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Gushing Laboratory Tests as Successful Methods for Obtaining New Cognitions on Gushing

This work investigated the gushing phenomenon in wort samples by applying commonly used gushing tests (Weihenstephaner and modified Carlsberg test) in order to identify conditions for the development of gushing. The mashing and boiling procedures were modified (Weihenstephaner test), and the amount of wort and time of shaking of the bottle were increased (modified Carlsberg test). For the experiments one malt sample was applied that was identified as gushing-positive (Weihenstephaner test). A changing pattern of the mashing process revealed that gushing was induced after heating up to 97 °C while no gushing was observed until 80 °C. Heating up the mash from 80 °C to 100 °C and boiling it for 5 min afterwards sufficed for gushing to be induced in the wort sample. From the applied malt sample it was possible to produce not only a gushing-positive wort but also a gushing-negative one. By mixing these wort samples, gushing could be reduced, or even entirely suppressed, applying a volume of only 10 % v/v of the gushing-negative sample. The gushing-positive wort could only induce gushing in amounts of above a critical level. Gushing started to develop after a mild shaking for 10 h (modified Carlsberg test) and increased significantly after 20 h of shaking. The results demonstrated that the temperature of the mash is a decisive parameter for the development of gushing.

Descriptors: gushing, beer, Weihenstephaner test, modified Carlsberg test, mashing and boiling procedure, wort concentration, shaking duration

1 Introduction

Gushing or spontaneous overfoaming of filled carbonized beverages is still a quality problem for the brewing and beverage industry. From experience it is known that gushing is a multicausal phenomenon, as the raw materials and diverse interacting factors during the production process influence the gushing affinity of the beer products [1–5]. These circumstances make it difficult to introduce effective measures to at least get along with this problem. Research in the past has therefore strived to get gushing under control by considering areas in physico-chemistry, biology, microbiology, agriculture and brewing or beverage technology [6–12]. Gushing research has shown so far that proteins become more important. Molecular research thereby focuses mainly on two classes of proteins, the plant-typical non-specific lipid transfer proteins (ns-LTPs) and the mould specific hydrophobins. Both classes can serve as indicators for cereal and malt grains infection by mould and, therefore, are interesting for assessing the risk of gushing prior to the beer production [6,13–18]. Hydrophobins possess a remarkable surface activity enabling them to accumulate at hydrophobic/hydrophilic interfaces and to self-assemble into highly organized structures. They are able to stabilize air bubbles and foams [19], which makes them interesting as gushing inducing substances. In a previous study [15] it was found that proteins, e.g. ns-LTP1, induce gushing when they are glycosylated and degraded by proteolysis during the brewing process. These surface active peptides are most likely responsible for gushing.

For the brewer it is important that gushing can be prevented beforehand. Therefore, estimating the gushing characteristics of malt is an important quality assurance measurement to assess the gushing risk of the subsequent beer products. For this purpose, two industry-acknowledged gushing tests are applied, the Weihenstephaner test and the modified Carlsberg test. By these tests the gushing potential of malt can be characterized [20–23]. For this, malt extract containing no hop products is carbonized (Weihenstephaner test) without further process steps for the production of beer such as fermentation, or the malt extract is added to table water already containing CO_2 (modified Carlsberg test). The overfoaming amount of the CO_2 containing wort after shaking the bottles is determined afterwards and serves as a measure for the gushing potential of the tested malt sample. Therefore, both tests are interesting for research as gushing can be observed in practice by overfoaming of the bottle content containing CO_2. The aim of this work, by applying both gushing laboratory tests, is to gain more insights on the gushing phenomenon, which occurs during the production of wort and during the shaking of bottles. New cognitions on gushing reducing effects allow for the brewing industry to implement effective counter-measures against gushing.

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Tables and figures see Appendix

2 Materials and Methods

2.1 Raw material

The malt sample used for the experiments (malt A) was barley-malt from a German malt house.

2.2 Weihenstephaner test

The Weihenstephaner test was based on the analytical instruction of the gushing rapid test according to MEBAK [20]. The wort produced from the malt sample was investigated for its gushing behavior. For this, fine-grist mash was boiled after a mashing process starting from 20 °C up to 100 °C (see Fig. 1 for a mashing diagram). The solid material of the mash was separated using pleated filter, the wort obtained thereby was carbonized (2.8 bar overpressure, at 4 °C) and filled afterwards into bottles (0.5 l). The bottles were then rotated by 360 degrees prior to opening using the bottle turning device with a frequency of 0.66 Hz (Dr. Thiedig & Co.).

The varied experiments were performed once. For the Weihenstephaner test five or six bottles were applied ($n = 5$ or $n = 6$) in order to obtain statistical significance. The results in figures 5 and 6 are illustrated as arithmetic means including confidence intervals with a probability of $P = 0.95$ (applying Student`s *t*-test) [24].

From malt A, the wort samples A0, A1, A2 and A3 were produced according to the Weihenstephaner test. More details on the produced wort samples are mentioned when explaining the results. The process steps for producing wort A0 were in accordance with the standard mashing process of the Weihenstephaner test as described in the MEBAK instructions (Fig. 1). For wort A1 the mashing (heating phase) was stopped at 80 °C (Fig. 1). Wort A2 was produced in the same way as A1 (mashed at 80 °C); after the separation of the solid material, wort A1 was boiled for 1 h. For obtaining wort A3, the process steps were similar to A1; after the separation of the solid material, a filtration step was performed additionally, applying a membrane with 0.2 µm pore size.

2.3 Modified Carlsberg test

Wort was added to Bonaqa® water bottles containing carbon dioxide (in a ratio of 50 ml to 280 ml). The bottles were then shaken horizontally for 3 days and turned 3-times manually towards the head shortly prior to opening. In figures 2, 3 and 4 one measurement point corresponds to one bottle ($n = 1$).

3 Results and Discussion

3.1 Influence of the amount of wort used in the modified Carlsberg test

The following experiments were designed to investigate the effect of dilution of wort in Bonaqa® water, as it is the case for the modified Carlsberg test. For this, the wort sample (A0) which exihibited strong gushing (according to the Weihenstephaner test, Fig. 6 – 1st bar), was added to Bonaqa® (50 ml of wort, 280 ml of Bonaqa® water). After shaking the bottles the samples showed gushing.

To determine the amount of the wort sample that is needed for gushing to be induced, an array of amounts was applied starting from 1 ml and going up to 50 ml (Fig. 2).

A significant increase of the overfoaming with increasing wort amount, particularly from 5 ml onwards, was observed. Gushing was induced rapidly between 5 and 10 ml, which means that a minimal amount of wort is necessary for gushing to be induced.

During the following set of experiments, the same wort sample (wort A0) was prepared in fixed amounts of 1, 5, 10, 25, and 40 ml and mixed with a non-gushing wort sample by adding up to 50 ml each. These wort samples (50 ml) were then added to Bonaqa® water (280 ml) and the bottles were shaken according to the modified Carlsberg test. The purpose here was to figure out whether the induction of gushing depends on the amount of wort or rather on the concentration of gushing inducing substances which are present in the original gushing-positive wort. As can be seen from figure 3 by comparison with figure 2, the two curves are similar.

Therefore, applying the non-gushing wort shows no influence on the gushing behavior, neither in terms of increasing gushing nor in hampering gushing, which proves in this case that not the amount of wort, but the concentration of respective substances contained in the original wort A0 is responsible for gushing to be induced. The observation of gushing only from a certain concentration of gushing-relevant substances was made in a previous research, investigating hydrophobins. *Home et al.* [25] examined that hydrophobins needed to be present in a minimal concentration before gushing could be induced.

3.2 Influence of the duration of shaking in the modified Carlsberg test

To determine the influence of the shaking duration on the induction of gushing, 50 ml of the content of Bonaqa® bottles (0.33 l) were substituted with 50 ml of wort A0 to run the shaking experiments (modified Carlsberg test). Gushing starts to develop after 10 h shaking and increases significantly after 20 h as can be seen from figure 4. The final overfoaming amount seems to be reached after approximately 3 days (72 h) of shaking.

These experiments point out that, besides of the sufficient presence of gushing inducing substances, gushing in the wort/Bonaqa® solution is only induced after a certain shaking time, which in this case is between ca. 5 and 10 h minimum.

3.3 Modification of the mashing and boiling process (Weihenstephaner test)

In the following the mashing procedure of the Weihenstephaner test was changed by applying different mashing temperatures and boiling times (compare also with Fig. 1, the mashing diagram).

For these experiments, malt A was used and subjected to different mashing programs (Fig. 5):

- Starting from 20 °C, the temperature program for one sample was halted at 80 °C (compare with Fig. 1, wort A1)

- For another two samples, the mashing temperature was increased up to either 97 °C, or 100 °C, applying a boiling time of 5 min for the second sample
- The mashing temperature program between 20 to 100 °C and with increasing the boiling time for up to 1 h. This is the standard mashing process as described in the MEBAK instructions (compare with Fig. 1, wort A0)
- One sample was processed for 5 min boiling, but the mashing temperature started at 80 °C.

If the mashing process is conducted between the temperature interval of 20 °C and 80 °C, then no overfoaming is observed while performing the Weihenstephaner test – figure 5, 1st bar, wort A1. However, once the mash is heated up to 97 °C, the wort sample distinctly exhibits gushing (2nd bar). During the following experiments, the mash was heated up to boiling temperature and the boiling time was varied. Boiling the mash for 5 min (3rd bar) resulted in an overfoaming amount of more than 100 g (3rd bar). After extending the boiling time for up to 1 h, the overfoaming amount increased significantly and reached about 280 g (4th bar). To figure out if the premashing step (mashing between 20 °C and 80 °C) is necessary in order for gushing to be induced, the mashing started already at 80 °C and the mash was boiled for 5 min (5th bar). As can be seen from figure 5, it is sufficient for malt A to heat up the mash from 80 to 100 °C (with a boiling time of 5 min), in order to produce a wort sample that exhibited gushing. The resulting overfoaming amount (5th bar) corresponded to the amount when the mashing started at 20 °C and the mash was boiled also for 5 min (3rd bar). This demonstrates that by mashing at both temperature ranges of 20–100 °C and 80-100 °C (boiling for 5 min, respectively), wort samples are obtained showing nearly identical gushing behavior. Therefore, the pre-mashing step for malt A was not crucial for gushing to be induced, but its subjection to a temperature of above 80 °C was crucial.

During the following experiment it should be investigated, if gushing for malt A is only induced once the mash is exposed to temperatures of above 80 °C, or if it is sufficient that only the wort is subjected to this high thermal exposure. Thereupon, the mashing process was conducted only until 80 °C, and the wort produced thereby, which is known from previous experiments did not show gushing (Fig. 5, 1st bar, wort A1), was boiled subsequently for 1 h (wort A2). The advantage of this subsequent boiling step is that the influence of the thermal exposure (> 80 °C) on the induction of gushing in wort can be evaluated. The additional boiling step for 1 h did not result in gushing to be induced (results not shown here). Another experiment confirmed that gushing in the wort sample produced is only detected once the malt-grist is sufficiently exposed to hot water (T > 80 °C). If entire grains (not milled) instead of malt-grist were added to water already heated at ca. 95 °C and boiled for 1 h, then the liquid obtained thereby did not show gushing upon proceeding with the Weihenstephaner test – only a slight overfoaming could be observed. This demonstrates again the entire content of the grain needs to be freed and exposed to hot water (> 80 °C) in order to develop gushing, as shown here by the example of malt A.

During further experiments, it could also be demonstrated that for the development of gushing, not only the temperature of the mash is one of the driving parameters, but also the amount of malt-grist that is applied for producing the wort. If only 10 % of the usual amount of malt A is used for working with the Weihenstephaner test, then overfoaming is not observed after an extended boiling time of the mash for 3 h (results not shown here). For this procedure, it could be assumed that gushing-relevant substances were present in the wort samples, but not in concentrations above the critical value so that gushing did not occur.

3.4 Reduction of gushing by mixing using a gushing-negative wort sample

In the following, the question should be answered as to what happens if two wort samples are mixed, with one of them exhibiting strong gushing, while the other one shows no sign of gushing at all.As demonstrated previously, the same malt sample (malt A) can be used to produce wort samples that either exhibit strong gushing (wort A0, Fig. 6, 1st bar), or no gushing at all (wort A1, Fig. 5, 1st bar). In figure 6 it is shown how the overfoaming is influenced by mixing gushing-negative wort samples (A1, A2, and A3) in equal amounts together with the gushing-positive wort sample (wort A0). For this, each 10 % v/v were mixed with wort A0 prior to carbonization. The wort mixtures were further processed according to the Weihenstephaner test.

The first bar in figure 6 shows the overfoaming amount of wort A0 without mixing (plain sample) for comparison. By mixing with 10 % of wort A1, gushing was entirely suppressed (2nd bar). If the wort sample A1 is boiled for 1 h, then this results in wort A2, which also did not show any signs of gushing. The mixing of A2 with A0 resulted in clear overfoaming (Fig. 6, 3rd bar). This demonstrated that the gushing reducing characteristic of wort A1 is not remained through the boiling process – gushing is only reduced. If the gushing suppressing wort sample (A1) is not boiled but filtered using a membrane with 200 nm pore size, then the wort sample A3 is produced. By mixing wort A3 with A0, gushing is not suppressed but reduced (Fig. 6, 4th bar). Therefore, not only through boiling but also through membrane filtration, the gushing suppressing effect of wort A1 is at least partly lost.

These results assume that the gushing suppressing wort sample, A1, contains substances with respective characteristics. The suppressing effect can be explained that gushing-repressive substances initiate certain reactions with gushing inducing ones. It could be that fine fractions, e.g. albumins, denaturate during boiling and, thus, loose their characteristics to react with gushing inducing substances. This explains that through boiling of the gushing suppressing wort sample the respective effect is gone and that gushing is only reduced. Not only through boiling but also through separating particles in sizes larger than 200 nm, the gushing suppressing effect was not apparent, but gushing could be reduced significantly, which demonstrates that respective particles (> 200 nm) contribute to the reduction of gushing. Particles below 200 nm are also in capacity to reduce gushing. A suppression of gushing occurs only by a combination of both stated class of particles.

4 Conclusion

The aim of this study was to investigate influencing factors for the development of gushing by focusing on the wort production process. By varying the mashing and boiling procedures of the Weihenstephaner test, it could be demonstrated that gushing can be influenced by the temperature of the mash. For the applied malt sample, gushing can only occur above the mash temperature of 80 °C. Therefore, it was possible to generate worts with gushing-positive and gushing-negative characteristics deriving from the same malt. With these worts it was possible to suppress gushing entirely by mixing low amounts of gushing-negative wort (10 % v/v) to the gushing-positive wort. Other results indicated that gushing can only occur above a critical concentration of gushing-relevant substances as a minimal amount of gushing-positive wort was necessary to observe gushing. Furthermore, it could be demonstrated by the modified Carlsberg test that gushing was only induced in the wort/Bonaqa® solution after a certain time of mild shaking. The obtained new findings on gushing can be utilized for further investigation from the perspective to work out technological measures for the brewing industry in order to control or even prevent gushing in beer.

Acknowledgement

The financial support by „Die Wissenschaftsförderung der Deutschen Brauwirtschaft e.V." is gratefully acknowledged.

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Received 26 May, 2009, accepted 17 June, 2009

Appendix

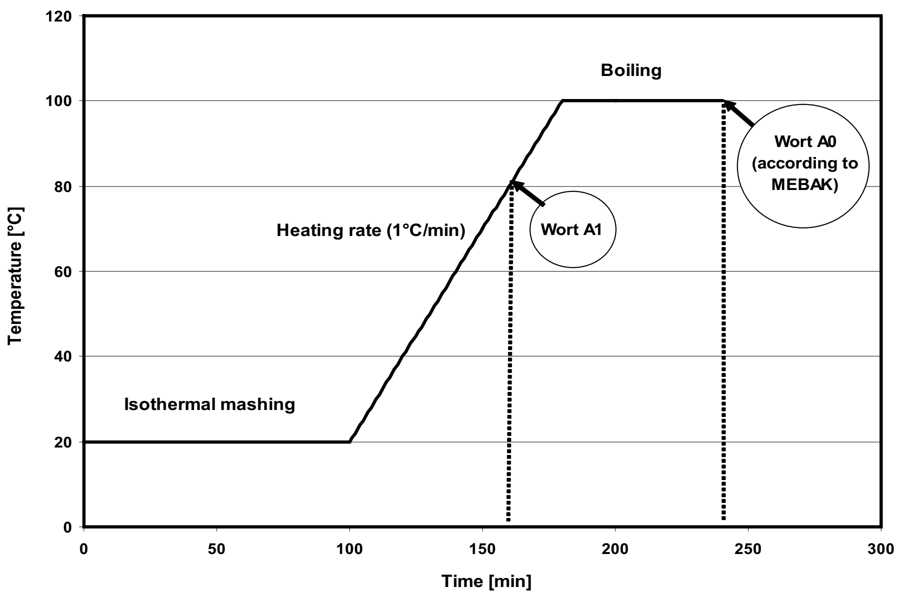

Fig. 1 **Mashing diagram of the Weihenstephaner test as temperature-time course. For the wort sample produced according to MEBAK instruction [20], the entire mash was boiled for 1 h, and designated as wort A0 for malt A. Mashing until 80 °C leads to wort A1 produced also from malt A**

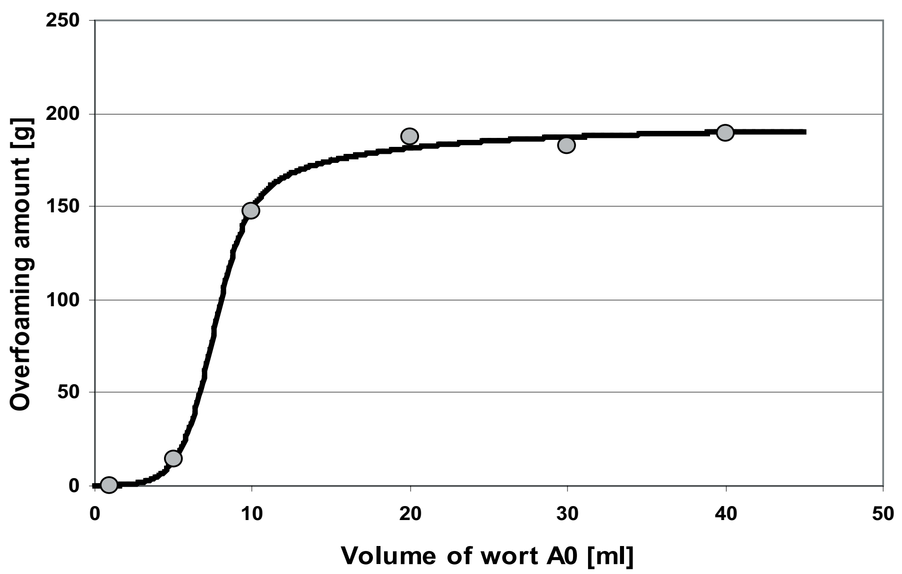

Fig. 2 **Overfoaming amount related to the wort volume (A0) according to the modified Carlsberg test**

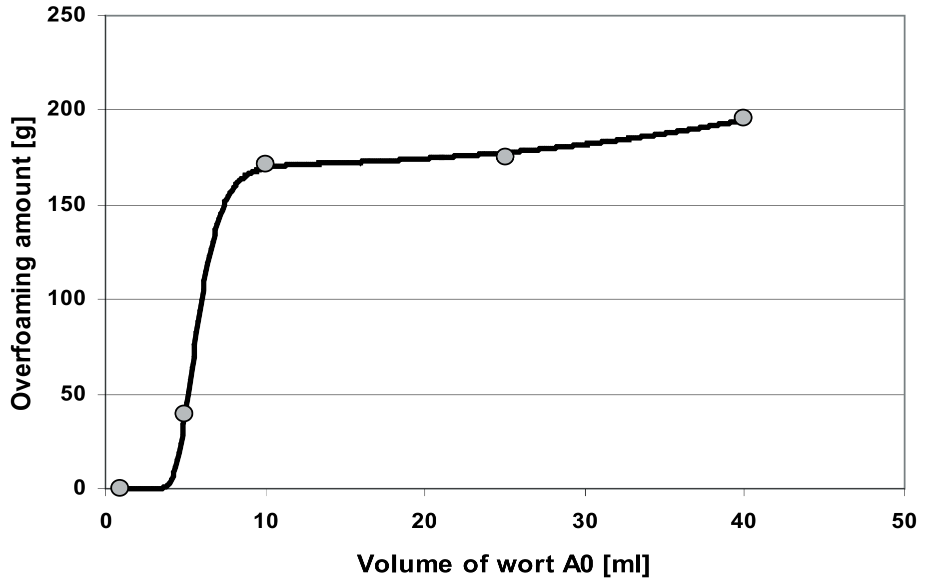

Fig. 3 **Overfoaming amount related to the volume of wort A0 (modified Carlsberg test). Wort A0 was prepared in fixed amounts (1, 5, 10, 25, and 40 ml) and was added up to 50 ml by a non-gushing wort sample**

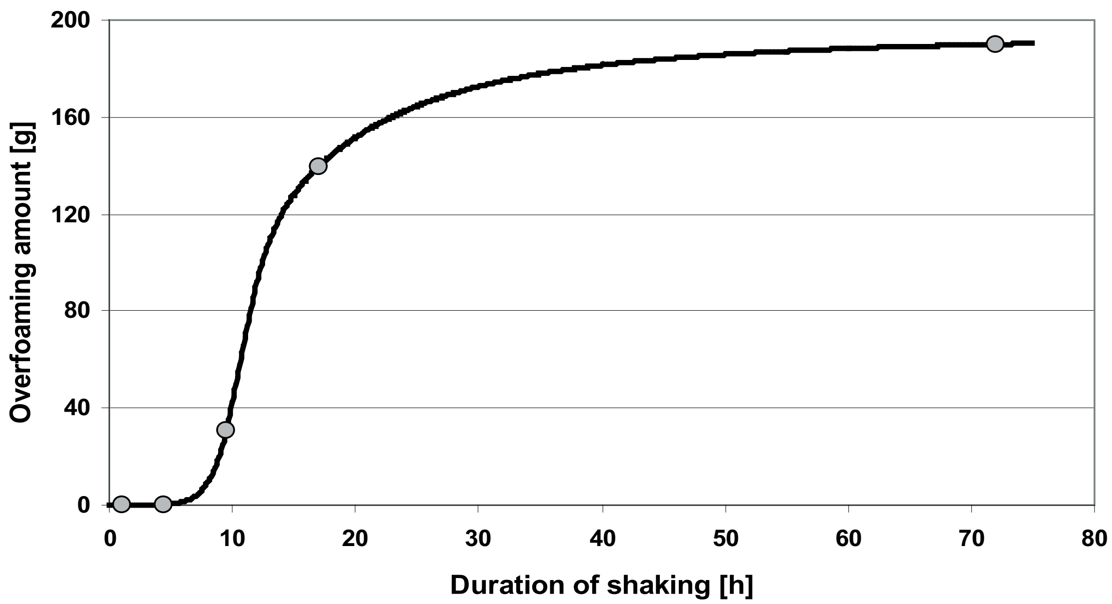

Fig. 4 **Overfoaming amount of wort A0 (50 ml added in 280 ml Bonaqa® water) in relation to the shaking duration (modified Carlsberg test)**

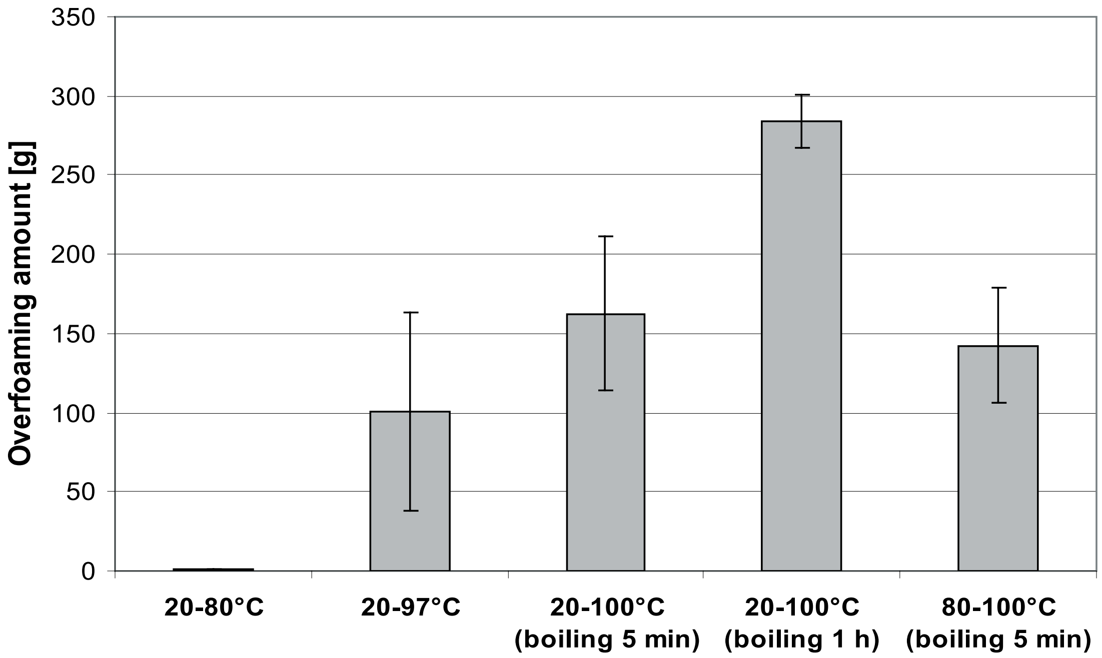

Fig. 5 **Overfoaming amount of worts produced from malt A at different mashing temperatures and boiling times (Weihenstephaner test)**

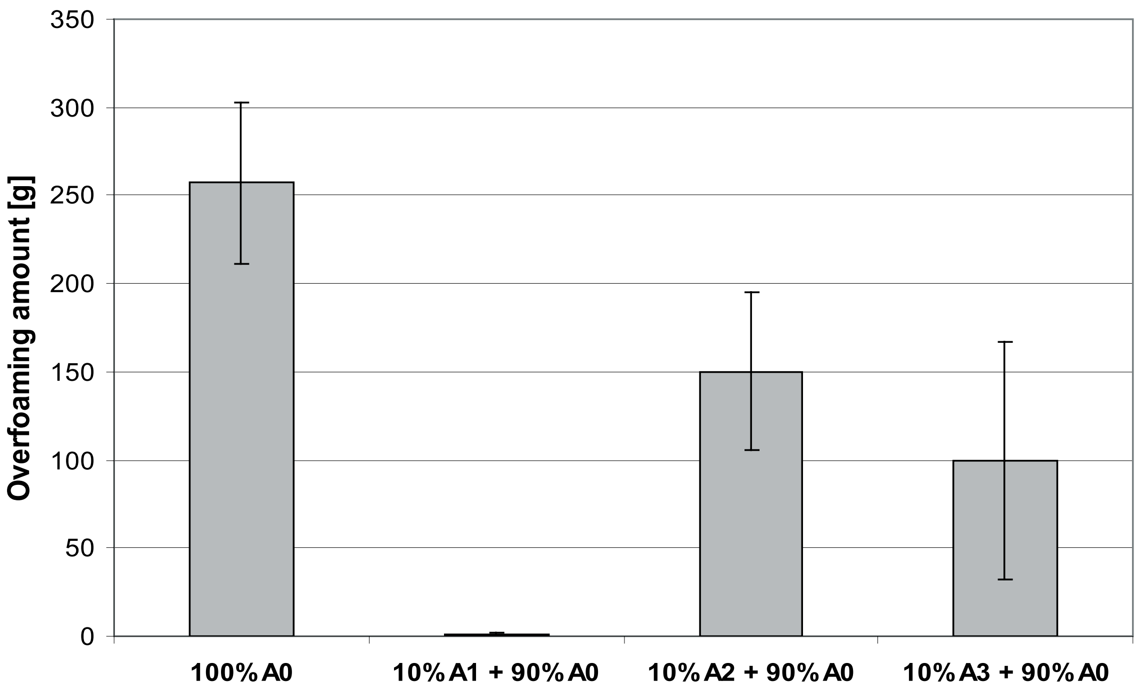

Fig. 6 **Overfoaming amount according to the Weihenstephaner test, using the gushing-positive wort sample (wort A0) either in plain, or in mixture with the wort samples A1, A2, and A3**