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Yearbook 2022 BrewingScience

A life devoted to Brewing Science

I am pleased to present you with the BrewingScience Yearbook 2022. A Yearbook full of new insights – ranging from hop and practical yeast matters all the way to use of new methods such as CrispR-Cas9 in the brewing industry. Contributions extending beyond the horizons of the brewing industry round off the range of topics.

However, in past weeks, our pleasure was dampened by the demise of one of the world's most famous brewing scientists. As you certainly will have heard, Prof. Ludwig Narziß passed away on November 29th, 2022 aged 97. In the eight decades of his brewer's life, he wrote myriads of contributions for our and other scientific journals and thus acquired an international reputation, in particular for German brewing science. We are intimately committed to honour his achievements and pass on the legacy, using his name as a sponsor of the "Ludwig-Narziß-Award for Brewing Science" of the BrewingSciene Journal, honouring – thus following in his footsteps – research work relevant to practical applications.

The contribution which received the Ludwig-Narziß Award in 2022 covers an area that Prof. Narziß felt strongly about: the influence of malt on beer aroma. At the EBC Congress that took place in Madrid at the end of May 2022, we were able to honour in persona Michael Féchir, one of the three authors of the contribution "Identification of marker volatiles in malt to predict malt-derived aroma properties of bottom-fermented beers" (BrewingScience 74 (2021), no. 1/2, pp. 17-26).

I wonder who will come out on top next time and will take home the award at "Trends in Brewing" in Ghent in April. And there is no doubt about it – it will certainly be one of the contributions on the following pages ...

L. Juliersfild



Dr. Lydia Junkersfeld (r) and Dr. Frank Braun (l) presented the Ludwig-Narziß Award for Brewing Science to Michael Féchir in Madrid





BrewingScience Yearbook 2022

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BrewingScience Yearbook 2022

This yearbook is sponsored by



























IMPRINT

Yearbook BrewingScience
(ISBN 978-3-418-00934-6)
Publisher: Fachverlag Hans Carl GmbH,
Andernacher Str. 33 a
Germany-90411 Nuremberg
Telephone +49 (9 11) 9 52 85-0
Telefax +49 (9 11) 9 52 85-48
subscription@hanscarl.com
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Volume 75

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This publication is a compilation of the articles published in the BrewingScience bimonthly online journal in 2022.

The articles are also available for subscribers at http://www.brewingscience.de (ISSN 1613-2041).

Annual subscription price (1.1.2023) incl. Yearbook EUR 172,00 (incl. VAT) Yearbook EUR 99,00 (incl. VAT)

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BEST PAPERS / BEST POSTERS OF THE 38 EBC CONGRESS IN MADRID



Barley proteins: relation to malt quality in a context of climate change and need of sustainability – The new challenge

Proteins in raw materials play an important part in both malting and brewing processes, as actors of raw material biotransformation, contributors to foam, haze or even nitrogen source for the yeast.

Nonetheless, a comparable total protein content of barley can lead to various malt qualities and different proteolysis levels. In a context of climate change, of inputs reduction and need of more sustainable raw materials, there is a need to improve knowledge of proteins in barley. The collaborative "PROsIT" project (barley PROteins of InTerest) proposed to draw up the qualitative profile of the different barley proteins and tried to identify the possible relation to malt quality. The project is supported by FSOV (Found to support breeding selection in France) and all barley to beer chain (CTPS, Breeders, ARVALIS, French Maltsters & Brewers)

The analysis of barley protein fractions was developed on a sequential isolation inspired by Schalk et al. (2017) [1] followed by capillary electrophoresis analysis (Labchip, PERTEN). In more details, protein fractions are first isolated according to Osborne [2] classification: albumin, globulin, hordein, glutelin. Each of them are further separated according to molecular weight to collect, respectively 18, 10, 10 and 15 peaks.

A collection of 321 barley samples of different crops, locations, varieties and nitrogen fertilizations produced by project partners was analysed. All the samples were micro-malted at IFBM micromalting plant and malts were characterized (extract, friability, total

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& soluble proteins, wort viscosity & beta glucans content, diastatic power, etc.). A database of 1284 profiles (more than 16 000 peaks) and the corresponding malt parameters was built.

The first data treatment indicates that there is a very large diversity of barley protein composition for the same total nitrogen content with a 2 up to 3-fold factor depending the protein fraction. In other words, several barley batches could exhibit the same protein content but contain some proteins of interest and some less useful for the maltsters or brewers.

Besides, a PCA analysis on nitrogen fertilization trials on 220 samples shows an important varietal effect on protein profile. However, the crop, location or fertilizing modalities have no significant effect on protein composition in our sampling.

This major result demonstrates that the barley variety is the most significant factor triggering different protein compositions. But the different clusters gather independently some 6 row winter lines can be with some 2 row spring lines. This means that the barley specie does not drive, alone, the protein profile.

The data treatment needs to be completed but this work shows the importance of barley protein composition to assess malt quality. And the results gathered in the project is the beginning of a larger study investigating the proteins of interest for the barley to beer chain.

- Schalk, K., Lexhaller, B., Koehler, P., & Scherf, K. A. (2017). Isolation and characterization of gluten protein types from wheat, rye, barley and oats for use as reference materials. PLoS ONE, 12(2), 1–20. https:// doi.org/10.1371/journal.pone.0172819.
- Osborne, T.B., (1907). The proteins of the wheat kernel. Publication 84. Carnegie Institute of Washington, Washington DC.

Brewers' spent grain for organic thin-film transistor application

Brewer's spent grain (BSG) represents the main by-product of the brewing industry, accounting for $\sim 85\,\%$ of the total by-products. The annual global production of BSG is massive, that is ~ 40 million tons. In the European Union, the production is ~ 3.4 million tons/year [1]. Currently, BSG is mainly sold to farmers ($\sim 30\,\%$ in EU) as animal

feed, with a low market value of \sim 35 Euro/ton or landfilled. Finding alternatives, higher value uses for BSG is therefore particularly attractive from the point of view of brewery economics [1].

Besides, disposable electronics applications such as in smart food and beverage packaging require environmentally safe devices with low cost and large volume processability. Therefore, switching from nonrenewable manufacturing to sustainable processes is a major challenge for next generation electronics, and organic materials offer a unique opportunity to drive the electronic industry in an environmentally safe direction [2].

Among various organic electronic devices, thin film transistors (OTFTs) are fundamentals. They are multilayered-structured

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devices that comprises conducting electrodes, dielectrics and semiconductors. We investigated for the first time the use of two no starch polysaccharides components of BSG, β -glucans and arabinoxylans, as green dielectric materials for OTFTs.

Since such components possess rich polar functional groups, they can be in principle effectively employed as dielectric layers for OTFTs due to their good polarization under electric field, which would lead to high capacitance and, ultimately, to promising possibilities and applications for green devices.

The thin films were prepared by spin coating technique and, indeed, encouraging capacitance values of ~1820 nFcm $^{-2}$ at 1kHz were obtained for both components. β -Glucans revealed to be particularly promising when implemented in OTFTs, enabling

devices giving good response, i.e. a charge mobility of $1.5 \text{ cm}^2/\text{V}$ s with average current on/off ratio of $\sim 10^4$ and a threshold voltage of -1 V. The Ph-BTBT-C10 benchmark semiconductor was employed as active layer.

Further developments along this line are currently in progress and will include the use of the devices as platform to realize smart food and beverage package demonstrators.

- A. Trusek et al. "Brewer's Spent Grains Valuable Beer Industry By-Product" Biomolecules 2020, 10, 1669.
- C. Kim et al. "Sustainable approaches in the design of dielectric materials for organic thin-film transistors" in A. Marrocchi (Ed) "Sustainable Strategies for Organic electronics", Woodhead publishing Elsevier, 2022

Are small starch granules ruining the benefits of high gravity brewing?

The mashing process has to maintain a complex balance between starch gelatinisation and thermal inactivation of starch-hydrolysing enzymes. This balance can be affected by the intrinsic starch granule properties, extrinsic factors such as the presence of sugars and other wort components and the process parameters such as mashing thickness and the temperature-time profile. Nowadays, breweries can use high gravity brewing, which improves brewhouse efficiency. However, large proportions of small starch granules in barley malt seem to endanger this benefit. While in the past, the impact of small starch granules on the mashing process was considered neglectable because their levels in barley malt were deemed low, recent findings showed small starch granule proportions of up to 27 V/V%. Therefore, their importance during mashing has to be reconsidered. In this work, the intrinsic starch gelatinisation behaviour of barley malt starch and the impact of mash thickness on the gelatinisation of small and large starch granules was assessed. Small starch granules isolated from barley malt had a higher peak gelatinisation temperature (62.5 °C) than large starch granules (59.7 °C). In addition, mainly water-extractable, non-starch components from barley malt caused an elevation of the intrinsic starch gelatinisation temperatures by 4.6 °C. During the mashing process, additional water-extractable components such as sugars are produced. We, therefore, hypothesised that the impact of these components would be of great importance, especially when performing high gravity brewing. Mashing was performed with malt to water ratios varying between 1:6 to 1.2.5. Thicker mashes resulted in less efficient sugar production (a sugar yield of 79 % for 1:6 mashing compared to 66 % for 1:2.5 mashing), mainly due to a decrease in maltose production. These results oppose previous findings in literature. We hypothesise that this is due to delayed gelatinisation of the small starch granules during mashing, caused by wort components such as sugars. The addition of 24 °P wort to isolated starch granules resulted in a 10 °C increase in the gelatinisation temperature of starch indeed. In the case of small starch granules, this resulted in a peak gelatinisation temperature of 72.5 °C. This is problematic considering that malt β-amylase, which produces maltose, will be thermally inactivated rapidly at this temperature. Considering these results, it is clear that the small starch granule proportion in barley and malt should be introduced as a selection criterium.

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Development of a bacterial biocontrol process applicable in the barley-malt-beer industry

Pathogenic fungi represent a generic problem for cereals as they can produce a variety of toxic secondary metabolites such as mycotoxins that represent a significant concern for the malting and brewing industries, and may affect the quality and safety of barley, malt, and beer. Besides, this situation is worsening due to the highly variable climatic conditions that favor pathogenic fungi development and the societal desire to reduce the use of phytosanitary products, including fungicides. In this context, this communication describes the development of an innovative biocontrol process applicable in malting facilities, that would contribute to guaranteeing a better hygienic and technological quality of malt, despite the increasingly complex and variable conditions for barley production. The process is based on technological bacteria, isolated from infection-resistant barley cultures, that can reduce the development of spoilage fungi and the associated mycotoxin production. The experimental approach consists of: i) determining the growth kinetics of the bacterial and fungal strains by co-culturing in order to evaluate the impact of the bacteria on the fungal pathogens; ii) carrying out a micro-malting process in order to develop

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the aforementioned process, and iii) evaluating the technological and sanitary properties of the generated barley malts in order to validate the process developed. The findings highlight the ability of a barley-associated novel bacterial strain, Erwinia gerundensis, to inhibit the growth of fungal species and to reduce their toxigenic potential. E. gerundensis exhibited a significant fungistatic activity against pathogenic fungi by reducing their growth by up to 80 %, and their mycotoxin production by 70 to 100 % in liquid medium and on barley matrix. In addition, micro-malting assays carried out using naturally contaminated barley kernels have revealed that the bacterial strain was capable of reducing the fungal load and mycotoxin (enniatin) content of malt by 70 % and 50 % respectively, without any degradation of its technological quality. Based on these results, our study supports the use of Erwinia gerundensis as a biocontrol agent in strategies aiming at reducing the presence of pathogenic fungi and mycotoxins in cereal-based products, or as a food and feed supplement for the bio-detoxification of mycotoxins. The biocontrol process based on this bacterial strain is therefore expected to make it possible to guarantee an irreproachable hygienic and technological quality of the malt obtained from barley, thus significantly reducing the setbacks related to pathogenic fungi and mycotoxins in the brewing industry. The use of this process would also contribute to the reduction of contamination levels of malting plant effluents. Finally, future works are required to effectively evaluate the impact of Erwinia gerundensis during brewing and on beer quality.



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I. Weishaupt, P. Neubauer and J. Schneider

Approach to an inline monitoring of the heat impact in a high temperature short time treatment (HTST) of juice with the help of a chemical marker

The conventional method for the determination of the lethal heat load during pasteurisation (expressed in so-called pasteurisation units (PU)) by measuring temperature and flow rate provides known inaccuracies and requires safety margins in terms of a planned over-pasteurisation to the detriment of the product quality. Based on the hypothesis that chemical conversions correlate with applied heat input, despite the differences in reaction kinetics between chemical conversion and microbiological inactivation, inline near infrared spectroscopy (NIRS) was investigated to identify and quantify applied PU. Acid hydrolytic sucrose degradation was confirmed a favourable marker reaction. In a first step by still using offline analytics (HPLC) and a calculation the feasibility and plausibility in principle could be proved. Compared with conventional PU deviation of only 0.3 % were found when using the chemical marker reaction. However, the inline application using NIRS showed too high variations. The too low accuracy of the NIRS model for the sucrose measurement was identified of being the cause for failing the overall goal. Improvements in the inline determination seem to be promising.

Descriptors: near infrared spectroscopy, apple juice, pasteurisation, acid hydrolytic sucrose degradation, inline measurement of heat input, pasteurisation units

1 Introduction

Beverages, such as fruit juices, are often preserved by thermal treatment in continuous pasteurisation plants. The most important objective of this step is to guarantee microbiological safety, whereby large safety margins and known inaccuracies must be applied [1, 2]. The usage of empirical-based formulas for calculating the amount of required heat input (expressed in pasteurisation units (PU)) leads to rough generalisations because of inconsideration of heating and cooling sections as well as product-specific properties. The general formula for calculating the PU value is given in equation 1 with the time (t [min]) for which a certain temperature (T [°C]) was hold [1]. The theoretical basis of this simplified formula is the so-called D-value. It is a measure of the heat resistance of microorganisms and is given in minutes. This time indicates how long it takes at a defined temperature to reduce the initial microorganism count to one tenth. The D-value is specific for the various microorganism species and also for the environment in which they are found. For certain product groups, however, they can be generalised, as has

https://doi.org/10.23763/BrSc21-20weishaupt

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been done with regard to fruit juices, for example. Here, specific constants are assumed and specified, which in turn contribute to a more unspecific pasteurisation, as the product properties are left out as mentioned above [3, 4].

$$PU=t\cdot 10^{\frac{\vartheta-\vartheta_B}{z}} \tag{Eq. 1}$$

The z-value defines the temperature increase compared to the reference temperature $(\vartheta-\vartheta_{\scriptscriptstyle B})$ which provokes a decrease of the D-value by one order of magnitude. The kind of microorganism (strain) and the habitat conditions influence the z-value. However, for fruit juices a z-value of 10 K is typically used in practice by convention (so-called fruit juice formula) [3, 5, 6]. The applied temperature is $\vartheta,$ the reference temperature $\vartheta_{\scriptscriptstyle B}$ for fruit juices is by convention 80 °C [7].

The use of this formula with globalised values leads on the one hand to a high degree of safety in terms of microbiological stability, but on the other hand is also accompanied by adverse product changes regarding nutritive and sensory properties. With the knowledge of the negative effect of heat on the value-giving ingredients of fruit juices, there is no need to explain that such safety margins should be reduced as far as possible. Otherwise vitamins and antioxidants are degraded unnecessarily, colour changes and other physiochemical modifications occur [8–12]. Many studies deal with the observation of changes in fruit juices during thermal treatment, such as ascorbic acid degradation, colour and flavour changes or the formation of hydroxymethylfurfural [13].

The aforementioned facts give reasons for the goal of turning away from the current method of PU determination. A prior attempt aimed to make latent, nonspecific chemical changes, caused by heating, directly measurable using inline near infrared spectroscopy (NIRS). In combination with chemometric methods, an attempt was made to determine the thermal load expressed in PU [14]. However, using this method, the microbiological impact could only be determined with sufficient accuracy limited to a case specific application. For the use of, for example, a different beverage, the underlying model must be adapted. The limitation of this approach leads now to a modified approach undertaken in the present study. Here, also previous research on the sucrose hydrolysis as a chemical marker in pasteurisation was brought in [15]. The earlier studies on sucrose hydrolysis showed that the chemical marker reaction (sucrose degradation to fructose and glucose) can be used to measure heat input accurately in the course of thermal treatment [2, 15, 16]. The studies also showed that the traditional method of PU determination using temperature and time are comparably inaccurate.

However, the use of these markers is limited to studies outside of the real production operation (offline) and using artificial test solutions that are not comparable with real products (especially pH). In the present work now, the attempt is made to apply this chemical marker system in the process as inline method in a real product (apple juice). The challenges are hence that real products are used and thus the marker conversion cannot be artificially adjusted by the matrix properties (particular pH value) as in the former work. NIRS is used as the inline measurement method. Many studies work on with NIRS in combination with chemometric methods as analytical tool, but mostly in offline mode. Thus, the use of NIRS in combination with chemometrics has already proven to be a suitable method for such applications, such as fruit juice analysis both in-line and off-line [12–17].

As a part of the working hypothesis, NIRS is assumed to be capable for an inline sufficient quantification of sugar composition changes that take place and to assign them to a specific heat impact based on previously created models. In the next step, this must then be converted into the PU relevant for microorganism inactivation

with the aid of kinetic data (z-values). With the knowledge of the changes in chemical composition, particularly the sucrose content, caused by a certain thermal stress, the heat impact, expressed in the "chemical" PU value, is supposed to be predictable. For the transformation from chemical reaction data to those of microbiological, also the reaction kinetics of the acid hydrolytic sucrose degradation are used. Finally, the hypothesis is that the lethal heat input in terms of the "microbiological" PU value, can be determined with the help of the chemical marker reaction of the sucrose degradation in an inline setup (called "chemical" PU). Consequently, the work of this study comprises two research questions. The first question is, if the extent of the chemical conversion of sucrose (acidic hydrolysis) in the real juice is sufficient (high enough) for an adequate indirect determination of the lethal heat impact (PU). Sufficient means here in comparison to the conventional PU derived from the process data temperature and flow rate (residence time). In this first part of the investigation, the sucrose is still analysed off-line in the laboratory by HPLC. The second research question derivated from the working hypothesis wants to know how the accuracy of the finally determined PU is impaired when the sucrose determination is transferred into an inline measurement with the help of NIRS and a chemometric model.

2 Material and Methods

2.1 General approach

Apple juice was pasteurised in a in a pilot scale flash pasteuriser. In this high temperature short time process (HTST) selected different settings for flow rate and temperature enabled the variation of the heat impact. Process data as temperatures, flow rates, system volume were measured and samples were taken beforehand the heat treatment and afterward in order to analyse the sucrose concentration changes. The correlation of sucrose concentration and heat impact provided a regression model that allowed to determine the here newly introduced "chemical" Pasteurisation Units (PU_{chem}) from the sucrose content. The PU_{chem} are dedicated to serve as an auxiliary calculation figure. For the calculation of the PU_{chem}, equation 1 is taken as for the "microbiological" PU. However, for

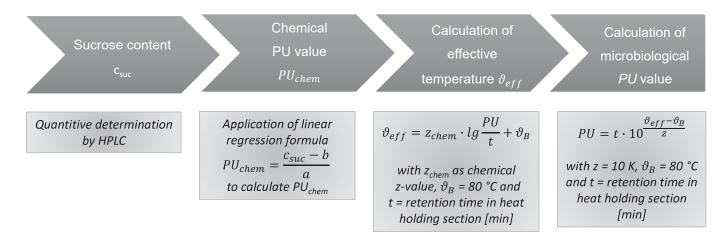


Fig. 1 Approach to the first research question: Indirect calculation of "microbiological" PU values by using the (offline) sucrose concentration measurement with a HPLC; with c_{suc} as sucrose content in g/L and with a and b as slope parameter and axis intercept of a regression function