F. F. Jacob, M. Hutzler, M. Michel and F.-J. Methner

Boosting yeast propagation via yeast extract supplementation

Yeast extracts are usually used as nutritional supplements to improve the fermentation performance of brewing yeast. In this work, the potential of yeast extract to boost yeast propagation performance was investigated. Therefore, mechanically (via ultrasonic sonotrode) and autolytically produced yeast extracts from brewer's spent yeast, already characterised in recent studies, were supplemented in comparison to a pure nitrogen source (L-glutamine). The propagation process of the yeast strain *Saccharomyces pastorianus* TUM 34/70 was simulated in small-scale fully equipped bioreactors using standard all-malt (12 °P) and high-gravity adjunct (D-(+)-glucose) wort (16 °P, 18 °P). Process data (apparent extract, individual sugars (fructose, glucose, maltose, maltotriose), free-alpha amino nitrogen, ethanol, cell count) were monitored and harvested yeast was checked for viability and vitality (test of fermentative capacity). As a result, supplementing a propagation process with yeast extract increased the yield and quality of propagated yeast. In detail, a maximum cell count of 260, 170 and 130 million/mL was propagated using 12, 16 and 18 °P wort supplemented with yeast extract to an initial FAN concentration of 500 mg/L. The vitality of the propagated yeast was also improved despite the increased final ethanol concentration. No significant difference was found between the mechanically and autolytically produced yeast extract but both were more effective than the pure nitrogen source glutamine. Ensuring the practical benefit for commercial brewing, a pilot high-gravity (16 °P) fermentation process was conducted, that was pitched with yeast harvested from a high-gravity (16 °P) propagation process supplemented with yeast extract. The fermentation performance increased while the concentration of fermentation by-products (higher alcohols, esters), the sensory quality and the proteinase activity of the finished beer were not affected.

Descriptors: yeast propagation, yeast extract supplementation, high gravity wort, *Saccharomyces pastorianus*, vitality, yeast cell concentration

1 Introduction

The quality of pitching yeast is one of the key factors for a satisfactory fermentation process in brewing. There are quality consequences for the end product beer [35] but also effects on the productivity of the fermentation and maturation procedure [33]. Continued serial repitching of yeast in brewing provides opportunities for contaminants in the form of bacteria and wild yeast to be introduced to the fermentation process [5]. Apart from this, a gradual deterioration in yeast condition occurs and yeast performance decreases [18]. Typical signs of yeast degeneration are decelerated and incomplete fermentations, increased or reduced flocculation behaviour, reduced foam and taste stability of the beer [5]. Therefore, it is important to introduce fresh propagated yeast into the brewery. The broad range of different propagation plants vary in their sophistication, capacity and yield [1]. However, they all have the same objective: to supply a high concentration of pitching yeast with high viability and vitality in compliance with high hygienic standards.

In brewing practise, it has proven advantageous to use the same wort for both fermentation and propagation [5]. The reasons are a permanent availability of sterile wort in the brewery and the adaption of the yeast physiology to the nutritional composition of the wort [5]. Furthermore, adverse flavour effects in the first generation beer are reduced when pitching propagation yeast together with its propagation medium [5]. Conversely, the nutritional composition of wort is not appropriate for yielding high yeast biomass concentration, especially when high-gravity adjunct worts are used [33]. A high concentration of sugars causes the "Crabtree effect" resulting in a reduction of pure biomass formation in favour of the formation of ethanol [33, 37]. In addition, both osmotic pressure and ethanol adversely affect yeast vitality and viability [5, 27, 35]. A limited supply of key growth factors in wort, especially free assimilable nitrogen (FAN) and zinc, also inhibits the formation of high yeast concentration in the propagation plant [1, 5, 38]. To increase the sustainability of the malting and brewing process, by-products can be used as additives for nutritional supplementation [38]. Possible supplements could be malt radicles [24, 38], liquid fractions of spent grain [38], trub [20] and spent yeast [19].

In previous studies, we investigated the nutritional potential of brewer's spent yeast and different industrially applicable extraction

https://doi.org/10.23763/BrSc19-22jacob

Authors

Friedrich Felix Jacob, Frank-Jürgen Methner, Technische Universität Berlin, Institut für Lebensmitteltechnologie und Lebensmittelchemie, Fachgebiet Brauwesen, Berlin, Germany; Mathias Hutzler, Maximilian Michel, Forschungszentrum Weihenstephan für Brau- und Lebensmittelqualität, Technische Universität München, Freising-Weihenstephan, Germany; corresponding author: f.jacob@campus.tu-berlin.de

Table 1 Wort composition

Parameter	Amount
Original gravity (°P)	12.00
pH	5.15
Zinc (mg/L)	0.15
FAN (mg/100 mL)	18.30
Total AS (mg/100 mL)	176.46
EBC-Bittering units (EBU)	20.00
Glucose (g/L)	13.61
Fructose (g/L)	2.61
Maltose (g/L)	62.01
Maltotriose (g/L)	15.95

methods to produce nutrient-rich yeast extract [15-17]. The positive impact of yeast extract supplementation on high gravity and adjunct fermentation was already studied in detail [15]. However, the results varied because of the different nutritional composition of used yeast extracts [15]. In this work, it was shown for the first time how yeast extract supplementation boosted the brewing yeast propagation process. Therefore, the propagation process of the yeast strain *Saccharomyces pastorianus* TUM 34/70 was conducted in small-scale bioreactors vessels using 12 °P, 16 °P and 18 °P wort. To simulate high-gravity adjunct wort, glucose was added to 12 °P all-malt wort to achieve higher gravities (16 °P, 18 °P). Yeast propagation performance is reduced using high-gravity adjunct wort that contains high proportions of glucose [33] and therefore yeast extract supplementation was assumed to be beneficial. Different amounts of autolytically or mechanically produced yeast extract from brewer's spent yeast were compared for supplementation. Their nutritional composition were already examined in detail [15, 17]. In order to demonstrate the beneficial effect of non-nitrogen sources of yeast extract, a pure nitrogen source (L-glutamine) was supplemented for comparison. Cell number, apparent extract, sugar (fructose, glucose, maltose, maltotriose) utilisation, FAN and ethanol concentration were analysed to monitor the propagation process. Yeast viability and vitality (test of fermentative capacity) were assessed at the end of the propagation procedures. To ensure the practical benefit for commercial brewing, we performed a high gravity (16 °P) fermentation process that was pitched with yeast harvested from a high-gravity (16 °P) propagation process supplemented with yeast extract. Therefore, fermentation by-products and the sensory quality of the finished beer were evaluated.

2 Material and Methods

2.1 Yeast strain, nutritional supplements, propagation and fermentation media

The bottom-fermenting yeast strain *S. pastorianus* TUM 34/70 was sourced on agar slant from the Yeast Center at the Weihenstephan Research Center for Brewing and Food Quality of the Technical University of Munich. A sterilised, hopped and standardised all-malt wort concentrate was used as propagation and fermentation medium (Döhler GmbH, Darmstadt, Germany). The all-malt wort concentrate was diluted to an original gravity of 12 °P. For the precise composition, refer to Table 1. To adjust wort gravity to 16 and 18 °P, D-(+)-glucose anhydrous (Merck KGaA, Darmstadt, Germany) was added to 12 °P all-malt wort as an adjunct. Before use, worts were heat treated at 100 °C for 10 minutes for sterilisation. L-glutamine was obtained in powder form (Merck KGaA, Darmstadt, Germany). Yeast extracts (produced via ultrasonic sonotrode or autolysis) were manufactured from brewer's spent yeast (*S. cerevisia*e TUM 68) according to our previous studies [15, 17]. For application in the brewing propagation process, yeast extract production methods had to be slightly modified without changing the nutritional composition of the finished yeast extract: no sodium chloride was used during autolysis and both yeast extracts (via sonotrode and autolysis) were heated to 95 °C for 10 min before freeze-drying. An overview of checked nutritional key parameters according to our previous studies [15, 17] is shown in Table 2.

2.2 Propagation and fermentation vessel

The yeast propagation processes were conducted in sterile small-scale (380 mL) bioreactors (BioBLU® 0.3f single-use vessels; Eppendorf AG, Hamburg, Germany). The vessels had a Rushton-type stirrer with a magnet-coupled drive, and connections with air filters for gas supply and exhaust. Furthermore, the vessels were equipped with ports for submerse aeration, medium addition, cell harvest, sampling, and temperature and pH probes. Standardised laboratory-scale brewing trials were performed according to Meier-Dörnberg et al. in stainless steel vessels of 10 cm diameter x 33 cm height (2.5 L) with 20 % headspace and clamped-down lids [23].

2.3 Propagation procedure

An inoculation loop of a pure agar slant colony was transferred to 60 mL standardised wort in a 100 mL Erlenmeyer flask and incubated for 48 h at 15 °C and ambient pressure on an orbital

Table 2 Nutritional composition of the autolytically and mechanically produced yeast extract (YE) per dry weight (dw)

Parameter	Autolytically produced YE	Mechanically produced YE
Protein (N × 5.5) (mg/g dw)	420	430
FAN (mg/g dw)	45	29
Total AS (mg/g dw)	440	450
Free amino acids (mg/g dw)	303	150
Zinc (mg/kg dw)	21.1	21.2
Magnesium (mg/100 g dw)	550	630
Pantothenic acid (mg/100 g dw)	15	18
Fat (mg/g dw)	5	11
Ash (mg/g dw)	130	130
Antioxidative potential (mg Trolox/g dw)	1	25

shaker (80 rpm; Witeg Labortechnik GmbH, Wertheim, Germany). The generated yeast suspension after the inoculation procedure was used to pitch the wort (250 mL) of the main propagation process into the small-scale bioreactors, adjusting a living cell count of 10 million CFU/mL at the beginning. The wort composition used in the inoculation and the main propagation process corresponded. The main propagation process was conducted at 15 °C with moderate stirring (80 rpm) and was unpressurised. A sufficient air supply was ensured by submerse aeration with a dispenser and constant stirring. The aeration was adjusted to 1 vvm. Mechanically (sonotrode) or autolytically produced yeast extract or glutamine were supplemented to the wort, adjusting an initial FAN concentration of 250 or 500 mg/L. Small-scale bioreactors were placed in a tempered cooling chamber to guarantee a constant process temperature. Sterile samples were taken via a fixed pipe whose inlet was submerged in the middle between the surface of the suspension and the bottom of the bioreactor to guarantee constant sampling.

2.4 Fermentation procedure

Propagated yeast suspension according to 2.3 (16 °P wort; with or without yeast extract supplementation) was pitched in aerated (10 mg O_2/L) standardised wort (2 L; 16 °P) attaining a living cell count of 15 million CFU/mL in a stainless steel fermentation vessel. Fermentation took place at 15 °C and was unpressurised until final attenuation. After specific gravity remained constant for two days, an additional five days for maturation were given at the same fermentation temperature, and seven days for lagering at 0 °C. The fermentation vessels were placed in a tempered cooling chamber to guarantee a constant process temperature.

2.5 Analytical methods

2.5.1 Physical and chemical parameters

Ethanol, pH, specific gravity, and the degree of attenuation were measured using an Anton Paar DMA 5000 Density Meter with Alcolyzer Plus measuring module, and pH measuring module (Anton-Paar GmbH, Ostfildern, Germany). Free α-amino nitrogen was determined photometrically according to the ninhydrin method and derived from MEBAK (Mitteleuropäische Brautechnische Analysenkommission) method 2.6.4.1. [14]. Sugar composition was measured according to MEBAK Method 2.7.1 using high-performance liquid chromatography (HPLC) [14]. Concentrations of fermentation by-products were analysed using gas chromatography (GC) according to MEBAK Method 2.21. [14].

2.5.2 Yeast cell number

Cell counts were determined using a Thoma cell counting chamber with a chamber depth of 0.1 mm and an area of 0.0025 mm² (Brand GmbH & Co. KG, Wertheim, Germany). Each sample was counted twice within 10 min and the deviation was less than 5 %.

2.5.3 Yeast viability and vitality

Yeast viability was measured according to MEBAK using the methylene blue method [29]. Yeast vitality was determined according to a modified method of *Müller-Auffermann* et al. and *Michel* et al. [25, 28]. At the end of the propagation processes, harvested yeast slurry was centrifuged at 750 g for 5 min at 20 °C and the supernatant was discarded. The remaining yeast was washed with tap water (20 °C) and vortexed for 30 sec. The yeast slurry was centrifuged (750 g; 5 min; 20 °C), the supernatant was discarded and the washed yeast was added to tap water (4.5 g/10 mL). 10 mL of the resulting yeast suspension was added to a Duran glass bottle (100 mL) containing 80 mL of tempered (20 °C) maltose solution (7.5 %). The bottle was sealed with a wireless automatic system for fermentative gas production monitoring (GMP, ANKOM Technology, Macedon, NY, USA) to detect the rise in pressure over 60 min.

2.5.4 Protease activity

Protease activity was measured using the Azocasein method. The principle of this method is that an azo dye, which is linked to casein, is split by proteinase. The amount of azo free azo dye is measured spectrophotometrically at 360 nm. Negative extinctions correspond to a proteinase concentration below 100 ppb [31]. The detailed procedure can be found in the work of *Stamm* [31].

2.5.5 Sensory evaluation

Beer samples were evaluated by a sensory panel of seven DLG-certified tasters according to the DLG scheme (Deutsche Landwirtschafts-Gesellschaft). A triangle test was conducted to demonstrate a significant difference between two beer samples (DIN EN ISO 4120:2007-10). Environmental conditions during sensory evaluation were implemented according to the MEBAK sensory handbook.

2.6 Yeast quality control

The purity of the yeast culture was checked by incubating 1 mL of the thick yeast slurry in 20 mL NBB Broth (Döhler GmbH, Darmstadt, Germany). The sample was incubated for seven days at

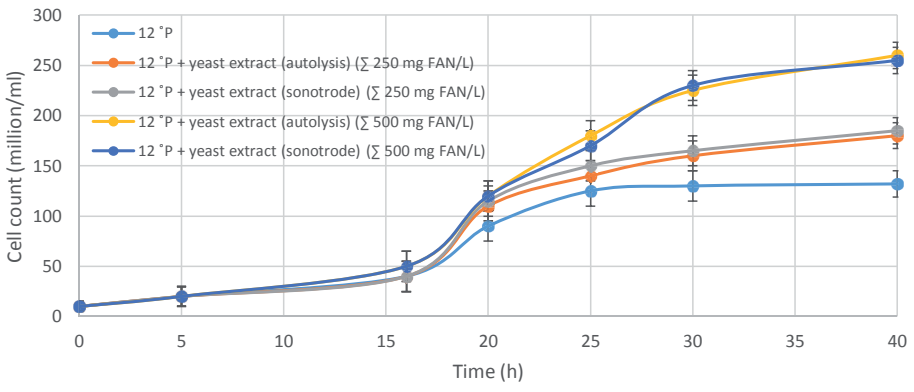

Fig. 1 **Development of cell concentration (million/mL) throughout a propagation process with 12 °P wort supplemented with different amounts of various yeast extracts (produced via autolysis or sonotrode) attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

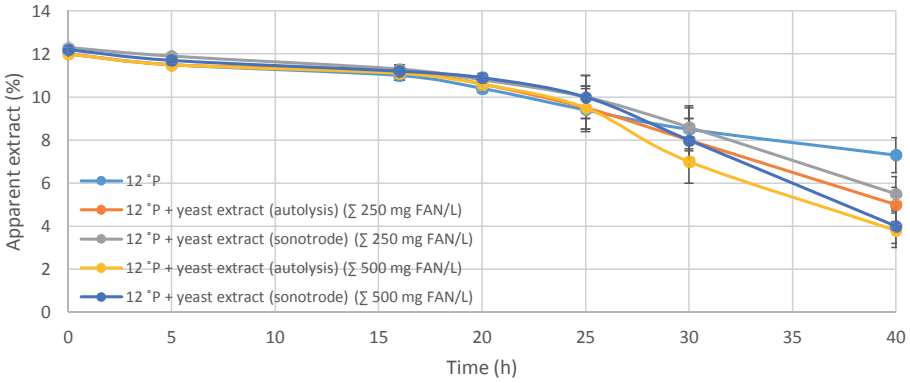

Fig. 2 **Development of apparent extract (%) throughout a propagation process with 12 °P wort supplemented with different amounts of various yeast extracts (produced via autolysis or sonotrode) attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

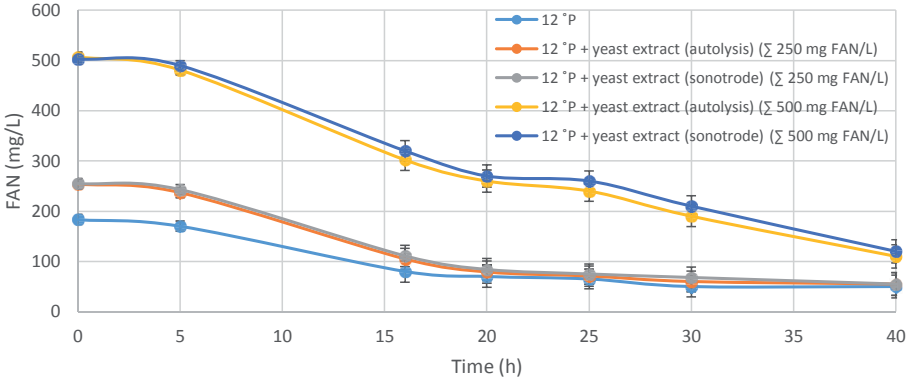

Fig. 3 **FAN concentration (mg/L) throughout a propagation process with 12 °P wort supplemented with different amounts of various yeast extracts (produced via autolysis or sonotrode) attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

27 °C to detect beer-spoilage bacteria [2]. 1 mL yeast slurry was then inoculated into 50 mL YM Broth, which was then incubated at 37 °C to detect potential top-fermenting yeast contamination (see [26] for a detailed description).

2.7 Statistical evaluation

All experiments were performed in triplicate and the relevant results given as arithmetic means with bars representing the standard deviation. A single factor analysis of variance (ANOVA) and a paired t-test were performed to demonstrate differences between the results. “Significant” differences were described as having a p-value < 0.05. A test by Dixon was used to evaluate the results.

3 Results and discussion

3.1 Yeast propagation in standard wort with yeast extract supplementation

In the first tests, we compared the impact of an autolytically and a mechanically (via sonotrode) produced yeast extract on a yeast propagation process in 12 °P all-malt wort. During the first 15 h, there was no significant difference between the cell concentration (Figure 1) of the standard (12 °P) and the supplemented propagation processes (12 °P + yeast extract). In the further course of the experiments (15–20 h), the cell concentration of all trials supplemented with yeast extract significantly increased compared with the standard but did not differ from each other. After 25 h, all propagation processes supplemented with yeast extract had a yeast cell concentration of between 140 and 180 million cells/mL while the standard process (12 °P) had a concentration of 120 million cells/mL. After 30 h, yeast cell concentration was significant higher (230 million cells/mL) in the propagation process that had a starting concentration of 500 mg FAN/L compared with the propagation processes, which had a concentration of 250 mg FAN/L (160 million cells/mL). At that time, yeast cell concentration of the standard propagation trial reached its maximum of 130 million cells/mL. At the end (40 h), yeast extract (via autolysis) supplementation (500 mg FAN/L) increased yeast cell concentration to 260 million cells/mL. Yeast extract supplementation attaining an initial FAN concentration of 250 mg/L still boosted yeast cell concentration to 180 million cells/mL. In terms of yeast biomass formation, no significant difference was found between the mechanically and autolytically produced yeast extract. In a comparison of figures 1 and 2, a positive correlation can be assumed between increased yeast biomass formation and increased extract consumption. After 30 h, the propagation process supplemented with autolytically produced yeast extract (500 mg FAN/L) had an apparent extract of 7 % while the standard process (12 °P) had 8.5 %. At the end (40 h), propagation trials supplemented with yeast extract (via autolysis) that had an initial concentration of 250 and 500 mg FAN/L had an apparent extract of 5 % and 3 %, respectively. No significant difference was found between the mechanically and autolytically produced yeast extract (Fig. 2). FAN consumption as a part of the overall extract consumption was also higher for propagation processes supplemented with yeast extract (Figure 3). After 40 h, a FAN decrease of 199 and 397 mg/L was evaluated for propagation processes supplemented to initial concentrations of 250 and 500 mg/L, respectively. Furthermore, fructose and glucose were completely consumed at the end of all propagation processes in 12 °P wort (Table 3). Maltose and maltotriose consumption increased, depending on the amount of yeast extract added. An initial FAN concentration of 500 mg/L achieved by yeast extract supplementation resulted in a complete metabolisation of all analysed sugars. Significantly increased final ethanol contents of 2.6 and 4.3 vol. % were measured for propagation processes supplemented with yeast extract (250 and 500 mg FAN/L) compared with the standard propagation process (1.8 vol. %) (Fig. 3). Mechanically and autolytically produced yeast

Table 3 **Sample overview, final alcohol content and percentage sugar consumption of all propagation processes; data are expressed as mean values ± standard deviation of three independent experiments**

Sample name: initial wort gravity + supplements (initial FAN content of wort), propagation time	Final ethanol content (vol. %)	Percentage (%) consumption of			
		Fructose	Glucose	Maltose	Maltotriose
12 °P, 24 h	0.8 ± 0.2	98 ± 2	98 ± 2	18 ± 5	< 2
12 °P, 40 h (30 h)	1.8 (1.2) ± 0.2	98 ± 2	98 ± 2	25 ± 8	< 2
16 °P, 30 h	1.3 ± 0.2	< 2	28 ± 3	17 ± 3	< 2
18 °P, 30 h	1.5 ± 0.3	< 2	61 ± 3	29 ± 2	< 2
12 °P + yeast extract (autolysis)					
(∑ 250 mg FAN/L), 40 h	2.6 ± 0.3	98 ± 2	98 ± 2	68 ± 5	73 ± 3
12 °P + yeast extract (sonotrode)					
(∑ 250 mg FAN/L), 40 h	2.5 ± 0.3	98 ± 2	98 ± 2	60 ± 4	69 ± 2
12 °P + glutamine					
(∑ 250 mg FAN/L), 40 h	2.3 ± 0.3	98 ± 2	98 ± 2	61 ± 3	63 ± 3
12 °P + yeast extract (autolysis)					
(∑ 500 mg FAN/L), 40 h	4.3 ± 0.2	98 ± 2	98 ± 2	98 ± 2	98 ± 2
12 °P + yeast extract (sonotrode)					
(∑ 500 mg FAN/L), 40 h	4.4 ± 0.3	98 ± 2	98 ± 2	98 ± 2	98 ± 2
12 °P + glutamine					
(∑ 500 mg FAN/L), 40 h	2.7 ± 0.3	98 ± 2	98 ± 2	81 ± 2	71 ± 2
16 °P + yeast extract (autolysis)					
(∑ 500 mg FAN/L), 30 h	1.8 ± 0.2	< 2	70 ±	19 ± 3	< 2
18 °P + yeast extract (autolysis)					
(∑ 500 mg FAN/L), 30 h	2.5 ± 0.2	< 2	98 ± 2	31 ± 2	< 2

extract had the same impact on FAN and sugar consumption but also on ethanol formation (Fig. 3, Table 3).

Various yeast extract products can be used to enhance the fermentation performance of yeast [12, 15]. In this context, the positive impact of yeast extract on the fermentation of high-gravity worts with high proportions of unmalted grains has already been investigated [21, 36]. To the best of our knowledge, the application of yeast extract, especially that produced from brewer's spent yeast, to boost the brewing propagation process has not been examined before. During a conventional brewing propagation process in 12 °P wort, it was shown that yeast cell count could be increased (Fig. 1) until the FAN is completely consumed by the yeast (Fig. 3). However, the vitality of propagated yeast was higher at low ethanol (0.8 %) and cell concentration (100 million/mL) after 24 h than at high ethanol (1.8 %) and yeast cell concentration (130 million/mL) at the end of the propagation process (40 h) (Fig. 10, see page 164). The deteriorating effect of ethanol on yeast metabolism has already been shown [27]. One reason is that ethanol destroys the integrity of the yeast cell membrane and enzymes by affecting hydrogen bonds [11, 13]. Yeast extract supplementation remarkably increased yeast cell concentration of a propagation process in 12 °P wort (Fig. 1) but final ethanol concentration (Table 3) also rose. Similar results were found by *Weigert*, supplementing an extract of malt radicles having high FAN and zinc concentration [38]. However, despite the high final ethanol concentration, the vitality of the propagated yeast at the end of the propagation process supplemented with yeast extract was higher than that for the propagation process without yeast extract supplementation, which had a lower final ethanol concentration. There can be several reasons for this increased vitality, considering the different nutrients in the supplemented yeast extract. Proline, for example, is not usually metabolised but may have a protective role against ethanol stress by increasing the stability and the solubility of hydrophobic macromolecules and soluble proteins [10, 32]. Zinc also enhances the tolerance of yeast cells to ethanol by promoting the accumulation of membrane-protecting substances like trehalose and ergosterol [39]. Furthermore, zinc is presumably involved in the alteration of membrane fluidity by compensating for the fluidifying effect of ethanol via rigidification [3]. An increased magnesium concentration prevents yeast cells from the toxic effect of ethanol by increasing the cell membrane permeability elicited by ethanol-induced stress [4]. In addition, at the end of the propagation process, a deficiency in essential nutrients in the propagation wort may lead to nutrient depletion in the yeast cells. Several studies have provided evidence that nutrient limitation is the main reason for poor fermentation performance that is related to yeast vitality [8, 9, 13]. Yeast extract supplementation (500 mg FAN/L) provided a wide range of different additional nutrients for yeast propagation. The final FAN concentration of 110 mg/L suggests that there were still nitrogen sources left (Fig. 1). In contrast, the propagation process without yeast extract supplements already had a final FAN concentration of 50 mg/L after 30 h. The positive impact of zinc, magnesium, calcium and free amino acid supplementation on yeast metabolism has already been shown [7, 12, 15]. Complex sources of nitrogen (casamino acids, peptides) may be considered

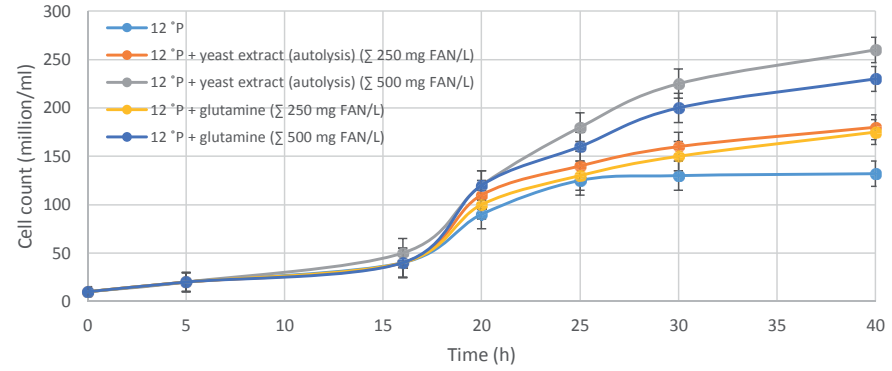

Fig. 4 **Development of cell concentration (million/mL) throughout a propagation process with 12 °P wort supplemented with different amounts of yeast extract (produced via autolysis) or glutamine attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

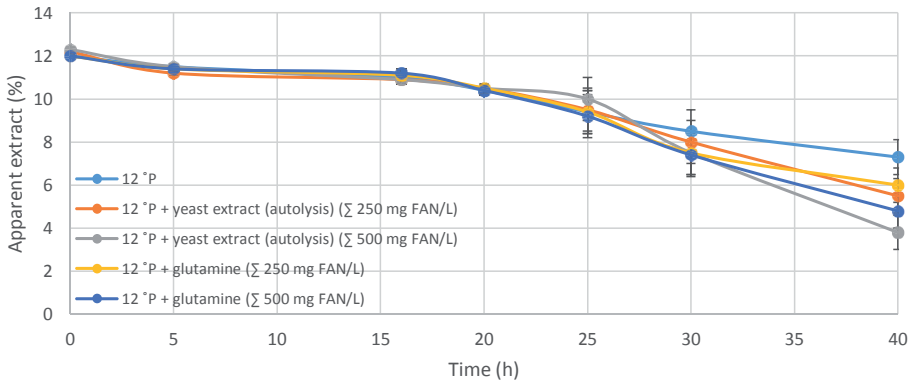

Fig. 5 **Development of apparent extract (%) throughout a propagation process with 12 °P wort supplemented with different amounts of yeast extract (produced via autolysis) or glutamine attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

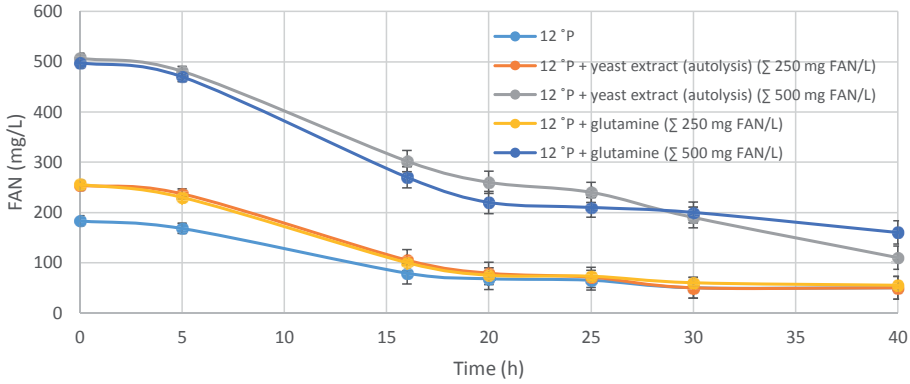

Fig. 6 **FAN concentration (mg/L) throughout a propagation process with 12 °P wort supplemented with different amounts of yeast extract (produced via autolysis) or glutamine attaining an initial FAN concentration of 250 or 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

to have higher bioavailability than simple nitrogen sources such as ammonium sulphate [10]. Furthermore, yeast is able to transport minerals like zinc via zinc-transporters into the cell, store them in der cell vacuole and mobilise them when required by releasing them into the cytoplasm [22].

To our surprise, when comparing the growth-stimulating effect of the autolytically and mechanically produced yeast extract, no significant difference was found (Fig. 1). To achieve an initial FAN concentration of 250 or 500 mg/L wort, 2.6 g/L or 12.3 g/L of the mechanically produced yeast extract was added and 1.4 g/L or 7.0 g/L of the autolytically produced yeast extract, respectively. Considering the fact that the autolytically produced yeast extract had a higher nitrogen content, more non-nitrogen nutrients were therefore added when supplementing with the mechanically produced yeast extract [17]. Furthermore, the mechanically produced yeast extract had more vitamins and a higher antioxidative potential [17]. However, on the other hand the availability of FAN of the mechanically produced yeast extract could presumably be reduced because part of its FAN is linked to fractions of greater peptide molecules compared with the autolytically produced yeast extract [15].

3.2 Yeast propagation supplemented with yeast extrac compared to L-glutamine

The objective of further tests was to demonstrate that the effect of yeast extract supplementation on yeast propagation performance could not only be attributed to an increase of FAN. Therefore, a propagation process in 12 °P wort was supplemented with a pure nitrogen source (L-glutamine) to an initial level of 250 or 500 mg FAN/L. Figure 4 shows that after 25 h processing time there was a significant difference between the yeast cell concentration of trials supplemented with yeast extract and glutamine (500 mg FAN/L). After 40 h, the yeast cell concentration of the propagation process supplemented with glutamine (500 mg FAN/L) was 30 million cells/mL lower than the corresponding trial in which yeast extract was supplemented. The final apparent extract (Fig. 5) was lower for glutamine supplementation than for yeast extract supplementation, both with an initial FAN concentration of 500 mg/L. Yeast biomass formation, FAN and extract consumption of propagation processes supplemented with glutamine and yeast extract (Fig. 6), both with an initial FAN concentration of 250 mg/L, did not differ from each other but were higher compared with the standard process (12 °P). During the first 20 h of a propagation process with an initial FAN concentration of 500 mg/L, the FAN decease was significantly higher when using glutamine as a supplement than yeast extract (Fig. 6). In contrast, after 40 h, FAN consumption was higher using yeast extract as a

supplement than using glutamine. Maltose consumption and final ethanol formation were lower using glutamine as a supplement instead of yeast extract (Table 3).

Ingledew et al. noted the positive effect of yeast extract on fermentation performance is not solely a result of increased nitrogen availability [12]. Our results showed the same situation regarding yeast propagation performance. Propagation trials supplemented with glutamine (500 mg/L) had a lower final yeast cell number (Fig. 4) and yeast vitality (Fig. 11, see page 164) compared with trials in which yeast extract was supplemented. It was also noticeable that FAN consumption was faster in the first 20 h of the propagation process supplemented with glutamine than that with yeast extract (Fig. 6), presumably because glutamine was directly metabolised as being part of the central nitrogen metabolism [34]. In contrast, only a small percentage (4.6 %) of all free amino acids of the autolytically produced yeast extract was glutamine and therefore all other amino acids had to first be enzymatically converted to glutamate and glutamine before being metabolised further [34]. However, the final FAN concentration of the propagation process supplemented with yeast extract (500 mg/L) was lower (Fig. 6), presumably due to the metabolic-promoting effect of non-nitrogen sources such as minerals or vitamins. The zinc concentration of the propagation wort (12 °P) was increased from a low level initially of 0.15 mg/L to 0.18 mg/L or 0.30 mg/L as a result of yeast extract (produced autolytically) supplementation (initial FAN concentration of 250 mg/L or 500 mg/L). Comparing propagation processes supplemented with glutamine and yeast extract and with an initial FAN concentration of 250 mg/L, no significant difference could be found for any of the investigated propagation parameters (Table 3, Fig. 4, Fig. 5, Fig. 6). Presumably, the increase of non-nitrogen sources like zinc was not sufficient here. Weigert investigated the impact of different FAN, zinc and pantothenic acid concentration on yeast propagation in a synthetic wort medium and stated that increasing the zinc concentration increases yeast cell formation at high FAN levels [38]. In contrast, at low FAN levels increasing the zinc concentration is not beneficial in increasing yeast biomass formation [38]. However, a lack of zinc always slows down the yeast metabolism during propagation regardless of high or low FAN levels [38]. This was also substantiated by the fact that a lower final apparent extract (Fig. 5), a higher sugar consumption and a higher final alcohol content (Table 3) was measured in the yeast extract compared with the propagation process supplemented with glutamine, which had an initial FAN concentration of 500 mg/L. However, similar values were not measured in the trials that had an initial FAN concentration of 250 mg/L. Weigert used an extract of malt radicles to increase the FAN and zinc concentration of propagation wort [38]. According to this study, yeast biomass formation and extract consumption were slower than for a propagation process supplemented with

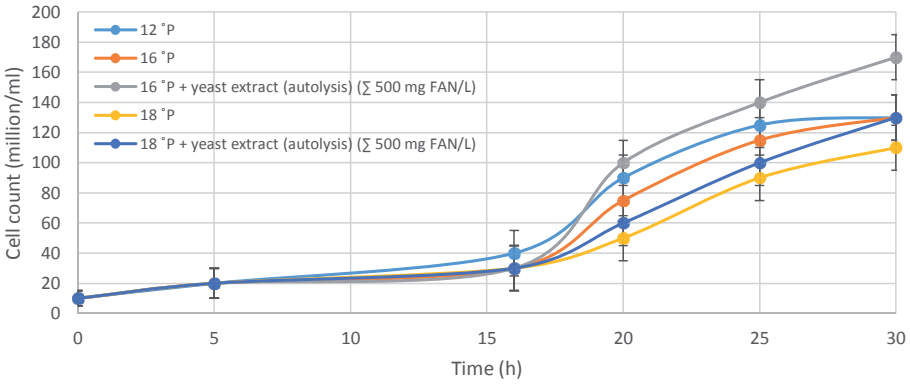

Fig. 7 **Development of cell concentration (million/mL) throughout a propagation process with 12, 16, 18 °P wort supplemented with or without yeast extract attaining an initial FAN concentration 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

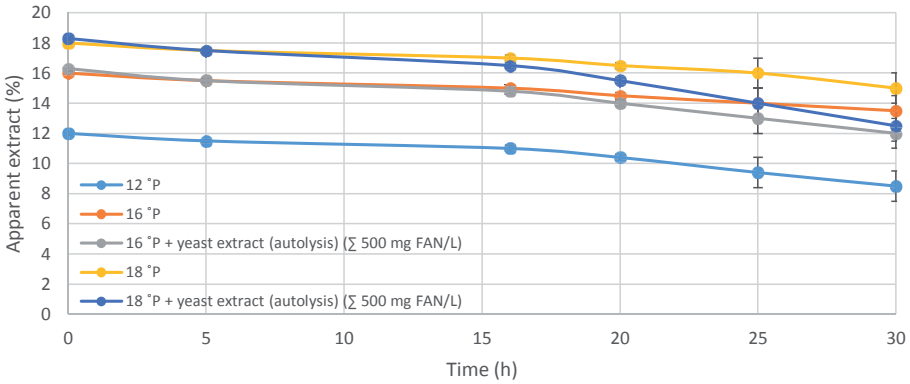

Fig. 8 **Development of apparent extract (%) throughout a propagation process with 12, 16, 18 °P wort supplemented with or without yeast extract attaining an initial FAN concentration 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

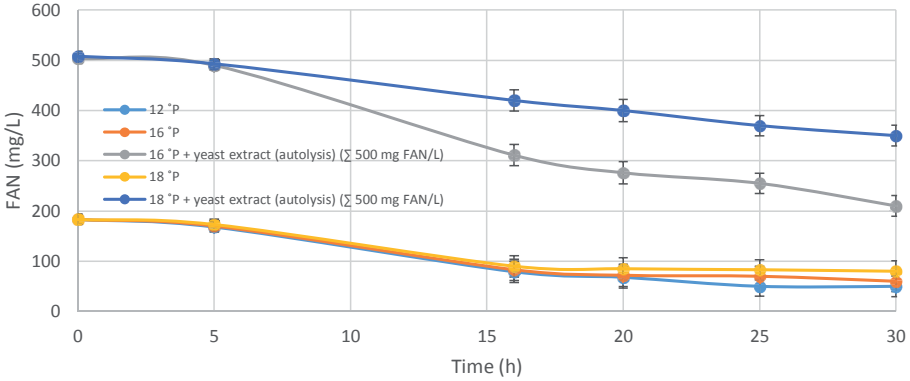

Fig. 9 **FAN concentration (mg/L) throughout a propagation process with 12, 16, 18 °P wort supplemented with or without yeast extract attaining an initial FAN concentration 500 mg/L; data are expressed as mean values ± standard deviation of three independent experiments**

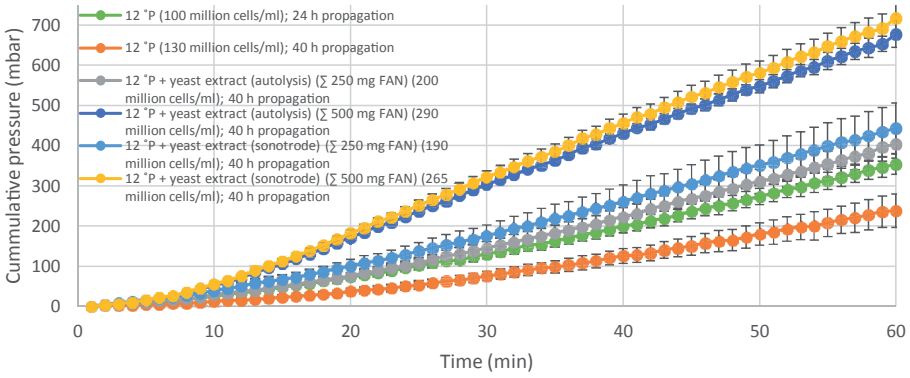

Fig. 10 **Yeast vitality test: cumulative pressure (mbar) of different propagation yeasts (supplemented with different amounts of various yeast extract (produced via autolysis or sonotrode) during propagation procedure) in maltose solution; data are expressed as mean values ± standard deviation of three independent experiments**

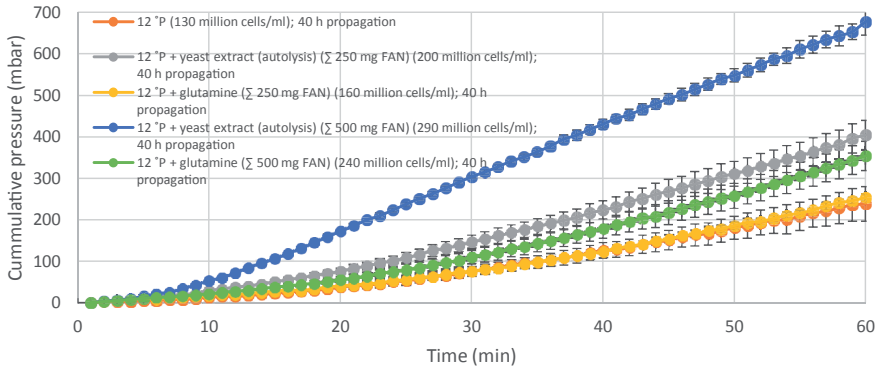

Fig. 11 **Yeast vitality test: cumulative pressure (mbar) of different propagation yeast (supplemented with yeast extract (produced via autolysis) and glutamine during propagation procedure) in maltose solution; data are expressed as mean values ± standard deviation of three independent experiments**

glutamine [38]. It was presumed that rise in the apparent extract (15 %) caused a higher osmotic pressure and extended the initial growth phase of the yeast propagation process [38]. In contrast, yeast extract did not increase the apparent extract significantly and the initial phase was not extended as was the case for glutamine supplementation (Fig. 8).

3.3 Yeast propagation in high-gravity adjunct wort with yeast extract supplementation

Additional experiments were conducted to evaluate the effect of yeast extract supplementation on yeast propagation in high-gravity adjunct wort. Figure 7 shows that yeast propagation in 16 °P wort did not differ from yeast propagation in 12 °P wort with regard to final yeast biomass formation. However, the final yeast cell count was reached 4 h earlier in 12 °P wort. In contrast, using 18 °P wort for yeast propagation yeast biomass formation was significantly lower compared to 12 °P and 16 °P wort. A cell concentration of 110 million/mL was measured after 30 h. Yeast extract supplementation that achieved an initial FAN level of 500 mg/L wort, increased yeast biomass formation for both wort gravities (16 °P, 18 °P). While the increase of yeast cell concentration in 16 °P was 40 million cells/mL, the rise in 18 °P wort was 20 million cells/mL after 30 h. Supplementing yeast propagation in 16 °P and 18 °P wort with yeast extract (500 mg FAN/L) caused a lower final apparent extract compared to propagation in 12 °P wort. In detail, the decrease of the apparent extract, the final ethanol content and glucose consumption was greater in 18 °P compared to 16 °P wort (Fig. 8, Table 3). The FAN decrease of both high-gravity propagation processes (16 °P, 18 °P) without yeast extract supplementation was 110 mg/L on average and did not differ significantly (Fig. 9). In contrast, using yeast extract as a supplement, the FAN consumption of the yeast propagation in 16 °P and 18 °P wort was 294 mg/L and 160 mg/L, respectively.

High-gravity adjunct worts (16 °P, 18 °P) adjusted via glucose addition were less suitable for yeast propagation than standard (12 °P) all-malt wort because yeast biomass formation was slower in both 16 °P and 18 °P wort. In detail, yeast cell formation was lower and glucose consumption was higher during propagation in 18 °P compared to 16 °P wort. These observations can presumably be attributed to the Crabtree effect. The high glucose concentration presumably suppressed the respiration, which is energetically disadvantageous for the yeast, resulting in slower growth and formation of biomass [33]. Nevertheless, yeast extract supplementation that increased several nutrients such as FAN, zinc, vitamins was presumably beneficial for all metabolic processes of the yeast and boosted therefore respiration and fermentation. *Tenge* at al. observed a prolonged lag phase and a slower growth in the exponential phase of yeast propagation in high-gravity wort [33]. After measuring the activity of enzymes with key roles in sugar metabolism, it was concluded that a change in yeast metabolism was due to the Crabtree effect [33]. Furthermore, it was shown that the yeast propagated in wort with the addition of glucose syrup had the lowest growth rates and the yeast metabolism more strongly followed the fermentation pathway via acetaldehyde instead of acetyl-CoA. They excluded a lack of nutrients as an influencing factor stating there was a sufficient supply in 12 °P wort [33]. However, our results show that yeast extract supplementation improves yeast propagation performance with regard to yeast cell formation (Fig. 7) but also boosts the anaerobe metabolism of yeast during propagation. Tenge et al. also conducted pilot fermentations in 12 °P wort using yeast of high-gravity propagations to test yeast vitality [33]. They observed that fermentation performance was unsatisfactory because of a slower fermentation speed and concluded that it was caused by a longer adaptation phase of the yeast to its new milieu [33]. Measuring the vitality of propagation yeast according to our test by recording the cumulative pressure, a lower value was found for yeast propagated in high-gravity wort compared to propagation in standard 12 °P wort, which confirmed the results of Tenge et al.

[33]. According to our results, it was shown that the vitality of yeast of a high-gravity propagation process could be improved by yeast extract supplementation (Figure 12). Presumably, at the end of the propagation process, yeast extract addition prevented a depletion of relevant nutrients. It was probably the same beneficial effect as in the case of supplementing a 12 °P propagation process with yeast extract.

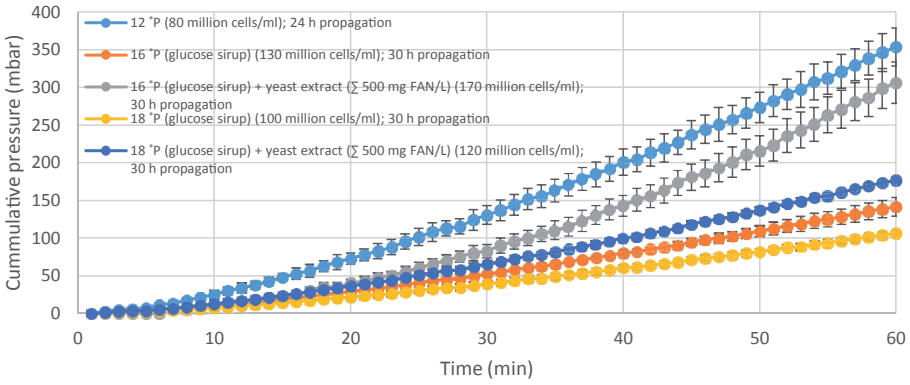

Fig. 12 **Yeast vitality test: cumulative pressure (mbar) of different propagation yeast (supplemented with yeast extract (produced via autolysis) during propagation procedure in high-gravity wort) in maltose solution; data are expressed as mean values ± standard deviation of three independent experiments**

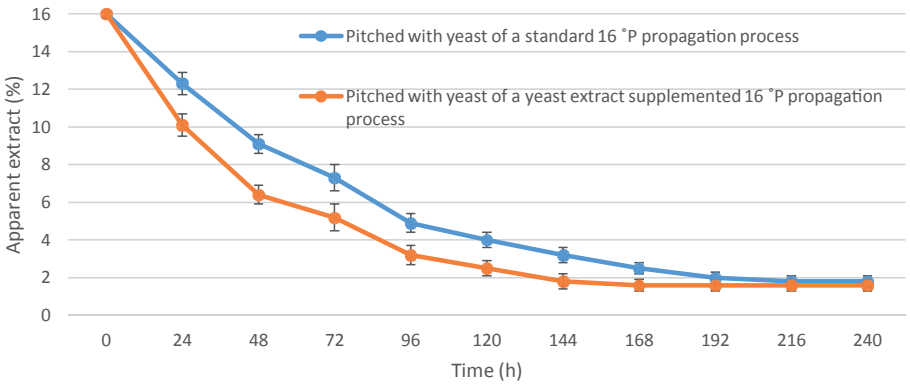

Fig. 13 **Development of apparent extract during a 16 °P fermentation pitched with yeast harvested from a 16 °P propagation process with or without yeast extract supplementation (500 mg FAN/L); data are expressed as mean values ± standard deviation of three independent experiments**

3.4 Effect of yeast extract supplementation on vitality of propagation yeast

Yeast viability describes the percentage share of living yeast cells based on the entire yeast population. The viability of all propagation yeast was 98 ± 1.5 % and no significant difference was determined between any of the conducted propagation processes. Yeast vitality characterises the physiological condition of yeast. According to the vitality test, yeast vitality was linked to the formation of CO_2 that was calculated as a cumulative pressure value. A high cumulative pressure value represented high vitality. Figure 10 shows that propagation yeast of a 24 h propagation process (12 °P) produced more CO_2 than yeast of a 40 h propagation (12 °P) process. Propagation yeast of a propagation process supplemented with yeast extract (250 mg FAN/L) (40 h) generated a higher cumulative pressure value in comparison with the propagation yeast of the 24 h propagation process (12 °P) without yeast extract supplementation. Yeast of the 40 h propagation process supplemented with an initial FAN concentration of 500 mg/L generated the highest cumulative pressure value and therefore had the highest vitality. According to the vitality test, autolytically and mechanically (sonotrode) produced yeast extract had the same beneficial effect on yeast vitality. Supplementing the propagation process with glutamine (500 mg FAN/L) increased the vitality of the propagation yeast but was not as beneficial as yeast extract (500 mg FAN/L) (Fig. 11). Yeast extract (500 mg FAN/L) supplementation of a high-gravity propagation process also increased the vitality of the propagation yeast (Fig. 12). After a propagation process in 16 °P wort, yeast produced more CO_2 compared with yeast supplemented to a process in 18 °P. Boosting yeast vitality via yeast extract supplementation was more effective for a 16 °P than for an 18 °P propagation process.

3.5 Fermentation with yeast from yeast extract supplemented propagation

To evaluate the practical benefit for commercial brewing, pilot high-gravity fermentation (16 °P) trials were pitched with yeast that was harvested from a high-gravity propagation process (16 °P) supplemented with or without yeast extract (500 mg FAN/L). Figure 13 shows the development of the apparent extract. The extract consumption of the trial that was pitched with propagation yeast of the process supplemented with yeast extract was significantly faster (36 h). In contrast, the final apparent extract (1.6 % ± 0.5 %) and the resulting ethanol content (7.8 % ± 0.3 %)

Table 4 **Concentration (mg/L) of selected flavour and aroma components in the finished beer that was fermented with yeast from a propagation process that was supplemented with and without yeast extract (YE); the values were reported to the same ethanol content of 5 % (v/v); data are expressed as mean values ± standard deviation of three independent experiments**

Flavour and aroma component (mg/L)	Without YE	Supplemented with YE
Ethyl acetate	28.45 ± 0.9	30.12 ± 1.2
Ethyl decanoate	< 0.01	< 0.01
Ethyl hexanoate	0.10 ± 0.05	0.10 ± 0.05
Isoamyl acetate	2.13 ± 0.10	2.2 ± 0.10
Ethyl octanoate	1.20 ± 0.05	1.10 ± 0.05
Acetaldehyde	6.75 ± 0.25	6.53 ± 0.25
Isobutanol	17.76 ± 1.22	16.48 ± 1.46
3-methyl-butanol	66.82 ± 2.86	63.43 ± 2.49
Propanol	21.75 ± 1.02	22.17 ± 1.13

did not differ. All finished beers were given a sensory rating of 4.5 with regard to flavour, body and bitterness according to the DLG scheme and no sensory defects were detected. According to the sensory triangle test, the finished beers did not differ significantly. Analysed beer volatiles (esters and higher alcohols) did not differ either (Table 4). Using the Azocasein method according to Stamm to determine the proteinase activity, the measured extinctions of all tested beers were negative and therefore, proteinase concentration was below 100 ppb [31]. Here, no significant difference between all tested beers was found.

As shown above, yeast extract supplementation of high-gravity adjunct wort was beneficial for yeast vitality and yeast propagation performance. Yeast was propagated faster and higher yeast biomass formation was achieved at the end of the propagation process. It is advantageous for a brewery that has only high-gravity wort at its disposal to use the same wort for yeast propagation and fermentation because brewhouse operations do not have to be changed and subsequent wort dilution holds the risk of biological contamination [33]. To improve high-gravity fermentation performance yeast extract is usually supplemented into the fermentation medium [9, 10, 15]. However, any benefit of fermentation performance should not be at the expense of beer quality. The impact of yeast extract on product quality is the subject of controversial debate. Different nutrients of yeast extract influence yeast metabolism and change the production of higher alcohols, esters and vicinal diketones [10]. Furthermore, the nutritional composition [10, 15], especially of amino acids [15], of yeast extract often varies greatly and changes the organoleptic profile of the beer [10]. According to our experiments, there was no impact on the production of higher alcohols and esters or the resulting beer flavour. Concentration of the selected aroma and flavour compounds approximately corresponded to data of *Piddocke* et al. who measured these compounds in beers, which were produced via high-gravity fermentations [30]. They determined increased concentrations for ethyl acetate and isoamyl acetate when using high-gravity adjunct wort [30]. The residual and unmetabolised yeast extract nutrients of the propagation medium that were pitched together with the propagation yeast into the fermentation medium did not influence the fermentation process. Their concentration was presumably too low to have a significant impact. Reduced yeast vitality also causes an increase of proteinase concentration during lagering resulting in a reduced foam stability [31]. During high gravity fermentations, where osmotic pressures and ethanol concentrations are higher, the yeast releases more proteinase [6]. A decrease of proteinase concentration as a result of increased yeast vitality was not detected in our experiments because proteinase concentration in the finished beer was already at a low level. According to our results, proteinase concentration in the finished beer was not significantly influenced by supplementing yeast extract during yeast propagation.

4 Conclusion

This study showed that yeast extract produced from brewer's spent yeast boosted yeast propagation performance. The final yeast cell concentration was increased to 260 million/mL when supplementing 12 °P wort with yeast extract to an initial FAN concentration of 500 mg/L. The vitality of the propagation yeast was higher than for a standard propagation process although alcohol concentration reached a final level of 4.4 vol. %. Supplementing a propagation process in high-gravity (16 °P, 18 °P) adjunct wort with yeast extract, yeast biomass formation and vitality were also improved. No significant difference was found in any of the experiments between the mechanically and the autolytically produced yeast extract. The beneficial impact of yeast extract supplementation on yeast propagation could not only be attributed to an additional supply of nitrogen sources but also to non-nitrogen sources, presumably minerals and vitamins. In pilot brewing trails, the practical benefit was shown for high-gravity fermentation pitched with yeast of a high-gravity propagation process supplemented with yeast extract. Primary fermentation was accelerated, but flavour formation, organoleptic profile and proteinase activity of the finished beer were not affected. In conclusion, supplementing a propagation process with yeast extract increased the yield and quality of the propagated yeast and had a subsequent beneficial impact on fermentation performance.

5 References

1. Annemüller, G.; Manger, H. J. and Lietz, P.: The yeast in the brewery, 1st edition: VLB Berlin, 2011.
2. Back, W.: Ausgewählte Kapitel der Brautechnologie, 2nd edition: Fachverlag Hans Carl GmbH, 2008.
3. Binder, H.; Arnold, K.; Ulrich, A. S. and Zschornig, O.: Interaction of Zn2+ with phospholipid membranes, Biophysical Chemistry, **90** (2001), no. 1, pp. 57-74.
4. Birch, R. M. and Walker, G. M.: Influence of magnesium ions on heat shock and ethanol stress responses of *Saccharomyces cerevisiae*, Enzyme and Microbial Technology, **26** (2000), no. 9-10, pp. 678-687.
5. Boulton, C. and Quain, D.: Brewing Yeast & Fermentation, edition: Blackwell Science Ltd, 2001.
6. Brey, S. E.; Bryce, J. H. and Stewart, G. G.: The loss of hydrophobic polypeptides during fermentation and conditioning of high gravity and low gravity brewed beer, Journal of the Institute of Brewing, **108** (2002), no. 4, pp. 424-433.
7. Bromberg, S. K.; Bower, P. A.; Duncombe, G. R.; Fehring, J.; Gerber, L.; Lau, V. K. and Tata, M.: Requirements for zinc, manganese, calcium, and magnesium in wort, Journal of the American Society of Brewing Chemists, **55** (1997), no. 3, pp. 123-128.
8. Casey, G. P.; Chen, E. C. H. and Ingledew, W. M.: High-gravity brewing: production of high levels of ethanol without excessive concentrations of esters and fusel alcohols, Journal of the American Society of Brewing Chemists, **43** (1985), no. 4, pp. 179-182.
9. Casey, G. P.; Magnus, C. A. and Ingledew, W. M.: High-gravity brewing – effects of nutrition on yeast composition, fermentative ability, and alcohol production, Applied and Environmental Microbiology 48 (1984), no. 3, pp. 639-646.
10. Gibson, B. R.: 125th Anniversary Review: Improvement of Higher Gravity Brewery Fermentation via Wort Enrichment and Supplementation, Journal of the Institute of Brewing, **117** (2011), no. 3, pp. 268-284.
11. Hallsworth, J. E.: Ethanol-induced water stress in yeast, Journal of Fermentation and Bioengineering, **85** (1998), no. 2, pp. 125-137.
12. Ingledew, W. M.; Sosulski, F. W. and Magnus, C. A.: An assessment of yeast foods and their utility in brewing and enology, American Society of Brewing Chemists, **44** (1986), no. 4, pp. 166-170.
13. Ingram, L. O. and Buttke, T. M.: Effects of alcohols on microorganisms, Advances in Microbial Physiology, **25** (1984), pp. 253-300.

14. Jacob, F.: MEBAK Brautechnische Analysemethoden Würze Bier Biermischgetränke, edition: Selbstverlag der MEBAK, 2012.
15. Jacob, F. F.; Michel, M.; Zarnkow, M.; Hutzler, M. and Methner, F.-J.: The complexity of yeast extracts and its consequences on the utility in brewing: A review, BrewingScience, **72** (2019), no. 3/4, pp. 50-62.
16. Jacob, F. F.; Striegel, L.; Rychlik, M.; Hutzler, M. and Methner, F. J.: Spent Yeast from Brewing Processes: A Biodiverse Starting Material for Yeast Extract Production, Fermentation, **5** (2019), no. 2.
17. Jacob, F. F.; Striegel, L.; Rychlik, M.; Hutzler, M. and Methner, F. J.: Yeast extract production using spent yeast from beer manufacture: influence of industrially applicable disruption methods on selected substance groups with biotechnological relevance, European Food Research and Technology, **245** (2019), no. 6, pp. 1169-1182.
18. Jenkins, C. L.; Kennedy, A. I.; Hodgson, J. A.; Thurston, P. and Smart, K. A.: Impact of serial repitching on lager brewing yeast quality, Journal of the American Society of Brewing Chemists, **61** (2003), no. 1, pp. 1-9.
19. Kieninger, H.: Abfallhefe-Verwendung im Sudhaus, BRAUWELT, **118** (1978), no. 24, pp. 872-880.
20. Kreder, G. C.: Yeast assimilation of trub-bound zinc, Journal of the American Society of Brewing Chemists, **57** (1999), no. 4, pp. 129-132.
21. Le Van, V. M.; Strehaiano, P.; Nguyen, D. L. and Taillandier, P.: Microbial protease or yeast extract – Alternative additions for improvement of fermentation performance and quality of beer brewed with a high rice content, Journal of the American Society of Brewing Chemists, **59** (2001), no. 1, pp. 10-16.
22. MacDiarmid, C. W.; Gaither, L. A. and Eide, D.: Zinc transporters that regulate vacuolar zinc storage in *Saccharomyces cerevisiae*, Embo Journal, **19** (2000), no. 12, pp. 2845-2855.
23. Meier-Dörnberg, T.; Michel, M.; Wagner, R. S.; Jacob, F. and Hutzler, M.: Genetic and Phenotypic Characterization of Different Top-fermenting *Saccharomyces cerevisiae* Ale Yeast Isolates, BrewingScience, **70** (2017), no. 1/2, pp. 9-25.
24. Methner, F. J.: Optimierte Hefepropagation mittels kontinuierlicher Belüftung, Proceedings of the 27th EBC Congress, 1999, Cannes.
25. Michel, M.; Meier-Dörnberg, T.; Jacob, F.; Schneiderbanger, H.; Haselbeck, K.; Zarnkow, M. and Hutzler, M.: Optimization of beer fermentation with a novel brewing strain *Torulaspora delbrueckii* using response surface methodology, Master Brewers Association of the Americas, **54** (2017), no. 1, pp. 23-33.
26. Michel, M.; Meier-Dornberg, T.; Kleucker, A.; Jacob, F. and Hutzler, M.: A New Approach for Detecting Spoilage Yeast in Pure Bottom-Fermenting and Pure *Torulaspora delbrueckii* Pitching Yeast, Propagation Yeast, and Finished Beer, Journal of the American Society of Brewing Chemists, **74** (2016), no. 3, pp. 200-205.
27. Müller-Auffermann, K.; Hutzler, M.; Schneiderbanger, H. and Jacob, F.: Scientific Evaluation of Different Methods for the Determination of Yeast Vitality, BrewingScience – Monatsschrift für Brauwissenschaft, **64** (2011), no. 9/10, pp. 107-118.
28. Müller-Auffermann, K.; Silva, W.; Hutzler, M. and Jacob, F.: Evaluation and Development of an alternative Analysis Method for rapid Determination of Yeast Vitality, BrewingScience – Monatsschrift für Brauwissenschaft, **67** (2014), no. 5/6, pp. 72-80.
29. Pfenninger, H.: MEBAK Brautechnische Analysemethoden Band 3, edition: Selbstverlag der MEBAK, 1996.
30. Piddocke, M. P.; Kreisz, S.; Heldt-Hansen, H. P.; Nielsen, K. F. and Olsson, L.: Physiological characterization of brewer's yeast in high-gravity beer fermentations with glucose or maltose syrups as adjuncts, Applied Microbiology and Biotechnology, **84** (2009), no. 3, pp. 453-464.
31. Stamm, M.: Enzymchemische und technologische Untersuchungen über den Einfluss von Hefeenzymen – speziell Hefeproteinasen auf den Bierschaum, Lehrstuhl für Technologie der Brauerei I, Technische Universität München, 2000.
32. Takagi, H.; Takaoka, M.; Kawaguchi, A. and Kubo, Y.: Effect of L-proline on sake brewing and ethanol stress in *Saccharomyces cerevisiae*, Applied and Environmental Microbiology, **71** (2005), no. 12, pp. 8656-8662.
33. Tenge, C.; Nagel, M.; Wellhoener, U. and Jacob, F.: Influence Of Yeast Propagation In High-Gravity Wort On Subsequent Fermentation Performance, BrewingScience – Monatsschrift für Brauwissenschaft, **59** (2006), no. 1/2, pp. 41-44.
34. ter Schure, E. G.; van Riel, N. A. W. and Verrips, C. T.: The role of ammonia metabolism in nitrogen catabolite repression in *Saccharomyces cerevisiae*, Fems Microbiology Reviews, **24** (2000), no. 1, pp. 67-83.
35. Thiele, F.: Einfluss der Hefevitalität und der Gärparameter auf die Stoffwechselprodukte der Hefe und auf die Geschmacksstabilität, TU München, 2006.
36. Van Zandycke, S. M. and Fischborn, T.: The impact of yeast nutrients on fermentation performance and beer quality, Master Brewers Association of the Americas, **45** (2008), no. 3, pp. 290-293.
37. Walker, G. M.: Yeast Physiology and Biotechnology, Auflage: John Wiley & Sons Ltd, 1998.
38. Weigert, C.: Erhöhung der Zelldichte während der Propagation von Bierhefe, TU Berlin, 2010.
39. Zhao, X. Q.; Xue, C.; Ge, X. M.; Yuan, W. J.; Wang, J. Y. and Bai, F. W.: Impact of zinc supplementation on the improvement of ethanol tolerance and yield of self-flocculating yeast in continuous ethanol fermentation, Journal of Biotechnology, **139** (2009), no. 1, pp. 55-60.

Received 10 September 2019, accepted 2 October 2019