increase in FAN by 375 mg/L	Extract decomposition with yeast extract additive (up to 4 g/100 mL) within 4 days; without yeast extract additive within 6 days (up to 4 g/100 mL)	[76]
1.0 %	Complex medium (1.94 M glucose, 10 mM $(NH_4)_2SO_4$, 50 mM KH_2PO_4, 50 mM $MgSO_4$; 35 g dissolved solids/100 mL)	Unknown	Extract decomposition with yeast extract additive (up to 1 g/100 mL) within 9 days; without yeast extract additive within 9 days (up to 20.2 g/100 mL)	[103]
2 g/L	Rice adjunct (percentage unknown) wort (11 °P)	Increase in FAN by 74 mg/L	Extract decomposition with yeast extract additive (up to 3 °P) within 4 days; without yeast extract additive within 8 days (up to 4 °P)	[61]
12 g/L	Complex medium (glucose 300 g/L, $(NH_4)_2SO_4$ 3.0 g/L, KH_2PO_4 2.0 g/L, $MgSO_4$ 1.0 g/L, $CaCl_2$ 0.1 g/L, NaCl 0.1 g/L and yeast extract 3.0 g/L;	Increase in total N by 0.141 mol/L	Glucose decomposition with yeast extract 95 %; glucose decomposition without yeast extract additive 79 %	[6]
8 mg/L	All malt wort (11.9 °P)	Increase in zinc concentration by 4 mg/L	Extract decomposition with Servomyces additive (up to 2 °P) within 7 days; without Servomyces additive within 10 days (up to 2 °P)	[34]
14.4 %	Very-high-gravity wheat mash (37.6 g dissolved solids/100 mL)	Increase in FAN by 550 mg/L	Alcohol production (22.3 %) with yeast autolysate within 4 days; alcohol production (18.5 %) without yeast autolysate within 9 days	[56]
Unknown	All malt wort (12 °P); adjunct (40 % maltose syrup) wort (18 °P)	Unknown	All malt wort (six generation yeast): Extract decomposition with yeast extract additive (up to 1.70 °P) within 3 days; without yeast extract additive within 4 days (up to 4.27 °P) Adjunct wort (first generation yeast): Extract decomposition with yeast extract additive (up to 2.63 °P) within 4 days; without yeast extract additive within 5 days (up to 5.03 °P)	[107]

hydrolysis and the alpha-amino nitrogen [7, 22]. A change in the amino acid composition could take place when producing rice wine, glutamic acid is converted from autolyzed yeast cells into gamma-aminobutyric acid by bacteria during the post-fermentation [40].

3 Use of yeast extracts in the brewing process

3.1 Improved fermentation performance

The use of yeast extract or yeast food was already considered as a supply of nutrients for various microorganisms and cell cultures. The bacterial formation of various metabolic substances could be influenced by using yeast extract [87, 99, 116]. *Lopes-Solis* et al. investigated different inactive dry yeast products with regard to their use in wine production [65]. Other studies addressed the use of yeast extracts for the optimized growth of CHO (Chinese hamster ovary) cells and bacteria [90, 96]. Ingledew et al. conducted the first detailed study on the comparison of different yeast extracts and yeast foods for wine and beer production, and analyzed a fraction of the nutrient composition (available nitrogen sources and minerals) of commercially available products [53]. They were able to note differences in the extract decomposition of a wort to which this yeast food was added [53]. *Van Zandycke* et al. compared various commercially available yeast foods in beer brewing [107]. They described the extract decomposition over time and the alcohol content achieved at the end of fermentation as a function of the used yeast extracts and different worts [107]. No details were provided on the composition and used quantities of the yeast extracts [107]. Nevertheless, they stated that a nutrient-specific yeast food adapted to the fermentation requirements would achieve the best results [107]. The balance of the individual nutrients plays a key role in fermentation [107]. As was shown in the above sections 2.1, the physical composition of yeast extracts can vary greatly. The following intends to show which components of the yeast extract are currently regarded in the literature as relevant to the brewing process. In addition, the studies that have been performed on the use of yeast extract in the brewing process are summarized and compared (Table 2). As part of this summary, inconsistent results will be discussed, which may be related to the varying composition of the yeast extracts specified above.

90 % of globally produced beer is brewed with a blend of malt, unmalted grains and/or sugar syrup [61]. For profit reasons, the industry also uses high-gravity processes [86]. An increased extract content can be achieved either by producing a concentrated wort or by also adding sugar syrup to the fermentation medium [19, 20]. The associated nutrient composition of the worts (all nutrients are diluted by the high carbohydrate content) causes problems with the yeast fermentation performance [38]. These include incomplete fermentation of the extract and slow fermentation processes [20, 61]. Reasons for this poor fermentation performance are cited as being nutrient-induced growth problems in the yeast population [19, 20, 61], ethanol toxicity [23], and high osmotic pressure [79]. It is generally known that a growing yeast population has a more rapid and better sugar utilization than a non-growing population [58]. A limiting factor for yeast growth and fermentation performance for high-gravity and unmalted grain worts includes an insufficient quantity of free alpha-amino nitrogen (FAN) [19, 20]. *Le Van* et al. were able to significantly increase the FAN content for a wort with a high rice proportion (40 %) by adding yeast extract (2 g/L) [61]. This increased the fermentation performance (1.5 °P lower final attenuation, reduction of fermentation time by four days) and was equally as high as for a 100 % malt wort [61]. By using microbial protease in the mash, the authors also increased the FAN in this study, but not as high as when adding the yeast extract [61]. An "excess" of FAN is beneficial for high-gravity worts and can stimulate the yeast cell metabolism [76]. *Casey* et al. used yeast extract to increase the FAN for a high-gravity wort that had a proportion of unmalted grains [20]. Under the semi-anaerobic conditions, the extract was decomposed more quickly by adding yeast extract than without the additive [20]. The level of glycogen, protein and sterols in the cells increased [20]. A synergistic effect was registered when adding yeast extract and lipids in the form of ergosterol and unsaturated fatty acids and resulted in a greater decomposition of the FAN and quicker extract breakdown than with the yeast extract additive alone [20]. Under anaerobic conditions, the addition of yeast extract as a source of FAN did not improve the fermentation performance [20]. However, the protein content in the yeast cells did increase while the glycogen and sterol levels dropped [20]. In comparison, the combined use of lipids and yeast extract, also under anaerobic conditions, evidenced a quicker and greater extract decomposition than without these two additives [20]. The yeast extract's improved tolerance of yeast cells to a high ethanol content could not be proven to be a reason for improved fermentation performance [20]. In the fermentation of agave tequilana juice by *Kloeckera africana*, addition of yeast extract had a positive effect on cell growth and the fermentation performance. In this case, an increased alcohol tolerance by nutrients in the yeast extract could be proven [28]. In addition, *Kloeckera africana* did not assimilate any inorganic nitrogen sources, though it did assimilate organic sources in the yeast extract [28]. *O'Connor-Cox* et al. also reported improved extract decomposition and higher ethanol content by using yeast extract for high-gravity worts [76]. The increase in the FAN of the wort by adding yeast extract did not result in a higher yeast cell viability, but in higher yeast growth rates and cell mass, which was also related to an increased protein content in the yeast cells [76]. O'Connor-Cox eliminated other non-FAN nutrients as contributors to the increase in fermentation performance for high-gravity worts but showed, for fermentations of pure glucose solutions, that cell division and growth were stimulated [76].

Neither study reported the precise nutrient composition of the yeast extracts [20, 76]. By using a yeast extract proportion of 1 % (m/v), Casey et al. increased the FAN content of wort by 400 mg/L [20]. In comparison, O'Connor-Cox et al. used 1 % (m/v) and achieved 500 mg/L [76]. Accordingly, the used yeast extracts had a different FAN content. The results for the optimum yeast extract dosage and the resulting fermentation performances varied in both studies [19, 20]. In one study at a pitching yeast count of 20×10^6 CFU per mL, the starting extract of 27 mg dissolved solid per 100 mL degraded to 10 mg/100 mL in eight days when adding 1 % yeast extract [20]. In the other study, a lower pitching yeast count of 2×10^6 CFU per mL and with less yeast extract added (0.75 %) only four days were needed (extract content at the start was 24 mg dissolved solid per 100 mL) [76]. The starting composition of the wort topped up with sugar syrup (16 °P with unknown corn-unmalted grain proportion [76] and 11.5 °P at 22.5 % corn unmalted grain [20]), might also have had an influence on the fermentations. In a full factorial experimental design, *Dragone* et al. determined the optimum parameters for the fermentation of a high-gravity wort with *S. cerevisiae* in order to achieve high ethanol productivity [31]. They showed that the original wort content and temperature play a role in addition to the yeast extract dosage [31]. *D'Amore* et al. confirmed the results of studies by O'Connor-Cox et al. and Casey et al. in detail in a separate study [27]. A yeast extract addition could increase the fermentation performance at high osmotic pressure or high alcohol content [27]. However, this fact could not be associated with a reduced alcohol content in the yeast cells but was attributed to a nutrient restriction [27, 98].

Thomas et al. speculated in their study that yeast extract and other complex additives such as tryptone and peptone exhibit a dual mechanism at very high-gravity fermentations [103]. Consequently, these not only supply growth factors for an insufficiently equipped medium, they also stimulate the growth and fermentation with high-gravity worts [103]. For individual substances such as glycine betaine, glycine and proline, they could demonstrate that these did increase the fermentation performance, but also keep cell viability at 80 % and act as osmoprotectants [103]. In the investigations, glycine was the most effective [103]. The effect of glycine betaine, which is also a component of yeast extracts [32], was only moderate and was associated with difficulties in transport in the cell on account of its positive charge [103]. In addition, it was possible to show that the proline produced via arginine catabolism acts as an osmoprotectant in the fermentation of very high-gravity wheat mashes [102]. The use of nucleic acids also increased yeast growth and fermentation performance for Thomas et al. in a high-gravity medium, although the cell viability could not be maintained [103]. As a result of the referenced effectiveness of individual amino acids or nucleic acids in high-gravity fermentations, the precise composition of the nitrogen sources such as free amino acids or peptides should be known in addition to FAN. This can vary greatly as already discussed in previous sections.

The minerals in a yeast extract did not stimulate the fermentation performance in the study of Thomas et al. [103]. However, this could be a result from the medium having an adequate quantity of minerals in this study [103] or indicate insufficient bioavailability. Conversely, the fact that yeast extract can increase the bioavailability of minerals was already proven using nickel and cobalt in

anaerobic bioreactors in waste water treatment [41]. *Fischborn* et al. showed that a higher alcohol content and more rapid extract decomposition of a wort can be achieved if yeast that contains zinc under the manufacturer's trade name "Servomyces" is dosed in the wort kettle [34]. Based on the better bioavailability of zinc from the Servomyces cells, the fermentation performance results were better than those when adding pure $ZnCl_2$ [34]. Magnesium was also defined as a critical factor when fermenting very high-gravity worts that were topped up with yeast extract [114].

In their small-scale fermentations with various yeast strains, *Haukeli* et al. concluded that yeast extract probably does not contain any components that possess similar stimulating properties such as ergosterol [45]. This fact corresponds to the low total fat content of the yeast extract, which was mentioned in 2.1. In contrast, if hulls are added to a fermentation, the lipids contained in these can stimulate fermentation [73].

Bafrncova et al. compared a yeast extract addition with casamino acids (peptide mixture made from hydrolyzed casein), urea and fresh yeast autolysate additive in the fermentation of a synthetic very-high-gravity medium of glucose and yeast extract [6]. The yeast extract also induced a high alcohol production rate in this case, and the other additives showed a similar effect [6]. Cell viability was not increased by the additives [6]. Using a yeast from a continuous fermenter to produce a yeast autolysate was the focus of a study by Harris et al. [44]. Adding the amino-nitrogen-rich yeast autolysate back into the fermenter increased the fermentation performance [44]. Jones et al. also established an increased fermentation performance by using a fresh and clear yeast autolysate, as a result of an increase in FAN with very-high-gravity wheat mashes [56].

Van Zandycke et al. compared various yeast foods (composition and used quantity unknown) in the brewing process [107]. For a normal 12 °P all-malt wort, particularly after repeated use of the yeast (six times), the yeast decomposed more quickly and produced a higher alcohol content at the end of fermentation. The individual yeast foods each differed in their effect on the fermentation performance [107]. They made the same observations for high-gravity unmalted grain worts and were also able to increase the fermentation performance for these [107]. A deactivated zinc-enriched yeast as a yeast food delivered similar fermentation results to a commercial yeast food with added zinc [107]. In terms of the composition of yeast foods, Van Zandycke et al. mentioned a balanced, complex nutrient composition, which was to align specifically to the wort requirements [107]. *White* postulated the same and cited the process environment in addition to the yeast strain as a criterion that determines the nutrient requirements of the yeast [117]. These statements are supported by research by *Marchetti* et al., who verified that different nutrient components such as glucose, NH_4^+, K^+, $H_2PO_4^-$ and Mg^{2+} influence each other [66].

It is also possible to add spent yeast directly into the unmalted grain or mash kettle [48]. *Hernandes-Pinerua* et al. describe how extract from the yeast cells is transferred into the wort in the brewhouse and this causes fermentation to proceed more quickly [48], whereas the lautering process was delayed [48]. This observation was also made by *Kawa-Rygielska* et al. for the addition of brewer's spent yeast to very-high-gravity corn mashes, which were more viscous based on hydrocolloids (β-glucan, polypeptides) [57]. The highest ethanol content was achieved when fermenting mashes both when adding yeast extract and when using wet yeast [57]. Thomas et al. also presumed, during the fermentation of very-high-gravity wheat mashes, that the yeast growth was stimulated due to lysis and therefore the release of nutrients from non-vital cells [104]. This was especially the case when increasing the pitching yeast cell count [104].

Bourdaudhui et al. and Bastin et al. proved the possibility to increase the stress tolerance of yeasts by adding an extract produced from *Saccharomyces cerevisiae* with antioxidative properties [8, 13]. A positive influence of the produce yeast extract on the metabolism of oxidatively stressed cells was shown to be a result of the catalase activity and the vitality [13]. In particular, ninhydrin-positive compounds with a low molecular weight were good inhibitors for lipoxigenases and good radical scavengers [8].

3.2 Impact on the sensory properties

The yeast extract flavorings can be cited as being primarily responsible for influencing a product produced by adding yeast extract. Lin et al. showed that meat and roasted flavors dominate the overall flavor in a yeast extract [63]. The compounds responsible are aldehydes, acids, ketones, pyrazines and furan derivatives [63]. According to Zhang et al., there are also undesirable off-flavors in the yeast extract that were described as "yeasty" [122]. Münch evidenced various flavorings produced via Strecker reactions when heating yeast extract [72]. In wine production, the influence of yeast extract and yeast autolysate on product flavor has already been studied. A low yeast extract dosage, floral and fruity notes are achieved whereas a higher dosage produced yeasty, herby and cheese-like flavors [24]. In the research for this review, no study could be found that explicitly discusses yeast extract flavors as being primarily responsible for the sensory properties of beer. The quantity of yeast extract used and the addition time/place during beer manufacture could also have an impact.

A secondary influence on product quality could be attributed to the various components of the yeast extract that influence the flavor formation by stimulating or inhibiting yeast metabolism. There are a very small number of publications on this topic. *Kruger* et al. studied the synthesis of different flavorings in a beer, brewed from a 14.4 °P wort with 35 % maltose syrup, with the addition of 0.04 g/L protein-based yeast food (composition unknown) [60]. They were able to establish that a greater uptake of amino acids and carbohydrates in the fermentations with yeast food additive formed larger quantities of higher alcohols than for the control fermentations [60]. In addition, lower quantities of acetaldehyde were formed with yeast food additive, although this depended on the yeast strain [60]. Casey et al. determined lower values of higher alcohols in some cases for high-gravity worts with yeast extract addition, while the ester concentrations increased [18]. If beer is brewed with a high proportion of rice (40 %), Le Van et al. reported diacetyl notes and less of a full body [61]. Adding yeast extract remedied those off-flavors and the beer was classified as being equivalent to an all-malt wort beer [61]. In addition, lower quantities of vicinal diketones were found in the finished beer with added yeast extract [61]. However, *McCaig* et al. determined higher

quantities of vicinal diketones in the undiluted beer of a high-gravity wort with yeast extract [68]. These beers were less palatable and, in this case, the relatively poor effect of the yeast extract on the fermentation performance should be taken into account [68]. If a multitude of different essential yeast nutrients (minerals, essential amino acids, lipids and vitamins) is dosed into a wort at an amount of 40 ppm, variations in the wort-nutrient composition can be compensated according to *Hsu* et al. and therefore this positively influences product uniformity [50]. The effect of different amino acids present in the yeast extract on the flavor development of yeast has already been discussed in detail [97].

4 Conclusion and outlook

The use of yeast extracts as a yeast food is a complex challenge as it is essential to know both the nutrient composition and the effect in the relevant process environment. Based on various manufacturing practices and a different yeast starting material, the quantity of metabolically effective substances in the yeast extracts varies greatly. The nutrient requirements of the yeast are different for normal, high, and very-high-gravity fermentations and therefore need to be defined. The process environment and the used yeast strain also require an adequate supply of nutrients. The bioavailability and the interactions of individual nutrients also play a role. Only with this knowledge can a yeast extract be used successfully to facilitate an efficient and profitable fermentation process and also ensure a consistently high product quality. The aim is to obtain, as far as possible, a yeast food that is tailored to the relevant fermentation requirements and balanced in nutrients. The challenge for yeast food manufacturers is to maintain a cost-effective manufacturing process, but still supply a product quality that is as consistent as possible. Research into the various nutrient requirements of individual brewer's yeast strains is needed to further improve. Knowledge on the interaction of individual components in the yeast extract would also be useful in determining the optimized nutrient balance in yeast food. The use of yeast extracts in the area of yeast propagations could also be investigated in more detail. It ought to be examined whether in addition to increasing the cell count as a result of higher FAN quantities, the vitality could also be positively influenced by other physiologically effective substance groups.

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Received 9 January 2019, accepted 15 March 2019