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Evaluation of the Acidic Sucrose Hydrolysis as Time-Temperature Integrator in Flash Pasteurizers

In the practice of flash pasteurization, the applied heat load is not exactly known. It is rather estimated by measuring the holding tube outlet temperature and the flow rate (theoretical mean holding time). Microbiological methods, such as the Count Reduction Test (CRT), include certain disadvantages and basic unavoidable inaccuracies. A chemical reaction, the acidic sucrose hydrolysis, was investigated as a Time-Temperature Integrator (TTI) for the determination of the heat load expressed as Pasteurization Units (PU). For this purpose, the reaction was already calibrated as reported in a prior article [1]. Here the application and verification in terms of a comparative plausibility test of the TTI in a semi-technical scale are presented. For the TTI test, the conversion rate of the reaction had to be adjusted with a sufficient acid concentration, which can be calculated with the help of the previous calibration. The acidic sucrose solution is pasteurized under realistic conditions. The conversion ratio is mathematically transposed into a statistical figure, the effective temperature (ϑ_{TTI}). The effective PU can then be derived from the ϑ_{TTI} and the residence time. This TTI test was performed at four different temperature levels using three different target residence times, 5-fold respectively. The resulting effective temperatures were compared with the measurements of thermocouples (inlet and outlet of the holding tube). The results indicate that the TTI provides plausible results. In the following step, the TTI was compared with a microbiological Count Reduction Test. *Lactobacillus hilgardii* was chosen as the test microorganism, therefore, thermal death kinetics of *L. hilgardii* were determined in terms of the $D_{60\,°C}$-value of 0.75 min and a z-value of 5.4 °C. Both methods, the TTI and the CRT, were performed under the same process conditions. The comparison with the measurements of the temperature probes in the holding tube revealed a significant higher reliability of the TTI test.

Descriptors: flash pasteurization, Time-Temperature Integrator, residence time distribution, Count Reduction Test, D- and z-values, *Lactobacillus hilgardii*

1 Introduction

Currently employed pasteurization intensities follow guidance derived from a series of publications by *Del Vecchio, Dayharsh* and *Baselt* from the 1950s [2; 3; 4]. They state 15 min, at 60 °C (15 Pasteurization Units; PU) as sufficient beer treatment for pasteurization to achieve a microbiological stable beer. To date many breweries are pasteurizing with 15 PU or more. With ever increasing significance on faster microbiological methods, an individual adjustment of the pasteurization corresponding to the actual microorganism count and species could become possible in the near future. The thermal death kinetics of the microorganisms and the applied heat load has to be known exactly, for this purpose.

A satisfactory method to measure the effective (applied) PU (PU_{eff}) during flash pasteurization is not available yet. However, a well-known method to estimate the applied PU, which is also used as performance qualification, is the Count Reduction Test (CRT). This test employs a definite microorganism suspension, which is pasteurized, and the reduction of the cultivable cell count is taken to determine the efficacy and to derive the PU applied respectively. This method has several disadvantages [5]: the thermal death rate of microorganisms depends among others on the conditions during cultivation and pasteurization, the origin of the microorganism, and the growth phase. In addition, a possible result could be a microorganism count below the detection limit. Furthermore, the z-values of the test microorganism must correspondent to the targeted microorganism occurring in the product or rather to the z- value used in the PU-formula (6.9 °C [2; 6; 7; 8]). Otherwise, the calculated PU (Eq. 1) differs if the count reduction of the entire plant (inclusive heating and cooling section) is used.

$$PU = t \cdot 10^{\frac{\vartheta - 60\ °C}{z}} \qquad 1$$

Finally, the exact destruction parameters, the D- and z-value, which can vary comparably widely, are not known or not taken into account in case of the common CRT. Moreover, using microorganism in a high concentration may spoil the pasteurization plant.

Because of insufficiencies in the standard plate count method, another method to determine the PU_{eff} accurately was investiga-

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ted. The problem of the standard plate count method is due to the inevitable disadvantage of microorganisms, hence substitution of them by a chemical integrator seems to be promising. Those Time-Temperature Integrators (TTI) of which several have been examined in the past [9; 10], include among others the destruction of indigotine [11], the melibiase activity [12; 13] and the sucrose hydrolysis [14; 15; 16; 17]. However, none of these attempts succeeded in the way that their results could be applied in practice. In the presented paper, sucrose hydrolysis is investigated as TTI. One innovation is the mathematical combination of the conversion ratio with the results of a residence time distribution (RTD). With this procedure, the PU_{eff} can be included with the help of the effective temperature. This effective temperature is an auxiliary measure that can be derived from the residence time distribution and the TTI conversion ratio if the dependency of the TTI reaction rate from the process conditions is known. The reaction rate of the TTI can be controlled by the adjustment of the acid concentration thus the TTI test can be adapted to fit any realistic pasteurization intensity.

Employed sucrose hydrolysis has been thoroughly investigated with a large amount of available kinetic data [1; 18]. However, for a successful application it became necessary to perform own kinetic studies in order to obtain data for exactly the required conditions, which includes especially the sucrose and acid concentrations, defined by the conditions of the real pasteurization process which the test intends to simulate. The sugar serves as mean to simulate the viscosity of a real product. The rheological properties have an influence on the RTD, which is a part of the test. Hence, calibrations were carried out ad hoc and published in a previous paper [1]. The calibration were conducted with the help of the Arrhenius equation:

$$k = k_0 \cdot \exp\left(\frac{-E_A}{R \cdot T}\right) \quad 2$$

With the reaction rate constant k [s^{-1}], the frequency factor k_0 [s^{-1}], the ideal gas constant R (8.314 J/mol*K) and the activation energy E_A [J/mol]. Hereafter, a sugar concentration of 5 $\%_{(w/w)}$ (corresponding viscosity to beverages such as beer) is considered in this paper. The acid concentration had to be adjusted to the experimental conditions to ensure a conversion ratio between 40 % and 70 %. The following dependency was found for the standardized acid concentration c* ($\frac{c}{1\frac{mol}{L}}$) [1]:

$$c^*(H^+) = \left(\frac{\ln\left(\frac{1}{1-\chi}\right)}{t \cdot 1s^{-1} \cdot \exp\left(\frac{-E_A}{R \cdot T}\right) \cdot 4.361 \cdot 10^{14}}\right)^{\frac{1}{1.103}} \quad 3$$

The activation energy (105.09 kJ/mol) [1] describes the dependency of the reaction rate from the temperature, χ is the conversion ratio of the sucrose (range 0–1) and T the temperature [K] which impacts a specific time t [s].

A crucial question of the application of a chemical integrator is, whether it is allowed to transfer the results of the chemical reaction to the inactivation kinetics of microorganism, which could be followed by a problem in the differences in the activation energies. The activation energies of the thermal death of vegetative microorganisms are higher (250–400 kJ/mol) than those of chemical reactions (acidic hydrolysis of sucrose 105.09 kJ/mol ± 1.07 kJ/mol (confidence interval with a probability of 95 %); own measurements [1]). As long as the pasteurization takes place at an almost constant temperature, transformation from chemical reaction kinetics to microbial inactivation is possible. Nevertheless, in case of a distinct temperature profile as in the heating or cooling section, the different activation energies lead to significant deviations in the resulting PU_{eff}. Therefore, in this investigation the holding section is measured separately.

Besides the heat treatment in the holding section, there is a thermal impact in the heating and cooling sections, which is neglected in the common pasteurization. The influence of the heating and cooling zone was investigated by *Franklin* et al. [19]. They compared the effect of an indirect and a direct UHT plant by treating a spore suspension. To achieve the same sterilizing effect, by heating temperatures to around 140 °C, the process temperature of the direct heating plant had to be 4 °C higher than by the indirect heating plant. This difference originates in longer heating and cooling phases during indirect heating. Whereas, due to fast heating at the beginning and slower temperature rising at higher temperatures the heating section has a bigger effect compared to the cooling section. This effect has to be proved for the flash pasteurization.

As described in earlier publications [20; 21; 22; 23], the residence time distribution could have an influence on the resulting microorganism reduction. In practice, theoretical mean residence time is calculated out of the holding section volume V and the flow rate ($\dot{V}$):

$$\tau = \left(\frac{V}{\dot{V}}\right) \quad 4$$

The volume of the holding section is often not exactly known and the measurement of the flow rate in the cold side, fouling and channeling can lead to an overestimated mean residence time. On the other side neglected sections of the holding tube [24] induces underestimated holding times. These inaccuracies lead to wrongly calculated PU_{eff}. Therefore, in this investigation the residence time distribution will be measured additionally to determine the PU_{eff}.

The purpose of this work is to evaluate the acidic sucrose hydrolysis in a flash pasteurization as TTI to prove the plausibility. For this purpose, the calculated effective temperature from the chemical TTI test will be compared with the measured inlet and outlet temperatures and with the CRT.

2 Materials and methods

2.1 General Approach

The selection of the chemical TTI system and its calibration are presented in a previous paper [1]. In order to prove the ability of the TTI to become a tool for the determination of the heat load (PU) in flash pasteurizers two comparative verifications in the sense of comparative plausibility tests are carried out on a pilot plant scale. At first the TTI test including the residence time distribution is executed in a comparably wide range of the temperature and the holding time by several repeatings (5 time each setting). For the evaluation, not the PU itself but the corresponding effective temperature as a result of the TTI test is compared with the

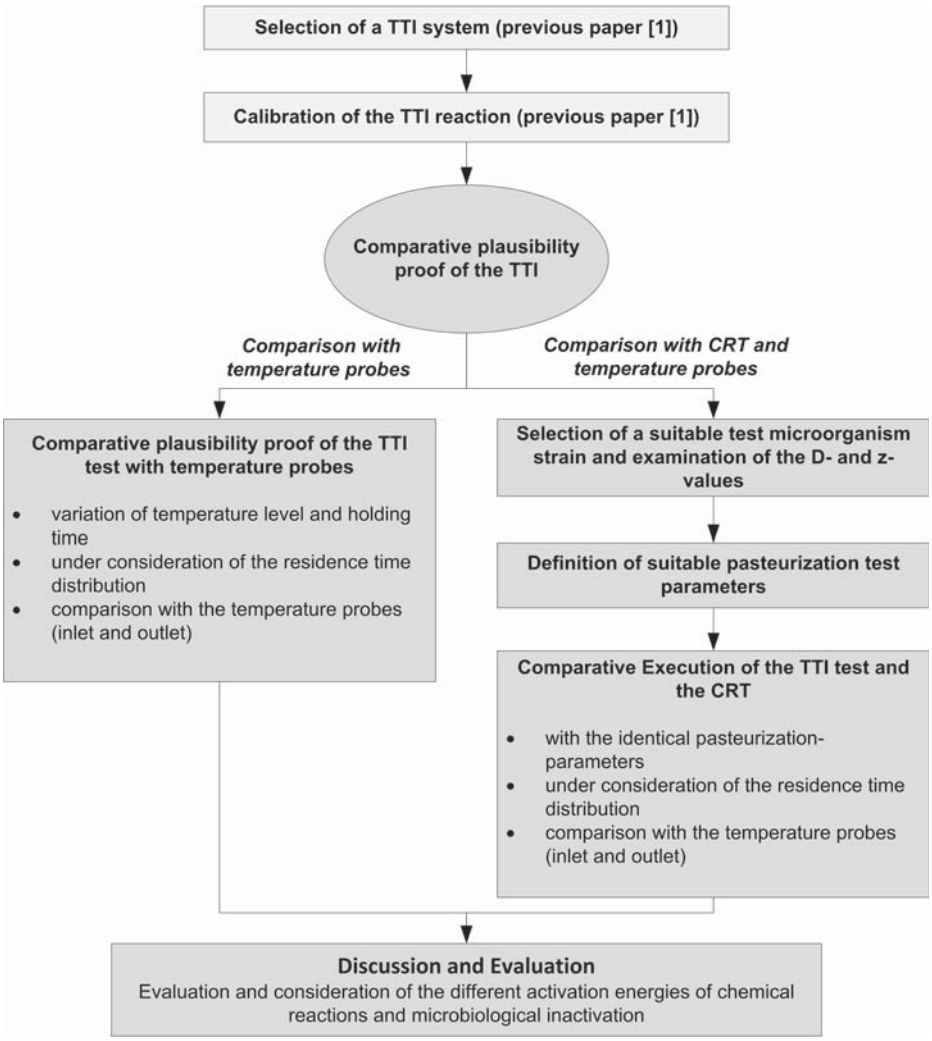

Fig. 1 Workflow of the ability tests of the Time-Temperature Integrator (TTI) test. The results of the TTI test are compared with the temperature probes and the common used Count Reduction Test (CRT)

measurements of temperature probes in the holding tube (Fig. 1). In a second step, the TTI test is compared with the microbiological Count Reduction Test (CRT). For that purpose, a suitable test strain has to be selected. The thermal vulnerability must allow, for a precise mathematical evaluation, surviving cells above the detection limit under pasteurization test conditions. The D- and z-values of the test strain are therefore investigated. The pasteurization test conditions are then adapted to the microbiological inactivation parameters. Finally, the CRT and TTI test with the residence time distribution measurements are executed with these identical conditions. For both tests, the PU and the corresponding effective temperature are calculated and compared with the results of the temperature probes. The results are discussed under consideration of the different activation energies of chemical reactions (TTI) and microbiological inactivations.

2.2 Application of the Time-Temperature Integrator and comparison with temperature probes

For the examination of the plausibility of the calibrated Time-Temperature Integrator (TTI), the TTI was applied in a semi-technical scale flash pasteurizer. The holding section consists out of 5 insulated loops (together 24 m) with an inner tube diameter of 0.015 m and connecting pieces with diameters from 0.015 to 0.025 m, together results in a volume of about 5.2 L. The heating and cooling section have a volume of 3.1 L respectively. The TTI test was executed at 5 temperature levels (70, 75, 80, 85, 90 °C) and 3 mean residence times (45, 60, 75 s). All trials were carried out in a 5- fold determination. It was necessary to adapt the conversion rate of the sucrose hydrolysis to the pasteurization process parameters time and temperature with the acid concentration. The needed acid concentration was determined by equation 3 for a target conversion ratio of 60 %. The resulted acid concentration of the solution was measured by titrating with a 0.1 molar sodium hydroxide solution. A conventional cleaning solution (Leracid 169, Stockmeier) containing concentrated nitric acid served as H^+ source. The sucrose-acid solution with 5 $\%_{(w/w)}$ sucrose was prepared in a sufficient quantity to allow the process of approximately 15 min with the determined flow rate (260, 320 and 430 L/h). The acid was added just before the experiment was started and was mixed by a stirrer (Fig. 2). The TTI test starts with cycling water until adjustment of the temperature. Then the product flow is changed to the sugar-acid solution.

The inlet and outlet of the holding tube are equipped with sampling ports consisting diaphragms through which small quantities of liquid can be taken by means of a syringe (Fig. 3). The syringes are prepared with cold sodium hydroxide solution (concentration 1.5 times of the molar acid concentration in the sample). The sucked sample volume (sugar acid solution) is equal to the prepared sodium hydroxide solution in the syringe. Thus a quick neutralization and cooling stops the reaction. Furthermore, samples were taken from the receiver tank and the outlet of the cooling section.

The sucrose conversion was measured by means of polarimetry (Krüss, P32002 RS). From additional unneutralized samples the acid concentration and, after heating, the end polarizing angle (α_∞) was determined. The conversion ratio χ follows from equation 5:

$$\chi = 1 - \left(\frac{\alpha - \alpha_\infty}{\alpha_0 - \alpha_\infty}\right) \qquad 5$$

Whereat α_0 is the rotation angle of the sample at the beginning of the investigated section, which was the sample of the tube inlet in the case of the holding section, and α is the rotation angle of the sample at the end. The holding times were determined by means of the weighted mean of the residence time distribution (Eq. 7). For this purpose, a saturated sodium nitrite solution was used as a tracer for the measurements. The concentration of the tracer was measured inline at the end of the holding tube by means of the conductivity with an inductive sensor (Bürkert, Type 8226; 10 s^{-1}). A quantity of 5 mL saturated NaCl-solution was injected instantly (injection was completed in one second) through a septum at the inlet of the holding tube (Fig. 3 left). From the mean conductivity

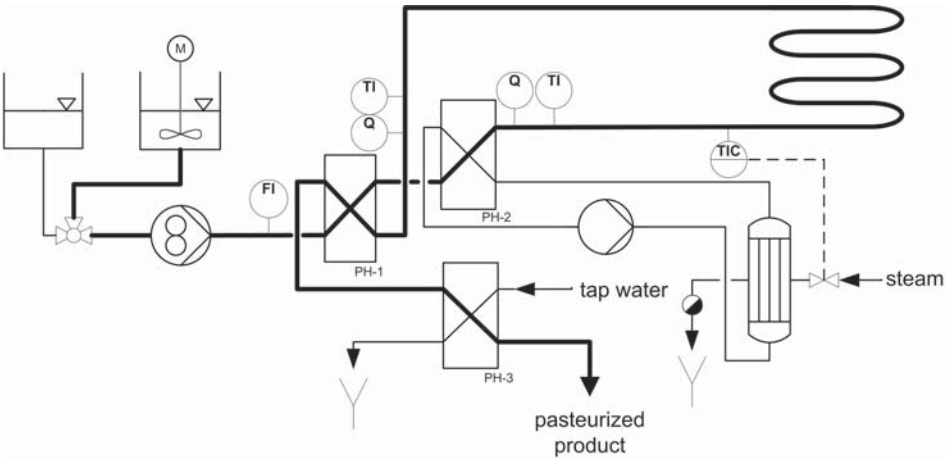

Fig. 2 **Simplified flow chart of the flash pasteurization plant modified for the application of the Time-Temperature Integrator examination. Q flags the sample ports consisting of diaphragms to take the samples by means of a syringe and to measure the temperature. The thick line indicates the track of the product, PH 1–3 are the several steps of the plate heat exchanger.**

(salt concentration) of the measured intervals c(t_i) and the interval width Δt the time distribution function E(t) results:

$$E(t_i) = \frac{c(t_i)}{\sum_{i=0}^{i_{max}} c(t_i) \cdot \Delta t} \quad 6$$

The weighted mean of the residence time distribution $\bar{t}$ was calculated with equation 7:

$$\bar{t} = \sum_{i=0}^{i_{max}} t_i \cdot E(t_i) \cdot \Delta t \quad 7$$

The mean temperature T_{TTI} [K] can be calculated by the conversion ratio χ between the inlet (α_0) and outlet (α) of the holding tube and the mean residence time by means of equation 3 solved for T:

$$T_{TTI} = \frac{-E_A}{R \cdot \ln\left(\frac{\ln\left(\frac{1}{1-\chi}\right)}{c^*(H^+)^{1.103} \cdot 4.361 \cdot 10^{14} \cdot \bar{t} \cdot 1s^{-1}}\right)} \quad 8$$

In this equation, only the mean residence time calculated using the residence time distribution is considered. In reality, there is a residence time distribution, which could influence the calculated effective temperature. In order to consider the residence time distribution, equation 9 (see below) can be applied to determine the T_{TTI} iterative.

Hereby t_i is the mean time of the measured residence time distribution intervals, and ΔF is the area of the interval. The equation underlies the assumption, that all volumes have a constant but varying velocity.

The temperatures were simultaneously measured by thermocouples (± 0.15 °C) which were inserted through the membranes (Fig. 3, right). The temperatures were recorded with a sampling rate of 2 s^{-1}. The statistical analyses were performed software aided by Statistica.

$$\chi = \sum_{i=0}^{i_{max}} \left(1 - \frac{1}{\exp\left[c^*(H^+)^{1.103} \cdot 4.361 \cdot 10^{14} \cdot \exp\left(\frac{-E_A}{R * T_{TTI}}\right) \cdot t_i \cdot 1s^{-1}\right]}\right) \cdot \Delta F_i \quad 9$$

2.3 Preparation of the *Lactobacillus hilgardii* suspension

A wild strain of *Lactobacillus hilgardii* isolated from apple juice (provided by VLB Berlin e. V.) was used. The culture grew in MRS bouillon at 30 °C for 48 h to stationary phase. Then the culture was stored in a refrigerator for 12 h. The suspension for the D- and z-values were diluted 1 : 10 and the solution for the CRT 1 : 100 with physiologic salt solution (distilled water, 8.5 % NaCl, 1 % peptone) right before the experiments. A possible change of the D-values induced by the different dilutions was excluded by further capillary experiments with 1 : 100 dilutions. Furthermore, $D_{60\,°C}$-values were determined from samples taken from the receiver tank.

2.4 Measurement of the D- and z-value

In order to determine the effective PU by means of the effective temperature from the CRT the exact D- and z-values for the used

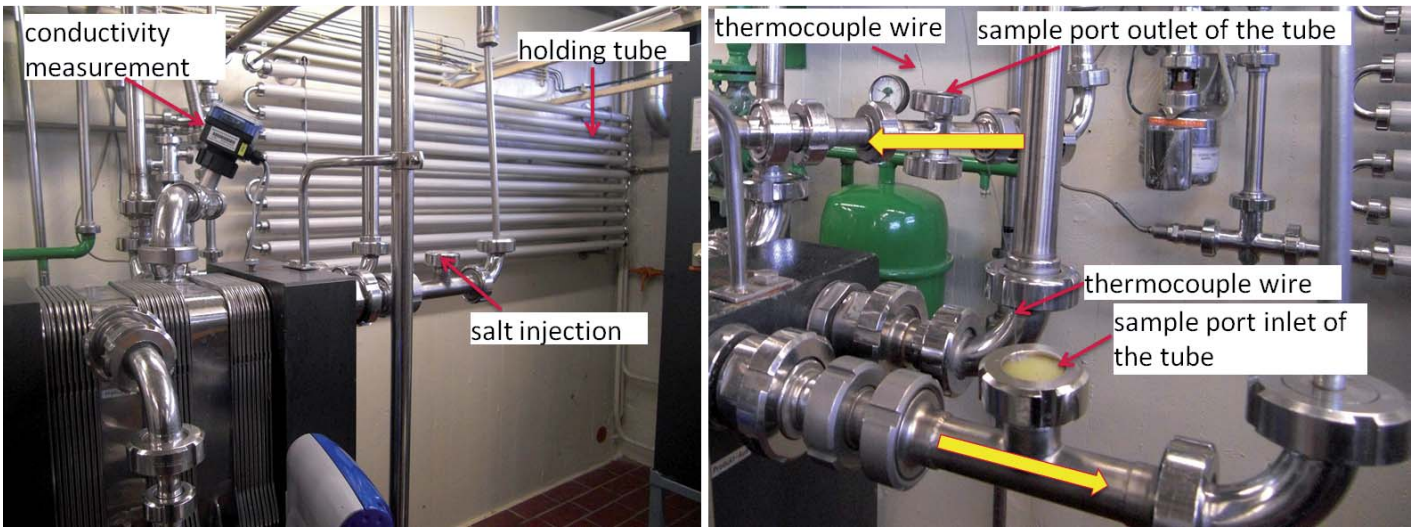

Fig. 3 **Flash pasteurizer pilot plant equipped for the residence time distribution measurement (left) and for the TTI test with additional temperature probes (right)**

microorganism have to be known. The D-value derives from the thermal death rate during a constant temperature. The z-value is built from the D-values at different temperatures. For this, the capillary method was applied. An amount of 10 µL of the diluted suspension of *L. hilgardii* was transferred in a glass capillary (length: 100 mm inner diameter: 1.15 mm external diameter: 1.55 mm) and closed on both sides over a flame. Up to 24 capillaries were immersed in a tempered water bath and after definite time intervals, two of the capillaries were transferred into cold water. The survived microorganisms were determined by means of the standard plate count technique on MRS agar. For this purpose, each capillary was rinsed with 1 mL of dilution solution. The 1 mL rinsing liquid was plated without a further dilution for the determination of the limit of detection. For the serial dilution, additional liquid was added before plating twice on MRS agar. The viable cell count is plotted on a logarithmic scale versus time. The D-value is calculated from the linear part of the graph. The z-value results from the plot of the logarithmic D-values against the corresponding temperatures. For each temperature, a 5-fold determination was carried out.

2.5 Application of the Count Reduction Test

The Count Reduction Test was carried out analogous to the Time-Temperature Integrator test. Before the test was started, hot water (80 °C) was circulated to decontaminate the pasteurization plant. To prove the decontamination step, samples were taken from the receiving tank as well as from the outlet of the plant and incubated on MRS agar. A pasteurization temperature about 61 °C was selected in order to assure measurable surviving cells after the pasteurization. To measure the cell count reduction, samples were taken with syringes, which were prepared with 9 mL cold dilution solution. An amount of 1 mL of the microorganism suspension was sucked into the syringe. Thus, a quick stop of the heat exposition and the reaction was assured. The sampling was done analogous to the Time-Temperature Integrator test of the storage tank, the inlet and the outlet of the holding tube and at the outlet of the plant. Targeted residence times of 45, 60 and 75 s were set by means of the flow rate (430, 330, 260 L/h) for each of these times a 5-fold determination were carried out. The temperature was recorded in the same way as during the TTI test. For a correct comparison of the CRT with the TTI test, the TTI test was carried out with the corresponding parameter settings to the Count Reduction Test, too.

The resulting effective temperature measured with the CRT (ϑ_{CRT}) can be calculated from the microorganism count at the inlet (N_0) and at the outlet (N) of the holding tube, the z- and $D_{60\,°C}$-value of the used microorganism and the mean residence time $\bar{t}$:

$$\vartheta_{CRT} = \log\left(\frac{\log\frac{N_0}{N}}{\bar{t}} D_{60\,°C}\right) \cdot z + 60\,°C \qquad 10$$

In order to consider the RTD, equation 10 is solved for the measured microorganism count N; the RTD is included due to the mean time of the measured residence time distribution intervals t_i and the area of the intervals ΔF, thus equation 11 can be applied to determine ϑ_{CRT} iterative:

$$N = \sum_{i=0}^{i_{max}} \frac{N_0}{10^{\left(\frac{t_i}{\frac{10^{\frac{\vartheta_{CRT}-60\,°C}{z}}}{D_{60\,°C}}}\right)}} \cdot \Delta F_i \qquad 11$$

3 Results

Since all available methods for the measurement of the heat application in a flash pasteurizer are considered deficient, the best way to verify the TTI test is a plausibility proof. For this purpose, a comparison of the TTI test with temperature measurements in the process line and with the microbiological Count Reduction Test (CRT) was conducted. For both tests, the TTI test was made on a semi-technical scale flash pasteurizer (Fig. 2).

3.1 Comparison of the TTI test with temperature probes

The TTI test was carried out in an experimental design of 5 different temperature levels between 70 °C and 90 °C and 3 different targeted residence times (45, 60, 75 s). Each combination has been performed as a 5-fold trial and the effective temperatures ϑ_{TTI} were calculated as described above (Eq. 9). Simultaneously with the TTI test, the temperatures at the inlet and outlet of the holding tube were measured. The result of the TTI test would be plausible if the ϑ_{TTI} is inside of the span between the measured temperatures at the inlet and the outlet of the holding tube. The spans ranged between 1 and 4 °C; as expected the span increases at higher temperature levels and longer holding times. For a better comparability, all temperatures were standardized:

$$\vartheta_{stand.} = \left(\frac{\vartheta_{TTI} - \vartheta_{outlet}}{\vartheta_{inlet} - \vartheta_{outlet}}\right) \qquad 12$$

The standardization allows a comparison of all temperature levels and all residence times. The summarized results are shown in figure 4. Almost all temperatures derived from the TTI test (ϑ_{TTI}) are in between the inlet and the outlet temperature and thus the TTI test results are considered plausible and repeatable. Furthermore, the mean effective temperatures (ϑ_{TTI}) are slightly below the arithmetic mean temperature of the inlet and outlet. The effective temperatures (ϑ_{TTI}) are thus closer to the outlet temperature. This might be a plant dependent phenomenon.

3.2 D- and z-values for the comparison of the TTI with a microorganism count reduction

The next step is the comparison of the TTI test with the conventional CRT. For this, initially a suitable microorganism species have to be chosen which enables an evaluable CRT. To ensure this, the number of surviving (cultivable) cells may not fall below the detection limit of 10 CFU/mL. Since *Zufall et al.* [25] determined $D_{60\,°C}$-values of 2 min for obligate beer-spoiling *Lactobacillus brevis* and *Pediococcus damnosus* in wheat beer these strains seemed to be suitable. For high accuracy the exact D- and z-values of the used strain had to be determined using the same medium as in the CRT. These trials with the capillary method resulted in a $D_{60\,°C}$-value of approximately 2 s in MRS bouillon or rather in dilution solution (data not shown). This would mean that the pasteurization temperature has to be considerably below 60 °C to get a measurable surviving cell count after the pasteurization. Because a temperature as low as this does not accord to practical conditions of beer pasteurization a different Lactobacillus species, a wild strain of *Lactobacillus hilgardii*, was examined. The $D_{60\,°C}$-value of *L. hilgardii* ranges at 0.75 min ± 0.09 (95 % confidence interval). The regression lines of the logarithmic cell count versus the time have a coefficient of

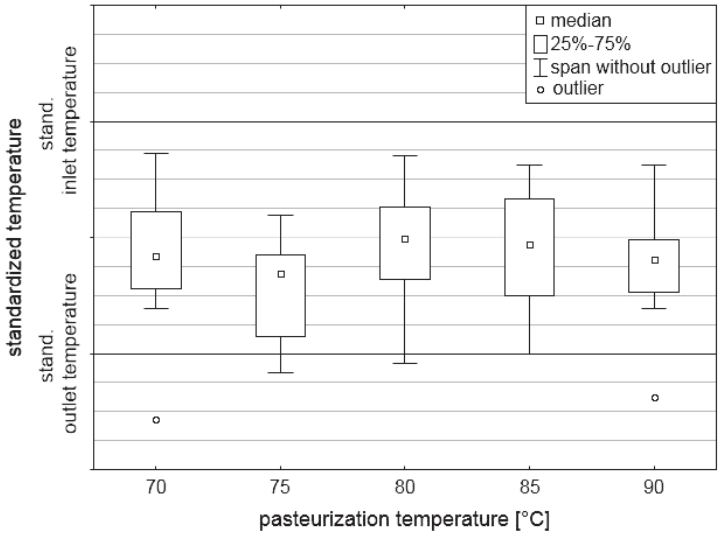

Fig. 4 **The Time-Temperature Integrator was tested in the holding tube of a semi-technical flash pasteurization plant at different temperatures while the inlet and outlet temperatures were recorded. The standardized temperature as ($\frac{\vartheta_{TTI}-\vartheta_{outlet}}{\vartheta_{inlet}-\vartheta_{outlet}}$) for 5 pasteurization temperature levels is pictured (each temperature n = 15). Represented is the box and whisker plot with indicated quartiles**

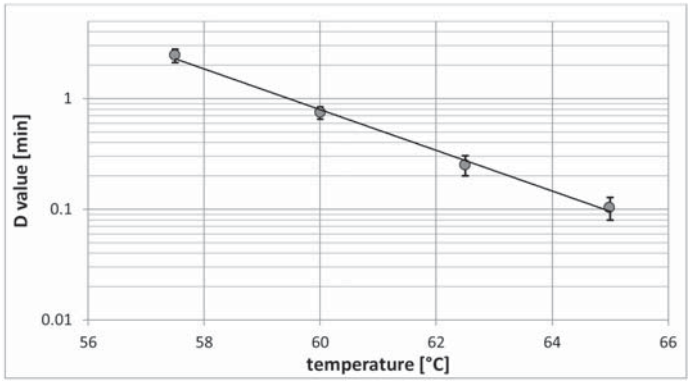

Fig. 5 **D-values of *Lactobacillus hilgardii* at different temperatures. The D-values were determined with the capillary method. The z-value with 5.4 °C follows from the gradient, the regression analysis was performed by the method of least squares (r^2 = 0.996). For each temperature a 5-fold determination was performed, the corresponding 95 % confidence intervals are plotted with vertical lines**

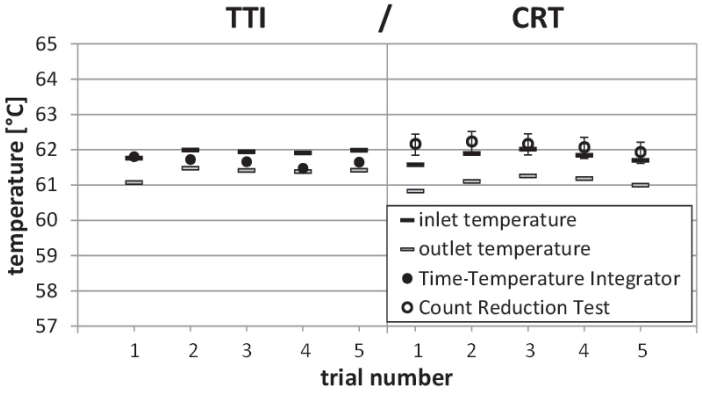

Fig. 6 **Comparative application of the Time-Temperature Integrator (TTI) test (left) and the Count Reduction Test (CRT) (right).The effective temperatures were calculated via the conversion of the sucrose or rather the death ratio of *L. hilgardii* in the holding tube (dots). The comparative temperatures were measured at the inlet and outlet of the holding tube with thermocouples (lines)**

determination between 0.93 and 0.99 thus the reaction first order, is a good match with the thermal death in the investigated ranges (temperature and cell counts).

The D-values plotted versus the temperature is shown in figure 5. In the measured temperature range of 57.5 °C to 65 °C the logarithmic D-values show a linear dependency on the temperature (r^2 = 0.996); a z-value of 5.4 °C results. The determined z-value correlates to an activation energy of rounded 390 kJ/mol. With this, a pasteurization of 45 to 75 s at 61 °C results in a reduction within the holding tube of about 2–3 decades respectively. Hence, with a starting cell count of 10^7 CFU/mL, a surviving cell count above the detection limit of the method is ensured.

3.3 TTI test in comparison with the Count Reduction Test

The TTI test and the Count Reduction Test with *L. hilgardii* were carried out at a (target) temperature of 61 °C to compare the accuracy of the TTI and CRT, respectively. Both methods were executed with holding times of 45, 60 and 75 s (260, 320, 430 L/h corresponding to Reynolds numbers between 13,000 and 21,000). With both methods – TTI and CRT – a plausibility test was made in the way as described previously. The results of the TTI again points out that the effective temperatures are in between the inlet and outlet temperatures measured with the probes (Fig. 6). However, all mean temperatures calculated from the CRT are above the measured inlet temperatures and thus outside of the plausible span; data for one exemplary flow rate are shown in figure 6.

The resulting PUs can be calculated with equation 1. For comparison purposes, the common method of PU calculation (state of the art) is calculated additionally. For this, the measured outlet temperature and the z-value from the beer formula (6.9 °C) were used. This PU is compared with the PU derived from the temperature resulting from the TTI test and the CRT respectively, shown in figure 7. The PU from the TTI is 18 % higher than calculated with the outlet temperature and the PU from the CRT is 32 % higher.

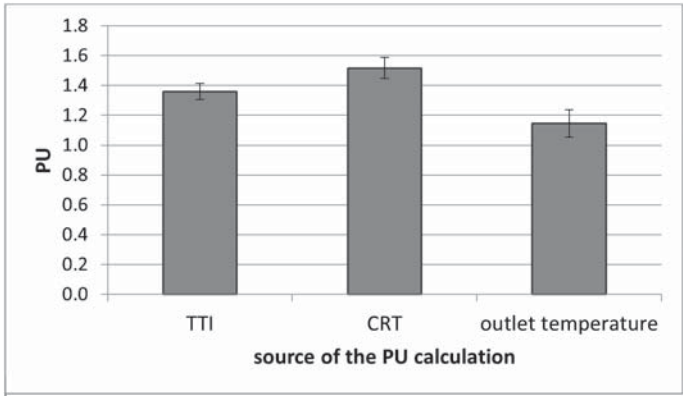

Fig. 7 **Pasteurization Units (PU) calculated from the Time-Temperature Integrator test, the Count Reduction Test (CRT) and the simultaneously measured outlet temperature**

4 Discussion

As mentioned, it is not possible to examine the TTI test with a real verification as long as there is no method that can serve as reliable measure. However, the plausibility test with the temperature probes and the comparative plausibility test with the CRT indicate that the TTI provides results that are more plausible than the CRT. Even though, the CRT was calculated with D- and z-values which were determined ad hoc. The results of the CRT show that the thermal death kinetics of microorganisms are influenced by many unpredictable factors. A chemical reaction is influenced by fewer factors and is thus generally more reliable. Of course, this also means that the transmission of a TTI result to the microorganism inactivation is afflicted with uncertainties. However, this applies to the CRT also. Hence, it can be concluded that the CRT is not always more suitable, solely on the basis that it is a microbiological method.

A crucial issue and a general question that needs to be discussed is whether it is at all possible to compare the reaction kinetics of chemical reactions and microbiological inactivation processes. This question arises due to the differing activation energies (E_A) for thermal death of microorganisms and the sucrose hydrolysis in the TTI (250–400, 105 kJ/mol respectively). As long as the temperature remains approximately constant, as is the case in the holding section, the differences in the E_A have no consequences and the results of the TTI can be transposed to microbiological conclusions under considerations of the above mentioned uncertainties. The temperature deviates here to a small extent, which does not affect the result significantly (maximal 0.2 %). However, in the heating and cooling section the situation is different. The reaction rate constant (k) depends beside others on E_A. Exemplary the consequences for the rate constant k is illustrated for a linearly increasing temperature (40 to 72 °C) in figure 8. The calculated mean temperatures with E_A corresponding to the TTI and microbiological inactivation differ (60.5 and 63.2 °C respectively) as indicated in the figure as single dots.

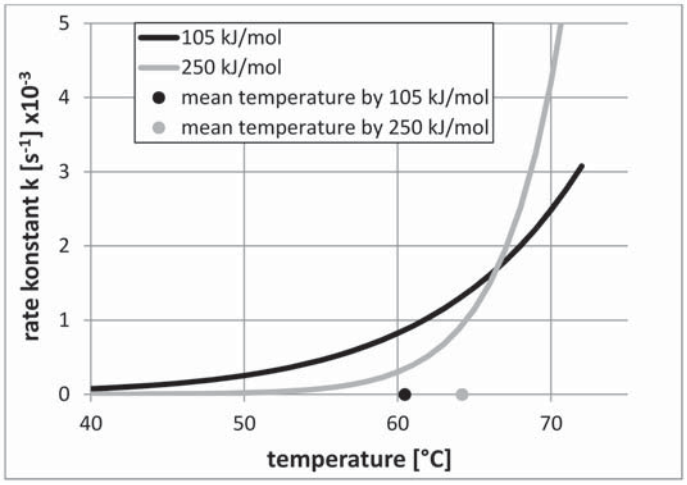

Fig. 8 Impact of different activation energies on the reaction rate constant k and effective temperature. Shown is a simplified example calculated with a theoretical linearly increasing temperature (40 to 72 °C) and two different E_A levels (105 kJ/mol and 250 kJ/mol representing TTI and CRT respectively). For the calculation of the effective temperature, first the total conversion ratio is calculated. From the result, a constant temperature is estimated that would result in the same conversion ratio

The example points out that the TTI would underestimate the real effect on the thermal death of microorganism and thus a TTI test provides conservative results even if the heating section (no constant temperature) is taken into account. Only the faster rise at higher temperatures of the reaction rate constant with higher activation energies induces this deviation, thus it is independent from the temperature level. The relevance of this unavoidable inaccuracy depends on the relative contribution of heating and cooling section to the total heat load. This contribution ranged in respect of the pilot plant at 61 °C, of between 8 and 15 % of the PU occurring in the heating section. The percentage depends on the total PU, the pasteurization temperature and the volume ratio. Pasteurization plants differ widely in the relative volume of the heating and cooling section to the volume of the holding section. In the used pasteurization pilot plant the heating section was comparably small, heating : holding ratio (3 : 5) compared to the ratio in industrial scale plants of up to 4 : 1. The same also counts for the cooling section. This must be considered in the application of the TTI test in large-scale plants.

5 Conclusion

The acidic hydrolysis of sucrose has been revealed as a plausible method to determine the real heat load in a flash pasteurizer. In comparison with the common microbiological test method, the Count Reduction Test, the TTI is significantly more reliable. The theoretical consideration indicates that the TTI test results represent the best available approach to measure the heat load. This can be useful in the practice for performance tests of plants and for challenging purposes in the sense of a more gentle pasteurization.

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