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Yeasts from the Styrian orchard meadow and their potential for the production of alcohol-free beer

Alcohol-free beer has become an asset even for small-scale breweries, since the consumption of low and non-alcoholic beverages is increasing steadily. Hence, the search for new, cost-effective and efficient methods of producing non-alcoholic beers that can also be realised by small breweries has also intensified. Maltose-negative yeast species play a key role in some of these low-threshold strategies to establish new and innovative alcohol-free beer products on the market. Due to this, the quest for new maltose-negative yeast species and strains becomes increasingly important. The ideal yeast species for this application shows an aroma profile with high consumers' acceptance and a low degree of attenuation, preferably accompanied by a fast fermentation rate. Since the large part of already known yeast species associated with trees, flowers and fruits are maltose-negative, fruit plantations seem the appropriate hunting grounds for viable candidates. Meadow orchards, which are extensively cultivated and traditionally tilled, promise a rich bounty of yeast species diversity. In addition, local breweries may also be able to utilise yeasts from their own region as a valuable fermentation and marketing asset to refine and to promote their own products. The meadow orchard investigated in this study harbors at least ten different microfungal species, of which three were tested in fermentation trials: *Torulaspora delbrueckii*, *Candida peoriensis* and *Filobasidium wiringae*. The species were inoculated and enriched in culture media, suspended in a standardized mixture (Pilsner Malt (10 % extract w/w), 20 IBU/L) and incubated for seven days at 25 °C and 60 % relative humidity. During the fermentation, the extract and pH-value were documented continuously. After this, afterwards the sugar utilisation of *T. delbrueckii* and *C. peoriensis* was analysed. The isolated strain of *Torulaspora* shows promising properties for producing alcohol-free beer, whereas *C. peoriensis* may be utilised in co-fermentations or in the production of fermented beverages other than beer.

Descriptors: wild yeast, yeast hunt, *Torulaspora delbrueckii*, *Candida peoriensis*, *Filobasidium wieringae*, *Saccharomyces* var. *chevalieri*, alcohol free beer, orchard meadow (Streuobstwiese)

This work is dedicated to the memory of Prof. Dr. Ludwig Narziß, in honour of his 100th birthday.

1 Introduction

The consumption of non- and low alcoholic drinks is increasing in various countries of Europe and the European Union [1, 2]. In numbers, the production volume of alcohol-free beer sold more than doubled from 5.9 million to 13.8 million hectolitres in the years from 2013 to 2019, which represents 3.8 % of all beer volume produced. Kokole et al. (2022) also report that Germany, the Netherlands, Spain, Poland and Czechia are the main promoters of alcohol-free beer in Europe, whereby Germany accounts for approximately 81 % of the sold production volume and Czechia shows the highest average consumption [2]. The reason for the consumption of alcohol-free beers and similar beverages may vary, and the motivation of consumers to choose alcohol-free beer over conventional beers has been the focus of ongoing consumer research [3–5]. The foregoing notwithstanding, based on the recent changes in consumer behaviour, it also makes economic sense for breweries to include so-called alcohol-free or non-alcoholic beers in their product portfolio. There are different ways to produce non-alcoholic beer. The available methods can generally be divided into physical and biological ones [6]. Ethanol can be removed procedurally from the fermented beer using a falling film evaporator or by vacuum distillation. Pervaporation and reverse osmosis are also ways of removing the alcohol from the final product. These physical methods can be combined to

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further reduce the content of ethanol [7]. The efficiency of these methods in removing ethanol from beer differs, and so does their impact on the sensory quality of the resulting beverage [6–8]. The biological methods include the selection of special brewing grains, adjuncts and mashing procedures, as well as the usage of specific yeast strains, which produce low amounts of alcohol. The latter, more recent approach is based on the fermentation characteristics of maltose-negative yeasts, which do not ferment maltose, the main fermentable sugar of beer wort [9, 10]. This strategy may seem especially intriguing to small-scale breweries, which cannot afford the additional investment in de-alcoholisation systems [6]. To inoculate beerwort with an adequate low level of extract with a maltose-negative yeast strain can also meet the legal requirements of many countries regarding alcohol-free beverages, for example those of Germany and Austria (0.5 % of alcohol by volume) [6, 11, 12]. Nevertheless, this seemingly elegant method has its own challenges. Firstly, the original gravity must be low enough to ensure that the alcohol concentration is still within the legal limits after the fermentation. Secondly, the unfermented sugar also poses a considerable risk of contamination, which is why pasteurisation or comparable preservation methods are mandatory. Flash pasteurisation followed by hygienic filling, chamber pasteurisation and tunnel pasteurisation are currently common technologies for ensuring microbiological safety, with the latter two offering even greater safety as recontamination cannot occur during filling. The handling of these specialised yeast strains for brewing applications also requires appropriate experience with their fermentation characteristics. Finally, the available selection of suitable maltose-negative yeasts is also limited, whereby the requirements regarding the sensory properties of the resulting alcohol-free beer further restrict this selection. This limited selection of suitable yeast species includes among others *Naumovia dairenensis*, *Hanseniaspora uvarum*, *Saccharomycodes ludwigii* and *Zygosaccharomyces rouxii* [13]. All these species are non-*Saccharomyces* yeasts. *Hanseniaspora uvarum* is found in abundance on fruits, and especially on grapes, where it is associated with the initial phase of the alcoholic fermentation [14]. *Saccharomycodes ludwigii* is particularly noteworthy, since on the one hand it is predominantly known as a spoilage organism of wine and fruit juices but on the other hand is used in the production of alcohol-free beers since 1933 [15]. This yeast species is highly resistant to high ethanol concentrations and high temperatures and does not ferment maltose [16, 17]. *Zygosaccharomyces rouxii* is also recognized as a wine and fruit juice spoilage organism, and, like *S'codes ludwigii*, exhibits a high tolerance to osmotic stress and high levels of ethanol. Notably, *Z. rouxii* does metabolise ethanol and can lower the ethanol content of beverages under aerobic conditions [18]. Hence, this species can also cause the refermentation of wines and the unwanted production of CO_2. This specialised metabolic characteristic is also observed in strains of *Torulaspora delbrueckii* [18]. Certain strains of both *S'codes ludwigii* and *Z. rouxii* are known to produce concentrations of diacetyl exceeding the sensory threshold in beer while other strains of the same species are also capable of diacetyl production in a maturation phase. This strain-dependent nature of diacetyl production requires the screening for low-diacetyl-producing strains of both species to ensure the sensory quality of alcohol-free beer [16, 18]. The aforementioned *Torulaspora delbrueckii* is a ubiquitous yeast, occurring on plants and their fruits, in the soil, and even in insects; it is also considered highly osmo- and cryotolerant [19–22]. Mistletoe was found to be a major source of *T. delbrueckii* in nature [13]. Regarding the production of non-alcoholic beers, *Torulaspora delbrueckii* is gaining increasing attention in the recent years [19, 23, 24]. Furthermore, this yeast was investigated in its capability to enhance the aroma profile

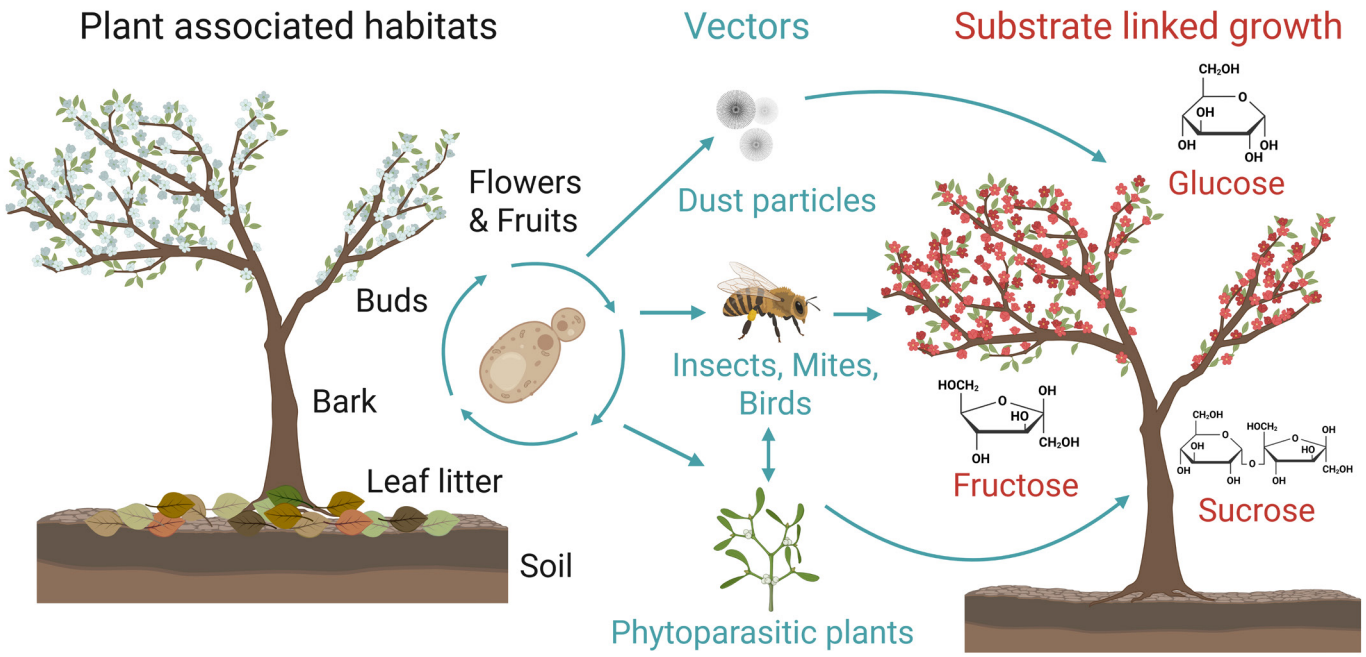

Fig. 1 **The life cycle of yeasts, associated with pom and stone fruit trees according to Hansen and Hutzler [13, 30, 31]. Yeast cells grow on buds, flowers and fruits of fruit trees in orchard meadows and can overwinter in secondary habitats such as the bark, the soil and in leaf litter. During the vegetation period yeast cells can be transferred to other fruit trees in the nearby vicinity by vectors such as airborne dust particles, insects, mites, and even by bird droppings after the digestion of fruits of phytoparasitic plants that grow on fruit trees, such as Viscum album L. The growth of these yeasts is linked to substrates which occur abundantly in fruit trees of orchard meadows: glucose, fructose and sucrose. Created in BioRender. Rehorska, R. (2025) https://BioRender.com/ao0jp7x**

in wheat beers in co- fermentation together with *Saccharomyces cerevisiae* and is also mentioned as a possible tool to enhance the aroma profile of wines [19, 22, 25–27]. One of the few available *Saccharomyces* species used for the commercial production of non-alcoholic beer is *Saccharomyces cerevisiae* var. *chevalieri*, which occurs in the fermenting phloem sap of the coconut palm, where it is the dominant yeast species [9, 28]. This yeast variety is not strictly maltose-negative, but ferments 25 % of the available maltose and it is reported to produce beers with fruity, floral and herbal aromas [29]. Since many yeast species suitable for the production of alcohol-free beer are maltose- and maltotriose-negative but capable of fermenting glucose, fructose and sucrose, it seems plausible that such yeasts preferably inhabit ecological niches characterised by high concentrations of these fermentable sugars, such as it is the case with vineyards and orchards. Orchard meadows in particular are traditionally and extensively cultivated and represent a habitat of abundant and rich microbial diversity. Orchard meadows provide a wide range of substrates, advantageous environmental conditions in comparison to conventionally tilled orchards and, in addition, a variety of secondary habitats for yeasts to hibernate and to survive. These secondary habitats are tree barks, moss, leaf litter and the soil [13]. These anthropogenically created groves therefore represent an ideal habitat for many yeast species, in which the immediate vicinity of pom and stone fruit trees ensures the availability of glucose, fructose and sucrose as substrate. The postulated lifecycle of these yeasts, first proposed by Emil Christian Hansen [30, 31], encompasses their substrate conjugated growth on fruits, their distribution by various vectors such as insects and airborne dust particles and their dormancy in the aforementioned secondary habitats, as shown in figure 1 [13].

This study presents the results of an initial screening of yeast biodiversity in a traditional orchard meadow located in Styria, Austria, with the aim of identifying yeast species suitable for producing alcohol-free beer. Orchard meadows, in German Streuobstwiesen, are a defining element of the Styrian cultural landscape and despite the widespread use of their fruits for fermentation products such as must, sparkling wines, and distilled spirits, their yeast biodiversity remains largely unknown, promising an uncharted terrain of microorganisms with biotechnological potential [32–34]. The aim of the study was to isolate and identify yeast strains on a species level in this specific habitat. Afterwards, the isolated yeast strains should be examined for their fermentation properties with regards to the production of non-alcoholic beer in fermentation tests, and the most suitable candidates should be evaluated further. The introduced initial screening of a selected traditional orchard meadow led to the identification of ten microfungal species, three of which exhibit fermentative capabilities: *Filobasidium wieringae*, *Candida peoriensis* and *Torulaspora delbrueckii*. At least one of these species, *Torulaspora delbrueckii*, shows promising potential in the production of alcohol-free beer, whereas *Candida peoriensis* may be a promising candidate for the co-fermentation of beer.

2 Material and methods

2.1 The sample site and the methodology of sampling

The sample site is a 100-year-old orchard meadow, located in St. Nikolai im Sausal, 8505. Styria, Austria (the GPS coordinates are given in Supplementary Material). The following varieties of apple trees and pear trees are cultivated there: Red Boskoop, Maschanzker, Rote Sternrenette, Bonapfel, Kronprinz, cider pears, and butter pears. In addition, this orchard also includes cherry trees, sloes and mirabelle plums. The sampled trees belong to a 50-year-old stock. In total, four apple trees (*Malus domestica L.*) and one pear tree (*Pyrus communis L.*) were sampled on February 9, 2025 at an average ambient temperature of 6 °C. The positions of these sampled trees within the orchard are marked on an aerial image of the sampling site in figure 2.

The sampling was performed as follows: At least three buds, the bark and the leaf litter were sampled from each of the 5 trees; thereafter the bark and the leaf litter of each sampled tree were swabbed at three different positions. The sampling procedure was performed with a customised wild yeast harvesting kit, received from the Research Center Weihenstephan for Brewing and Food Quality (85354 Freising, Germany) consisting of sterile sampling bags, sterile 50 ml centrifuge tubes, sterile swabs for sampling specific surfaces, such as greasy or mucilaginous areas, pointed scissors, forceps and disinfectant. The pointed scissors and the forceps were sanitised and flamed before sample collection. The collected sample material then was stored in sterile 50 ml centrifuge tubes in 25 ml of sterile buffered peptone water (BPW) until the subsequent transfer onto Yeast Glucose Chloramphenicol Agar (YGC), which is a selective culture medium for yeasts and other fungal species. YGC was prepared according to the instructions of

Fig. 2 **The aerial image of the orchard meadow, captured by drone, indicating the five sampled trees: four different varieties of apple trees Malus domestica (A1 - A4) and one pear tree Pyrus communis (B1). The varieties are: A1 "Red Boskoop", A2 "Rote Sternrenette", A3 "Maschanzker", A4 "Boskoop" and B1 "Kärntner Speckbirne"**

the manufacturer (Carl Roth GmbH + Co. KG). The sample tubes containing the plant material were vortexed for one minute, then 100 µl of the BPW was pipetted onto YGC and evenly distributed using a sterile Drigalski spatula under a laminar flow sterile bench. Two YGC plates were opened and placed on each of the sampled trees for a duration of 24 hours to collect present airborne yeasts and other microfungi. Control plates were also opened and placed accordingly next to the sampled trees on April 22nd, during the season of the apple blossom.

2.2 Isolation and Identification of the isolated organisms

The inoculated plates were incubated at 20 °C for four days in an incubator. The relatively low inoculation temperature was chosen to focus on mesophilic target yeast species and to reduce temperature stress for the microorganisms, which were sampled in winter. After four days, based on colony morphology and light microscopy, yeast colonies were preselected and transferred with an inoculation loop to YGC and, again, incubated at 20 °C for four days. The initial identification, using Species Specific *Saccharomyces* Real-Time PCR Assays and ITS, D1/D2 26S rDNA Sequencing, was performed at the Research Center Weihenstephan for Brewing and Food Quality (Freising, Germany). To this purpose, yeast DNA was isolated using a modified InstaGene Matrix (Biorad, Feldkirchen, Germany) protocol for the extraction of yeast DNA as published by Hutzler (2009) [35]. Yeast DNA of the single colonies were analysed using different species-specific *Saccharomyces* Real-Time PCR assays [36–39]. Sequencing of ITS and D1/D2 26S rDNA loci was performed according to White et al. (1990) and Kurtzman et al. (2003) [40, 41]. The protocols used were modified according to Kurtzmann and Robnett and Hutzler [35, 42, 43]. Sequences were analysed using the NCBI Blast tool (NCBI) and DNAStar, MegAlign Software (DNASTAR, Inc., Madison, Wisconsin).

2.3 Fermentation trials

The yeast colonies were transferred to 9 petri dishes of Yeast Glucose Chloramphenicol Agar (YGC) with an inoculation loop and incubated for 7 days. For the fermentation trials, liquid unhopped pilsner malt extract (Weyermann, Germany) was dissolved in tap water and adjusted to 10 % extract (w/w). Pre-isomerised super critical CO_2 hop extract with 51 % iso-alpha-acids was added after the boil to achieve 20 international bitterness units (IBU) per L of medium. The pre-isomerised extract was derived from Nateco2 - Hopfenveredelung St. Johann GmbH, Germany. For each fermentation trial, ten autoclaved bottles (Duran® Youtility®) were prepared with 1 L of the above-mentioned wort. The cell mass of approximately 2 grams was harvested directly from nine petri dishes using a Drigalski spatula. These 2 grams then were transferred into 200 ml of sterile buffered peptone water (BPW) in a wide-neck Erlenmeyer flask, and the exact cell mass was then determined using a precision balance. The harvested cells were kept in suspension by gently shaking the Erlenmeyer flask. Subsequently, nine bottles were inoculated with 20 ml of the respective yeast cell suspension; one bottle was not inoculated to serve as a negative control. Thus, each yeast strain was tested in nine biological replicates, each bottle was capped with a gas lock, and all inoculated replicates were incubated at 25 °C in a WTB Binder KFB-S 720 climate chamber at 60 % relative air humidity (Binder GmbH, Germany). The extract and the pH-value were measured with a DMA 35 portable density meter (Anton Paar GmbH, Austria) and an AL10 pH-meter (Aqualytic, Germany). The temperature of the climate chamber was monitored with a wireless data logger thermometer. In addition, two fermentation trials were conducted for comparison with one commercially available maltose- and maltotriose-negative strain of *Saccharomyces* var. *chevalieri* (Fermentis SafBrew LA-01) and with one conventional top fermenting *Saccharomyces cerevisiae* ale strain (Fermentis SafAle US-05). After seven days of fermentation four batches of each trial were pasteurised at 75 °C for 10 seconds, which equals approximately 20 Pasteurisation Units (PU), and the alcohol by volume (ABV), the Apparent and the Real Degree of Fermentation were measured with the modular Anton Paar Packaged Beer Measurement Systems (PBA) at Anton Paar in Graz, Austria. The difference in the apparent residual extract and the final pH-values among the tested yeast strains on the last day of the fermentation experiment was statistically evaluated by conducting a non-parametric Kruskal-Wallis test, followed by Dunn's post-hoc test with Bonferroni correction. Statistical analyses were conducted with DATAtab [44].

2.4 Sugar profile analyses

To compare the sugar uptake of the isolated yeast strains with conventional brewing yeasts, aliquots of the unfermented malt extracts were frozen in duplicates in sterile 50 ml Falcon® conical tubes. Samples of the same fermented malt extract, after the end of the fermentation trials performed with two of the promising isolated yeast strains, were also frozen in sterile 50 ml Falcon® conical tubes. Analogous to this, samples of the conventional *S. cerevisiae* strain Fermentis US-05 were prepared. These samples were neither degassed nor pasteurised and were taken with sterile serological pipette tips before freezing. The sugar profile analyses of the unfermented extract and the fermented samples were performed with LS-HPLC at the Research Center Weihenstephan for Brewing and Food Quality in Freising, Germany, using the accredited analysis method LS-HPLC 002_2 2018-07 (Code number 23230). In brief, the samples were degassed in an ultrasonic bath, and diluted with acetonitrile (1/5, v/v). After the addition of an internal standard solution, samples were centrifuged. The supernatant was used for analysis. Separation was achieved via a Luna Omega Sugar column (3 µm, 150 x 4,6 mm, Phenomenex on a HPLC system (Ultimate 3000, Thermo Fisher Scientific). Detection was performed using an evaporative light scattering detector (Sedex LT-ELSD 90, Sedere). The concentrations of fructose, glucose, sucrose, maltose and maltotriose were determined in two technical replicates of each sample, using triple measurements.

Table 1 Applied methods in the analysis of the samples according to MEBAK

Method [45, 46]	Application
MEBAK WBBM 2.9.1	Degassing of samples
MEBAK WBBM 2.9.2.3	Original Gravity [% w/w], Extract [% w/w]
MEBAK WBBM 2.9.6.3	Alcohol by volume [% vol.], Apparent and Real degree of Attenuation [% w/w]
MEBAK WBBM 2.13	pH-value

2.5 List of methods applied according to MEBAK standards

Table 1 provides an overview of applied standard methods in sample preparation.

3 Results and discussion

3.1 Identified yeast and microfungi species and their related location of detection in the orchard meadow

In total, ten fungi were identified. Three of them are ascomycetous yeast species (*Candida peoriensis*, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*) or at least occur as (basidiomycetous) yeasts in their anamorphic developmental stage (*Filobasidium wieringae*, *Cystofilobasidium macerans*). Among the other isolates, Tremellomycetes (*Vishniacozyma tephrensis*), ubiquitous plant-colonising fungi (*Aureobasidium pullulans*), saprobic fungi (*Dothiora prunorum*) and phytopathogenic fungi (*Rhizosphaera macrospora*) were found. One mould fungus was identified as the ubiquitous species *Aspergillus niger*. Only *Filobasidium wieringae* was isolated twice, from two different apple trees, where it was found on buds and in the leaf-litter. *Torulaspora delbrueckii* and *Candida peoriensis* were isolated from apple trees once, found in the leaf litter and the bark respectively. Only moulds could be found on the sampled pear tree. Moulds also dominated the petri dishes placed open and next to the sampled trees as control for airborne fungi. *Rhizosphaera macrospora* and *Aspergillus niger* were among the isolated species from these controls at the date of sampling. *Metschnikowia pulcherrima* was isolated from the petri dishes placed open next to the sampled trees during the apple blossom in April. The isolated and identified fungi, including a short description, and their related location on the respective tree, supplemented by the cultivar, are given in table 2.

Candida peoriensis, also referred to as *Candida peoriaensis* is an ascomycetous yeast, which was first isolated in the United States from the trunk of an elm tree (*Ulmus* sp.), and was attributed to the *Pichia anomala* (now *Wickerhamomyces anomalus*) clade by Kurtzman et al. (2001) [47]. It may also have been isolated from a veterinary sample derived from a horse by Garner et al. (2010) and from chernozem soil samples of the Privolzhskaya forest-steppe in Russia, by Glushakova et al. (2017) [48, 49]. In addition, *C. peoriensis* was isolated in a more recent study by Ghanbarzadeh et al. (2021) from grapes, who investigated the mycobiont associations on immature grape berries in Iran [50]. Apart from this, reports on *Candida peoriensis* are scarce.

Torulaspora delbrueckii is a ubiquitous non-*Saccharomyces* yeast, which belongs to the family of the Saccharomycetaceae, and which can be found both in natural and in anthropogenically influenced habitats. At least five strains of this species are utilised and commercialised in wine making on a regular basis, and there are also promising studies on its capability in the co-fermentation of wheat

Table 2 Molecular identification of isolated fungi from cultivar varieties of *Malus domestica* based on ITS-PCR and based on BLASTn search. Samples were isolated from an orchard meadow in Styria. The percentages of the sequence identities are given in brackets. The samples derived from Pyrus communis (pear) at the time of sampling consisted only of mould

Sample designation	*Malus domestica* cultivar	Location of Detection	Identified Species/Species with closest alignment hit	Short Profile
A1	*Red Boskoop*	Tree bark	*Candida peoriensis* [99.08 %]	Ascomycetous yeast
A2	*Rote Sternrenette*	Buds	*Vishniacozyma teprhensis* (formerly *Cryptococcus tephrensis*) [99.37 %]	Basidiomycetous yeast
		Leaf litter	*Torulaspora delbrueckii* [100.0 %]	Ascomycetous yeast
A3	*Maschanzker*	Buds	*Filobasidium wieringae* [100.0 %]	Basidiomycetous yeast
		Leaf litter	*Cystofilobasidium macerans* [97.74 %]*	Basidiomycetous yeast
A4	*Red Boskoop*	Buds	*Aureobasidium pullulans* [99.63 %]	Ascomycetous yeast-like mould
		Buds	*Dothiora prunorum* [99.61 %]	Saprobic ascomycetous microfungus
		Leaf litter	*Filobasidium wieringae* [100.0 %]	Basidiomycetous yeast
Airborne environmental microfungi (Sampling in February)				
A1	*Red Boskoop*	YMC placed open for 24 h	*Aspergillus niger*	Ascomycetous mould
A3	*Maschanzker*	YMC placed open for 24 h	*Rhizosphaera macrospora* [100.0 %]	Ascomycetous phytopathogenic microfungus
Airborne environmental microfungi (Apple blossom in April)				
A4	*Red Boskoop*	YMC placed open for 24 h	*Metschnikowia pulcherrima* [99.21 %]	Ascomycetous yeast

*closest DNA sequence Blast/Alignment hit Cystofilobasidium macerans with 97.74 % sequence identity; very likely a strain belonging to a novel species (ITS sequence identity below 98.5 %)

beer together with *Saccharomyces cerevisiae* [24, 26, 51]. Although the fermentation properties and aroma profiles of this yeast have been very well studied regarding winemaking, and although it is already known that there are major intraspecies differences in these properties in the same context, there are very few reports in the recent literature of other habitats where this species can be found besides those associated with wine making or grape vines [52, 53]. Regarding this, *T. delbrueckii* was found to belong to the diversity of yeasts occurring in naturally fermented Ecuadorian cacao, together with *Kluyveromyces marxianus* and *Candida tropicalis* [54]. It was also isolated from agaves (*Agave tequilana*) and at least one more recent study reports the isolation of *T. delbrueckii* from soil samples adjacent to fruit trees belonging to the Rosaceae family in Slovakia [54, 55]. This latter investigation of the yeast diversity of a very similar habitat as it is described in the present work was conducted by Vadkertiová et al. in 2019. In addition to *T. delbrueckii* they also found *Metschnikowia pulcherrima*, *Hanseniaspora uvarum*, and *Saccharomyces cerevisiae* among a variety of other mycobionts [55]. Wang et al. (2025) did find, among other yeast species, *Kluyveromyces marxii*, *Hansenioaspora uvarum*, *Candida tropicalis*, *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* in apple orchards in the province of Shaanxi, but *T. delbrueckii* was not among their isolates [56]. Hutzler found in a study published in 2021 *Torulaspora telbrueckii* on *Quercus* sp., *Acer plananoides*, *Loranthus europaeus*, *Viscum* spp. And in abandoned beer cellars in Central Europe. The comprehensive available data on *T. delbrueckii* regarding the production of beverages and even bread make this isolated strain the most promising and safe candidate for the production of non-alcoholic beers found in the present study, even though there is evidence that the utilisation of maltose may occur under specific conditions and pasteurisation of the final product will be mandatory in any case [57].

Filobasidium wieringae is a basidiomycetous yeast which was found to be the predominant epiphytic mycobiont on the surfaces of fruits of *Malus domestica* and *Pyrus communis* trees in a sample-site in Moscow [58]. It can also be isolated from other plant species, such as *Allium cepa*, and it is investigated in this context as biocontrol agent against the so-called purple blotch disease, a fungal infection caused by *Alternaria porri* which causes serve economic losses in onion production [59]. The genus *Filobasidium* (Classis Tremellomycetes, Familia Filobasidiaceae) encompasses yeast-like fungi, but also filamentous species which are producing hyphae with clamp connections and haustorial branches, which points to parasitic life stages, such as the albidus clade in the *Filobasidium* lineage [60]. At least one specific member of the teleomorphic genus *Filobasidium* is known as a fungicide-resistent human pathogen, which causes meningitis: *Filobasidium uniguttulatum*. Regarding this, the similarity to infections caused by the anamorphic human pathogens *Cryptococcus neoformans* and *Cryptococcus neoformans* was reported by Pan et al. (2011) [61]. For this reason, *F. wieringae* was investigated for its fermentative capabilities, but it was not considered safe to produce non-alcoholic beers.

Both *Vishniacozyma teprhensis* and *Cystofilobasidium macerans* belong to the Tremellomycetes, a taxonomic classis of dimorphic basidiomycetous fungi. This class of fungi includes the orders *Cryptococcus* and *Papiliotrema*, both of them encompassing human pathogen species [60, 62]. *Vishniacozyma teprhensis*, former *Cryptococcus tephrensis*, is a psychrotolerant and psychrophilic basidiomycetous yeast, that was found to be associated with apple fruits in Estonia and was also isolated from seeds of *Triticale* plants in the Canadian prairies [63–65]. In this respect, it may also occur frequently as phytopathogen on wheat plants [66]. Yurkov et al. (2014) showed that *V. tephrensis* did not assimilate mucic and saccharic acids as sole carbon sources, in contrast to other members of the *Cryptococcus victoriae* clade [67].

Cystofilobasidium macerans is the teleomorphic life stage of *Cryptococcus macerans* and was described as such and isolated from an oligotrophic lake in north-western Patagonia, Argentina, by Libkind et al. in 2009 [68]. The anamorphic *C. macerans* was isolated also from frozen environmental samples in Iceland and it is reported to produce cold-active polygalacturonases. These are enzymes which are breaking down the pectin-containing layers of the cell walls of plants [69]. At least one isolated strain from *Cystofilobasidium macerans* shows high cellulolytic and proteolytic enzyme activity, which makes it a viable candidate for biotechnological applications [70].

Aureobasidium pullulans, a member of the Dothioraceae family, is an ubiquitous yeast-like and melanin-producing fungus that occurs epiphytic on the phylloplane on plants, such as the leaves of apple trees, or endophytic in plants [71, 72]. This fungus produces a wide variety of different enzymes, ranging from amylases, xylanases and mannanases to lipases and proteases, and it is therefore regarded as biotechnologically important fungal species [73]. In addition, *A. pullulans* is utilised to produce Pullulan, a water soluble and non-toxic polysaccharide, a polymer consisting of maltotriose-subunits, which is used in pharmaceutic applications due to its properties [74]. In rare cases, *Aureobasidium pullulans* may also cause opportunistic mycotic infections in humans [72].

Dothiora prunorum, initially described as *Aureobasidium prunorum*, is a cosmopolitan saprobic ascomycetous microfungus which was first isolated from *Prunus domestica* in 1973 by Dennis and Buhagiar [75]. It is also known as an endophyte, occurring frequently in crops [76]. In general, the genus *Dothiora* can be found on dead plants in tropical and temperate regions[77].

Aspergillus niger was one of the many mould-like mycobionts, which was isolated and identified representatively from the YGC petri dishes placed open and adjacent to the sampled trees to collect environmental airborne fungi. This species is ubiquitous; it can both act as a phytopathogen and human pathogen, causing aspergillosis in humans. It is also utilised on a large scale in the biotechnological production of various organic compounds [78–80].

Rhizosphaera macrospora (family *Venturiaceae*) is a phytopathogenic, endophytic microfungus that causes needle browning in silver firs (*Abies alba*), but the genus is also known to infect other coniferous trees [81]. According to Butin (1995), *R. macrospora* is one of the most important phytopathogenic fungi infecting coniferous trees [82]. The occurrence of *R. macrospora* at the sampling site can be explained by spruce trees (*Picea abies*), adjacent to the sampled orchard meadow.

Metschnikowia pulcherrima is a ubiquitous, ascomycetous non-*Saccharoymces* yeast species, which can be found on a large variety

of fruits and on fruit flies (genus *Drosophila*), which are attracted and used by these yeasts as vectors [83]. This is only one example out of other, more complex interactions of this yeast with insects [84]. *M. pulcherrima* shows antagonistic effects against other yeast species, which has led to in-depth research on this species regarding its viability as a biocontrol agent in wine making [85, 86]. According to Drosou et al. (2022), at least one specific strain of *M. pulcherrima* (Flavia 365) has the ability to ferment glucose, fructose, and maltose and can be used to ferment wort [87]. *M. pulcherrima* was the only yeast isolated from the YGC petri dishes placed open in April, during the apple blossom, to collect environmental airborne fungi. These petri dishes were otherwise dominated by moulds, comparable to the petri dishes placed during sampling in February. The occurrence of *M. pulcherrima* during the flower pollination seems plausible.

3.2 Fermentation trials

According to the literature research in section 3.1 *Candida peoriensis*, *Filobasidium wieringae* and *Torulaspora delbrueckii* were chosen for subsequent fermentation trials. All tested yeasts showed fermentation activities to a certain degree, with *C. peoriensis* and *F. wieringae* showing the lowest activity, reaching Apparent Degrees of Fermentation (ADF) of 4 % (*C. peoriensis*) and 6 % (*F. wieringae*) respectively (see fig. 3 A; low extract decrease correlates with a low ADF). It is noteworthy that both *C. peoriensis* and *F. wieringae* started fermentation and acidification of the wort only after a lag-time of 24 hours in all nine batches, which may be explained by the necessity of adaptation to the available carbon sources. On the other hand, the isolated *Torulaspora delbrueckii* strain displayed vigorous and fast fermentation activity already within the first two days after inoculation, resulting in an average ADF of 22 % at the end of the fermentation trials (see fig. 3 A; low extract decrease correlates with a low ADF), which may indicate a faster adaptation regarding the induction of glycolytic enzymes and wort sugar specific transporters. The commercial maltose-negative *Saccharomyces cerevisiae* var. *chevalieri* strain (Fermentis LA-01) performed similarly, but still lower, resulting in an average ADF of 17 %, which corresponds to the manufacturer's specifications. In comparison, the conventional *Saccharomyces cerevisae* Ale strain (Fermentis US-05) produced, as expected, the highest average ADF of 69 %. The pH-value decreased accordingly to the amount of extract degraded by *T. delbrueckii* and both tested commercial yeasts but was exceptionally distinctive in batches fermented with *F. wieringae* and *C. peoriensis* (see fig. 3, B). This may be caused by the production of metabolites such as lactic or acetic acid, or by increased activity of the plasmamembrane H^+-ATPase Pma1p, a regulator of intracellular pH-values in fungi and a mechanism to maintain pH-homeostasis by hydrolysing ATP to facilitate the export of protons from the cytoplasma [88, 89]. All respective individual measurement points of the fermentation trials are given in table 4, 5 and 6 in the Supplementary Material.

As shown in table 3, measurements conducted with the Packaged Beverage Analyzer for Beer (PBA-B) (see 2.3) were in accordance with results of the fermentation trials with minor deviations, revealing that the highest amount of alcohol by volume (ABV) was produced in the malt extract fermented with the conventional top fermenting *Saccharomyces cerevisiae* ale strain (3.85 % v/v), whereas the lowest ABV were produced by both *F. wieringae* (0.33 % v/v) and *C. peoriensis* (0.51 % v/v). The commercial maltose-and maltotriose-

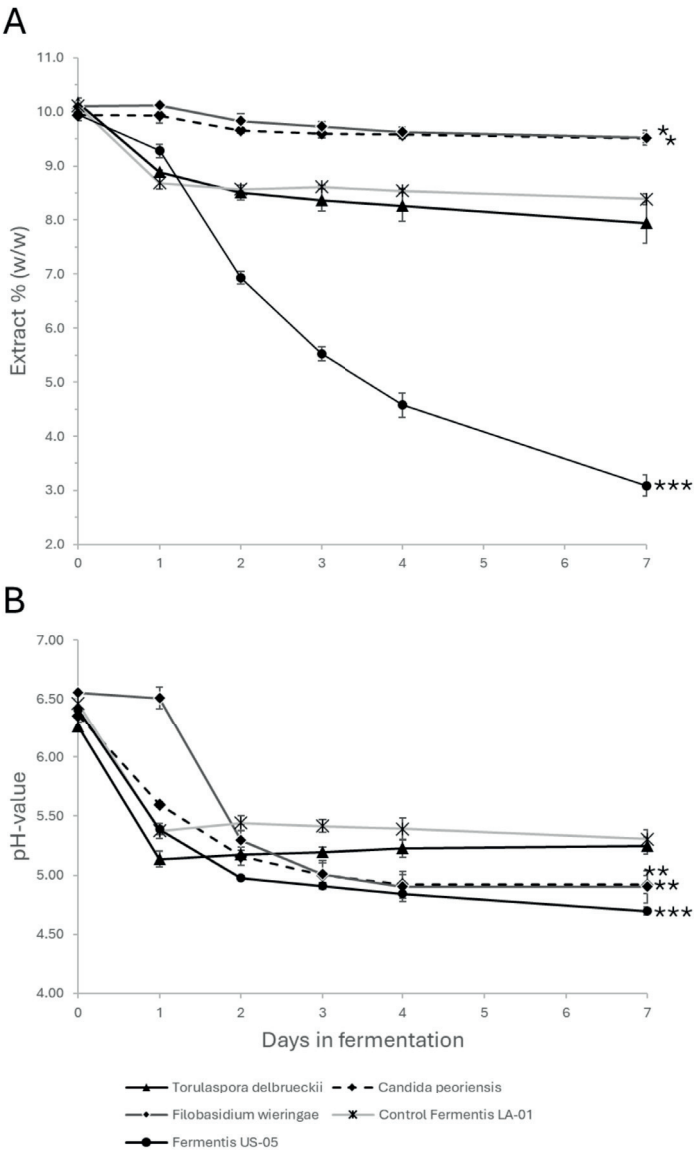

Fig. 3 Extract degradation (A) and pH-decrease (B) in the course of the fermentation. Nine batches of each yeast were fermented for 7 days at a constant temperature of 25 °C. Shown are means (n = 9), error bars indicate (±) standard deviations All respective individual measurement points of the fermentation trials are given in Table 4 and 5 in the Supplementary Material. Kruskal-Wallis test, followed by Dunn's post-hoc test was performed. Significant, highly significant and very significant differences to Fermentis LA-01 are shown as *p < 0.05; ** p < 0.01; p < 0.001

negative strain of *S. cerevisiae* var. *chevalieri* both displayed similar fermentation profiles, with *T. delbrueckii* achieving 1.29 % (v/v) and *S. cerevisiae* var. *chevalieri* achieving 0.92 % (v/v). This may indicate a more extensive sugar utilisation by *T. delbrueckii* in comparison to LA-01. Since Fermentis states an Apparent Degree of Fermentation of 17 % for LA-01, and recommends to use original gravities of 6.5 to 7.5 °P to target 0.5 % ABV, it seems plausible to state that 0.5 % ABV could be achieved with *T. delbrueckii* in worts of 7.0 °P [90].

A non-parametric Kruskal-Wallis test, followed by Dunn's post-hoc test revealed that the maltose-negative *Saccharomyces cerevisiae* var. *chevalieri* strain Fermentis LA-01 differed highly significantly from the conventional *S. cerevisiae* strain US-05 (p = 0.005), significantly

Table 3 **Alcohol in percentage by Volume (ABV) produced by the three isolated yeast species in comparison to ABV produced by one conventional top fermenting *Saccharomyces cerevisiae* yeast strain (Fermentis US-05) and one commercial maltose-negative *Saccharomyces cerevisiae* var. *chevalieri* strain, used for the production of non-alcoholic beers. Given is also Alcohol in percentage by weight, the apparent extract (AE) and the real extract (RE). Shown are means and standard deviations. The measurements were conducted with the PBA-B system**

Tested Yeast	Alcohol (v/v %)	Alcohol (w/w %)	AE (w/w %)	RE (w/w %)
Candida peoriensis	0.51 (±SD 0.10)	0.39 (±SD 0.08)	9.47 (±SD 0.15)	9.65 (±SD 0.11)
Filobasidium wieringae	0.33 (±SD 0.04)	0.25 (±SD 0.04)	9.48 (±SD 0.06)	9.60 (±SD 0.06)
Torulaspora delbrueckii	1.29 (±SD 0.25)	0.99 (±SD 0.19)	7.72 (±SD 0.43)	8.19 (±SD 0.33)
Saccharomyces cerevisiae var. *chevalieri* (Fermentis LA-01)	0.92 (±SD 0.07)	0.70 (±SD 0.05)	8.37 (±SD 0.05)	8.71 (±SD 0.04)
Saccharomyces cerevisiae (Fermentis US-05)	3.85 (±SD 0.16)	3.01 (±SD 0.12)	2.95 (±SD 0.21)	4.37 (±SD 0.16)

from *C. peoriensis* (p = 0.022) and from *F. wieringae* (p = 0.018). Only *T. delbrueckii* did not differ significantly from Fermentis LA-01.

Regarding the final pH-values, again compared to Fermentis LA-01, the yeasts *C. peoriensis* (p = 0.002), *F. wieringae* (p = 0.001) and Fermentis US-05 (p < 0.001) showed highly significant differences. Only *T. delbrueckii* did not differ significantly.

It is worth mentioning that at the end of the fermentation trials *T. delbrueckii* showed a very neutral to fruity aroma, comparable to *S. cerevisiae* var. *chevalieri* LA-01, which is described as a phenolic off-flavour positive (POF+) yeast strain (sensorial sniffing data not shown). *F. wieringae* provided aromas which can be described as musty or mouldy, whereas the aromas found in batches fermented with *C. peoriensis* were found to be pleasantly citric, tart and tangy (sensorial sniffing data not shown). This makes *C. peoriensis*, which otherwise did not show remarkable fermentative capabilities in malt extract, particularly interesting for co-fermentations and for applications in other fermented beverages apart from (non-alcoholic) beer. However, it must be clearly stated that these descriptions are based on initial impressions and that thorough aroma profile analyses would still need to be carried out if this yeast were to be further characterised for beer fermentation.

3.3 Sugar profile analyses

The worts fermented by *T. delbrueckii* and *C. peoriensis* were chosen for subsequent sugar profile analyses. The sugar utilisation revealed, at first that *T. delbrueckii* did ferment maltose, but to a far lesser extent than the conventional *S. cerevisiae* strain did. Whereas almost 90 % of maltose was utilised by *S. cerevisiae*, *T. delbrueckii* only fermented approximately 50 % of this sugar (fig. 4). The analyses indicate that also maltotriose was fermented, but to a very small extent. These findings may explain that *T. delbrueckii* achieved a higher apparent degree of fermentation than the used reference *S. cerevisiae* var. *chevalieri* strain. Nevertheless, a considerable concentration of maltose and maltotriose remained unfermented after 7 days of fermentation at 25 °C. If this yeast strain is to be used for the production of non-alcoholic beers, pasteurisation is mandatory, because stress-induced fermentation has to be expected. This is interesting insofar as Pater et al. report 25 % of maltose-utilisation by the same *S. cerevisiae* var. *chevalieri* strain used in the present study as reference [29]. However, based on these findings it can be stated that the isolated strain of *T. delbrueckii* should be further tested in the production of non-alcoholic beers. Regarding this, it is vital to mention that the utilisation of maltose and maltotriose in *T. delbrueckii* is highly strain dependent, which is clearly shown in the work of Michel et al. [24]. In contrast to *T. delbrueckii* and the conventional *S. cerevisiae* strain, *C. peoriensis* fermented only minimal quantities of fructose, sucrose and obviously also of maltose. It is also extremely remarkable that *C. peoriensis* did barely utilise glucose.

4 Conclusion

The Styrian orchard meadow is a regional ecological niche influenced by specific factors such as the Illyrian climate and the Opok soil type. Although numerous fruits from these orchard meadows are used

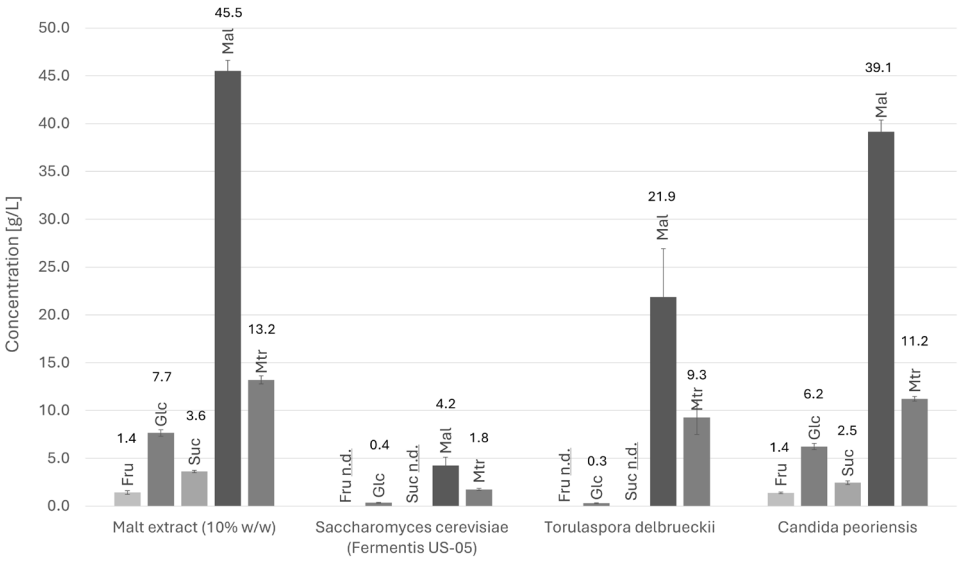

Fig. 4 **Sugar profile analysis after 7 days of fermentation at 25 °C of *S. cerevisiae*, *T. delbrueckii* and *C. peoriensis* in comparison to unfermented malt extract. Given concentrations are means, error bars indicate standard deviations. Abbreviations are as follows: fructose (Fru), glucose (Glc), sucrose (Suc), maltose (Mal), maltotriose (Mtr), and not detectable (n. d.), at a limit of determination of 0.1 g/L and 0.5 g/L (maltotriose) respectively**

to produce fermented beverages, their microbiological diversity has not been extensively investigated. It was shown in this study that even a superficial sampling of one orchard meadow resulted in the isolation and identification of ten different fungi, of which at least one, *T. delbrueckii*, proved to be a promising candidate for the production of non-alcoholic beers. The fermentation performance of this isolated cryotolerant yeast strain was comparable to the performance of the commercially used maltose- and maltotriose-negative *S. cerevisiae* var. *chevalieri* strain Fermentis LA-01 [57]. This is not surprising, since *T. delbrueckii* has already been extensively investigated in this regard and is also used commercially to produce non-alcoholic beers. In contrast, a very little researched yeast, *C. peoriensis*, although fermentatively underactive, showed promising characteristics to be used in co-fermentations regarding the aromas it produced. Especially the observed capability of this yeast, to lower the pH-values while not reducing the sugar concentrations substantially, make it also an interesting candidate for fermented, non-alcoholic beverages aside from beer. Unpublished preliminary experiments with *C. peoriensis* in a ginger-based beverage containing sucrose showed promising results. To the knowledge of the authors, it is the first time that *Candida peoriensis* was tested regarding its fermentative capability. This species will be investigated in further fermentation trials for the biological preservation of novel, low-alcohol beverages based on mead and on fruit juices. Although *F. wieringae* shows comparable capabilities in terms of pH reduction, the potential of this yeast is rather low due to its unpleasant aromas and potential human pathogenicity. *Metschnikowia pulcherrima*, that has already been investigated regarding its fermentation properties, may not be suitable for the production of non-alcoholic beers, but it is certainly interesting as a non-conventional yeast for conventional beers and other fermented beverages [87]. As an ecological sidenote regarding biodiversity it should be mentioned that the studies conducted by Glushakova and Kachalkin, by Vadkertiová et al. and by Wang et al. are of particular importance, as they examine the mycobionts of a habitat very similar to the one presented in this work: The surface and inner tissues of *Malus domestica* and *Pyrus communis* fruits under high anthropogenic impact and the environment of these trees [55, 56, 58]. Hence, it can be assumed that the composition of species is at least comparable. In this study, species that are likely to be mycobionts were found, which were also isolated by the previously mentioned authors in habitats that are very similar to the orchard meadow presented, such as *Torulaspora delbrueckii*, *Aureobasidium pullulans*, *Metschnikowia pulcherrima* and *Filobasidium wieringae*. Although these likely mycobionts can be described as ubiquitous, the frequent occurrence of these species in one and the same ecological niche at least testifies that the living conditions here are favourable for them. Referring to this, investigations in the regions of origins of cultivated fruit trees of the Rosaceae family, such as *Malus domestica*, may promise enlightening findings [91]. In conclusion, the outlook is as follows: experimental small-scale brews will be done with the isolated strain of *T. delbrueckii* and with *C. peoriensis* in co-fermentation, focusing on aroma profiles in no and low alcohol beer. The isolated strain of *M. pulcherrima* will be assessed in the same manner, focusing on its viability as non-conventional brewing yeast. To the best of the author's knowledge, this would be the first (non-alcoholic) beers brewed with wild yeasts isolated from Styria. Regional yeast strains can be an important, innovative factor as regional raw material for regional beers.

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Supplementary materials

The GPS coordinates of the sampled orchard meadow are 46°47'47.7"N 15°27'37.6"E.

Table 4 Results of Extract and pH-measurements during the fermentation. Shown are means and (±) standard deviations (n = 9)

	S. cerevisiae		*S. cerevisiae* var. *chevalieri*		*T. delbrueckii*		*C. peoriensis*		*F. wieringae*	
Day	**Extr. % (w/w)**	**pH-value**	**Extr. % (w/w)**	**pH-value**	**Extr. % (w/w)**	**pH-value**	**Extr. % (w/w)**	**pH-value**	**Extr. % (w/w)**	**pH-value**
0	10.0	6.41	10.1	6.46	10.2	6.36	9.9	6.36	10.1	6.55
±SD	±0.1	±0.02	±0.0	±0.08	±0.1	±0.04	±0.0	±0.02	±0.0	±0.01
1	9.3	5.39	8.7	5.38	8.9	5.14	9.9	5.60	10.1	6.51
±SD	±0.1	±0.05	±0.1	±0.06	±0.0	±0.07	±0.1	±0.02	±0.0	±0.09
2	6.9	4.98	8.6	5.44	8.5	5.18	9.7	5.16	9.8	5.30
±SD	±0.1	±0.02	±0.1	±0.07	±0.1	±0.03	±0.0	±0.08	±0.1	±0.13
3	5.5	4.91	8.6	5.41	8.4	5.20	9.6	5.00	9.7	5.01
±SD	±0.1	±0.03	±0.1	±0.05	±0.2	±0.04	±0.1	±0.11	±0.1	±0.11
4	4.6	4.84	8.5	5.39	8.3	5.23	9.6	4.92	9.6	4.90
±SD	±0.2	±0.04	±0.0	±0.09	±0.3	±0.08	±0.1	±0.08	±0.1	±0.12
7	3.1	4.70	8.4	5.30	7.9	5.25	9.5	4.92	9.5	4.90
±SD	±0.2	±0.03	±0.1	±0.08	±0.4	±0.07	±0.1	±0.08	±0.1	±0.14

Table 5 Raw data of all fermentation trials, showing the extract degradation per day in each batch

		Extract % (w/w)									
Yeast	**Day**	**N1**	**N2**	**N3**	**N4**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**
S. cerevisiae	0	9.8	9.8	9.9	9.9	9.9	10.1	10.1	10.1	10	9.8
	1	9.1	9.1	9.3	9.2	9.2	9.4	9.4	9.4	9.4	9.1
	2	6.8	7	7.1	7	6.9	7	6.9	7	6.7	6.8
	3	5.6	5.6	5.7	5.6	5.5	5.3	5.6	5.5	5.3	5.6
	4	4.5	4.8	4.8	4.6	4.6	4.6	4.6	4.7	4	4.5
	7	3.1	3.3	3.3	3.2	2.8	3.1	3.1	3.2	2.7	3.1
S. cerevisiae var. chevalieri	0	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.2	10.1	10.1
	1	8.8	8.8	8.7	8.8	8.7	8.7	8.7	8.6	8.4	8.8
	2	8.7	8.5	8.7	8.5	8.5	8.5	8.6	8.6	8.5	8.7
	3	8.6	8.7	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
	4	8.5	8.6	8.6	8.5	8.5	8.6	8.6	8.5	8.5	8.5
	7	8.3	8.3	8.5	8.5	8.4	8.4	8.4	8.5	8.2	8.3
T. delbrueckii	0	10.1	10.1	10.1	10.1	10.3	10.3	10.3	10.1	10.1	10.10
	1	8.9	8, 7	8.9	8.9	8.8	8.9	8.9	8.9	8.9	8.9
	2	8.4	8.2	8.6	8.6	8.6	8.5	8.7	8.5	8.5	8.4
	3	8.3	7.9	8.6	8.3	8.5	8.5	8.6	8.3	8.3	8.3
	4	8.1	7.6	8.5	8.2	8.5	8.5	8.6	8.2	8.2	8.1
	7	7.8	7.1	8.2	7.7	8.4	8.2	8.3	7.8	8.0	7.8
C. peoriensis	0	9.9	10	9.9	10	10	9.9	9.9	10	9.9	9.9
	1	9.8	10	10	9.9	10	9.6	10	10	10.1	9.8
	2	9.6	9.7	9.6	9.7	9.7	9.7	9.6	9.7	9.6	9.6
	3	9.7	9.7	9.6	9.5	9.5	9.7	9.6	9.6	9.5	9.7
	4	9.6	9.7	9.6	9.6	9.6	9.6	9.6	9.5	9.4	9.6
	7	9.5	9.6	9.5	9.6	9.5	9.6	9.5	9.5	9.3	9.5
F. wieringae	0	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.1
	1	10.2	10.1	10.1	10.1	10.1	10.2	10.1	10.1	10.1	10.2
	2	9.9	9.9	9.6	9.9	10.0	9.8	9.7	9.7	10.0	9.9
	3	9.8	9.8	9.6	9.9	9.7	9.7	9.7	9.6	9.8	9.8
	4	9.7	9.7	9.7	9.7	9.5	9.6	9.7	9.5	9.6	9.7
	7	9.7	9.5	9.5	9.7	9.5	9.2	9.6	9.5	9.5	9.7

Table 6 Raw data of all fermentation trials, shown is the decrease of pH-values per day in each batch

		pH values									
Yeast	**Day**	**N1**	**N2**	**N3**	**N4**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**
S. cerevisiae	0	6.39	6.4	6.39	6.41	6.4	6.41	6.45	6.41	6.4	6.39
	1	5.45	5.46	5.44	5.40	5.38	5.34	5.33	5.37	5.33	5.45
	2	4.97	5.00	4.99	4.98	5.01	4.95	4.96	4.97	4.96	4.97
	3	4.90	4.91	4.93	4.93	4.94	4.92	4.90	4.92	4.84	4.90
	4	4.84	4.89	4.88	4.86	4.85	4.81	4.83	4.87	4.75	4.84
	7	4.64	4.73	4.71	4.74	4.68	4.71	4.72	4.69	4.64	4.64
S. cerevisiae var. chevalieri	0	6.58	6.51	6.51	6.51	6.52	6.39	6.38	6.39	6.34	6.58
	1	5.51	5.31	5.41	5.36	5.41	5.31	5.29	5.38	5.40	5.51
	2	5.53	5.37	5.51	5.37	5.45	5.36	5.39	5.51	5.48	5.53
	3	5.46	5.38	5.50	5.49	5.40	5.34	5.35	5.40	5.41	5.46
	4	5.55	5.40	5.46	5.42	5.49	5.31	5.30	5.27	5.32	5.55
	7	5.37	5.41	5.40	5.35	5.31	5.22	5.23	5.16	5.29	5.37
T. delbrueckii	0	6.30	6.24	6.24	6.23	6.23	6.28	6.35	6.24	6.24	6.30
	1	5.29	5.12	5.06	5.14	5.06	5.12	5.20	5.10	5.14	5.29
	2	5.21	5.11	5.18	5.20	5.18	5.20	5.20	5.13	5.18	5.21
	3	5.28	5.12	5.24	5.18	5.16	5.22	5.19	5.17	5.20	5.28
	4	5.40	5.09	5.29	5.18	5.23	5.22	5.23	5.21	5.20	5.40
	7	5.30	5.06	5.27	5.31	5.31	5.20	5.26	5.27	5.25	5.30
C. peoriensis	0	6.39	6.32	6.35	6.34	6.36	6.35	6.37	6.38	6.35	6.39
	1	5.64	5.6	5.6	5.58	5.57	5.59	5.57	5.62	5.61	5.64
	2	5.25	5.11	5.12	5.12	5.12	5.12	5.08	5.17	5.33	5.25
	3	5.19	5.00	4.91	4.88	5.00	4.91	4.90	5.08	5.13	5.19
	4	4.98	4.86	4.86	4.92	4.89	4.84	4.85	4.99	5.11	4.98
	7	5.07	4.92	4.88	4.93	4.94	4.90	4.96	4.91	4.76	5.07
F. wieringae	0	6.57	6.55	6.55	6.54	6.54	6.55	6.56	6.54	6.58	6.57
	1	6.61	6.54	6.48	6.55	6.53	6.55	6.26	6.49	6.56	6.61
	2	5.35	5.16	5.16	5.23	5.52	5.48	5.19	5.20	5.38	5.35
	3	5.22	5.10	4.98	4.82	5.02	5.03	4.87	4.98	5.07	5.22
	4	5.13	5.00	4.89	4.72	4.87	4.98	4.72	4.86	4.96	5.13
	7	5.10	5.10	4.90	4.70	4.87	4.96	4.70	4.86	4.94	5.10